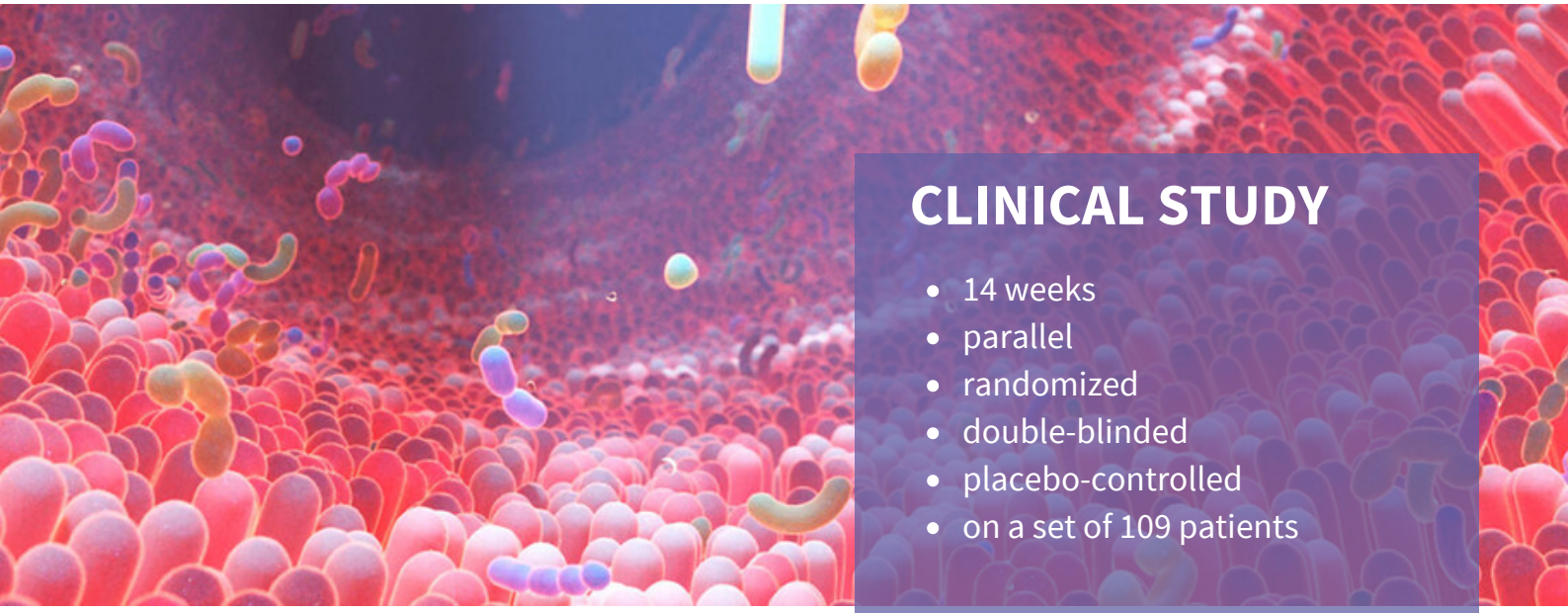
Functional digestive disorders and the microbiome

Targeted combination of 5 immunomodulating probiotic strains and OMEGA3 fatty acids



CLINICAL STUDY

- 14 weeks
- parallel
- randomized
- double-blinded
- placebo-controlled
- on a set of 109 patients

A targeted combination of 5 immunomodulating probiotic strains and OMEGA3 fatty acids

The probiotic bacterial strains in this study were carefully selected based on their scientifically proven positive effects in the management of symptoms of functional digestive disorders.

In 2018, a clinical study of a targeted combination of 5 probiotic bacteria was conducted to evaluate the effect on alleviating the symptoms of functional digestive disorders, primarily irritable bowel syndrome (IBS). The results showed that the investigated probiotic mixture is effective in improving the severity of IBS-type symptoms. After 6 weeks of treatment, a significantly higher rate of treatment success, a reduction in pain perception according to various clinical scales and a modification of the intestinal microbiota, characterized by an increase in bifidobacteria, which are still detectable 6 weeks after stopping probiotics, were found.

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CLINICAL STUDY

Introduction

Functional gastrointestinal disorders (FGID), known as disorders of **the gut-brain axis**, are characterized by significant changes at the level of the intestinal microbiota profile, namely a decrease in biodiversity, a significant decrease in the number of bifidobacteria and lactobacilli, as well as an increase in the number of potentially pathogenic bacteria (1).

The resulting **dysbiosis** often leads to a violation of the integrity of the intestinal epithelium, to visceral hypersensitivity and abnormalities of intestinal motility, which increases **the dysfunction of the intestinal barrier**.

FGID includes, among other things, **irritable bowel syndrome (IBS),** functional dyspepsia (FD), or **a spectrum of symptoms** such as abdominal pain/burning, bloating, nausea, fullness, vomiting, and altered bowel habits such as diarrhea, constipation, or both.

This clinical study revealed that **a probiotic mixture of lactobacilli and bifidobacteria** is effective in reducing the severity of IBS-like symptoms by modifying the gut microbiota, primarily by increasing the number of bifidobacteria and lactobacilli. The severity of IBS symptoms according to selected rating scales was significantly reduced in the probiotic group compared to the placebo group as well as at the beginning and end of the clinical trial.

Goals

The primary objective of the clinical trial was to evaluate the efficacy and safety of the probiotic mixture in patients with symptoms of **irritable bowel syndrome.**

Methodology

Patients with IBS-type symptoms entered a prospective, double-blinded, randomized, placebo-controlled, fourteen-week study.

The six-week treatment period (3-8 weeks) was preceded by a two-week adaptation phase and followed by a 6-week observation phase.

Clinical data were monitored throughout the study with validated questionnaires: **IBS Severity Scoring System (IBS-SSS); Gastrointestinal Symptom Rating Scale (GSRS) and Bristol Stool Form Scale (BSFS).** Faecal microbiota was assayed by culture and 16S rRNA sequence analysis (1).

Table 1. *Basic characteristics of study participants*
**Median (range)*
†Normal TTG-IgA value <10 IU/ml
BMI, body mass index; CI, confidence interval; NS, not significant; TTG-IgA, tissue transglutaminase - immunoglobulin-A. Adapted from Francavilla et al., 2019

	Probiotic (n = 54)	Placebo (n = 55)	P
Age	43.3± 19	44.6± 19	NS
Sex (m/f)	6/35	9/46	NS
BMI kg/m2	22.8±3.5	23.4±2.9	NS
TTG-IgA (IU/mL)*†	0.8 (0-1.2)	0.5 (0-2.1)	NS
IBS-SSS	295± 84.9 (95% CI, 26.9-32.0)	237.6±86.5 (95% CI, 13.4-27.5)	0.01
GSRS	18.7± 5.8 (95% CI, 14.6-26.1)	14.9± 5.1 (95% CI, 13.4-27.5)	0.02
BSFS	2.6± 1.2	2± 1.5	NS

Results

1) IBS Severity Scoring System (IBS-SSS)

Severity of IBS symptoms according to the IBS-SSS rating scale in the probiotic group was significantly reduced compared to placebo (-21.4%± 15.5% vs. -6.8%± 21.7%; p <0.01) (Figure 1) (1).

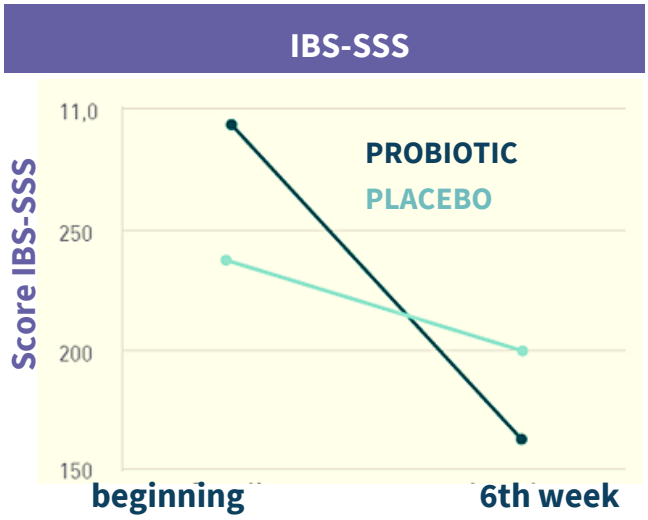


Figure 1. *Assessment of IBS symptom severity.*

2) Gastrointestinal Symptom Rating Scale (GSRS) and Bristol Stool Form Scale (BSFS)

GSRS IBS symptom severity was significantly **reduced** in the probiotic group compared to placebo (-19.8% ± 16.6% vs. 12.9% ± 31.6%; p < 0.001)(Figure 2) (table 2).

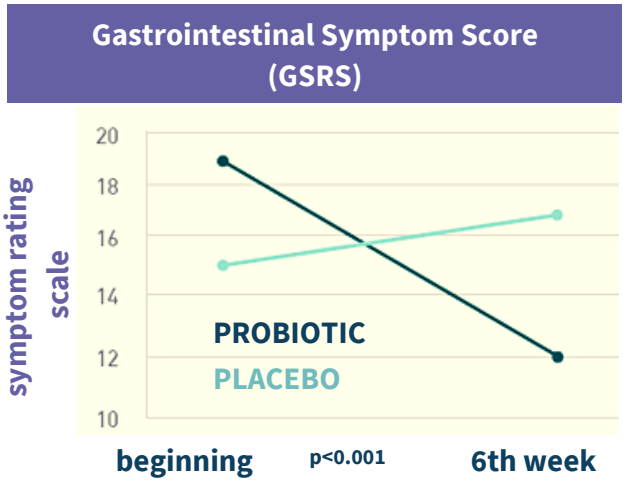


Figure 2. *Evaluation of the severity of gastrointestinal symptoms.*

At the end of treatment, BSFS scores were significantly reduced in the probiotic group compared to placebo (-3.3% ± 41.6% vs. 51.5% ± 101.1%; P < 0.01) (1).

Clinical score	Probiotic (n=54)	Placebo (n=55)	P
IBS-SSS	170.1± 53.4 (95% CI, 154-187)	200.8± 74.4 (95% CI, 179-223)	0.008
ΔoZ	-15.9%± 14,8% (95% CI, −20.4 - −11.4)	8.2%±25.9% (95% CI, 0.3-16.2)	0.001
GSRS	12.2±5.5 (95% CI, 11.6-14.9)	16.7±6.7 (95% CI, 14.5-18.8)	0.007
ΔoZ	−19.8%±16.6% (95% CI, −24.8 - −14.8)	12.9%±31.6% (95% CI, 3.2-22.6)	0.001
BSFS	2.2 ±1.3	3.1 ±1.9	NS
ΔoZ	−3.3%±41.6% (95% CI, −16 - 9.3)	51.5%±101.1% (95% CI, 20.4-82.6)	0.01

Table 2. *Clinical score at the end of treatment; CI, confidence interval; NS, not significant; ΔoZ change from baseline, adapted from Francavilla et al., 2019.*

(3) Evaluation of fecal culturable bacteria and microbiome

Compared to baseline, total anaerobes increased from 7.02 to 8.35 log CFU/g (mean values, P=0.018) after 6 weeks of probiotic treatment. Treatment with probiotics also leads to an increase in putative lactic acid bacteria (Lactobacillus, Lactococcus, Streptococcus, Staphylococcus and Bifidobacterium) (Figure 3). Compared to the baseline value, a higher level of putative bifidobacteria was also detected after 6 weeks of probiotic treatment (Figure 4).

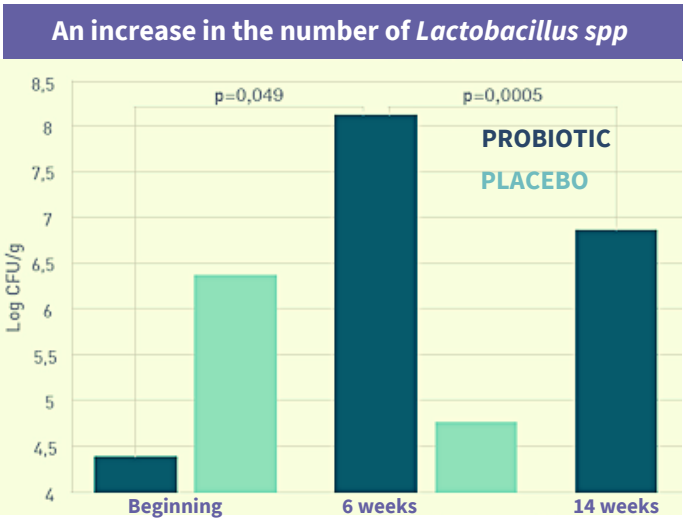


Figure 3. *Increase in the number of lactobacilli in the placebo group vs. in the group with probiotics.*

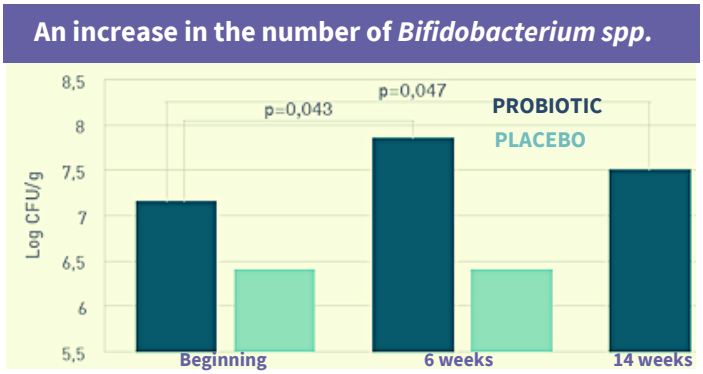
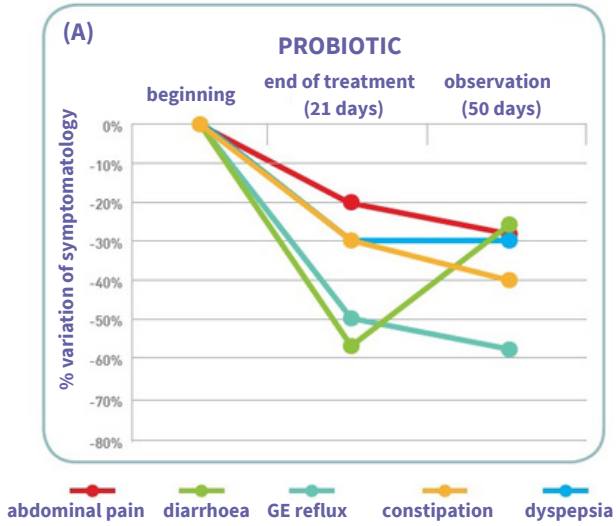


Figure 4. *Increase in the number of bifidobacteria in the placebo group vs. in the group with probiotics.*

Treatment success

Treatment success was significantly higher in patients taking probiotics compared to placebo in an intention-to-treat analysis (14.8% vs. 3.6%; $p < 0.04$) and according to protocol (15.3% vs. 3.8%; $p < 0.04$) (Figure 5 A and B) (1).



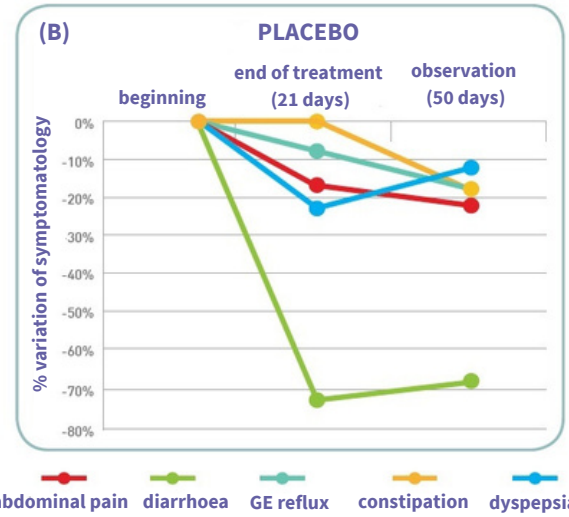


Figure 5. *Evaluation of symptomatology variation in the probiotic group (A) and the placebo group (B).*

IN-VITRO STUDIES

Celiac disease is an autoimmune enteropathy caused by intolerance to gluten proteins. An in vitro study from 2020 is aimed at evaluating the ability of a targeted combination of 5 probiotic bacteria to hydrolyze gluten peptides after simulated gastrointestinal digestion of gliadin in Caco-2 cells.

The ability of bacterial hydrolysates to counteract the toxic effects of gliadin-derived peptides in Caco-2 cells was also evaluated. The results of this study indicate that these probiotic strains are able to reduce the toxicity of gliadin, which remains after peptic-tryptic digestion by degrading immunodominant gliadin peptides, thereby inhibiting their harmful effects on intestinal epithelial cells and thus may have a protective role in celiac disease.

Goals

The primary objective was to evaluate the ability of a targeted combination of 5 probiotic bacteria to digest gliadin peptides in vitro and to modify gliadin-induced pro-inflammatory response and changes in epithelial structure in human intestinal Caco-2 cell lines (2).

Methodology

An in vitro study observed the proteolytic activity of selected probiotic strains in aliquots with a gliadin/enzyme complex (PT-gliadin, P: pepsin, T: trypsin or chymotrypsin w/w, ratio 1:40) as well as with raw gliadin compared to a control sample without probiotic bacteria (2).

Results

1) Proteolytic activity of a targeted combination of 5 probiotic bacteria

The results of **gliadin analysis** by **SDS-PAGE** indicate that after the first enzymatic cleavage by a combination of pepsin and trypsin (PT, lane 3) or chymotrypsin alone (chymo-, lane 4), the selected probiotic bacteria are able to further cleave gliadin into fragments with a molecular weight of less than 35kDa (track 6,7). Moreover, gliadin untreated with digestive enzymes (lane 2) appears to be partially digested by bacteria (lane 5), which speaks in favor of their proteolytic activity even without prior digestion (Figure 6). Thus, the results suggest that probiotics can help with the digestion of gliadin (2).

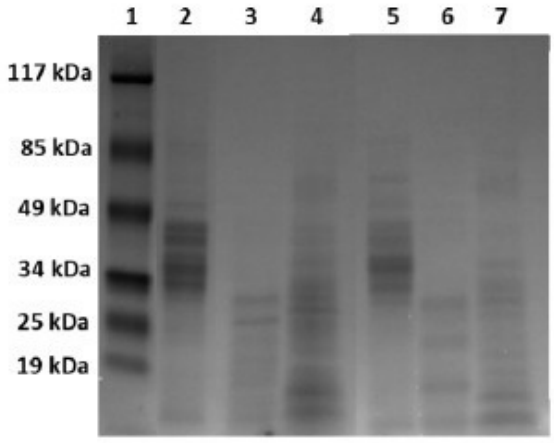


Figure 6. *Analysis of gliadins by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). (1) Molecular weight (MW) markers; (2) Gliadin standard; (3) PT-gliadin; (4) chymo-gliadin; (5) gliadin + bact.; (6) PT-gliadin + bact; (7) Chymo-gliadin + bact. Adapted from Giorgi et al., 2020.*

2) Effect of PT-Gliadin and PT-Gliadin/Probiotics on Caco-2 tight junctions

The results show that untreated Caco-2 monolayers had the typical localization of ZO-1 and occludin at the cell periphery (3), whereas PT gliadin treatment for 3 h induced a marked decrease in ZO-1 fluorescence and a redistribution of occludin at the cytosolic level, which may lead to weakening the tight junctions of the barrier and increasing its permeability. When cells were treated with PT-gliadin/probiotics, neither ZO-1 release nor occludin redistribution was observed, suggesting the ability of probiotics to protect tight junctions (Figure 7).

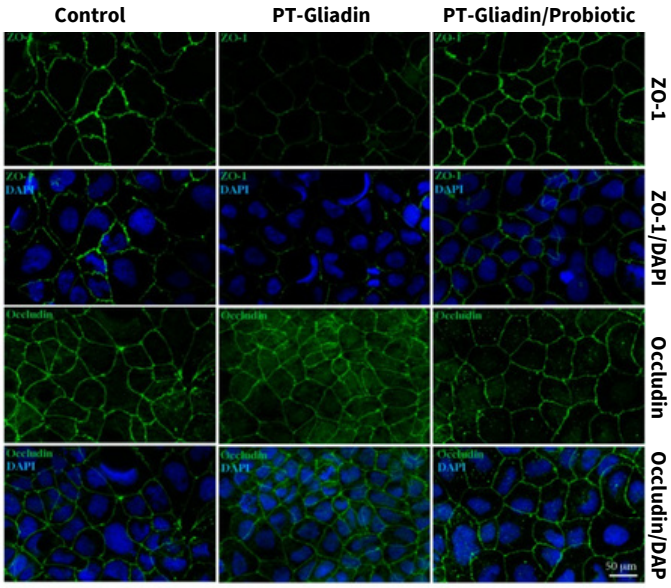


Figure 7. *Caco-2 cells stained to detect zonulin (**ZO-1**) or **occludin.** Cells were treated with 1 mg/ml PT gliadin or PT-gliadin/probiotic, and after 3 hours of treatment, cells were fixed and stained with antibodies against ZO-1 or occludin and 4',6-diamidino-2-phenylindole (**DAPI**) to counterstain nuclei. . Adapted from Giorgi et al., 2020.*

3) Modulation of IL-6 production and oxidative stress by Caco-2 cells exposed to bacterially digested or undigested PT-gliadin

PT-gliadin was able to cause a significant increase in IL-6 production compared to the control. In the presence of PT-gliadin/probiotics, no increase in IL-6 could be observed, instead a significant decrease in IL-6 production was observed compared to control cells, indicating an anti-inflammatory protective effect of probiotics (2).

OMEGA-3 PUFA:INCREASING PROBIOTICS EFFECTIVENESS

The combination of probiotics with synergistically acting components of natural origin is a suitable way **to increase the effectiveness of probiotic preparations**. It seems that a number of suitable components, such as **polyunsaturated fatty acids (PUFA)**, can be used to potentiate the effect of probiotics. Their presence supports the adhesion of probiotic bacteria (e.g. Lactobacillus spp.) to the receptors of the intestinal mucosa (4).

Omega-3 PUFAs may have a positive effect by altering the composition of the microbiota in these diseases and increasing the production of anti-inflammatory compounds such as short-chain fatty acids (acetate, propionate, butyrate). Furthermore, accumulating evidence in animal model studies suggests that the interplay between gut microbiota Omega-3 fatty acids and immunity helps maintain the integrity of the gut wall and interacts with host immune cells. Finally, human and animal studies have highlighted the ability of Omega-3 PUFAs to influence the gut-brain axis through the composition of the gut microbiota (5).

Conclusion

Current research indicates that the severity of the symptoms of functional digestive disorders, especially IBS, can be alleviated by supplementing with a probiotic mixture of lactobacilli and bifidobacteria by modifying the intestinal microbiota and correcting dysbiosis.

The results of a clinical as well as in vitro study indicate that selected strains of lactobacilli and bifidobacteria are suitable for patients with functional digestive disorders such as IBS as well as for celiacs due to their antioxidant and anti-inflammatory activities and their role in restoring the intestinal barrier. Additionally, the selected bacterial strains target directly **the symptoms associated with HIT, do not produce histamine** and so they are also suitable for people with histamine intolerance (HIT).

The effect of the purposefully selected 5 probiotic strains of bacteria can be strengthened by synergistically acting polyunsaturated fatty acids (PUFA) Omega3-EPA and Omega3-DHA, which support the production of anti-inflammatory compounds and help the probiotic bacteria to adhere to the intestinal mucosa.

References

(1) Francavilla R, *et al.* Clinical and Microbiological Effect of a Multispecies Probiotic Supplementation in Celiac Patients With Persistent IBS-type Symptoms: A Randomized, Double-Blind, Placebo-controlled, Multicenter Trial. J Clin Gastroenterol. 2019 Mar;53(3):e117-e125.

(2) Giorgi A, *et al.* A Probiotic Preparation Hydrolyzes Gliadin and Protects Intestinal Cells from the Toxicity of Pro-Inflammatory Peptides. Nutrients. 2020 Feb 14;12(2):495.

(3) Lei S, *et al.* Somatostatin ameliorates lipopolysaccharide-induced tight junction damage via the ERK-MAPK pathway in Caco2 cells. Eur. J. Cell Biol. 2014, 93,299-307.

(4) Bomba A, *et al.* Improvement of the probiotic effect of micro-organisms by their combination with maltodextrins, fructo-oligosaccharides and polyunsaturated fatty acids. Br J Nutr. 2002 Sep;88 Suppl 1:S95-9.

(5) Costantini L, Molinari R, Farinon B, Merendino N. Impact of Omega-3 Fatty Acids on the Gut Microbiota. Int J Mol Sci. 2017 Dec 7;18(12):2645.

Targeted probiotics

They bring positive results in studies for:

- **functional digestive disorders**
- **coeliac disease**
- **HIT**
- **GE reflux**
- **dyspepsia**
- **biodiversity**

HIT for Digestion+
contains probiotic strains purposefully selected on the basis of scientific research and clinical studies aimed at monitoring the symptoms of functional digestive disorders, celiac disease and HIT.

This information has not been evaluated by the Department of Health of the United Kingdom. Neither the information nor any formulas provided are intended to diagnose, treat, cure or prevent any disease.