Diet, Microbiota and Inflammatory Bowel Disease: Review

ABSTRACT

Propose: Verify the possible associations between dietary components and the intestinal microbiota in clinical parameters of inflammatory bowel disease.

Methodology: In this review, a search in PubMed and Bireme databases was performed. We included randomized clinical trials published between 2005 and 2017, only in adult humans with CD or UC.

Findings: Six articles were included by the end of the search. The most widely used intervention was the use of prebiotics, including fructooligosaccharides or fructooligosaccharides with inulin, followed by probiotics. The main findings regarding the microbiota were the increase in the total amount of bacteria and variability (phyla). Clinically there was improvement in inflammation, seen in parameters such as C-reactive protein, interleukins and tumor necrosis factor alpha.

Originality: Dietary interventions, especially from symbiotics, can modulate the microbiota, mainly in relation to time, when compared pre and post supplementation, and this positively interferes with clinical parameters of IBDs. However, the studies were quite heterogeneous in population, methodology, intervention, mycobiota analysis and inflammatory markers.

Keywords: microbiota, prebiotics, probiotics, inflammatory bowel disease, dietary supplements, review.

INTRODUCTION

Inflammatory Bowel Diseases (IBD) are a group of chronic gastrointestinal tract disorders, affecting more than 3.6 million people worldwide (Edward et al. 2004), with cases in North America and Europe doubling every decade. In developing countries, IBDs have expanded since the 1990s, with a higher incidence of ulcerative colitis (UC) than Crohn's disease (CD) (Molodecky et al., 2012). The IBDs mainly include UC and CD (Ye et al, 2015), these two phenotypes with distinct descriptions, UC being a continuous, mucosal limited inflammation located in the colon and CD characterized by discontinuous, transmural inflammation and may involve any part of the Intestine, but both tend to affect adolescents and young adults (Whelan and Quigley, 2015). Its pathogenesis is not yet well understood, but several factors have been related: genetic, environmental, immune and more recently intestinal microbiota and diet. The latter two are modifiable factors that are related to both prevention and treatment of the disease (Lee et al., 2015).

The bowel microorganisms have effects on gastrointestinal physiology, as well as on pathologies, but these mechanisms are still unclear. The human microbiota is composed of approximately one thousand different species, including bacteria, fungi, bacteriophages and viruses, which live synergistically with the host (Scaldaferri et al., 2013). Current studies suggest that the altered profile of the intestinal microbiota is related to pathogenesis of IBD, characterized by low density of beneficial genera and high density of invasive genes. (Zhou et al, 2017). Microbial predominance includes 4 different phyla, the *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria*, and the first two make up 90% of the intestinal flora (Dethlefsen et al., 2007; Barbara et al., 2016). Results from several studies have shown that commensal bacteria such as *Escherichia coli*, *Bacterioides*, *Enterococcus* and *Klebsiella* are involved in intestinal inflammation and in pathogenic proprieties (Lakatos et al., 2006; Yang and Jobin, 2014), whereas species of *Lactobacillus*, *Bifidobacterium* and *Lactococcus* promote intestinal barrier integrity, prevent bacterial translocation in the gut and reduces inflammation (DeGruttola et al, 2016). In addition, changes in the microbiological profile in these diseases include the reduction of the diversity of the microbiota and the phylum *Firmicutes* (especially in the *Clostridium leptum* group, highlighting the *Faecalibacterium prausnitzii*) and increase of *Proteobacteria* (Matsuoka and Kanai, 2015). Finally, recent evidence suggests that diet influences the composition of the

intestinal microenvironment, which plays a key role in the syntactic functions, that can affect the immunity and metabolism of individuals and, consequently, their susceptibility to disease (Barbara et al., 2016).

The diet and in particular some specific components such as vitamins, amino acids and short chain fatty acids may aid in regulating the immune function of the intestinal mucosa (Brestoff and Artis, 2013). Pre, pro and symbiotics can also be used in an attempt to modulate the intestinal microbiota and contribute to the treatment of IBDs. The WGO (2011) guidelines define probiotics as living microorganisms that when administered in appropriate amounts confer benefit the health of the host. The most widely used are Lactobacillus and Bifidobacteria that can be included in the preparations of products such as foods and supplements for dietary habits. In IBDs, probiotics may increase biodiversity and improve intestinal symptoms, and may suppress inflammation and / or activate innate immunity and assist in treatment (Saez-Lara et al., 2015). Prebiotics, such as oligofructose, inulin, galactooligosaccharides and lactulose, are food substances that nourish a specific group of microorganisms in the intestine, favoring a larger scale growth of beneficial bacteria and can be included as ingredients of food products. In this way, they can reestablish the balance of the intestinal microbiota and benefit the host's health. Within this context, there are still symbiotics, which are appropriate combinations of prebiotics and probiotics, and therefore exert the effects of both dietary components (WGO, 2011).

Several studies have questioned the influence of diet affecting the intestinal microbiota, but only in recent years have been obtained consistent data on this subject, investigating the use of probiotics, prebiotics and symbiotics to change the composition of the microbiota to replace or increase conventional IBD therapies (Ghouri et al, 2014), whereas evidence suggests that lifestyle and diet are determinants of intestinal function and composition (Barbara et al., 2016). Thus, the objective of this study was to verify the influence of dietary interventions on the intestinal microbiota and, consequently, on IBD, through a review.

MATERIALS AND METHODS

The review included original studies, published until January 2017, on dietary intervention in humans, whenever there was an evaluation of intestinal microbiota and

clinical parameters in IBD, necessarily both parameters should be evaluated to enter in our review. Non-original articles (revision, editorial and letter) were excluded, with a sample without IBD, pathology not associated with IBDs, and articles that did not address the outcomes of interest. Outcomes of interest were changes in the intestinal microbiota and clinical parameters associated with IBD in the study participants. In addition, there was no limitation on the language of the publication.

We performed a literature search in eletronic databases PubMed and Bireme, during the months January and February of 2017. The search terms used were “microbiota", "inflammatory bowel disease”, "food", "diet", therapy, diet" and "randomized controlled trial" and these terms of reference have been cross-referenced.

The articles were initially selected by two reviewers (BLR and AS) reading the titles and then the abstracts. After analysis of inclusion and exclusion criteria, the complete texts of the selected articles were interpreted, and the eligible studies were identified. If there were disagreements between the reviewers, they were discussed with a third reviewer (PBZ) and clarified by consensus with the group's senior reviewer (VDA).

In order to aid in the identification of information about the articles, the data were presented in summary form, in a table, including: authors' names, year of publication, number of individuals included, dietary component used, duration of treatment, time of follow-up, changes in the intestinal microbiota and in the clinical parameters of IBD.

RESULTS

In the initial search with the descriptors 196 articles were found. After the removal of duplicates remained 162 articles. Of these, 84 articles were excluded because they were not with humans or not published in the last decade, totalizing 78 studies for eligibility. Subsequently the titles and abstracts were read (28 were not RCTs and 44 did not evaluate outcomes of interest). Finally, 6 articles remained, which are described in Tables 1, 2, 3 and 4.

The impact factor of the selected publications ranged from 2.71 to 14.92 of which 3 were over 14 and all were in the English language. The year of publication varied from 2005 to 2013 and most of the studies were published in 2011 and 2012. All

articles were performed with humans and were RCT. The total human sample was 277, with the median human intervention time being 4 weeks. The most frequent intervention was with prebiotics (FOS or FOS with Inulin), 50.0%, followed by probiotic, symbiotic and enteral diet x NPT, 16.6% each.

All the studies analyzed the intestinal microbiota and clinical outcomes associated with IBD. Microflora analysis was done mainly on the phyla Firmicutes and Actinobacteria and the prevalent clinical parameters involved the inflammatory markers and disease activity. In addition, most samples included patients with CD.

For a better understanding of the results, they were grouped according to the type of intervention.

The alterations in intestinal microbiota

All studies evaluated the intestinal microbiota. The analysis of phyla was described in all articles and all included the Actinobacteria and in 5 articles the Firmicutes (1, 2, 3, 4 e 6). The most studied genus was Bifidobacteria, in 87.5% of the researches. For the analysis of the microbiota, 3 articles used qPCR (1, 4 and 5), 1 FISH (2), 1 qPCR and DGGE (3) and 1 qPCR and T-RFLP (6). The type of microbiota analyzed was different between the articles, investigations were carried out in the fecal microbiota in 4 articles (1, 2, 3 and 4) and in the mucosal microbiota only in 2 articles (5 and 6).

In general, probiotics, prebiotics and symbiotics showed changes in the intestinal microbiota of the patients, consequently promoting positive clinical results. Except for one study (2), which had no changes.

Inflammatory Markers

The total number of articles that analyzed inflammatory markers was three (2, 5 and 6). Two studies measured CRP (5 and 6) and another one analyzed the cytokines produced by CD (2). In addition, the trial (5) examined other non-specified inflammatory markers.

Clinical outcomes

In general, probiotics, prebiotics and symbiotics showed positive clinical results in patients. With the exception of one study (3), which did not report clinical alterations,

the others demonstrated a reduction of inflammation, when evaluated by indexes validated for IBDs and inflammatory markers.

DISCUSSION

In the last decade, we have observed the beginning of publications about the influence of dietary components on the microbiota's modulation in patients with IBD. The present review of the literature allowed recognizing the main dietary interventions used to improve clinical outcomes associated with signs and symptoms of IBDs. Most of the studies used prebiotics for treatment, and observed positive effects on inflammation, usually associated with disease activity indexes and inflammatory markers. Despite the heterogeneity of the intestinal flora analyses, there was a prevalence of investigation in the phyla *Actinobacteria* and *Firmicutes*, which are generally associated with IBDs.

The most effective intervention included in this review, was done with symbiotics, (*B longum* of 2 x 10^{11} and 12 g of oligofructose and inulin) over a period of 4 weeks, in patients of both sexes with active UC (Furrie et al., 2005).

Several studies using symbiotics as an intervention in patients with IBD have shown evidence that these dietary components can potentially be developed in therapies for acute or active disease (Saez-Lara et al., 2015). Treatment with TPN and ED was also effective, with decreased inflammation (CRP) and clinical remission in 88.23% of patients (CDAI <150). NPT treatment in active DC is used to contribute to intestinal rest of the inflamed tissue, less antigenic stimulation and stimulation of protein synthesis, which may aid in cell renewal and in healing wounds in the intestinal mucosa. In addition, the rate of remission after 3 months of onset of intervention ranges from 20 to 79% depending on population characteristics and administration. Enteral nutrition has also shown efficacy in the treatment of active CD, mainly related to anti-inflammatory mechanisms and has remission rates between 20 and 84.2% (Altmore et al., 2015).

The outcomes in the microbiota were divergent among the studies, mainly the increase of bifidobacteria and the diversity of intestinal flora after intervention. The phylum *Actinobacteria*, which includes the genus *Bifidobacterim*, increased after intervention with FOS and symbiotics. This genus is abundant in the colon of adult

humans, but appears to be decreased in IBDs. Other studies investigating the potential of prebiotics in the stimulation of bifidobacterium also found positive results (Scott et al., 2014). Likewise, symbiotics have been related to the increase of this genus in some studies (Zanten et al., 2012).

The relationship between the microbiota and the host is symbiotic, with benefit to both parties, in which the individual provides protection and nutrients and microorganisms assist in the digestion of food, conversion of harmful compounds to less toxic substances and production of bioactive molecules (Patterson et al., 2014). It is known that the microbiota of healthy individuals and those with IBDs are divergent and even between the CD and UC (Sokol et al., 2006) and that there are relations between these pathologies with alteration of the diversity and stability of the bacterial ecosystem (Yang and Jobin, 2014). Thus, if there is an imbalance of the intestinal flora the consequence will be dysfunctions related to microbial activities. Taxonomic changes include decreased *Firmicutes* and *Bacteroides* and increased levels of *Proteobacteria* and *Actinobacteria.* In the Firmicutes phyla specifically, there is a reduction of anaerobic protective bacteria, such as *F prausnitzii*, *Clostridium*, as well as in the phylum *Bacteroides*, as *B fragilis*. Thus, in general there is a greater amount of gram-negative bacteria (Yang and Jobin, 2014).

The bacteria are also associated with intestinal homeostasis, regulation of host immunity and tissue barrier function (Clemente et al., 2012). Several studies in the literature have debated this relationship and the consequent outcome in IBDs. The articles included in this study showed a relationship between IBDs and inflammatory markers, such as CRP, interleukins and TNF-α. The intestinal epithelial barrier is more sensitive and permeable in patients with IBD, and therefore there is a greater risk of bacterial translocation. Thus, antigens from the intestinal microbiota constantly stimulate the PRRs of immune cells, such as CD and macrophages, and activate them by inducing the production of inflammatory mediators (Basso et al., 2014), which triggers a chronic process. In this context, the reduction of inflammation by reduction of inflammatory cytokines has been seen in the studies of this review.

Despite the results in patients' intestinal microbiota outcomes, these beneficial effects were not necessarily reflected in clinical status. The findings inconsistent with the parameters analyzed in the studies of this review may have been related to differences in (a) sample size of studies, (b) supplementation period, (c) dosage, and (d)

types of supplementation given. The time of intervention varied from 4 to 52 weeks, and it was observed that the microbiota is very sensitive to the interventions and that this occurs relatively fast and in most cases, it has a positive impact on the patients' clinical picture.

This review points out that dietary interventions, especially from symbiotics, can modulate the microbiota, mainly in relation to time, when compared pre and post supplementation, and this positively interferes with clinical parameters of IBDs. However, the studies are heterogeneous, using different dietary components, doses and intervention time. The techniques obtained only partial results, without reflecting the variability of organisms that are present in the intestinal lumen. For more consistent results it is necessary more studies with a long-term treatment, this are needed to determine cause and effect relationships and to explain the mechanisms involved in this complex system involving diet, microbiota and IBD.

- The authors report no conflicts of interest.

- The lead author states that this manuscript is an honest, accurate, and transparent account of the study being reported, that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained.

REFERENCES

Altmore, R., Damiano, G., Abruzzo, A., Palumbo, V.D., Tomasello, G., Buscemi, S., Monte, A.I. (2015), "Enteral Nutrition Support to Treat Malnutrition in Inflammatory Bowel Disease", *Nutrients,* Vol. 7 No. 4, pp. 2125-33.

Barbara, G., Feinle-Bisset, C., Ghoshal, U.C., Santos, J., Vanner, S.J., Vergnolle, N., Zoetendal, E.G., Quigley, E.M. (2016), "The Intestinal Microenvironment and Functional Gastrointestinal Disorders", *Gastroenterology,* Vol. 50 No. 6, pp.1305-18.

Basso, P.J., Fonseca, M.T.C., Bonfá, G., Alves, V.B., Sales-Campos, H., Nardini, V., Cardoso, C.R. (2014), "Association among genetic predisposition, gut microbiota, and host immune response in the etiopathogenesis of inflammatory bowel disease" *Braz J Med Biol Res,* Vol. 47 No. 9, pp. 727-37.

Benjamin, J.L., Hedin, C.R.H., Koutsoumpas, A., Ng, S.C., McCarthy, N.E., Hart, A.L., Kamm, M.A., Sanderson, J.D., Knight, S.C., Forbes, A., Stagg, A.J., Whelan, K., Lindsay, J.O. (2011), "Randomised, double-blind, placebo-controlled trial of fructo-oligosaccharides in active Crohn's disease", *Gut,* Vol. 60 No. 7, pp. 923-29.

Brestoff, J.R. and Artis, D. (2013), "Commensal bacteria at the interface of host metabolism and the immune system", *Nat Immunol*. Vol. 14 No. 7, pp. 676–84.

Clemente, J.C., Ursell, L.K., Parfrey, L.W., Knight, R. (2012), "The Impact of the Gut Microbiota on Human Health: An Integrative View", *Cell*, Vol. 148 No. 6, pp.1258-70.

DeGruttola, A.K., Low, D., Mizoguchi, A., Mizoguchi, E. (2016). "Current understanding of dysbiosis in disease in human and animal models", *Inflamm Bowel Dis.,* Vol. 22 No. 5, pp.1137-50.

Dethlefsen, L., McFall-Ngai, M., Relman, D.A. (2007), "An ecological and evolutionary perspective on human–microbe mutualism and disease", *Nature*, Vol. 449, pp.811-18.

Edward, V. and Loftus, J.R. (2004), "Clinical Epidemiology of Inflammatory Bowel Disease: Incidence, Prevalence, and Environmental Influences", *Gastroenterology,* Vol. 126 No. 6, pp. 1504-17

Furrie, E., Macfarlane, S., Kennedy, A. (2005). "Synbiotic therapy (Bifidobacterium longum/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial", *Gut*, Vol. 54 No. 2, pp. 242–49.

Gentshew L. and Ferguson, L.R. (2012) "Role of nutrition and microbiota in susceptibility to inflammatory bowel diseases", *Mol Nutr Food Res*, Vol. 56 No. 4, pp. 524-35.

Ghouri Y.A., Richards D.M., Rahimi E.F., Krill, J.T., Jelinek K.A., Dupont A.W. (2014). "Systematic review of randomized controlled trials of probiotics, prebiotics, and synbiotics in inflammatory bowel disease", *Clin Exp Gastroenterol,* Vol.7, pp. 473-87.

Hanauer, S.B. (2006), "Inflammatory bowel disease: epidemiology, pathogenesis, and therapeutic opportunities", Inflamm Bowel Dis, Vol. 12, pp. 3-9.

Joossens, M., Peter, D.V., Ballet, V., Verbeke, K., Rutgeerts, P., Vermeire, S. (2012), "Effect of oligofructose-enriched inulin (OF-IN) on bacterial composition and disease activity of patients with Crohn's disease: results from a double-blinded randomised controlled trial", *Gut*, Vol. 61 No. 6, pp. 958.

Koleva, P.T., Valcheva, R.S., Sun, X., Gänzle, M.G., Dieleman, L.A. (2012), "Inulin and fructo-oligosaccharides have divergent effects on colitis and commensal microbiota in HLA-B27 transgenic rats", *Br J Nutr*, Vol. 108 No. 9, pp. 1633–43.

Lakatos, P.L., Fisher, S., Lakatos, L., Gal, I., Papp, J. (2006), "Current concept on the pathogenesis of inflammatory bowel disease-crosstalk between genetic and microbial

factors: Pathogenic bacteria and altered bacterial sensing or changes in mucosal integrity take "toll"?", *World J Gastroenterol*, Vol. 12 No. 12, pp. 1829-41.

Lee. D., Albenberg, L., Compher, C., Baldassano, R., Piccoli, D., Lewis, J.D., Wu, G.D. (2015), "Diet in the Pathogenesis and Treatment of Inflammatory Bowel Diseases", *Gastroenterology,* Vol. 148 No. 6, pp. 1087–106.

Lindsay, J.O., Whelan, K., Stagg, A.J., Gobin, P., Al-Hassi, H.O., Rayment, N., Kamm, M.A., Knight, S.C., Forbes, A. (2006), "Clinical, microbiological, and immunological effects of fructo-oligosaccharide in patients with Crohn's disease", *Gut*, Vol. 55 No. 3, pp. 348–55.

Matsuoka, K. and Kanai, T. (2015), "The gut microbiota and inflammatory bowel disease", *Semin Immunopathol*, Vol. 37 No. 1, pp. 47–55.

Molodecky, N.A., Soon, I.S., Rabi, D.M., Ghali, W.A., Ferris, M., Chernoff, G., Benchimol, E.I., Panaccione, R., Ghosh, S., Barkema, H.W., Kaplan, G.G. (2012), "Increasing Incidence and Prevalence of the Inflammatory Bowel Diseases With Time, Based on Systematic Review", *Gastroenterology,* Vol. 142 No. 1, pp. 46–54.

Patterson, E., Cryan, J.F., Fitzgerald, G.F., Ross, R.P., Dinan, T.G., Stanton, C. (2014), "Gut microbiota, the pharmabiotics they produce and host health", *Proc Nut Soc*, Vol. 73 No. 4, pp. 477–89.

Preter, V.T., Joossens, M., Ballet, V., Shkedy, Z., Rutgeerts, P., Vermeire, S., Verbeke, K. (2013), "Metabolic Profiling of the Impact of Oligofructose-Enriched Inulin in Crohn's Disease Patients: A Double-Blinded Randomized Controlled Trial", *Clin Transl Gastroenterol*, Vol. 4 No. 1, pp. 1-11.

Saez-Lara, M.J., Gomez-Lorente, C., Plaza-Diaz, J., Gil, A. (2015), "The Role of Probiotic Lactic Acid Bacteria and Bifidobacteria in the Prevention and Treatment of Inflammatory Bowel Disease and Other Related Diseases: A Systematic Review of Randomized Human Clinical Trials", *BioMed Res Int,* pp. 1-15.

Scaldaferri, F., Gerardi, V., Lopetuso, L.R. (2013), "Gut Microbial Flora, Prebiotics, and Probiotics in IBD: Their Current Usage and Utility", *BioMed Res Int*, pp. 1-9.

Scott, K.P., Martin, J.C., Duncan, S.H., Flint, H.J. (2014), "Prebiotic stimulation of human colonic butyrate-producing bacteria and bifidobacteria, in vitro", *FEMS Microbiol Ecol,* Vol. 87 No. 1, pp. 30-40.

Shiga, H., Kajiura, T., Shinozaki, J. (2012), "Changes of faecal microbiota in patients with Crohn's disease treated with na elemental diet and total parenteral nutrition", *Dig Liver Dis,* Vol. 44 No. 9, pp. 736–42.

Simpson, H.L. and Campbell, B.J. (2015), "Review article: dietary fibre–microbiota interactions", *Aliment Pharmacol Ther*, Vol. 42 No. 2, pp. 158–79.

Sokol, H., Seksik, P., Lepage, P., Lay, C., Lepage, P., Podglajen, I., Marteau, P., Doré, J. (2006), "Specificities of the fecal microbiota in inflammatory bowel disease". *Inflamm Bowel Dis,* Vol. 12 No. 2, pp. 106-11.

WGO. (2011), "Practice Guideline - Probiotics and Prebiotics".

Wildt, S., Nordgaard, I., Hansen, U., Brockmann, E. Rumessen, J.J. (2011), "A randomised double-blind placebo-controlled trial with Lactobacillus acidophilus La-5 and Bifidobacterium animalis subsp. lactis BB-12 for maintenance of remission in ulcerative colitis", *J Crohns Colitis,* Vol. 5 No. 2, pp. 115-21.

Whelan, K., Quigley, E.M.M. (2013). "Probiotics in the management of irritable bowel syndrome and inflammatory bowel disease", *Curr Opin Gastroenterol*, Vol. 29 No. 2, pp. 184–9.

Wu, H.J. and Wu, E. (2012), "The role of gut microbiota in immune homeostasis and autoimmunity", *Gut Microbes,* Vol. 3 No. 1, pp. 4-14.

Yang, Y. and Jobin, C. (2014). "Microbial imbalance and intestinal pathologies: connections and contributions", *Dis Model Mech,* Vol. 7 No. 10, pp. 1131-42.

Ye, Y., Pang, Z., Chen, W., Ju, S., Zhou, C. (2015). "The epidemiology and risk factors of inflammatory bowel disease", *Int J Clin Exp Med*, Vol. 8 No. 12, pp. 22529-42.

Zanten, G.C., Knudsen, A., Röytiö, H., Forssten, S., Lawther, M., Blennow, A., Lahtinen, S.J., Jakobsen, M., Svensson, B., Jespersen, L. (2012), "The Effect of Selected Synbiotics on Microbial Composition and Short-Chain Fatty Acid Production in a Model System of the Human Colon", PLoS ONE, Vol. 7; pp. e47212.

Zhou, M., He, J., Shen, Y., Zhang, C., Wang, J., Chen, Y. (2017). "New Frontiers in Genetics, Gut Microbiota, and Immunity: A Rosetta Stone for the Pathogenesis of Inflammatory Bowel Disease", *BioMed Res Int*, pp. 1-17.

Table 1 - Dietary interventions with prebiotics in the intestinal microbiota and clinical parameters of patients with Inflammatory Bowel Disease*.

Author, year	Design	Sample	Groups	Intervention and Time	Methodology of microbiota analysis	Microbiota outcome	Clinical outcome
Joossens et al., 2012 (1)	Randomized Clinical Trial	67 patients[#] with CD in remission or medium to moderate activity, mean age of group I 40 years old (SD 14.8) and group C 39 years old (SD 13.7)	I (n = 34): 10g oligofructose enriched with inulin BID C (n =33): 10g maltodextrin BID	Prebiotic/ 4 weeks	qPCR Actinobacteria: *Bifidobacterium adolescentes* and *Bifidobacteria longum* Firmicutes: *Faecalibacterium prausnitzii*	I: ↑ *B longum* and ↓ *Ruminococcus gnavus* *No alteration in control group	- ↓ inflammation in patients with active CD - ↓ HBI in 23.52% of patients
Benjamin et al., 2011 (2)	Randomized Cinical Trial	103 patient of both sexes with moderate CD and mean age in the group I 40 years old (SD 14.8) and group C 39 years old (SD 13.7)	I (n=54): normal diet + FOS (oligofructose) 7.5g BID C (n=49): normal diet + Maltodextrin 7.5g BID	Prebiotic/ 4 weeks	FISH Actinobacteria: *Bifidobactérias* Firmicutes: *F. prausnitzii*	No alteration in fecal microbiota in both groups.	↓inflammation (↓IL-6 + CD , ↑CD IL-10) Quality of life related to symptoms (↓IBDQ score) was lower than control group.

Preter et al., 2013 (3)	Randomized Clinical Trial	40 patients of both sexes with inactive CD or moderately active, mean age 31 – 52 years old	I (n = 21): FOS 10g BID (oligofructose + inulin) C (n = 19): maltodextrin 10g BID	Prebiotic/ 4 weeks	DGGE and qPCR Actinobacteria: *B longum, B adolescentis* Firmicutes: *F prausnitzii, R gravus*	I:↑ *B. longum* and ↓ *R. gravus* ↑ level of acetaldehyde and butyrate	No improvement in clinical outcome was reported.

[#]gender uninformed

* Only significant results are shown

I-Intervention group; C- control group; CD – Crohn's disease ; BID – *bis in die* (twice day); qPCR - Real-time polymerase chain reaction HBI – Harvey-Bradshaw Index; FOS – fructooligosaccharides; FISH – fluorescent in situ hybridisation; IL-6 – interleukin 6; DC – Dendritic cells; IL-10 – interleukin 10 ; IBQD – Inflammatory Bowel Disease Questionnaire; TLR4 – Toll like receptors 4; DGGE - Denaturing Gradient Gel Electrophoresis; SCFA – short chain fatty acids; IL-1β – interleucin 1β.

Table 2 - Dietary intervention with probiotics in intestinal microbiota and clinical parameters of patients with Inflammatory Bowel Disease*.

Author, year	Design	Sample	Groups	Intervention and Time	Methodology of microbiota analysis	Microbiota outcome	Clinical outcome
Wildt et al., 2011 (4)	Randomized Clinical Trial	32 patients of both sexes with UC in remission median age 37.5 years old	I (n = 20): 2 Capsule of Probio-Tec AB-25 (*L acidophilus* strain LA-5 1.25x10^{10} + *B animalis* subsp. *lactis* strain BB-12 1.25 x10^{10}) TID C (n = 12): 2 capsules TID *All patients before inclusion were stopped on 5-ASAs – intervention not as add-therapy	Probiotic/ 52 weeks	qPCR Firmicutes: *L acidophilus* Actinobacteria: *B animalis* subsp. *lactis*	All patients in I group had BB-12 or LA-5 in faecal samples *VS* 2 patients in C group, at week 4 and week 28.	Remission maintained after treatment: - I: 25% - C: 8% (p=0,37) The median time to relapse was 125.5 days in I group *VS* 104 days in C group (p = 0,68) Gastrointestinal symptoms were reported equally in both groups.

* Only significant results are shown

UC – Ulcerative colitis; TID – *Ter in die* (Three times a day) ; I-Intervention group; C- control group; qPCR – Real Time Quantitative PCR; *VS* – versus;

Table 3 - Dietary intervention with symbiotics in intestinal microbiota and clinical parameters of patients with Inflammatory Bowel Disease*.

Author, year	Design	Sample	Groups	Intervention and Time	Methodology of microbiota analysis	Microbiota outcome	Clinical outcome
Furrie et al., 2005 (5)	Randomized Clinical Trial	18 patients of both sexes with active UC mean age 24 – 67 years old	I (n = 9): Gelatin capsule (*B longum* $2x10^{11}$) + sachet with FOS 6g BID (oligofrcutose + inulin) C (n = 9): Potato starch capsule + sachet with maltodextrin 6g BID	Symbiotic/ 4 week	qPCR Actinobacteria: *Bifidobacterias*	↑ molecules of r RNA *bifidobacteria* (↑ 42x in intervention group VS 4.6x in control group)	↑ defensins (↑ mRNA of hBD2) ↓ inflammation (↓inflammatory markers TNF α, IL-1α, CPR) ↓ Disease activity (↓HBI - 44,44% of patients go into remission)

* Only significant results are shown

I-Intervention group; C- control group; UC – Ulcerative colitis; FOS – fructooligosaccharides; BID – *bis in die* (twice a day); VS – versus; hBD2 – beta-denfesin 2; TNFα – tumor necrosis factor α; IL-1α – interleukin 1 alpha; CPR – C-reactive protein; HBI – Harvey-Bradshaw Index.

Table 4 - Other dietary interventions in the intestinal microbiota and clinical parameters of patients with Inflammatory Bowel Disease*.

Author, year	Design	Sample	Groups	Intervention and Time	Methodology of microbiota analysis	Microbiota outcome	Clinical outcome
Shiga et al., 2012 (6)	Randomized Clinical Trial	17 patients of both sexes with CD activity, median age 30 years old (15-47) + 12 healthy subjects with median age 32 years old (28-44)	I= (8 EN) and (9 TPN) C (n = 12 healthy subjects): normal diet and without restriction	Elemental Nutrition Diet or Total Parenteral Nutrition 6 weeks	qPCR-T-RFLP Total bacteria Actinobacteria: *Bifidobacterium* Bacteroidetes: *Bacteroides fragilis* Firmicutes: *Clostriudium coccoides*, *Clostridium leptum, Enterococcus* e *Lactobacillus* Proteobacteria: *Escherichia coli*	In patients treated with TPN: ↓ TR-Fs (↓ diversity of bacteria species) and↑*Enterococcus* In patients treated with EN: ↓ *Bacteroides fragilis* Fecal microbiota in patients with CD were markedly different from healthy subjects * In the control group there was no change	Improvement of inflammation: ↓ CPR There was clinical remission in both intervention groups (CDAI<150 – in 88,23% of patients)

* Only significant results are shown
I-Intervention group; C- control group; CD – Crohn disease; ED – Elemental Diet ; TPN– Total Parenteral Nutrition; T-RFLP - Terminal Restriction Fragment Length Polymorphism; TR-Fs – terminal restriction fragments; CDAI – Crohn's Disease Activity Index.