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Developing Health Claims for Probiotics in Canada **A CASE STUDY**

Nutri-Net Canada
Dairy Processor's Association of Canada

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1.0 INTRODUCTION

Consultations held in recent years have concluded that health claims for foods should be authorized through the existing Food and Drug Act. Such claims are currently regulated as a component of the *nutrition labelling* and under the *drug claims* regulatory framework. In the past, Health Canada approved five health claims for food. These claims related to risk reduction required a regulatory amendment, a fairly long process in the current Canadian system. In this context, Health Canada recently revised its position on health claims for foods. In order to use health claims for foods, the existing regulatory process offers three options: *A*) general health claims (limited to making healthy food choices); *B*) function claims (maintenance of good health and healthy body development); and *C*) risk reduction claims (claims that bring food under the drug definition with specific proof). The former two do not require any regulatory amendment, however the latter falls under the drug definition and requires a regulatory amendment. This approach was presented by Ms Nora Lee, Chief of the Nutrition Evaluation Division at Health Canada on October 24, 2007. Highlights of this presentation appear in Appendix I.

In early 2008, Health Canada prepared the *Health-related claims about probiotics microorganisms in Food and NHPs in food formats Interim Policy* (Jan-14,2008 and Apr-30, 2008). At the same time, the INAF-AISA-Tonic workgroup undertook the task of building a case study for the use of health claims on food products containing probiotics. The first step of the case study consisted in reviewing the available body of evidence on the safety and demonstrated efficacy probiotics in order to establish which categories of claims companies could use for probiotic foods, and establish minimal acceptance criteria. Dr. Denis Roy (AISA), a renowned scientific in the field of probiotic research, was the leader of this section. He was assisted in this task by Dr Pierre Chevalier, senior microbiologist. The specific objectives of the work were:

Overview of available scientific evidence to support health claims

- Basic criteria to select and identify probiotics
- Safety considerations (clinical, observational, anecdotal and history of safe use of probiotics)
- Content claims
- Function claims (without amendment)
- Reduction of disease risk or risk factor claims

With the recent revised position of Health Canada on health claims for food, specifically function claims, much effort has been devoted to sorting out the evidence in order to decide which “assertions” may or may not qualify as function claims for probiotics.

Dr. Paul Paquin (Tonic) and Ms. Renée Michaud (INAF) of the workgroup have also reviewed the evidence in terms evaluating of how it can “fit” into the existing regulatory framework. This evaluation has been achieved in light of the different type of claims, i.e. content, function and risk reduction, although function claims have been analysed in more details with the objective of identifying which categories of health effects could actually qualify as function claims.

The following steps were part of the objectives:

- Examine the process for submitting a function claim for foods
- Meet with Health Canada for guidance and follow up
- Compile modifications required by the authority and assess a time frame
- Formulate specific recommendations on the process
- Comment Health Canada’s *Health-related claims about probiotics microorganisms in Food and NHPs in food formats interim policy*

2.0 GUIDELINES FOR THE EVALUATION OF PROBIOTICS IN FOOD (WORLD SITUATION)

The international scientific community recognizes that claims can broadly be categorized into four main groups: *Content*; *Function*; *Enhanced function* and, *Reduction of disease risk or disease risk factor*. The first two categories (*content* and *function*) may be considered *generic claims* whereas the latter (*enhanced function* and *reduction of risk*) fall into what are considered *specific claims* (strain dependent, specific to a unique food product).

2.1 BACKGROUND INFORMATION

For a better comprehension of this case study, the following paragraphs present a brief overview of works published in recent years on the topic of probiotics and health claims by organisations such as the Food and Agriculture Organization (FAO) and the World Health Organization (WHO).

A first study of health claims for probiotics was carried out in 2001.

Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria.

Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. American Córdoba Park Hotel, Córdoba, Argentina 1-4 October 2001

The FAO/WHO received a first report which described the different scientific challenges for the use of probiotics in foods and milk powder. That first report led to the development of guidelines on probiotics in foods in 2002.

Guidelines for the Evaluation of Probiotics in Food

Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food. London Ontario, Canada April 30 and May 1, 2002. Food and Agriculture Organization of the United Nations World Health Organization

These guidelines are the main reference for health claims for probiotics and should be the one countries and the industry in general, rely on for health claim regulation. They were presented to two committees of the CODEX Alimentarius for the purpose of establishing this document as an international reference base. This is presented in the publication:

Probiotic Bacteria: Legislative Framework—Requirements to Evidence Basis 1,2.

Maya Pineiro and Catherine Stanton, Food Quality and Standards Service, Nutrition and Consumer Protection Division, Food and Agriculture Organization of the United Nations (FAO), 00153 Rome, Italy and Teagasc, Dairy Products Research Centre, Moorepark, Fermoy, County Cork, Ireland.

“Following on from the FAO/WHO Expert Consultation and Working Group outputs on probiotics and their presentation to Codex, it is hoped that these will be used as a science-based risk assessment process for managerial decision on probiotics, and that the “Guidelines for the Evaluation of Probiotics in Food” will be used as a model for scientific criteria for evaluation of health claims. It is also hoped that this work will be incorporated or taken as example for the Codex draft being prepared on health and nutrition claims and as a scientific assessment of a novel food and that the probiotic guidelines will be adopted by the industry.”

Reference: J. Nutr. 137: 850S–853S, 2007.

2.2 FAO/WHO GUIDELINES

This section presents a brief description of the different items treated in the FAO/WHO guidelines with the final recommendations of the FAO/WHO consultation group.

The FAO/WHO states that: “In order to claim that a food exerts a probiotic effect, the guidelines set forth in this report should be followed. A scheme outlining these guidelines for the evaluation of probiotics for food use is shown in Fig. 1¹. This was the basis for discussions and details are specified in the following sections of this report”.

Genus/species/strain

The current state of evidence suggests that probiotic effects are strain specific. It is necessary to know the genus and species of the probiotic strains. The group recommends that all strains be deposited in an international recognized culture collection.

In vitro tests to screen potential probiotics

In vitro tests are useful to gain knowledge about strains and about the mechanism of the probiotic effects. The in vitro tests most currently used for the study of probiotic strains are:

- *Resistance to gastric acidity*
- *Bile acid resistance*
- *Adherence to mucus and/or human epithelial cells and cell lines*
- *Antimicrobial activity against potentially pathogenic bacteria*
- *Ability to reduce pathogen adhesion to surfaces*
- *Bile salt hydrolase activity*
- *Resistance to spermicides (applicable to probiotics for vaginal use)*

Safety considerations

Historically, Lactobacilli and Bifidobacteria associated with foods have been considered to be safe. The group recommends that probiotic strains be characterized with the following tests:

- *Determination of antibiotic resistance patterns*
- *Assessment of certain metabolic activities (e.g., D-lactate production, bile salt deconjugation)*
- *Assessment of side-effects during human studies*
- *Epidemiological surveillance of adverse incidents in consumers (post-market)*
- *If the strain under evaluation belongs to a species that is a known mammalian toxin producer, it must be tested for toxin production. One possible scheme for testing toxin production has been recommended by the EU Scientific Committee on Animal Nutrition (SCAN, 2000)*
- *If the strain under evaluation belongs to a species with known hemolytic potential, determination of hemolytic activity is required*

In vivo studies using animals and humans

In some cases, animal models exist to provide substantiation of in vitro effects and determination of probiotic mechanism. Where appropriate, the Working Group encourages use of these prior to human trials.

The principal outcome of efficacy studies on probiotics should be proven benefits in human trials, such as statistically and biologically significant improvement in condition, symptoms, signs, well-being or quality of life; reduced risk of disease or longer time to next occurrence; or faster recovery from illness. Each should have a proven correlation with the probiotic tested.

Probiotics have been tested for an impact on a variety of clinical conditions (see Expert Consultation Report, Section 5.3). Standard methods for clinical evaluations are comprized of Phase 1 (safety), Phase 2 (efficacy), Phase 3 (effectiveness) and Phase 4 (surveillance). Phase 1 studies focused on safety are discussed in Section 3.3 above. Phase 2 studies, generally in the form of randomized, double blind, placebo-controlled (DBPC) design, measure efficacy compared with placebo. In addition, phase 2 studies measure adverse effects. A general recommendation for the testing of probiotic foods is that the placebo would be comprized of the food

¹ This figure appears in Appendix II.

carrier devoid of the test probiotic. Sample size needs to be calculated for specific endpoints. Statistically significant differences must apply to biologically relevant outcomes.

Health claims and labelling

Currently in most countries, only general health claims are allowed on foods containing probiotics. The Working Group recommends that specific health claims on foods be allowed relating to the use of probiotics, where sufficient scientific evidence is available, as per the guidelines set forth in this report. Such specific health claims should be permitted on the label and promotional material. For example, a specific claim that states that a probiotic 'reduces the incidence and severity of rotavirus diarrhea in infants' would be more informative to the consumer than a general claim that states 'improves gut health'. This would better comply with Codex General Guidelines on Claims (CAC/GL 1-1979 (Rev. 1-1991) to avoid misleading information.

The Working Group recommends that the following information be described on the label:

Genus, species and strain designation. Strain designation should not mislead consumers about the functionality of the strain

- Minimum viable numbers of each probiotic strain at the end of the shelf-life
- The suggested serving size must deliver the effective dose of probiotics related to the health claim
- Health claim(s)
- Proper storage conditions
- Corporate contact details for consumer information

Final recommendations of the group

1. Adoption of the definition of probiotics as 'Live microorganisms which when administered in adequate amounts confer a health benefit on the host'.
2. Use and adoption of the guidelines in this report should be a prerequisite for calling a bacterial strain 'probiotic'.
3. Regulatory framework to allow specific health claims on probiotic food labels, in cases where scientific evidence exists, as per the guidelines set forth in this report.
4. Promotion of these guidelines at an international level.
5. Good manufacturing practices (GMP) must be applied in the manufacture of probiotic foods with quality assurance, and shelf-life conditions established.
6. Further development of methods (in vitro and in vivo) to evaluate the functionality and safety of probiotics.

2.3 EXAMPLES OF HEALTH CLAIMS RELATED TO PROBIOTICS AROUND THE WORLD

In **Japan**, The Japanese Foods for Specific Health Use (FOSHU) system allows several health claims for probiotics {Farnworth, 2008 #3246}. Prebiotics such as oligosaccharides, raffinose, lactulose, and arabinose and probiotics such as *Lactobacillus* and *Bifidobacterium* found in different types of products (Soft drink, yogurt, biscuit cookie, table sugar, soyabean curd, vinegar, chocolate, powdered soup, fermented milk, yoghurt, miso soup, cereal) are grouped in the health claim: Food that improve gastrointestinal conditions. (National Centre of Excellence in Functional Foods-Health Claims Regulatory System Japan available from: www.nceff.com.au/pdf/Japan.pdf). For example in Japan, probiotic claims for foods and supplements are not strictly regulated and hence claims such as "good for your health" are permitted. But a list of centralized FOSHU claims has potential for use in probiotics if Japanese regulators approve clinical trials. Existing claims include:

- Improves gastrointestinal conditions
- Modulates cholesterol
- Modulates blood pressure

In **France**, Afssa (Agence française de sécurité sanitaire des aliments) recommends the health claims below. Afssa is a public independent organism contributing through monitoring, alert, research and research instigation to the protection and improvement of public health, animal health and welfare, vegetal and environmental health.

Product: Fermented milk containing *Lactobacillus casei* DN-114 001

Health Claim: “contributes to the strengthening of the natural defense”.

Reference: AVIS de l'Agence française de sécurité sanitaire des aliments relatif à l'évaluation des justificatifs scientifiques concernant les allégations présentes sur l'étiquetage d'un lait fermenté contenant notamment du *Lactobacillus casei* DN-114 001 available from: www.afssa.fr/Documents/NUT2003sa0200.pdf

In **Sweden**, the SNF Swedish Nutrition Foundation (formerly Stiftelsen Svensk Näringsforskning) was established in 1961. The aim of the activities at SNF is to support scientific research within nutrition and adjacent fields. SNF also promotes the implementation of developments within this field of research. SNF has about 40 members consisting of food and pharmaceutical companies and organizations.

Product: Proviva Fruit Drink with *Lactobacillus plantarum* 299v

Health Claim: “reduces the formation of gas in the intestine, by influencing the intestinal flora.”.

Reference: Statement concerning evaluation of the scientific documentation behind the product proviva. Available from: www.hp-info.nu/prodsp/finalreport_proviva.pdf.

Product: Milk-based drink containing *L. rhamnosus* GG, *L. rhamnosus* Lc705, *P. freudenreichii* ssp. *Shermanii* JS and a *Bifidobacterium* strain.

Health claims:

“Calms a stress tummy/stomach”

“Calms an irritated tummy/stomach”

“Reduces daily abdominal bother/upset/distress”

“Promotes gastrointestinal well-being”

Reference: Statement concerning evaluation of the scientific documentation behind a milk drink Available from: www.hp-info.nu/prodsp/FinalreportLGG.pdf.

In the **Netherlands**, the Netherlands Nutrition Centre (Stichting Voedingscentrum Nederland). The Nutrition Centre is funded by the Netherlands Ministries of Health, Welfare and Sport (VWS) and Agriculture, Nature and Food Quality (LNV) to provide scientifically founded, honest information about healthy and safe food and food quality (www.voedingscentrum.nl/Voedingscentrum/English)

Product: Yakult® fermented milk product, containing *Lactobacillus casei* strain Shirota (LcS).

Health claims:

“Drinking at least one bottle (65 ml) of Yakult® per day

1. may improve bowel habit in subjects who are susceptible to constipation

2. may support a well-balanced gut microbiota through an increased number of *Lactobacilli*”

Reference: Assessment report on Yakult www.voedingscentrum.nl/NR/rdonlyres/4E6011C9-7FED-47B4-A31A-EDA304693947/0/ASSESSMENTREPORT061110.pdf.

Product: Danone Activia yoghurt containing Bifidus Essensis® (Bifidobacterium animalis DN-173 010).

Health claims:

1. Consumption of at least one portion (125 g) Danone Activia per day stimulates the intestinal transit in subjects with slow transit times
2. Consumption of at least one portion (125 g) Danone Activia per day increases the number of bifidobacteria in the large intestine. This increase of bifidobacteria may support a well-balanced gut flora.

Reference: Assessment report on Danone Activia. Available from www.voedingscentrum.nl/NR/rdonlyres/0FB74719-263F-477A-A384-C7A9FDEFCAA4/0/beoordelingsrapport_danone_activiapdf.pdf.

3.0 HEALTH RELATED CLAIMS FOR PROBIOTIC FOODS IN CANADA

The INAF-AISA-Tonic workgroup has considered three types of health claims for probiotics in food in the context of the existing Canadian regulatory framework. The group has looked at content claims as well as claims bringing foods into the definition of a drug on the basis of new information supplied by Health Canada and also information from recent scientific data. Function claims have been analysed in more details with the objective of identifying which categories of health effects can qualify as function claims.

3.1 CLAIMS FOR PROBIOTICS IN FOOD

3.1.1 Content claims

In the Interim guidance document, Health Canada proposes that content and/or implied claims should only be accompanied by a valid explicit claim. The following is an excerpt of this document.

**Health-Related Claims About Probiotic Microorganisms In Food And NHPs In Food Formats
Interim Policy, April 30 2008**

II Types of statements and representations considered as health-related claims

- Implied claims (comparable to content claims)
- Function claims
- Claims bringing a food into the definition of a drug

III Use of the term probiotics and related implied claims

5. According to the Expert Consultation conducted by the FAO and the WHO probiotics.
'Live microorganisms which, when administered in adequate amounts, confer a health benefit on the host.'
6. The term probiotics and related implied claims such as those described under paragraph 4(1) above should only be used when accompanied by a valid explicit claim outlining the benefits or effects of the probiotic. Without explicit claim, these statements are vague, uninformative and potentially misleading. The following sections outline the type of explicit claims that may be made for a probiotic and the conditions for making them.

Comments from the INAF-AISA-Tonic workgroup on *Content claims*

It is the opinion of our group that Health Canada should revise its position on this aspect. Canadian consumers already have access to 'content claims'-like information. Indeed, the omega-3 and omega-6 fatty acids content appear on nutritional labels under the food nutritional labelling regulation without any additional or more explicit claim on the product. This kind of information implies that the consumption of these lipids is beneficial for health.

It is the opinion of our group that probiotics should be considered on the same basis. Numerous studies show that the regular consumption of probiotics as part of a healthy diet is beneficial for consumer health in general. Scientific evidence and statements from several organizations recognizes this as a fact. Therefore, the use of a statement such as "contains probiotics" means that the product contains bacteria that are beneficial for the consumers' health, as supported by solid scientific evidence. Such a statement would not be misleading consumers.

A second important point concerns the fact that the Interim document contains no references on the minimum level of probiotics content in a food product or serving. Our workgroup has examined the scientific literature and reviewed the position adopted by several regulatory agencies and/or associations (ex: IDF) on this matter. We recommend that a level of 10^8 CFU/ml or gram for each bacterial strain be defined as the acceptable minimum.

In order to support our recommendation, the Interim document (part VII/19 on labeling) should be modified as follows.

VII.	Labelling of foods for which health-related claims for probiotics are made
19.	The following specific requirements will apply to the labelling of foods for which any type of validated health-related claim about probiotics referred to in this policy are made
	Implied claims (comparable to content claims)
	– Function claims
	– Claims bringing a food into the definition of a drug
III	Use of the term probiotics and related implied claims
	a- the term probiotic(s) or other implied health claims (withdraw the last part of the sentence)
	b- the claimed effect of the probiotics is clearly stated
	c- where a health-related claim is made, the genus, specific epithet and strain of the probiotic microorganism(s) or mixed culture should be declared in addition to the identification by the common name
	d- the level of probiotic strain in serving of stated size of the food should be declared and reach a minimum of 10^8 CFU/ml per serving for each bacterial strain

3.1.2 Function claims

The INAF-AISA-TONIC workgroup has reviewed the scientific body of evidence with the objective of determining which categories of health effects would allow probiotics to qualify for function claims in the context of the existing Canadian regulatory process. The following documents were also considered:

- Conference presented by Ms. Nora Lee, Chief of the Nutrition Evaluation Division at Health Canada on October 24, 2007
- Health-related claims about probiotics microorganisms in Food and NHPs in food formats interim policy – Jan-14, 2008

Health claims must be supported by scientific evidence (e.g. clinical trials) and should describe the effect of a probiotic strain on a structure or physiological function in the human body. They should include the proper name of the probiotic strain and support the source for each strain. The dosage form should be consistent with the route of administration (capsules, milk beverage, dairy product, food and oral administration), and the same dose (or dosage range) recommended. These requirements are mainly based on FAO/WHO's Guidelines for the evaluation of probiotics in food².

Articles (clinical studies) and additional relevant publications have been evaluated by conducting a literature search on the effects of probiotics in healthy individuals. A comprehensive literature search was carried out to identify published data on probiotic strains (from clinical trials) using standard databases: PubMed³, Biosis Previews, Google Scholar. We made sure that the literature search was broad enough to cover original research articles and other relevant documents in support of the claims. A combination of key terms such as “Bifidobacterium Bb-12”, “Bifidobacteria Bb and 12” “Bifidobacterium lactis milk” “Bifidobacteria fermented milk” “persistence probiotic Bifidobacterium” and likewise with other species

² Report of a joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food (2002)

³ www.ncbi.nlm.nih.gov/entrez/query.fcgi

of probiotics was conducted to assess the volume of scientific literature available. The most relevant papers were then referenced for function claims assessment.

Based on these guidelines, the first review of the scientific body of evidence led to considering four categories of function claims for probiotics in food:

- Bowel transit
- Modification of the intestinal microbiota
- Immune enhancement
- Improved nutritional value of food

In order to verify whether these effects would qualify as function claims according to Health Canada requirements, we proceeded to “grade” each category based on directions provided by the documents presented in Appendix III.

Comments from the INAF-AISA-Tonic workgroup on qualified *Function claims*

Based on a thorough review and analysis of available scientific data and, after carefully weighing and grading the type and strength of the evidence, we concluded that only two categories of effects had sufficient substantiation to qualify as function claims: A- *Enhancement of immunity* and, B- *Maintenance of healthy intestinal flora*. We consider Bowel transit to be included in the latter.

A- Enhancement of immunity

Eleven papers out of all papers consulted were retained as sound scientific evidence to substantiate the claim on enhancement of immunity. The details of this analysis are presented Appendix IV-Table 1. The expert advice of Dr. Yvan Boutin was sought in order to corroborate our conclusions.

Function claims for enhancement of immunity in scientific terms could be stated as follows:
(Name of probiotic) contributes to modulate the nonspecific immune response in healthy persons (re: Appendix 2, Table 1)

Criteria – Evidence should demonstrate that the product

- enhances the nonspecific immune phagocytic activity of circulating blood granulocytes
- enhances NK cell activity

Suggested industry application claim
The daily consumption of probiotics helps strength the body’s natural defenses

B- Maintenance of healthy intestinal flora (including bowel transit)

Eleven papers out of all papers consulted were retained as sound scientific evidence to substantiate the claim on maintenance of a healthy intestinal microbiota. The details of this analysis are presented Appendix IV-Tables 2a and 2b.

Health claims for modification of intestinal microbiota in scientific terms could be stated as follows.
(Name of probiotic) helps maintain a healthy intestinal microbiota (Appendix 2, Table 2)

Criteria – Evidence should demonstrate that the product

- shows persistence
- helps control transit time
- improves bowel habits
- helps keep a balanced colonic microbiota associated with control of nutrient bioavailability

Suggested industry application claim
The daily consumption of probiotics promotes regularity and helps maintain a healthy gut

Bowel transit was not retained to qualify as a function claim by itself because it is linked to maintenance of a healthy intestinal flora in most cases reported in the literature and we believe there is no added value in making two different claims on this effect. The *Improved nutritional value of food* assertion was dropped because the available scientific data to support this claim was deemed insufficient.

3.1.3 Claims which bring a food into a definition of a drug (risk reduction)

The next section reports Health Canada’s position on risk reduction claim.

Health-Related Claims About Probiotic Microorganisms In Food And NHPs In Food Formats Interim Policy, April 30 2008

V Claims bringing a food into the definition of a drug

16. However, exemptions from the drug regulations and, where applicable, section 3 of the act have been provided in the regulations (B.01601 to B.01.603) to permit certain claims that would otherwise bring a food into the definition of a drug. To enable such claims for a food product, a regulatory amendment to the table following section B.01.603 of the regulation is required. Manufacturers wishing to make such health claims on foods must make a submission to the food directorate of Health Canada..

This section of the interim document for probiotics shows no progress on Health Canada’s part on claims related to risk reduction. This statement still brings foods under the definition of a drug and provides no options that could facilitate or speed up the process of obtaining the right to use a health claim related to risk reduction for a food product.

The FAO/WHO expert group holds a different position on that subject. They showed concerns about consumers being misled by function claims that are vague or difficult to demonstrate, when a claim on risk reduction could be supported by existing or obtainable scientific data.

Excerpt from the FAO/WHO guidelines, section 3.5. *Health claims and labelling*

Currently in most countries, only general health claims are allowed on foods containing probiotics. The Working Group recommends that specific health claims on foods be allowed relating to the use of probiotics, where sufficient scientific evidence is available, as per the guidelines set forth in this report. Such specific health claims should be permitted on the label and promotional material. For example, a specific claim that states that a probiotic 'reduces the incidence and severity of rotavirus diarrhea in infants' would be more informative to the consumer than a general claim that states 'improves gut health'. This would better comply with Codex General Guidelines on Claims (CAC/GL1-1979 (Rev. 1-1991) to avoid misleading information.

Our group concurs with the FAO/WHO position on this matter. Despite the fact that Health Canada's guidelines for function claims will soon allow the industry to use claims for probiotics, this alone does not solve the issue risk of reduction claims having to go through a lengthy and inconvenient regulatory process. We believe that Health Canada should devote significant efforts to the development of a simpler and faster process for risk reduction claims for probiotic foods, and show a clear willingness to support the industry in this respect. Health Canada should develop clear procedures and define precise steps and time frames for the industry with respect to getting a product specific risk reduction claim which brings a food product into the definition of a drug. The experience developed in other countries for this type of application should be used as a reference. Also, the Interim guidance document: *Preparing a Submission for Foods with Health Claims: Incorporating standards of evidence for evaluating foods with health claims* was published in 2002 and has remained in its *interim* form for the past five years. It has become obsolete and is presently useless. Since there has been no progress on how to submit a health claim, Canada is running late compared to other countries in this matter (re: NutriNet Canada). Health Canada should rapidly produce a new document with clear and precise guidelines to this effect.

4.0 SUMMARY OF THE INAF-AISA-TONIC WORKGROUP RECOMMENDATIONS

4.1 Proposed Recommendation N° 1 – Content Claim –

- *Considering that, according to the World Health Organization, probiotics must be alive when administered and must be taken in adequate quantities to provide health benefits;*
- *Considering that a scientific consensus stipulates that a probiotic food product should contain viable probiotic microorganisms at a minimum concentration of 10^6 cfu ml⁻¹ or g⁻¹ at the end of the product's shelf-life period to have a beneficial health effect, and assuming that a typical faecal lactobacilli count of 10^5 g⁻¹ of faeces, and 10^6 g⁻¹ for bifidobacteria, a one-log increase in response to a probiotic product may be expected;*

We recommend that Health Canada should approve the concept of a **content claim** for probiotics.

Suggested statement: *The probiotic product XYZ is a healthy choice*

Examples: *Probiotic "Namebrand" Yoghourt is a healthy choice*

Le yogourt probiotique "Marque déposée" est un choix santé

The above statement is a good example of a content claim as it would read on a product label. We also recommend that when making such a claim, the product should:

- list the probiotic genus and species of each strain present in the product
- state the total count of probiotics per serving
- contain no less than 10^8 CFU of each strain/species per serving.

4.2 Proposed Recommendation N° 2 – Function Claim –

We recognize that given the present regulatory framework in Canada, the submission for function claims should remain a voluntary process. However, we strongly recommend that a plan of stewardship should be devised by the industry in conformity with Health Canada requirements. This plan should include standards and guidelines established by the industry and approved by Health Canada, and the industry should have the responsibility of stewardship.

- *Considering that in order to make a function claim for a probiotic food product, this product should meet all the requirements established for a content claim;*
- *Considering that a function claim has to be substantiated by scientific evidence obtained in human intervention studies using the product as normally consumed;*

We recommend that companies should be responsible for gathering the scientific evidence substantiating their claim for due diligence, either from existing studies or by conducting their own clinical studies. The committee recognizes two function claims that are substantiated by a convincing body of evidence in the scientific literature presently available.

4.2.1 Proposed Recommendation N° 2a

Function claims for enhancement of immunity could be stated as follows.

Scientific statement: *(Name of probiotic) contributes to modulate the nonspecific immune response in healthy persons (re: Appendix 2, Table 1)*

Suggested industry application claim: *The daily consumption of probiotics helps strength the body's natural defenses*

Criteria: Evidence should demonstrate that the product

- enhances the nonspecific immune phagocytic activity of circulating blood granulocytes
- enhances NK cell activity

Main markers: Functional capacity of immune system as measured by:

- Responses to challenges (antibody response, secretory IgA measured by response, DTH response)
- Incidence and severity of infections
- Measuring specific cell functions *ex vivo*

4.2.2 Proposed Recommendation N° 2b – Function Claim –

Function claims for modification of the intestinal microbiota could be stated as follows.

Scientific statement: *(Name of probiotic) helps maintain a healthy balance of the intestinal microbiota (Appendix 2, Table 2)*

Suggested industry application claim: *The daily consumption of (name of probiotic strains) may support a well-balanced gut microbiota through an increased number of (lactobacilli) and (or) (bifidobacteria)*

Criteria: Evidence should demonstrate that the product

- shows persistence (Composition and activity of gut microbiota)
- helps control transit time (bowel habit)

Main markers:

Composition and activity of gut microbiota as measured by:

- Plating faecal microorganisms
- Molecular techniques
- Model systems only for mechanistic research

Bowel habit as measured by:

- Frequency of bowel movement
- Stool consistency and form
- Stool weight
- Transit time

4.3 Proposed Recommendation N° 3 – Risk Reduction Claim –

Applications for *Risk reduction claims* are still a very long, complex and demanding process in Canada. We strongly believe that Health Canada should implement a procedure to authorize risk reduction claims other than a *regulatory modification*, since this approach is presently a major setback for innovation and will limit the development of novel health foods in the near future.

With this new procedure, Health Canada should produce a flowchart-like or checklist-like information document for the industry. The sensible and very practical approach developed by the European Food Safety Authority (EFSA) should be set as an example. The Canadian industry should be able to rely on a similar set of clear and simple guidelines in order to submit an application for Risk reduction claims in Canada.

Ref: Final scientific and technical guidance for applicants for preparation and presentation of the application for authorisation of a health claim (www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178623592471.htm)

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APPENDIX I

Excerpts from a conference presented by Ms. Nora Lee, Chief of the Nutrition Evaluation Division at Health Canada on October 24, 2007

Current regulatory framework definitions

General health claims

include broad “healthy choice” claims that promote overall health, healthy eating or provide dietary guidance. Do not refer to a specific health effect, disease, or health condition.

Function claims

- claims about the maintenance of body functions that are necessary to the maintenance of good health and normal growth and development
- claims about maintaining or supporting body functions associated with the maintenance of good health or performance
- claims about restoring, correcting, or modifying body functions*

Disease risk-reduction claims

claims that link the consumption of foods or food constituents to a reduced risk of disease in the context of the total diet*

* would be considered drug claims under the Canadian Food and Drug Regulations (FDR).

Food as defined in the Act

“includes any article manufactured, sold or represented for use as food or drink for human beings, chewing gum, and any ingredient that may be mixed with food for any purpose whatever”

Section 5 (1)

states that “No person shall label, package, treat, process, sell or advertise any food in a manner that is false, misleading or deceptive or is likely to create an erroneous impression regarding its character, value, quantity, composition, merit or safety”.

Drug includes:

“any substance or mixture of substances manufactured, sold or represented for use in

- (a) the diagnosis, treatment, mitigation or prevention of a disease, disorder or abnormal physical state, or its symptoms, in human beings or animals,
- (b) restoring, correcting or modifying organic functions in human beings or animals.
- (c) disinfection in premises in which food is manufactured, prepared or kept.”

Foods with health claims that do not require regulatory amendments

Function claims expressly permitted by current food regulations

- Function claims for nutrients and energy “to the effect that the food's energy value or a nutrient in the food is generally recognized as an aid in maintaining the functions of the body necessary to the maintenance of good health and normal growth and development“ are expressly permitted in B.01.311(3), D.01.006 and D.02.004 of the FDR (biological role claims)
- food generally must meet specified conditions: for vitamins and minerals, at least 5% of the RDI/serving (D.01.004 & D.02.002); for protein and amino acids, at least a source of protein (B.01.305(1); no general conditions
- examples of acceptable claims are provided in the Guide to Food Labelling and Advertising
- Claim is about the nutrient, not the food.
- “generally recognized” standard is not officially defined but is reasonably understood to be based on a well-established prior history of a high level of scientific evidence and broad scientific agreement that is unlikely to be reversed with new data

- "nutrient" is not defined in the Food and Drugs Act or Regulations.
- "Nutrient" is generally accepted as defined by Codex Alimentarius, 1991:
 - *"any substance normally consumed as a constituent of food (a) which provides energy; or (b) which is needed for growth and development and maintenance of healthy life; or (c) a deficit of which will cause characteristic biochemical or physiological changes to occur"*
 - *In practice, Health Canada and CFIA consider a food substance as a nutrient if it is recognized as such by the Institute of Medicine, for which dietary reference intakes (DRI) have been established*

Some function claims not expressly prohibited or permitted

“Other” Function Claims:

- Historically, few other function claims for foods or food constituents have been permitted in Canada.
- However, if they are not drug claims, i.e. falling within the parameters of “modifying, correcting, or restoring organic function”, such claims are permitted if they are truthful and not misleading as per Section 5(1) of the Act.
- Function claims for foods should refer to effects where the nature of the effect and the mode of action are consistent with physiological effects of or responses to foods and diets
 - *there has been very little attention paid to this option, either on the part of manufacturers or the government,*
 - *little experience with determining when a function claim might not be a drug claim,*
 - *there are no guidelines for establishing the validity of this type of claim or for setting out conditions and defining appropriate wording to help ensure they are truthful and not misleading*

Examples of claims considered acceptable in this category include:

- *for a particular sports drink (“absorbed up to 30% faster than water”)*
- *for coarse wheat bran as an ingredient providing 7 grams of dietary fibre (“promotes regularity or laxation”).*

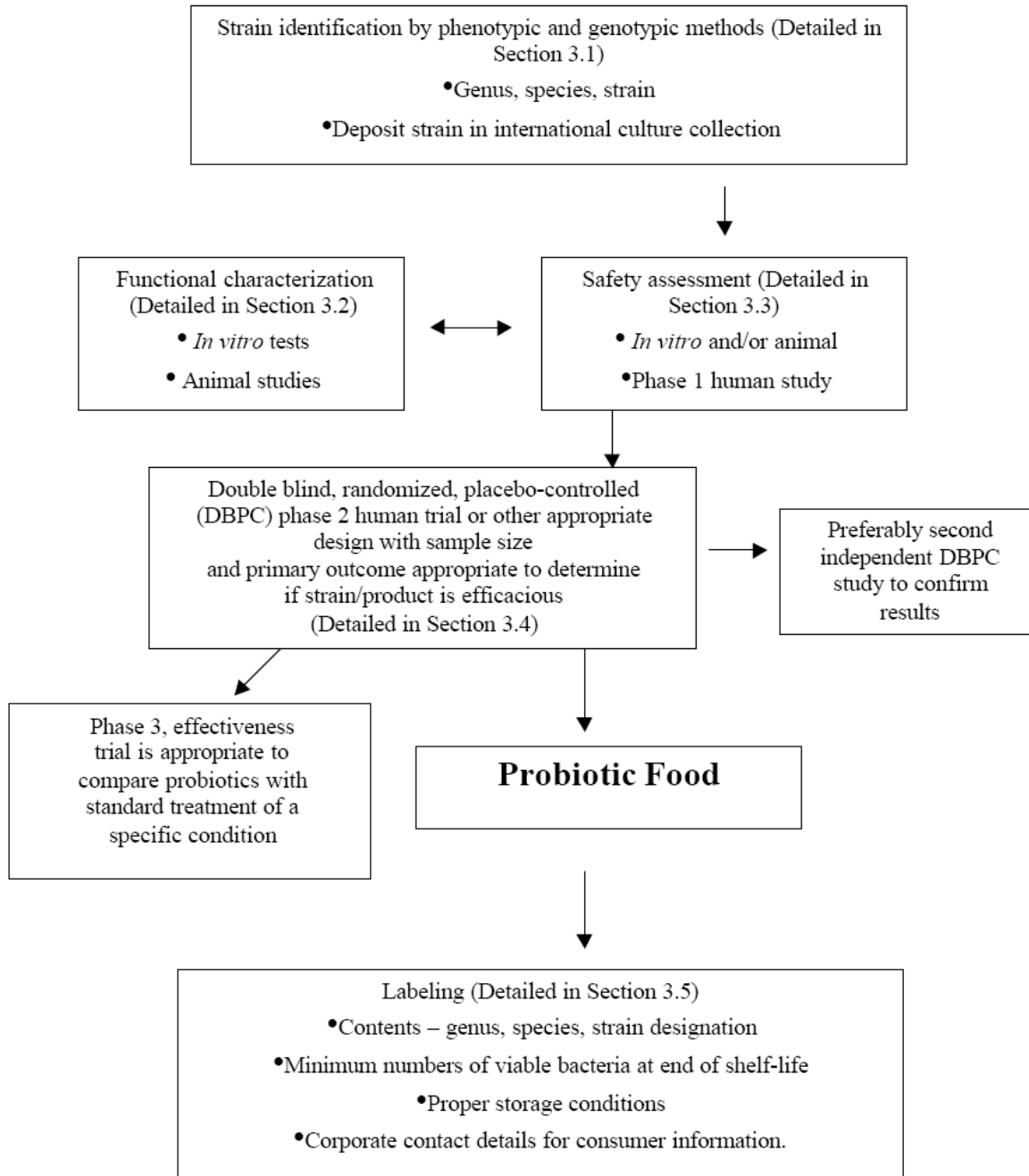
Foods with health claims that require regulatory amendments

- Claims that bring a product within the definition of a drug are then subject to the drug-related sections (Part C) of the FDR as well as, possibly, Section 3 of the Act
- To be permitted for foods, provisions have been included (Dec 12, 2002) in the FDR (B.01.601) to exempt food products with certain health claims from the drug regulations and Section 3 of the Act
- New claims and the conditions for their use can be added to the table by regulatory amendment following a review of the submission and approval of the claim by HC.
 - *ensures the continued and consistent application of food regulations and standards on food products.*
 - *transparent process with conditions of use for each approved disease risk-reduction claim listed in a table following section B.01.603 of the FDR.*

includes: prescribed wording for the claim; nutritional and eligibility criteria and conditions for the label or advertisement.

APPENDIX II

Figure 1. Guidelines for the Evaluation of Probiotics for Food Use



APPENDIX III

Summarizing the Evidence in the Submission

As per Health Canada’s requirements (Table below), the relevant evidence was summarized by briefly describing the following, as required:

- *the type of evidence;*
- *method (trial design);*
- *participants or subjects (number, inclusion criteria and if available exclusion criteria);*
- *daily dose and frequency;*
- *treatment characteristics (e.g. dosage form, formulation, methods of preparation, route of administration, etc.);*
- *duration of treatment;*
- *endpoints or measures of efficacy;*
- *effects or results (noting statistical significance, non-significance and trends);*
- *conclusions on the totality of the evidence; and*
- *in-text references (author-date style) for all evidence summarized*

Part C – Primary Evidence	
Information on the product for which a claim is sought (product research)	
<p>Summary of the studies conducted</p> <p>Using a systematic approach similar to that for literature review in Part B, for each study conducted on the product for which the health claim is sought, categorize the study into one of 3 categories</p> <p>(a) Experimental Studies in humans (b) Observational Studies in humans (c) Meta-analysis, if applicable</p>	<p>In a table format, summarize each study by study design under the following headings (use attached table to this section as a guide):</p> <p>(a) Study identification (author, year) (b) Study design and level (refer to Table 3B, Part I of this Guidance Document regarding categories of study design and levels) (c) Description of participants in control and intervention groups, including sample size (d) Description of treatment and control and duration (e) Amount of food and bioactive substance consumed (f) Identification of baseline (background) diet and/or use of control diet (g) Main results – provide actual data and statistical significance, include graphs where appropriate (h) Comments – statistical analysis (justify the types of statistics used), other factors affecting interpretation of results, methods of analysis of intake of food and bioactive substance, and outcome measures, general comment about study quality, deviations from protocol, adverse or side effects</p>

Strength of Evidence Grading System

Levels of Evidence Type of Evidence from Human Studies

- I Randomized controlled trials or other clinical trials, or at least one well-designed randomized controlled trial (preferably multicentred)
- II Well-designed clinical trials without randomization and/or control groups
- III Well-designed descriptive and observational studies, such as correlational studies, cohort studies and case-control studies
- IV Peer-reviewed published articles, conclusions of other reputable regulatory agencies or previous marketing experience, expert opinion reports, referenced textbooks.

Natural Standard evidence-based validated grading rationale™

- Grades reflect the level of available scientific evidence in support of the efficacy of a given therapy for a specific indication
- Expert opinion and folkloric precedent are not included in this assessment, and are reflected in a separate section of each monograph ("Strength of Expert Opinion and Historic/Folkloric Precedent").
- Evidence of harm is considered separately; the below grades apply only to evidence of benefit.

LEVEL OF EVIDENCE GRADE / CRITERIA
<p>A (Strong Scientific Evidence) <i>Statistically significant evidence of benefit from >2 properly randomized trials (RCTs), OR evidence from one properly conducted RCT AND one properly conducted meta-analysis, OR evidence from multiple RCTs with a clear majority of the properly conducted trials showing statistically significant evidence of benefit AND with supporting evidence in basic science, animal studies, or theory.</i></p>
<p>B (Good Scientific Evidence) <i>Statistically significant evidence of benefit from 1-2 properly randomized trials, OR evidence of benefit from >1 properly conducted meta-analysis OR evidence of benefit from >1 cohort/case-control/non-randomized trials AND with supporting evidence in basic science, animal studies, or theory.</i></p>
<p>C (Unclear or conflicting scientific evidence) <i>Evidence of benefit from >1 small RCT(s) without adequate size, power, statistical significance, or quality of design by objective criteria, * OR conflicting evidence from multiple RCTs without a clear majority of the properly conducted trials showing evidence of benefit or ineffectiveness, OR evidence of benefit from >1 cohort/case-control/non-randomized trials AND without supporting evidence in basic science, animal studies, or theory, OR evidence of efficacy only from basic science, animal studies, or theory.</i></p>
<p>D (Fair Negative Scientific Evidence) <i>Statistically significant negative evidence (i.e., lack of evidence of benefit) from cohort/case-control/non-randomized trials, AND evidence in basic science, animal studies, or theory suggesting a lack of benefit.</i></p>
<p>F (Strong Negative Scientific Evidence) <i>Statistically significant negative evidence (i.e. lack of evidence of benefit) from >1 properly randomized adequately powered trial(s) of high-quality design by objective criteria. *</i></p>
<p>Lack of Evidence† <i>Unable to evaluate efficacy due to lack of adequate available human data.</i></p>

* Objective criteria are derived from validated instruments for evaluating study quality, including the 5-point scale developed by Jadad et al., in which a score below 4 is considered to indicate lesser quality methodologically (Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ, McQuay HJ. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Controlled Clinical Trials* 1996; 17[1]:1-12).

† Listed separately in monographs in the "Historical or Theoretical Uses which Lack Sufficient Evidence" section. Available from: www.nlm.nih.gov/medlineplus/druginfo/natural/grading.html

APPENDIX IV

**Table 1. Listing of Evidence for function claim for probiotics in food:
(Name of probiotic) contributes to modulate the nonspecific immune response in healthy persons**

IMMUNE ENHANCEMENT: A (STRONG SCIENTIFIC EVIDENCE)

Type of evidence	Method (trial design)	Participants or subjects	Daily dose and frequency	Treatment characteristics	Duration of treatment	Measures of efficacy, Results, and Conclusions	Reference
I	Randomly assigned to one of two groups (A or B).	Healthy adult volunteers (females = 14, males = 16) aged from 19 to 59 years (mean 37.3)	3 × 125 g fermented milk per day of <i>Bifidobacterium lactis</i> Bb12 Viable counts of 1 × 10 ⁷ -10 ⁸ cfu g ⁻¹ of both La 1 and bifidobacteria throughout the study period.	All volunteers were requested to exclude fresh fermented products from their diet during an initial 2 week period (t - 21 to t - 8). Thereafter, Group A received 3 × 125 g fermented milk per day during 3 weeks (t - 7 to t + 13) while Group B continued the exclusion diet. On days 8, 10 and 12 (t 0, + 2 and + 4) of the fermented milk intake Period. All volunteers ingested the attenuated <i>S. typhi</i> Ty21a oral Vivotif vaccine capsule	3 weeks	<p>ENDPOINTS OR MEASURES OF EFFICACY: Specific antibody titres (anti-<i>S. typhi</i>) by immunoassays. Total IgG and IgA in sera were quantified by radial immunodiffusion and faecal flora analysis by conventional microbiological methods.</p> <p>EFFECTS OR RESULTS: The fermented milk group showed a 4.08-fold rise in specific IgA anti-Ty21a LPS antibody titres compared with a 2.48-fold rise (Wilcoxon P = 0.04) in the control group. In the fermented milk group, 15/16 volunteers had > 2 fold rise in specific IgA titres compared with 6/14 in the control group. Total serum IgA values increased significantly (P=0.014) in group receiving fermented milk. No significant changes in total IgG. On day 17 (t +9) of the fermented milk intake period there was almost a one log increase in both lactobacillus (P = 0.057) and bifidobacteria (P = 0.027) counts in Group A.</p> <p>CONCLUSIONS: During consumption of a fermented milk containing <i>L. acidophilus</i> and <i>Bifidobacterium lactis</i> Bb12, there were increased faecal counts of Lal and bifidobacteria which are indicative of a transient colonization of the intestine. Lactic acid bacteria taking along with fermented milk can act as adjuvants to humoral immune response.</p>	Amster, 1994
I	Randomized double-blind placebo-controlled.	Fifty healthy adults (31 males, 19 females) aged 22 to 56 years.	Each participant received a daily capsule (placebo or probiotic) with food.	<p>The placebo group consumed daily a capsule containing 200 mg of methylcellulose. Those in the probiotic group daily consumed a capsule containing 10¹⁰ CFU of the strain <i>Lactobacillus fermentum</i> CECT5716.</p> <p>An intramuscular vaccination for influenza was carried out to each participant (control and probiotic group) at midterm of experiment (day 14).</p>	28 days	<p>ENDPOINTS OR MEASURES OF EFFICACY: The primary endpoint of the study was to evaluate the immune response induced by the vaccination process against influenza and its modulation by the consumption of a probiotic. Lymphocytes subpopulations were determined by flow cytometry. Total and specific immunoglobulins in plasma were measured by immuno assays as well as cytokines.</p> <p>EFFECTS OR RESULTS: Effects on lymphocytes subpopulation demonstrated an increase in natural killer (NK) cells in the probiotic group but not in the placebo group (p < 0.05). Vaccination induced increase cytokines concentration (IL-10, IL-12, TNF-α and INF-γ) in both groups; the probiotic however showed a significant higher induction in TNF-α (p < 0.01). In the probiotic group, a significant increase in specific anti-influenza-IgA (p = 0.05) and a significant increase in total IgM was observed (p = 0.05). Finally, the number of influenza-like-illness episodes was smaller in the probiotic group during a 5 months follow-up (n = 25 vs 40; p = 0.05).</p> <p>CONCLUSIONS: Oral administration of a <i>Lactobacillus fermentum</i> CECT5716 potentiates the immunologic response of an anti-influenza vaccine and may provide enhanced systemic protection from infection by increasing the T-helper type 1 (Th1) response and virus neutralizing antibodies.</p>	Olivares et al., 2007

Type of evidence	Method (trial design)	Participants or subjects	Daily dose and frequency	Treatment characteristics	Duration of treatment	Measures of efficacy, Results, and Conclusions	Reference
I	Randomized.	28 healthy adults (12 females and 16 males) aged from 23 to 62 yrs (mean 36.9).	Daily intake of supplemented milk with 10^{10} cfu of <i>Bifidobacterium bifidum</i> Bb12 or 7×10^{10} cfu of <i>Lactobacillus</i> strain La1. There was no placebo control.	Participants were randomly assigned into two groups (<i>B. bifidum</i> Bb12 or <i>Lactobacillus</i> strain La1). Experimental design had 3 stages. For the first 3 weeks, all participants received milk only. During the following 3 wk (week 3 to 6), they received either Bb12 or La1 strain. During the final 6 wk (weeks 6 to 12), both groups received milk without probiotics.	3 weeks	<p>ENDPOINTS OR MEASURES OF EFFICACY: Blood samples taken at the beginning of the trial, before receiving probiotics (after wk 3), after the supplementation period (wk 6) and after the washout period (wk 12). Analysis of lymphocytes subsets (T cells CD3+, CD4+, CD8+, and NK cells) by flow cytometry, determination of phagocytic activity (granulocytes and monocytes) by flow cytometry. <i>Bifidobacteria</i> and lactobacilli fecal counts</p> <p>EFFECTS OR RESULTS: No major differences were observed in proportions of blood lymphocyte subsets or degree of T cell activation between groups. A significant increase in global phagocytic activity was observed in both groups after probiotic ingestion (comparison of week 6 to week 3); 86.5% activity vs 38.9% in Bb12 group; 84% vs 46% in La1 group ($p = 0.0001$). Covariance analysis showed that phagocytic activity increased more in granulocytes after fermented milk consumption than in monocytes ($p = 0.004$). Fecal counts of bifidobacteria increased significantly in Bb12 group ($p < 0.05$) while lactobacilli increased in La1 group ($p < 0.05$). However, 12 days after the end of fermented milk consumption, bacterial counts returned to initial levels.</p> <p>CONCLUSIONS: Oral administration of <i>Bifidobacterium bifidum</i> Bb12 or <i>Lactobacillus</i> strain La1 increased phagocytic activity of granulocytes and monocytes.</p>	Schiffriin et al (1995)
I	Randomized	28 healthy volunteers from 23 to 72 yr of age (mean 36.9).	Daily intake of milk supplemented with 10^{10} cfu of <i>Bifidobacterium bifidum</i> Bb12 or 7×10^{10} cfu of <i>Lactobacillus acidophilus</i> LA1.	Participants were randomly distributed into two groups for the three stages trial. During stage 1 (run-in diet; week 1 to 3), both groups received milk only. During stage 2 (wk 4 to 6) group 1 received <i>B. bifidum</i> Bb12 and group 2 received <i>L. acidophilus</i> LA1. During the wash-out stage (week 7 to 12), both groups received again milk without bacteria.	3 weeks.	<p>ENDPOINTS OR MEASURES OF EFFICACY: Blood samples were collected at the beginning of the study (time 0) and at the end of each of the three other periods (t3, t6 and t12). Lymphocytes subsets were analyzed: T lymphocytes CD3+; B lymphocytes CD19; suppressor-cytotoxic lymphocytes (CD3+ and CD8+); and natural killers cells (CD3-, CD16+ and CD56+). Phagocytic activity of monocytes and granulocytes was also quantified. Fecal colonization by probiotic bacteria was analyzed.</p> <p>EFFECTS OR RESULTS: No significant modifications in the proportion of the blood lymphocytes subpopulations or in the degree of T cell activation were detected in either group. An increment of phagocytic activity was however measured ($p < 0.0001$). This activity decreased for both groups six weeks after cessation of probiotic ingestion ($p = 0.001$ for group 1; $p = 0.05$ for group 2). Granulocytes had a higher increase in phagocytic activity than monocytes ($p < 0.004$). The increment in phagocytosis by monocytes was coincident with fecal colonization by the bacteria ($p < 0.001$) after stage 2, and persisted for 6 wk after ingestion; by this time, fecal lactobacilli and bifidobacteria had returned to concentrations prior to consumption.</p> <p>CONCLUSIONS: This study reports that at least one parameter of nonspecific immune activity (phagocytic activity in peripheral blood) was enhanced following the ingestion of a fermented milk containing either <i>Bifidobacterium bifidum</i> Bb12 or <i>Lactobacillus acidophilus</i> LA1 for 3 weeks.</p>	Schiffriin et al, (1995)

Type of evidence	Method (trial design)	Participants or subjects	Daily dose and frequency	Treatment characteristics	Duration of treatment	Measures of efficacy, Results, and Conclusions	Reference
I	Double-blind, placebo-controlled cross-over study.	17 students and staff members (13 women, 4 men, aged 22-50 y) from the University of Turku (Finland). Subjects with mild infections during the study were not excluded; three subjects had atopic dermatitis.	<i>Lactobacillus</i> GG (ATCC 53103) was given at a dose of 2.6 x 10 ⁸ cfu per day served in two 200 ml portions of milk.	Participants were divided in two groups, according to their tolerance or intolerance to milk. The first group (control) was composed of 9 subjects tolerant to milk. The second group was composed of 8 subjects nontolerant to milk. The study comprised 2 one-week challenge periods preceded and followed by a one-week washout period free from milk protein. During each one-week challenge period, subjects received milk (200 ml twice a day) with or without the probiotic.	2 one weeks	ENDPOINTS OR MEASURES OF EFFICACY: Expressions of complement receptors (CR1 and CR3), receptors for IgG (FcγRI, FcγRII, FcγRIII) and for IgA (FcaR) on neutrophils and on monocytes were measured to evaluate the expression of phagocytosis receptors. EFFECTS OR RESULTS: In milk intolerant subjects, milk challenge increased significantly receptors expression of CR1, FcγRI and FcaR in neutrophils and CR1, CR3 and FcaR in monocytes, while supplementation with <i>Lactobacillus</i> GG downregulated this increase in these subjects. A distinct pattern of immunomodulatory response was seen in controls (healthy subjects tolerant to milk): milk alone challenge did not influence receptor expression but milk with <i>Lactobacillus</i> GG significantly increased expression of CR1, CR3 FcγRIII and FcaR in neutrophils but not in monocytes. CONCLUSIONS: <i>Lactobacillus</i> GG appears to modulate the nonspecific immune response differently in healthy subjects and hypersensitive ones (milk intolerance in this case). An immunostimulatory effect was seen in healthy subjects while a downregulation response was observed in milk-hypersensitive subjects. In healthy subjects, it may stimulates the non specific immune response while in atopic or allergic subjects it may lead to improvement of hypersensitivity reactions.	Pelto et al (1998)
I	Double-blind, placebo-controlled, randomized cross-over study.	Fifteen healthy adults (mean 24 year; five males and 10 females) and 15 patients (mean 23 yrs; three males and 12 females) with atopic dermatitis (AD).	Each received 100 ml of yoghurt probiotic drink twice daily. The yoghurt drink (Zott GmbH, Germany) contained <i>Streptococcus thermophilus</i> (concentration not given) enriched with three probiotic cultures: <i>Lactobacillus paracasei</i> Lpc-37 (3.9 x 10 ⁸ cfu/g), <i>L. acidophilus</i> 74-2 (2.9 x 10 ⁴ cfu/g) and <i>Bifidobacterium lactis</i> DGCC-420 (5.9 x 10 ⁴ cfu/g).	At the beginning of the study, all subjects were randomly assigned to two groups. One group (8 patients, 7 healthy subjects) received probiotic drink and the other (7 patients, 8 healthy subjects) received the placebo drink over 8 weeks. After a 2-week washout period, the intervention was cross-over between the groups and the respective products were consumed for another 8 weeks.	8 weeks	ENDPOINTS OR MEASURES OF EFFICACY: At the beginning of the study and at the end of each intervention period, fresh stools samples were collected for quantification of bacteria; venous blood samples were taken to evaluate cellular and humoral immune responses. The SCORAD score after washout periods evaluated the severity of eczema. EFFECTS OR RESULTS: <i>Lactobacillus paracasei</i> (Lpc-37) and <i>Bifidobacterium lactis</i> (DGCC-420) increased significantly in feces after probiotic intervention period (p < 0.05) whereas <i>L. acidophilus</i> 74-2 marginally increased. Major lymphocytes subsets were not affected by the probiotic intervention; however CD57+ increased significantly in healthy subjects (p = 0.034) after probiotic intake, whereas CD4+ CD54+ decreased significantly (p = 0.031) in patients with AD but remained unchanged in healthy subjects. Phagocytic activity of monocytes and granulocytes significantly increased in healthy subjects after probiotic intake (p = 0.014). In patients, SCORAD tended to decrease by 15% but did not reach statistical significance (p = 0.081). CONCLUSIONS: Administration of <i>L.paracasei</i> Lpc-37, <i>L. acidophilus</i> 74-2 and <i>B. lactis</i> 420 stimulated phagocytic activity in healthy subjects. It appears that immune response was modulated differently in patients with AD. Possibly, different pathways of bacterial-intestinal enterocytes crosstalk may induce different immune response. Immune response is thus influenced by the immunological status of the host, but no significant improvement of the skin conditions could be shown	Roessler et al (2007).

Type of evidence	Method (trial design)	Participants or subjects	Daily dose and frequency	Treatment characteristics	Duration of treatment	Measures of efficacy, Results, and Conclusions	Reference
II	Not specified	10 healthy women and ten healthy men aged between 22 and 55 years.	<i>L. actobacillus paracasei</i> LTH 2579 at a concentration of 10 ⁸ cfu/g of fermented sausage. Each participant in the probiotic group consumed daily 50g of sausage (5 x 10 ⁹ cfu of bacteria/day)	During stage 1 (4 weeks), participants consumed 50 g of a conventional fermented sausage (without probiotic) in their daily diet. A 9-day period with a prescribed diet followed for transition. During the stage 2 (4 weeks), all participants consumed daily 50 g of sausage supplemented with the, followed by another 9-day period with a defined diet. Finally a washing out period (stage 3) of 7 days followed without any sausage.	4 weeks.	<p>ENDPOINTS OR MEASURES OF EFFICACY: Stool samples were collected on the 3rd and 9th day of defined diet at the end of each period and during the wash-out post-period for a PCR detection of <i>L. paracasei</i>. Four venous blood samples were taken, at the start of the study, at the end of each period and after 2 weeks of the probiotic sausage period, to evaluate blood cholesterol, triglycerides, some lymphocytes markers (CD) and phagocytic activity.</p> <p>EFFECTS OR RESULTS: The percentage of T-cells CD4 increased after the supplementation, as compared to the end of stage 2 period (p < 0.001) but decreased by the end of the washout period; a significantly positive correlation between the numbers of <i>L. paracasei</i> in the stool and the percentage of lymphocytes expressing CD4 was found (p < 0.001). The clearest effect was observed for CD54/ICAM-1 (an indicator of intestinal inflammation), which decreased significantly after the consumption of the probiotic food as compared to the beginning of the study (p < 0.001). Additionally, there was a 3.2% increase of phagocytic activity of monocytes and granulocytes (p < 0.05).</p> <p>CONCLUSIONS: <i>Lactobacillus paracasei</i> LTH 2579 included in sausage elevated the percentage of T-lymphocytes expressing CD4 and the phagocytic index of monocytes and granulocytes. <i>L. paracasei</i> also exerts an anti-inflammatory effect in bowel inflammation (decreased of CD54/ICAM-1 marker).</p>	Jahreis et al (2002).
I	Randomized double-blind, placebo-controlled study.	Twenty healthy men aged 40-65 years (mean 56).	Subjects in the probiotic group consumed daily 3 x 100 ml of fermented milk supplemented with <i>Lactobacillus casei</i> (Shirota strain; Yakult, Japan) at a concentration of 10 ⁹ cfu/ml (total daily intake of 3 x 10 ¹¹ <i>L. casei</i>).	During the 8-week study period, subjects were divided into a treatment group and a control group. The study consisted of a stabilisation period (2 weeks) with a strictly controlled diet. The test period (4 weeks) followed, during which probiotic group received the fermented milk with <i>L. casei</i> . A follow-up period (2 weeks) terminated the study, during which a control diet was given to all subjects.	4 weeks	<p>ENDPOINTS OR MEASURES OF EFFICACY: Endpoint measures included fecal microbiota analysis and some biochemical parameters (sterols, bile and short-chain fatty acids), bacterial enzyme activities, and immune parameters: lymphocytes subsets, natural killer (NK) cells activity, cytokines (interleukine 1β and γ-interferon), phagocyte functions and humoral parameters (immunoglobulins and complement factors).</p> <p>EFFECTS OR RESULTS: Consumption of <i>L. casei</i> Shirota resulted in a significant increase of this bacterium among the fecal microbiota (p < 0.01), reaching 10⁷ cfu/g faeces. A concomitant increase of total <i>Lactobacillus</i> species and a significant increase of <i>Bifidobacterium</i> species (p < 0.05) were also observed. The number of <i>Bacteroidaceae</i>, <i>Clostridium</i>, <i>Staphylococcus</i> and <i>Enterotoccus</i> decreased in both subjects groups. No statistically significant effects were observed in the percentage of B and T lymphocytes, NK cells, phagocyte functions and immunoglobulins.</p> <p>CONCLUSIONS: Consumption of <i>Lactobacillus casei</i> (Shirota strain) modulates the composition and metabolic activity of the intestinal microbiota but does not influence immune system parameters.</p>	Spanhaak et al (1998).

Type of evidence	Method (trial design)	Participants or subjects	Daily dose and frequency	Treatment characteristics	Duration of treatment	Measures of efficacy, Results, and Conclusions	Reference
I	Randomized double-blind, placebo-controlled study.	Fifty-four healthy volunteers aged 44- 80 years (mean 63.5).	Subjects received 5 x 10 ¹⁰ CFU of <i>Lactobacillus rhamnosus</i> HN001 (NZDRI) in of either low-fat milk (LFM) or lactose-hydrolyzed LFM in a daily basis.	All subjects consumed 200 mL of LFM (twice daily) for three weeks. After that, subjects were assigned in two groups: A) consumption of LFM supplemented with <i>L. rhamnosus</i> or B) consumption of lactose-hydrolyzed LFM supplemented with <i>L. rhamnosus</i> The test diet period lasted 3 weeks followed by a washout period of three weeks (consumption of LFM for all subjects).	3 weeks	<p>ENDPOINTS OR MEASURES OF EFFICACY: Blood collection at week 0, 3, 6, and 9) Phagocytic activity of polymorphonuclear (PMN) cells assessed via membrane-bound NADPH-oxidase activity. NK cell activity in mononuclear cell fraction assessed by specific target lysis (⁵¹Cr assay)</p> <p>EFFECTS OR RESULTS: The consumption of LFM alone had no effect on the studied immune parameters. The relative proportion of PMN cells showing phagocytic activity increased significantly (p<0.01) by 18.7% and 15.1% respectively in either group A or group B following consumption of HN001 (week 3 vs week 6). The degree of specific target cell lysis by NK cells increased significantly (p<0.01) following consumption of HN001 in both groups. The relative increase in NK cell activity due to consumption of HN001 was higher among the group B subjects (mean 147% increase) than group A subjects (mean 71.3% increase) but this difference between groups was not statistically significant (p=0.176).</p> <p>CONCLUSIONS: Dietary consumption of <i>L. rhamnosus</i> HN001 appears to enhance non specific cellular immune response.</p>	Sheih et al., 2001
I	Placebo-controlled, double-blinded, randomized crossover study	Twenty-six volunteers (13 males, 13 females). Age: 25±3.	Subjects received either 300 g of yoghurt or yoghurt supplemented with a combination of <i>Bifidobacterium animalis</i> subsp <i>lactis</i> DGCC 420 (3 x 10 ⁶ cfu/g) and <i>Lactobacillus acidophilus</i> 74-2 (9.3 x 10 ⁸ cfu/g)	After 3-week run-in period (yoghurt-free period), half of volunteers consumed 300 g/day of yoghurt supplement containing probiotic and other half received the placebo product for a period of 5 weeks. The two groups were crossed during the following 5-week period.	5 weeks	<p>ENDPOINTS OR MEASURES OF EFFICACY: Leucocyte populations were determined by flow cytometry, Phagocytic activity on polynuclear cells or mononuclear cells was assessed by flow cytometry. Oxidative burst activity was measured by flow cytometry. Blood lipids were also analyzed. FISH-based quantification of <i>L. acidophilus</i> and <i>B. lactis</i> was conducted on faecal samples.</p> <p>EFFECTS OR RESULTS: Faecal proportions of <i>L. acidophilus</i> and <i>B. lactis</i> increased significantly from 0.02 to 0.19 and 0.4 to 1.4% (P<0.05) respectively after consumption of probiotic yoghurt. After 5-week of placebo intake, percentage of active cells were 92±6% and increased to 95±6%. The oxidative burst activity remained unaffected by probiotic culture. No change in leucocyte population throughout the study.</p> <p>CONCLUSIONS: Supplementation of yoghurt with <i>L. acidophilus</i> and <i>B. lactis</i> increased significantly the percentage of monocytes and granulocytes showing phagocytic activity (P=0.044) and they are able to modulate unspecific cellular immune response.</p>	Klein et al., 2008

Type of evidence	Method (trial design)	Participants or subjects	Daily dose and frequency	Treatment characteristics	Duration of treatment	Measures of efficacy, Results, and Conclusions	Reference
I	Placebo-controlled, randomized study	Thirty-three young women of 22-29 years of age (mean 24.4)	<p>Subjects consumed 100g/day of either traditional yoghurt or probiotic yoghurt for 2 weeks and then, 200g/day for another 2 weeks.</p> <p>Probiotic yoghurt from Danone containing 3.7x10⁸ CFU/mL of live <i>Lactobacillus casei</i> (DN 114 001)</p>	<p>All participants had to refrain from consuming any fermented products 1 week before the beginning of yoghurt consumption (referred as T1). Women were divided in two groups: traditional or probiotic yoghurt. During the following two weeks, they consumed one portion of 100g/day of the respective product (T2). Then the portion of both products was doubled and the subjects had to take their respective product for another 2 weeks (T3) followed by a washout period of two weeks (T4).</p>	4 weeks	<p>ENDPOINTS OR MEASURES OF EFFICACY: Blood samples were drawn at the end of each period (T1, T2, T3, and T4). Activation of T lymphocytes was assessed ex vivo after PWM activation and CD69 expression (activation marker) was determined by flow cytometry. NK cell activity in mononuclear cell fraction was determined by flow cytometry.</p> <p>EFFECTS OR RESULTS: No major change in cell populations. The cytotoxic activity is augmented following intake of both products, this effect persisting after cessation of consumption. However, there were no significant difference between the probiotic and the conventional yoghurt group.</p> <p>CONCLUSIONS: Daily yoghurt intake has a stimulating effect on NK cell functions, but in this study the probiotic product did not perform better than the traditional one.</p>	Meyer et al. 2006

**Table 2a. Listing of Evidence for function claim for probiotics in food:
(Name of probiotic) helps maintain a healthy intestinal microbiota**

INTESTINAL SURVIVAL AND PERSISTENCE OF PROBIOTIC: A (STRONG SCIENTIFIC EVIDENCE)

Type of evidence	Method (trial design)	Participants or subjects	Daily dose and frequency	Treatment characteristics	Duration of treatment	Measures of efficacy, Results, and Conclusions	Reference
I	Feeding trial: two-week pre-feeding period during which the volunteers consumed a beaker of 125 mL plain low-fat, non-sugar yoghurt twice a day, followed by a two-week feeding period for which the volunteers were divided randomly to three groups each containing ten subjects	Three experimental groups in this study each consisted of six to eight adults undergoing routine diagnostic colonoscopy..	100 ml of a commercial drink based on lactose-hydrolyzed whey fermented with strain GG and flavored with a peach-apricot concentrate (Gefilus; Valio Ltd., Kouvola Dairy, Kouvola, Finland) twice daily	The daily dose of strain GG was approximately 6×10^{10} CFU. After administration of strain GG, the volunteers were divided into three groups those having undergone colonoscopy immediately after the 12-day GG administration period (one male, five females, 34 to 78 years old), those having undergone colonoscopy 1 week after stopping GG administration (five males, three females, 42 to 68 years old), and those having undergone colonoscopy 2 weeks after stopping GG administration (four males, three females, 27 to 73 years old)..	12 days	<p>ENDPOINTS OR MEASURES OF EFFICACY: Fecal samples were collected before, during, and after consumption. <i>L. rhamnosus</i> GG-like colonies were detected in both fecal and colonic biopsy samples. Strain GG was identified by its characteristic colony morphology, a lactose fermentation test, and PCR.</p> <p>EFFECTS OR RESULTS: The counts of strain GG-like colonies decreased as a function of time after discontinuation of GG administration. Strain GG was detected in biopsy specimens and final fecal samples of all volunteers in group A. GG-like colonies were 6×10^1 to 4×10^4 CFU per biopsy. In group B, <i>L. rhamnosus</i> GG-like colonies were detected in seven of eight biopsy samples, with counts varying between 2×10^3 and 1×10^6 CFU per biopsy. None of the seven subjects in group C had strain GG-like colonies in the final fecal samples</p> <p>CONCLUSIONS: <i>L. rhamnosus</i> GG is able to attach in vivo to colonic mucosae and to persist there for prolonged periods after discontinuation of administration of strain GG</p>	{Alander, 1999 #159}
I	Feeding trial: The volunteers were divided into three groups.	In Group I one individual withdrew from the study leaving two men (ages 36 and 46) and two women (ages 53 and 54); Group II: two men (ages 46 and 67) and three women (ages 63, 64 and 74); Group III one man (age 59) and four women (ages 40, 58, 72 and 74). The patients were asked to abstain from commercial products containing probiotic organisms during the trial.	One portion of 200 g d^{-1} 5×10^8 cfu/g of Bb12	low-fat, fermented milk product enriched with whey protein; fermented with a yoghurt starter together with three probiotic strains <i>B. animalis</i> subsp. <i>lactis</i> Bb-12, <i>L. acidophilus</i> NCFB 1748 and <i>Lactobacillus paracasei</i> subsp. <i>paracasei</i> LMG P-17806 (Lactobacillus F19).	10 days	<p>ENDPOINTS OR MEASURES OF EFFICACY: Based on the results of culture studies and strain-level analysis by randomly amplified polymorphic DNA (RAPD) fingerprinting; biopsy samples were collected from each volunteer at one sampling occasion as follows: Group I: at the end of the probiotic ingestion period, Group II: 8–9 d after discontinuation of probiotic ingestion, Group III: 18–19 d after discontinuation of probiotic ingestion</p> <p>EFFECTS OR RESULTS: Numbers of bifidobacteria in faecal samples increased during the probiotic ingestion period (comparison of baseline and end of ingestion; $p < 0.05$), and the numbers decreased to the initial level shortly (3–4 d) after probiotic ingestion discontinued. However, during a longer follow-up period the numbers of bifidobacteria tended to increase again.</p> <p>CONCLUSIONS: <i>B. animalis</i> subsp. <i>lactis</i> Bb-12 survived well through the human GI-tract. They were detected in reasonable numbers in the faeces of 79% of the study subjects. In addition to the transient colonization of the probiotic strains, the yoghurt increased the number of indigenous bifidobacteria.</p>	(Matto et al. 2006)

Type of evidence	Method (trial design)	Participants or subjects	Daily dose and frequency	Treatment characteristics	Duration of treatment	Measures of efficacy, Results, and Conclusions	Reference
I	Prospective, double-blinded, placebo-controlled randomized study	Twelve healthy volunteers	6-g bag. Each bag contained 5×10^9 of both <i>L. paracasei</i> strain B21060 and strain B21070 and 0.5×10^9 of <i>L. gasseri</i> strain B21090 [Flortec, Bracco SpA, Milan]. Each subject was instructed to take one bag three times a day (before breakfast, lunch and dinner) for 15 days. The content of the powder had to be dissolved in 50 ml of water before oral intake.	Three periods: 7-day screening and baseline period (day-7 till day 0), 15-day intake period (day 1-day 15) and 3-day post-treatment period (day +1-day+3)	15 days	<p>ENDPOINTS OR MEASURES OF EFFICACY: Faecal samples were collected at day-7 and day 0. During the administration period subjects returned to deliver stool samples collected at day 5, 10 and 15, a general daily questionnaire on daily well-being, stool consistency and frequency and verification of compliance to the study procedures. Endpoints: the ability to survive passage through the gastrointestinal tract was defined as successful if an increase of at least one log in the counts of <i>L. paracasei</i> group was observed in the stool sample at the end of treatment compared to baseline. Persistence was considered adequate if the concentration in the faecal sample after a 3-day discontinuation of intake was equal or only slightly decreased (max 1 log) compared to end of treatment.</p> <p>EFFECTS OR RESULTS (ONLY PERSISTENCE DATA): The microbiology examination of faecal samples showed an increase of at least 1 log of <i>L. paracasei</i> group in all subjects over the intake period. The increase in the counts was rapid and mostly evident after 5 day-administration. Six subjects who had a low number of counts of <i>L. casei</i> group at the baseline had an increase of even 3 log during the intake. Analysis of faecal samples after 3 days in the post-treatment period shows that strains tend to persist as the counts of <i>L. paracasei</i> group (which includes the administered strains) are similar or only slightly decreased compared to those achieved at the end of treatment and higher than those observed at baseline.</p> <p>CONCLUSIONS: These strains seems to persist in the colon for at least 3 days after discontinuation of the oral intake. During the study a favourable increase in total lactobacilli and bifidobacteria was also found.</p>	{Morelli, 2003 #80}
II	Feeding trial	Ten healthy adult volunteers, aged 24–49, three male seven female, were recruited. They were asked to refrain from consumption of <i>Bifidobacterium</i> containing products for three weeks prior to the intervention and during the intervention and follow up.	One bar (25 g) contained 5×10^9 colony forming units (CFU) <i>B. lactis</i> Bb-12.oat-based cereal bar	At the start of the intervention (day 0), a faecal sample was collected. For the following 7 days, the volunteers consumed one <i>B. lactis</i> Bb-12 containing cereal bar daily. At the end of the intervention period (day 7), a faecal sample was collected, two more samples were collected during follow-up (days 11 and 14)	7 days	<p>ENDPOINTS OR MEASURES OF EFFICACY: Of the faecal samples total bacterial counts and <i>B. lactis</i> Bb-12 counts were determined by fluorescent in situ hybridisation (FISH).</p> <p>EFFECTS OR RESULTS: At the end of the intervention period (day 7), <i>B. lactis</i> Bb-12 was detected in the faeces of four subjects, ranging from 1.8×10^6 to 6.4×10^6/g. During follow up, 4 days after consumption of the <i>B. lactis</i> Bb-12 containing cereal bar had stopped (day 11), the strain could be detected in five subjects at a range of 1.8×10^6–4.8×10^7/g. Also 1 week after consumption had seized (day 14), five subjects were still excreting <i>B. lactis</i> Bb-12 in the faeces, 1.8×10^6–8.1×10^7/g. There was no difference in the faecal levels of <i>B. lactis</i> Bb-12 between days 7, 11 and 14, Wilcoxon signed rank test. Consumption of the <i>B. lactis</i> Bb-12 containing cereal bar did not change the total faecal bacterial counts, Wilcoxon signed rank test.</p> <p>CONCLUSIONS: Dry food matrices, such as the oat-based cereal bar used in the current study, provide an equally efficient means of administering probiotics as dairy products</p>	{Ouwehand, 2004 #18}

Type of evidence	Method (trial design)	Participants or subjects	Daily dose and frequency	Treatment characteristics	Duration of treatment	Measures of efficacy, Results, and Conclusions	Reference
I	Randomized, double-blinded, placebo-controlled clinical trial	Twenty-four healthy individuals, 17 women and seven men, The mean age was 28 years (range 21–48 years).	250 mL twice daily for 14 days containing 10 ⁸ cfu/mL of the strains <i>L. acidophilus</i> NCFB 1748, <i>B. lactis</i> Bb12 and <i>Lactobacillus paracasei</i> subsp. <i>paracasei</i> F19.	All subjects received 150 mg clindamycin capsules four times daily for 7 days and were randomized into two groups, the active and the placebo group with reference to the probiotic product. Twelve subjects received a yogurt product. Individuals in the placebo group received a similar product but with no added microorganisms. The administration of the antimicrobial agent and the yogurts commenced on the same day.	7 days	<p>ENDPOINTS OR MEASURES OF EFFICACY: Stool specimens were taken before the administration of clindamycin and the yogurt products (days –3 and 0), during the clindamycin administration (days 2, 5 and 7) and after the administration (days 10, 14 and 21). Detection of the probiotic microorganisms by culture with confirmation of suspect colonies by randomly amplified polymorphic DNA (RAPD)-PCR to verify the identification.</p> <p>EFFECTS OR RESULTS The numbers of enterococci increased after treatment in the placebo group ($P \leq 0.05$) and in the active group, whereas other Gram-positive microorganisms decreased. In the placebo group, the numbers of lactobacilli ($P < 0.05$), bifidobacteria ($P < 0.005$), eubacteria, veillonella and bacteroides ($P < 0.05$) decreased during treatment and increased again at the end of the study period. In subjects receiving yogurt with added microorganisms, the numbers of lactobacilli and bacteroides remained stable throughout the study period, whereas numbers of bifidobacteria ($P < 0.005$) and veillonella ($P < 0.05$) decreased. <i>B. lactis</i> was recovered from four samples of three subjects (range log₁₀ 3.0–6.6 cfu/g faeces).</p> <p>CONCLUSIONS: The probiotic microorganisms tested in this study prevented ecological disturbances in the numbers of intestinal <i>B. fragilis</i> group species during clindamycin administration.</p>	{Sullivan, 2003 #42}

**Table 2b. Listing of Evidence for function claim for probiotics in food:
(Name of probiotic) helps maintain a healthy balance of the intestinal microbiota**

BOWEL TRANSIT TIME OR MOVEMENT

Type of evidence	Method (trial design)	Participants or subjects	Daily dose and frequency	Treatment characteristics	Duration of treatment	Measures of efficacy, Results, and Conclusions	Reference
II	One treatment group.	Eighteen male adult students (age 20-29 years) free of digestive disease.	Daily ingestion of 5×10^{10} cfu of two freeze-dried strains of <i>Propionibacterium freudenreichii</i> (St 26 and SI 41 – Standa-industrie, France), equivalent to the ingestion of 100 g Emmental cheese/day.	All enrolled student received the freeze-dried bacteria.	2 weeks	<p>ENDPOINTS OR MEASURES OF EFFICACY: Feces were collected weekly, twice before (day -8 and day -1), twice during (day 7; day 14) and twice after the end of supplementation (day 21; day 28). Segmental colonic transit time was determined twice before (starting day - 8) and from the end of the first week (starting day 7) of supplementation.</p> <p>EFFECTS OR RESULTS: Basal counts of propionibacteria increase in 15 subjects, from less than 5 log cfu/ml stools to 5.63 and 6.37 on day 7 and day 14 ($p < 0.01$) and returned to basal levels on day 21. Basal counts of bifidobacteria (mean 7.94 log cfu/ml stools to 8.39 on day 7, 8.36 on day 14 and 8.7 on day 21 ($p < 0.05$)). Transit time did not change in the right colon (17.4 h versus 17.3 h) or in the rectosigmoid area (12.8 h versus 13.3 h). Left colon transit was however significantly slowed (7.0 h versus 11.9 h).</p> <p>CONCLUSIONS: Propionibacteria (<i>Propionibacterium freudenreichii</i>) may survived the digestive transit but were not able to colonize the intestine. They however promoted the growth of colonic bifidobacteria and were associated with a deceleration of left colon transit time.</p>	(Bougle et al., 1999)
I	Parallel double blind study.	Seventy two healthy volunteers with a mean age of 30 (range 21-42 years; 36 males and 36 females)	Product A (test group) was a milk fermented by <i>Bifidobacterium animalis</i> (DN-173 010 strain used in the commercial BIO product – Danone) at a concentration of 2.6×10^8 cfu/g Product B (control group) was prepared as the product A heat-treated to contain no viable <i>Bifidobacterium</i> .	During a period preceding the intervention (D01 to D10), usual fermented milk and yoghurt consumption were excluded. Products A (treatment) and B (control) were added to the diet during D10 to D21 period (3 x 125 g daily). Treatment group (A) thus received 9.75×10^{10} cfu of the probiotic bacteria daily.	11 days	<p>ENDPOINTS OR MEASURES OF EFFICACY: The main marker was the total colonic transit time (CTT) measured with radio-opaque pellets. X-rays of abdomen was done on D10 and D21 and the radio-opaque markers were counted in eqach segment of the colon.</p> <p>EFFECTS OR RESULTS: Within the treated group, total colonic transit time was reduced by 20.6% ($p = 0.013$) and sigmoid transit time was reduced by 38.9% ($p = 0.02$) between D10 and D21 whereas right and left colonic transit times tented to be shorter, but not enough to reach statistically significant differences. Within the control group, there were no significant variations of transit times. The difference between initial and finaal total colonic transit time was significantly ($p < 0.05$) greater in the bifidus group (-6.8 hrs) than in the control group (+ 0.5 hr).</p> <p>CONCLUSIONS: A milk fermented with <i>Bifidobacterium animalis</i> DN-173 010 was able to shorten colonic transit time in humans, mainly due to improvement of sigmoid transit time.</p>	Bouvier et al (2001)

Type of evidence	Method (trial design)	Participants or subjects	Daily dose and frequency	Treatment characteristics	Duration of treatment	Measures of efficacy, Results, and Conclusions	Reference
I	Randomized double-blind, cross-over study.	36 healthy women aged 18-45 years without constipation.	The test product was "BIO", commercial semi-skimmed milk fermented by yogurt cultures (at least 10 ⁸ cfu/g) and <i>Bifidobacterium animalis</i> DN-173 010 (between 5 x 10 ⁷ and 10 ⁸ cfu/g), the Bifidus product. The control (placebo) product was the same fermented milk with yogurt cultures without bifidobacteria.	The study comprised four consecutive periods: a 10-day run-in period and two 10-day ingestion periods with an interval of 10 days between them. During the ingestion period, the subjects received three 125 g cups per day of either control product (3.75 x 10 ¹⁰ yogurt cultures) or the tested product containing yogurt cultures plus the Bifidus supplement (between 1.87 and 7.5 x 10 ¹⁰ <i>Bifidobacterium animalis</i>). Half of the subjects received the control before Bifidus (referred as group A) and half received the Bifidus before the control (group B).	Two 10 days with an interval of 10 days between them.	<p>ENDPOINTS OR MEASURES OF EFFICACY: Stool analysis was performed in 12 of the volunteers; all stools in the last 3 days of each study period were collected. In the last 3 days of the run-in and each test period, the subjects ingested every day 20 radio-opaque pellets.</p> <p>EFFECTS OR RESULTS: The total colonic transit time (CTT) and the sigmoid transit time were significantly shorter after Bifidus than after control consumption. In the whole population, CTT significantly decrease from 55.2 h to 51.5 (60.7 for the control group) and in the subgroup with an initial transit time above 40h, the CTT significantly decrease from 70.4 to 62.4 (71.9 for the control) (both p < 0.05). The number of stools per week did not significantly differ between the Bifidus, control and run-in periods (7.0 and 7.0 stools/week). In the 12 subjects analyzed, faecal weight did not differ significantly between the Bifidus and control periods and the products had no significant effect on the fecal bacterial mass.</p> <p>CONCLUSIONS: <i>Bifidobacterium animalis</i> DN-173 010 shortens the colonic transit time in healthy women, but it did not influence fecal excretion of secondary bile salts and it did not influenced fecal weight, bacterial mass.</p>	(Marteau et al., 2002)
I	Randomized.	100 suburban free-living elderly (age ranging between 60 and 75 years) with normal clinical and nutritional examinations and regular stool frequency.	The test product was a commercial dairy product (BIO, provided by Danone, France), a milk fermented by <i>Bifidobacterium animalis</i> DN-173 010 (at least 10 ⁸ cfu/g) and lactic acid cultures (at least 10 ⁸ cfu/g).	Two groups of subjects were constituted. One group of 50 subjects with initial stable oro-fecal transit time (TT) < 40 h and one group of 50 subjects with an initial TT > 40h. Those subjects were randomly assigned to consume during 2 weeks either 2 or 3 servings per day of 125g of bifidobacteria fermented milk (2.5 x 10 ¹⁰ or 3.75 x 10 ¹⁰ cfu total bifidobacteria).	2 weeks	<p>ENDPOINTS OR MEASURES OF EFFICACY: Three consecutive measurements of oro-fecal transit time were done for each subject before and at the end of the product intake period. Colored markers were used to assess oro-fecal transit time (TT).</p> <p>EFFECTS OR RESULTS: The consumption of either 2 or 3 servings of bifidus milk (BM) during 2 weeks decreased significantly the oro-fecal transit time (TT). In subjects with a TT < 40 h, the TT decrease 1.7 h with 2 BM and 2.9 h with 3 BM; in those with a TT > 40h, the TT decreased 24.6 h with 2 BM and 28.6 h with 3 BM. Post values statistically differed from pre values (p < 0.001) and 2MB values were significantly different from 3 BM (p < 0.05).</p> <p>CONCLUSIONS: A 2-week regular consumption of milk fermented with <i>Bifidobacterium animalis</i> DN-173 010 and lactic cultures shortened in a dose-dependant manner transit time in elderly, especially in those with longer transit time.</p>	Meance et al (2001)

Type of evidence	Method (trial design)	Participants or subjects	Daily dose and frequency	Treatment characteristics	Duration of treatment	Measures of efficacy, Results, and Conclusions	Reference
I	Randomized, controlled open study.	200 healthy volunteers aged between 50 and 75 years, with normal clinical and nutritional examination, and regular stool frequency as well as stable oro-fecal transit time.	A commercial test product marketed under the names of BIO or ACTIVIA (provided by Danone, France), a dairy product fermented by the strain <i>Bifidobacterium animalis</i> DN-173 010 plus yogurt cultures (BM). The population of bifidobacteria was equal or at least 10 ⁸ cfu/g. The population of yogurt cultures (<i>Streptococcus thermophilus</i> and <i>Lactobacillus bulgaricus</i>) was equal to at least 10 ⁷ cfu/g.	100 subjects with a medium oro-fecal transit time (MTT, 40-50 h) and 100 with a slow transit time (STT, > 50 h) were randomized to one of two groups (A or B). Subjects in group A, when not receiving treatment, acted when possible as a control for those in group B and vice versa. Following a 7-day run-in period, subjects in group A received 125 g of BM (1.25 x 10 ¹⁰ cfu of bifidobacteria) daily for 2 weeks, followed by a 6-week follow-up period. Subjects in group B received no active treatment for a control period of 4 weeks following the run-in period. At the end of this period, they received 250 g of BM daily (2.5 x 10 ¹⁰ cfu of bifidobacteria) for 2 weeks, followed by a 6-week follow-up period.	2 weeks	<p>ENDPOINTS OR MEASURES OF EFFICACY: Total oro-fecal transit time (TT) was measured in each subject every 2 weeks. Transit times were determined using colored markers.</p> <p>EFFECTS OR RESULTS: Consumption of BM significantly reduced TT in both MTT and STT groups. With one serving of BM (group A), mean TT was reduced by 20% (p < 0.05) compared with initial values and compared with the control group. In group B, TT was significantly reduced, with a 42.2% reduction to 26.9 h after days in MTT subjects. Similarly, statistically significant reductions in TT were observed in the STT group, with around a 27.2% reduction with one 125g serving of BM daily (group A), and a 38.1% reduction compared with initial values. Importantly, the positive effect of BM on intestinal TT continued during the follow-up period in both groups, from 2 to 6 weeks.</p> <p>CONCLUSIONS: This study demonstrated that consumption of fermented milk incorporating the probiotic strain <i>Bifidobacterium animalis</i> DN-173 010 for 2 weeks dose dependently reduced oro-fecal transit time in elderly subjects, especially in those with longer initial transit times.</p>	Meance et al (2003)
I	Randomized double-blind placebo-controlled trial.	30 healthy adult volunteers (15 females and 15 males) with ages ranging from 23 to 43 years old, without gastrointestinal disorders or metabolic diseases.	Yogurt group, used as a control, received on a daily basis 200 ml of a standard yogurt containing 10 ⁸ cfu of <i>Streptococcus thermophilus</i> and 4 x 10 ⁹ cfu of <i>Lactobacillus bulgaricus</i> (Puleva Food, Spain) The probiotic group ingested daily the same amount of yogurt but in which the <i>L. bulgaricus</i> strain had been replaced by 2 x 10 ⁹ cfu of each <i>Lactobacillus. coryniformis</i> (CECT5711) and <i>L. gasseri</i> CECT 5714.	The study consisted of three phases: a pre-treatment period (2 weeks), a treatment period (4 weeks) and a wash-out period (2 weeks).	4 weeks	<p>ENDPOINTS OR MEASURES OF EFFICACY: Once a week, fecal samples were collected. The participant were also asked to annotate the frequency and volume of stools and to compare their bowel function with that existing before the treatment by using a scale ranging from 0 to 10.</p> <p>EFFECTS OR RESULTS: The water content of the fecal samples increased significantly (p < 0.05) at the end of the treatment in the probiotic group and this effect was still observed after the wash-out period. In addition, the stool frequency was significantly higher (p < 0.05) in the probiotic group at the end of the consumption period and even after the wash-out period. The participants in the probiotic group reported a significant increase in the volume of their stools (p < 0.05). During the treatment, the consumption of the probiotic strains led to a significant increase in the number of fecal lactic acid bacteria (from 6.97 to 7.59 log cfu/g of feces). In contrast, in the control group, fecal counts decreased along the treatment period (from 6.82 to 6.48 log cfu/g of feces)</p> <p>CONCLUSIONS: The oral administration of two new probiotic strains, <i>Lactobacillus. coryniformis</i> (CECT5711) and <i>L. gasseri</i> CECT 5714 is well tolerated and exerts a beneficial effects on the bowel function of healthy adults.</p>	(Olivares et al., 2006)