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(54) Title: FERMENTED FORMULA WITH NON-DIGESTIBLE OLIGOSACCHARIDES

(57) Abstract: Administration to infants of an infant formula comprising non-digestible oligosaccharides that preferably also is partly fermented,results in an intestinal metabolomic profile and microbiota function which is more similar to that of breastfed infants than compared to infants fed a conventional infant formula.

FERMENTED FORMULA WITH NON-DIGESTIBLE OLIGOSACCHARIDES

FIELD OF THE INVENTION

The present invention relates to the field of nutrition for infants and young children for improving intestinal microbiota.

BACKGROUND OF THE INVENTION

It is universally accepted that the optimum nutrition for a new-born infant is human milk. When a mother is unable to breastfeed her infant, or chooses not to breastfeed, an infant formula (IF) developed based on the composition of mature human milk is recognised as the best alternative. Research to improve the quality of infant formulas is aimed not necessarily at mimicking the exact composition of human milk but at achieving the functional effects beyond merely the nutritional aspects that are observed in breastfed infants.

The human gut harbors a complex microbial ecosystem, the intestinal microbiota, which has been recognized as an essential part of our human physiology. In human adults the intestinal microbiota is considered to be a stable ecosystem, hence the microbial colonization process in early-life, which is heavily intertwined with the maturation of the gastrointestinal tract itself, can be considered as fundamental step in healthy development. Several environmental factors that can occur in early life have been shown to have long-lasting impact on the intestinal microbiota and its activity, thereby increasing the risk of diseases in later life. Early-life nutrition is a major factor that impacts the developing intestinal microbiota community. Bifidobacterium species typically dominate the intestinal microbiota of breastfed infants, while an intestinal microbiota that is richer in Firmicutes members is typically observed in infants that are fed conventional formulae. However, it is not merely the composition of the intestinal microbiota that is relevant. Also to a large extent it is the function of the microbiota and/or the presence of metabolites that will impact the intestinal physiology and being and have an effect on health now and later in life. Differences are observed between breast fed infants and conventional formula fed infants in these respects, which may explain the why breast fed infants have an improved health outcome on many aspects relating to gut, immune system, brain and metabolic health.

Formulae supplemented with a prebiotic scGOS/lcFOS mixture have been shown to modulate the gut microbiota composition and function, i.e. the metabolic activity and fermentation profile

was promoted towards what is found in human milk fed infants (Knol et al, 2005, JPGN 40:36-42). The effects of this scGOS/lcFOS mixture on the metabolic activity and fermentation profile was previously shown to be maintained to a large extent when added to a partly fermented, using *Bifidobacterium breve* and *Streptococcus thermophilus*, infant milk formulae (Huet, F. et al., 2016, JPGN 63:e43-53).

The above described prior art documents disclose interventions that modulate specific metabolic parameters for which targeted analyses was performed, e.g. the amount and relative contribution of specific short chain fatty acids, and pH, but has not focused on the entire metabolome.

WO 2017/021476 relates to a nutritional composition comprising fucosylated and N-acetylated oligosaccharides for promoting or inducing a global gut microbiota that is closer in function to the one of infants fed exclusively with human breast milk, in comparison to infants fed with a conventional nutritional composition.

However, there is a need for a nutritional composition for use in further improvement of the entire intestinal microbiota in infants or young children, even more similar to the intestinal microbiota of breast fed infants.

SUMMARY OF THE INVENTION

The inventors applied metabolomics, an -omics tool where hundreds of metabolites (often the small molecules, usually < 1,000-1,500 Da) are measured simultaneously, to investigate the functional picture of the gut microbiota in infants and the effect of feeding an experimental formula with non-digestible oligosaccharides, an experimental partly fermented formula with non-digestible oligosaccharides, or a control formula. Metabolites portray a more final phenotype that are the results of all the genetically encoded functions in an ecosystem, as they represent the final sum of all activated genes, epigenetic expression modifications and other transcriptional regulations, posttranslational protein modifications, and environmental factors (both biotic and abiotic). The inventors found that compared to the data on microbiota composition, the metabolomics data showed much more and more extreme differences between the study groups at nearly every time point (180 – 404 metabolites per comparison). At baseline the experimental and control arms were still relatively close to one other, but in time the patterns diverged, where the experimental groups stayed close to the pattern observed in breastfed

infants and the control group became more distinct from the breastfed reference group. This untargeted data set showed that the infant gut ecosystem functioning was highly dependent on and reactive to the diet. The significantly different metabolites originated from numerous of functional categories. One example was the different profile in secondary bile salts, where the pattern in the experimental groups remained more similar to, or deviated less from, the pattern of the breastfed reference group.

The characteristics of the faecal samples in this study reflected that the infants consuming the experimental formula with non-digestible oligosaccharides had more breastfed-like physiological conditions in the gut as compared to the control group. The effects were even more pronounced in the group receiving the formula that additionaly was partly fermented

DETAILED DESCRIPTION OF THE INVENTION

Thus the invention concerns a method for promoting the development in human subjects with an age of 36 months or below of an intestinal microbiota that is in function closer to the intestinal microbiota-function of human subjects at the same age fed with human milk, comprising administering a nutritional composition that comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide, when compared to the intestinal microbiota-function of human subjects with the same age fed a nutritional composition not comprising non-digestible oligosaccharides.

In one embodiment, the method according to the invention can be seen as a non-medical method for promoting the development of an intestinal microbiota-function.

The invention can also be worded as the use of non-digestible oligosaccharide for the manufacture of a nutritional composition that comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide, for use in promoting the development in human subjects with an age of 36 months or below of an intestinal microbiota that is in function closer to the intestinal microbiota-function of human subjects at the same age fed with human milk when compared to the intestinal microbiota-function of human subjects with the same age fed a nutritional composition not comprising non-digestible oligosaccharides.

The invention can also be worded as a nutritional composition that comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide, for use in promoting the development

in human subjects with an age of 36 months or below of an intestinal microbiota that is in function closer to the intestinal microbiota-function of human subjects at the same age fed with human milk when compared to the intestinal microbiota-function of human subjects with the same age fed a nutritional composition not comprising non-digestible oligosaccharides.

Preferably in the method or use according to the invention, the nutritional composition is at least partly fermented by lactic acid producing bacteria comprises 0.02 to 1.5 wt% based on dry weight of the sum of lactic acid and lactate. Moreover, preferable in the method or use according to the invention the promoting the development of an intestinal microbiota-function is when compared to the intestinal microbiota-function of human subjects with the same age fed a nutritional composition not being at least partly fermented by lactic acid producing bacteria and comprising 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight.

Thus the invention also concerns a method for promoting the development in human subjects with an age of 36 months or below of an intestinal microbiota that is in function closer to the intestinal microbiota-function of human subjects at the same age fed with human milk, comprising administering a nutritional composition that is at least partly fermented by lactic acid producing bacteria wherein the nutritional composition comprises 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight and wherein the nutritional composition comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide, when compared to the intestinal microbiota-function of human subjects with the same age fed a nutritional composition not being at least partly fermented by lactic acid producing bacteria and comprising 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight and not comprising non-digestible oligosaccharides.

The invention can also be worded as the use of a fermented composition and non-digestible oligosaccharide for the manufacture of a nutritional composition that is at least partly fermented by lactic acid producing bacteria wherein the nutritional composition comprises 0.02 to 1.5 wt% based on dry weight of the sum of lactic acid and lactate and wherein the nutritional composition comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide, for use in promoting the development in human subjects with an age of 36 months or below of an intestinal microbiota that is in function closer to the intestinal microbiota-function of human subjects at the same age fed with human milk when compared to the intestinal microbiota-function of human subjects with the same age fed a nutritional composition not being at least

partly fermented by lactic acid producing bacteria and comprising 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight and not comprising non-digestible oligosaccharides.

The invention can also be worded as a nutritional composition that is at least partly fermented by lactic acid producing bacteria wherein the nutritional composition comprises 0.02 to 1.5 wt% based on dry weight of the sum of lactic acid and lactate and wherein the nutritional composition comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide, for use in promoting the development in human subjects with an age of 36 months or below of an intestinal microbiota that is in function closer to the intestinal microbiota-function of human subjects at the same age fed with human milk when compared to the intestinal microbiota-function of human subjects with the same age fed a nutritional composition not being at least partly fermented by lactic acid producing bacteria and comprising 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight and not comprising non-digestible oligosaccharides.

Fermented composition

The nutritional composition in the methods or uses according to the present invention, hereafter also referred to as the present nutritional composition, or nutritional composition of the invention or final nutritional composition, is preferably at least party fermented. A partly fermented nutritional composition comprises at least for a part a composition that was fermented by lactic acid producing bacteria. It was shown that the presence of fermented composition in the final nutritional composition results, upon administration, in an intestinal microbiota-function more similar to the intestinal microbiota-function of breastfed infants.

The fermentation preferably takes place during the production process of the nutritional composition. Preferably, the nutritional composition does not contain significant amounts of viable bacteria in the final product due to heat inactivation after fermentation or inactivation by other means. Preferably the fermented composition is a milk-derived product, which is a milk substrate that is fermented by lactic acid producing bacteria, wherein the milk substrate comprises at least one selected from the group consisting of milk, whey, whey protein, whey protein hydrolysate, casein, casein hydrolysate or mixtures thereof. Suitably, nutritional compositions comprising fermented compositions and non-digestible oligosaccharide and their way of producing them are described in WO 2009/151330, WO 2009/151331 and

WO 2013/187764.

The fermented composition preferably comprises bacterial cell fragments like glycoproteins, glycolipids, peptidoglycan, lipoteichoic acid (LTA), lipoproteins, nucleotides, and/or capsular polysaccharides. It is of advantage to use the fermented composition comprising inactivated bacteria and/or cell fragments directly as a part of the final nutritional product, since this will result in a higher concentration of bacterial cell fragments. When commercial preparations of lactic acid producing bacteria are used, these are usually washed and material is separated from the aqueous growth medium comprising the bacterial cell fragments, thereby reducing or eliminating the presence of bacterial cell fragments. Furthermore, upon fermentation and/or other interactions of lactic acid producing bacteria with the milk substrate, additional bio-active compounds can be formed, such as short chain fatty acids, bioactive peptides and/or oligosaccharides, and other metabolites, which may also result in an intestinal microbiota-function more similar to the intestinal microbiota-function of breastfed infants. Such bioactive compounds that that are produced during fermentation by lactic acid producing bacteria or other food grade bacteria may also be referred to as post-biotics. A composition comprising such post-biotics is thought to be advantageously closer to breast milk, as breast milk is not a clean synthetic formula, but contains metabolites, bacterial cells, cell fragments and the like. Therefore the fermented composition, in particular fermented milk-derived product, is believed to have an improved effect compared to non-fermented milk-derived product without or with merely lactic acid producing bacteria on the intestinal microbiota-function.

Preferably the final nutritional composition comprises 5 to 97.5 wt% of the fermented composition based on dry weight, more preferably 10 to 90 wt% , more preferably 20 to 80 wt%, even more preferably 25 to 60 wt%. As a way to specify that the final nutritional composition comprises at least partly a fermented composition, and to specify the extent of fermentation, the level of the sum of lactic acid and lactate in the final nutritional composition can be taken, as this is the metabolic end product produced by the lactic acid producing bacteria upon fermentation. The present final nutritional composition comprises 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight of the composition, more preferably 0.05 to 1.0 wt%, even more preferably 0.1 to 0.5 wt%. Preferably at least 50 wt%, even more preferably at least 90 wt%, of the sum of lactic acid and lactate is in the form of the L(+)-isomer. Thus in one embodiment the sum of L(+)-lactic acid and L(+)-lactate is more than 50 wt%, more

preferably more than 90 wt%, based on the sum of total lactic acid and lactate. Herein L(+)-lactate and L(+)-lactic acid is also referred to as L-lactate and L-lactic acid.

Lactic acid producing bacteria used for producing the fermented ingredient

Lactic acid producing bacteria used for preparing the fermented ingredient, in particular for fermentation of the milk substrate are preferably provided as a mono- or mixed culture. Lactic acid producing bacteria consists of the genera *Bifidobacterium, Lactobacillus, Carnobacterium, Enterococcus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus and Weissella.* Preferably the lactic acid producing bacteria used for fermentation comprises bacteria of the genus *Bifidobacterium* and/or *Streptococcus*.

Preferably the *Streptococcus* is a strain of *S. thermophilus*. Selection of a suitable strain of *S. thermophilus* is described in example 2 of EP 778885 and in example 1 of FR 2723960. In a further preferred embodiment according to the present invention, the nutritional composition comprises 10^2-10^5 cfu living bacteria of *S. thermophilus*, per g dry weight of the final nutritional composition, preferably the final nutritional composition comprises 10^3-10^4 living bacteria of *S. thermophilus* per g dry weight.

Preferred strains of *S. thermophilus* to prepare the fermented ingredient for the purpose of the present invention have been deposited by Compagnie Gervais Danone at the Collection Nationale de Cultures de Microorganismes (CNCM) run by the Institut Pasteur, 25 rue du Docteur Roux, Paris, France on 23 August 1995 under the accession number I-1620 and on 25 August 1994 under the accession number I-1470. Other *S. thermophilus* strains are commercially available.

As *S. thermophilus* does not survive the stomach it is not considered a probiotic bacterium.

Bifidobacteria are Gram-positive, anaerobic, rod-shaped bacteria. Preferred *Bifidobacterium* species to prepare the fermented ingredient for the purpose of the present invention preferably have at least 95 % identity of the 16 S rRNA sequence when compared to the type strain of the respective *Bifidobacterium* species, more preferably at least 97% identity as defined in handbooks on this subject for instance Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989), Molecular Cloning, A Laboratory Manual, 2nd ed., Cold Spring Harbor (N.Y.) Laboratory Press. The Bifodobacteria preferably used are also described by Scardovi, V. Genus

Bifidobacterium. p.1418 - p.1434. In: Bergey's manual of systematic Bacteriology. Vol. 2. Sneath, P.H.A., N.S. Mair, M.E. Sharpe and J.G. Holt (ed.). Baltimore: Williams & Wilkins. 1986. 635 p. Preferably the lactic acid producing bacteria used for fermentation comprises or is at least one *Bifidobacterium* selected from the group consisting of *B. breve, B. infantis, B. bifidum, B. catenulatum, B. adolescentis, B. thermophilum, B. gallicum, B. animalis or lactis, B. angulatum, B. pseudocatenulatum, B. thermacidophilum* and B. *longum* more preferably *B. breve, B. infantis, B. bifidum, B. catenulatum, B. longum*, more preferably *B. longum* and *B. breve*, even more preferably *B. breve*, more preferably *B. breve* selected from the group consisting of *B. breve* Bb-03 (Rhodia/Danisco), *B. breve* M-16V (Morinaga), *B. breve* R0070 (Institute Rosell, Lallemand), *B. breve* BR03 (Probiotical), *B. breve* BR92 (Cell Biotech) DSM 20091, LMG 11613 and *B. breve* I-2219 deposited at the CNCM, Paris France. Most preferably, the *B. breve* is *B. breve* M-16V (Morinaga) or *B. breve* I-2219, even more preferably *B. breve* I-2219.

Most preferably the nutritional composition of the invention comprises fermented composition that is fermented by lactic acid producing bacteria comprising both *B. breve* and *S. thermophilus*. In one embodiment the fermentation by lactic acid producing bacteria is fermentation by *Streptococcus thermophilus* and *Bifidobacterium breve.* In one embodiment, the final nutritional composition comprises fermented composition wherein the lactic acid producing bacteria are inactivated after fermentation.

Preferably the fermented composition is not fermented by *Lactobacillus bulgaricus*. *L. bulgaricus* fermented products are considered not suitable for infants, since in young infants the specific dehydrogenase that converts D-lactate to pyruvate is far less active than the dehydrogenase which converts L-lactate.

Preferably the nutritional composition of the invention comprises inactivated lactic acid producing bacteria and/or bacterial fragments derived from lactic acid producing bacteria obtained from more than $1x10^4$ cfu lactic acid producing bacteria per g based on dry weight of the final composition, more preferably $1x10^5$ cfu, even more preferably $1x10^6$ cfu. Preferably the inactivated bacteria or bacterial fragments are obtained from less than $1x10^{12}$ cfu lactic acid producing bacteria per g based on dry weight of the final composition, more preferably $1x10^{10}$ cfu, even more preferably $1x10^9$ cfu. The correlation of inactivated lactic acid bacteria and cfu

can be determined by molecular techniques, known in the art, or by checking the production process.

Process of fermentation

Preferably the fermented composition is a milk-derived product, which is a milk substrate that is fermented by lactic acid producing bacteria, and said milk substrate comprising at least one selected from the group consisting of milk, whey, whey protein, whey protein hydrolysate, casein, casein hydrolysate or mixtures thereof. The milk derived product or milk substrate to be fermented is suitably present in an aqueous medium. The milk substrate to be fermented comprises at least one selected from the group consisting of milk, whey, whey protein, whey protein hydrolysate, casein, casein hydrolysate or mixtures thereof. Milk can be whole milk, semi-skimmed milk and/or skimmed milk. Preferably the milk substrate to be fermented comprises skimmed milk. Whey can be sweet whey, and/or acid whey. Preferably the whey is present in a concentration of 3 to 80 g dry weight per l aqueous medium containing milk substrate, more preferably 40 to 60 g per l. Preferably whey protein hydrolysate is present in 2 to 80 g dry weight per l aqueous medium containing milk substrate, more preferably 5 to15 g/l. Preferably lactose is present in 5 to 50 g dry weight per l aqueous substrate, more preferably 1 to 30 g/l. Preferably the aqueous medium containing milk substrate comprises buffer salts in order to keep the pH within a desired range. Preferably sodium or potassium dihydrogen phosphate is used as buffer salt, preferably in 0.5 to 5 g/l, more preferably 1.5 to 3 g per l. Preferably the aqueous medium containing milk substrate comprises cysteine in amount of 0.1 to 0.5 g per l aqueous substrate, more preferably 0.2 to 0.4 g/l. The presence of cysteine results in low redox potential of the substrate which is advantageous for activity of lactic acid producing bacteria, particularly *bifidobacteria*. Preferably the aqueous medium containing milk substrate comprises yeast extract in an amount of 0.5 to 5 g/l aqueous medium containing milk substrate, more preferably 1.5 to 3 g/l. Yeast extract is a rich source of enzyme co-factors and growth factors for lactic acid producing bacteria. The presence of yeast extract will enhance the fermentation by lactic acid producing bacteria.

Suitably the milk substrate, in particular the aqueous medium containing milk substrate, is pasteurised before the fermentation step, in order to eliminate the presence of unwanted living bacteria. Suitably the product is pasteurised after fermentation, in order to inactivate enzymes. Suitably the enzyme inactivation takes place at 75 °C for 3 min. Suitably the aqueous medium containing milk substrate is homogenised before and/or the milk-derived product is

homogenised after the fermentation. Homogenisation results in a more stable substrate and/or fermented product, especially in the presence of fat.

The inoculation density is preferably between $1x10^2$ to $5x10^{10}$, preferably between $1x10^4$ to $5x10^9$ cfu lactic acid producing bacteria/ml aqueous medium containing milk substrate, more preferably between $1x10^7$ to $1x10^9$ cfu lactic acid producing bacteria/ml aqueous medium containing milk substrate. The final bacteria density after fermentation is preferably between $1x10^3$ to $1x10^{10}$, more preferably between $1x10^4$ to $1x10^9$ cfu/ml aqueous medium containing milk substrate.

The fermentation is preferably performed at a temperature of approximately 20 °C to 50 °C, more preferably 30 °C to 45 °C, even more preferably approximately 37 °C to 42 °C. The optimum temperature for growth and/or activity for lactic acid producing bacteria, more particularly lactobacilli and/or bifidobacteria is between 37 °C and 42 °C.

The incubation is preferably performed at a pH of 4 to 8, more preferably 6 to 7.5. This pH does not induce protein precipitation and/or an adverse taste, while at the same time lactic acid producing bacteria such as lactobacilli and/or bifidobacteria are able to ferment the milk substrate.

The incubation time preferably ranges from 10 minutes to 48 h, preferably from 2 h to 24 h, more preferably from 4 h to 12 h. A sufficient long time enables fermentation and the concomitant production of immunogenic cell fragments such as glycoproteins, glycolipids, peptidoglycan, lipoteichoic acid (LTA), flagellae, lipoproteins, DNA and/or capsular polysaccharides and metabolites (postbiotics) to take place at a sufficient or higher extent, whereas the incubation time needs not be unnecessarily long for economical reasons.

Preferably, a milk derived product or milk substrate, preferably skimmed milk, is pasteurized, cooled and fermented with one or more lactic acid producing strains, preferably a strain of *S. thermophilus*, to a certain degree of acidity, upon which the fermented product is cooled and stored. Preferably a second milk-derived product is prepared in a similar way using one or more *Bifidobacterium* species for fermentation. Subsequently, the two fermented products are preferably mixed together and mixed with other components making up an infant formula, except the fat component. Preferably, the mixture is preheated, and subsequently fat is added

in-line, homogenized, pasteurized and dried. Alternatively the fermentation takes place having both *Bifidobacterium*, preferably *B. breve*, and *S. thermophilus* in the fermentation tank.

Procedures to prepare fermented composition suitable for the purpose of the present invention are known per se. EP 778885, which is incorporated herein by reference, discloses in particular in example 7 a suitable process for preparing a fermented ingredient. FR 2723960, which is incorporated herein by reference, discloses in particular in example 6 a suitable process for preparing a fermented ingredient. Briefly, a milk substrate, preferably pasteurised, containing lactose and optionally further macronutrients such as fats, preferably vegetable fats, casein, whey protein, vitamins and/or minerals etc. is concentrated, e.g. to between 15 to 50% dry matter and then inoculated with *S. thermophilus*, for example with 5% of a culture containing 10^6 to 10^{10} bacteria per ml. Preferably this milk substrate comprises milk protein peptides. Temperature and duration of fermentation are as mentioned above. Suitably after fermentation the fermented ingredient may be pasteurised or sterilized and for example spray dried or lyophilised to provide a form suitable to be formulated in the end product.

A preferred method for preparing the fermented composition to be used in the nutritional composition of invention is disclosed in WO 01/01785, more particular in examples 1 and 2. A preferred method for preparing the fermented composition to be used in the nutritional composition of invention is described in WO 2004/093899, more particularly in example 1.

Living cells of lactic acid producing bacteria in the fermented composition are after fermentation preferably eliminated, for example by inactivation and/or physical removal. The cells are preferably inactivated. Preferably the lactic acid producing bacteria are heat killed after fermentation of the milk substrate. Preferable ways of heat killing are (flash) pasteurization, sterilization, ultra-high temperature treatment, high temperature/short time heat treatment, and/or spray drying at temperatures bacteria do not survive. Cell fragments are preferably obtained by heat treatment. With this heat treatment preferably at least 90 % of living microorganisms are inactivated, more preferably at least 95 %, even more preferably at least 99 %. Preferably the fermented nutritional composition comprises less than $1x10^5$ colony forming units (cfu) living lactic acid bacteria per g dry weight. The heat treatment preferably is performed at a temperature ranging from 70 to180 °C, preferably from 80 to 150 °C, preferably for about 3 minutes to 2 hours, preferably in the range of 80 to 140 °C for 5 minutes to 40 minutes. Inactivation of the lactic acid bacteria advantageously results in less post acidification

and a safer product. This is especially advantageous when the nutritional composition is to be administered to infants or toddlers. Suitably after fermentation the fermented ingredient may be pasteurised or sterilized and for example spray dried or lyophilised to provide a form suitable to be formulated in the end product.

Non-digestible oligosaccharides

The present nutritional composition comprises non-digestible oligosaccharide and preferably comprises at least two different non-digestible oligosaccharides, in particular two different sources of non-digestible oligosaccharide. It was shown that the presence of non-digestible oligosaccharides improve the functioning of intestinal microbiota in making it more similar to the functioning of intestinal microbiota of breastfed infants. Hence, the presence of both the non-digestible oligosaccharide and the fermented composition, in particular the milk-derived product obtained by fermentation with lactic acid producing bacteria, synergistically and advantageously results in intestinal microbiota that is in function more similar to the intestinal microbiota-function of infants that are predominantly or exclusively breastfed.

The term "oligosaccharide" as used herein refers to saccharides with a degree of polymerization (DP) of 2 to 250, preferably a DP 2 to 100, more preferably 2 to 60, even more preferably 2 to 10. If oligosaccharide with a DP of 2 to 100 is included in the present nutritional composition, this results in compositions that may contain oligosaccharides with a DP of 2 to 5, a DP of 50 to 70 and a DP of 7 to 60. The term "non-digestible oligosaccharide" as used in the present invention refers to oligosaccharides which are not digested in the intestine by the action of acids or digestive enzymes present in the human upper digestive tract, e.g. small intestine and stomach, but which are preferably fermented by the human intestinal microbiota. For example, sucrose, lactose, maltose and maltodextrins are considered digestible.

Preferably the present non-digestible oligosaccharide is soluble. The term "soluble" as used herein, when having reference to a polysaccharide, fibre or oligosaccharide, means that the substance is at least soluble according to the method described by L. Prosky et al., J. Assoc. Off. Anal. Chem. 71, 1017-1023 (1988).

The non-digestible oligosaccharide included in the present nutritional compositions in the methods or uses according to the present invention preferably include a mixture of non-digestible oligosaccharides. The non-digestible oligosaccharide is preferably selected from the

group consisting of fructo-oligosaccharide, such as inulin, non-digestible dextrins, galactooligosaccharide, such as transgalacto-oligosaccharide, xylo-oligosaccharide, arabino-oligosaccharide, arabinogalacto-oligosaccharide, gluco-oligosaccharide, gentio-oligosaccharide, glucomanno-oligosaccharide, galactomannooligosaccharide, mannan-oligosaccharide, isomalto-oligosaccharide, nigero-oligosaccharide, glucomanno-oligosaccharide, chito-oligosaccharide, soy oligosaccharide, uronic acid oligosaccharide and mixtures thereof. Such oligosaccharides share many biochemical properties and have similar functional benefits including improving the intestinal microbiota-function. Yet is understood that some non-digestible oligosaccharides and preferably some mixtures have an even further improved effect. Therefore more preferably the non-digestible oligosaccharides are selected from the group consisting of fructo-oligosaccharide, such as inulin, galacto-oligosaccharide, such as betagalacto-oligosaccharide, and mixtures thereof, even more preferably betagalacto-oligosaccharide and/or inulin, most preferably betagalacto-oligosaccharide. In one embodiment in the nutritional composition according to the present invention, the non-digestible oligosaccharide is selected from the group consisting of galacto-oligosaccharide, fructo-oligosaccharide and mixtures of thereof, more preferably betagalacto-oligosaccharides, fructo-oligosaccharides and mixtures thereof.

The non-digestible oligosaccharide is preferably selected from the group consisting of β-galacto-oligosaccharide, α-galacto-oligosaccharide, and galactan. According to a more preferred embodiment non-digestible oligosaccharide is β-galactooligosaccharide. Preferably the non-digestible oligosaccharide comprises galactooligosaccharide with β(1,4), β(1,3) and/or β(1,6) glycosidic bonds and a terminal glucose. Transgalacto-oligosaccharide is for example available under the trade name Vivinal®GOS (Domo FrieslandCampina Ingredients), Bi2muno (Clasado), Cup-oligo (Nissin Sugar) and Oligomate55 (Yakult). These oligosaccharides improve the intestinal microbiota-function to a larger extent.

The non-digestible oligosaccharide preferably comprises fructo-oligosaccharide. A fructo-oligosaccharide may in other context have names like fructopolysaccharide, oligofructose, polyfructose, polyfructan, inulin, levan and fructan and may refer to oligosaccharides comprising β-linked fructose units, which are preferably linked by β(2,1) and/or β(2,6) glycosidic linkages, and a preferable DP between 2 and 200. Preferably, the fructo-oligosaccharide contains a terminal β(2,1) glycosidic linked glucose. Preferably, the fructo-oligosaccharide contains at least 7 β-linked fructose units. In a further preferred embodiment

inulin is used. Inulin is a type of fructo-oligosaccharide wherein at least 75% of the glycosidic linkages are β(2,1) linkages. Typically, inulin has an average chain length between 8 and 60 monosaccharide units. A suitable fructo-oligosaccharide for use in the compositions of the present invention is commercially available under the trade name Raftiline®HP (Orafti). Other suitable sources are Raftilose (Orafti), Fibrulose and Fibruline (Cosucra) and Frutafit and Frutalose (Sensus).

Preferably the present nutritional composition comprises a mixture of galacto-oligosaccharide and fructo-oligosaccharide. Preferably the mixture of galacto-oligosaccharide and fructo-oligosaccharide is present in a weight ratio of from 1/99 to 99/1, more preferably from 1/19 to 19/1, more preferably from 1/1 to 19/1, more preferably from 2/1 to 15/1, more preferably from 5/1 to 12/1, even more preferably from 8/1 to 10/1, even more preferably in a ratio of about 9/1. This weight ratio is particularly advantageous when galacto-oligosaccharide has a low average DP and fructo-oligosaccharide has a relatively high DP. Most preferred is a mixture of galacto-oligosaccharide with an average DP below 10, preferably below 6 and a fructo-oligosaccharide with an average DP above 7, preferably above 11, even more preferably above 20. Such a mixture synergistically improves the intestinal microbiota-function in infants by making it more similar to the intestinal microbiota-function of breastfed infants.

Preferably the present nutritional composition comprises a mixture of short chain fructo-oligosaccharide and long chain fructo-oligosaccharide. Preferably the mixture of short chain fructo-oligosaccharide and long chain fructo-oligosaccharide is present in a weight ratio of from 1/99 to 99/1, more preferably from 1/19 to 19/1, even more preferably from 1/10 to 19/1, more preferably from 1/5 to 15/1, more preferably from 1/1 to 10/1. Preferred is a mixture of short chain fructo-oligosaccharide with an average DP below 10, preferably below 6 and a fructo-oligosaccharide with an average DP above 7, preferably above 11, even more preferably above 20.

Preferably the present nutritional composition comprises a mixture of short chain fructo-oligosaccharide and short chain galacto-oligosaccharides. Preferably the mixture of short chain fructo-oligosaccharide and short chain galacto-oligosaccharides is present in a weight ratio of from 1/99 to 99/1, more preferably from 1/19 to 19/1, even more preferably from 1/10 to 19/1, more preferably from 1/5 to 15/1, more preferably from 1/1 to 10/1. Preferred is a mixture of

short chain fructo-oligosaccharide and galacto-oligosaccharides with an average DP below 10, preferably below 6.

The present nutritional composition comprises 2.5 to 20 wt% total non-digestible oligosaccharide, more preferably 2.5 to 15 wt%, even more preferably 3.0 to 10 wt%, most preferably 5.0 to 7.5 wt%, based on dry weight of the nutritional composition. Based on 100 ml the present nutritional composition preferably comprises 0.35 to 2.5 wt% total non-digestible oligosaccharide, more preferably 0.35 to 2.0 wt%, even more preferably 0.4 to 1.5 wt%, based on 100 ml of the nutritional composition. A lower amount of non-digestible oligosaccharide will be less effective in improving the intestinal microbiota-function, whereas a too high amount will result in side-effects of bloating and abdominal discomfort.

Nutritional composition

The nutritional composition used according to the present invention may also be considered as being a pharmaceutical composition and is preferably suitable for administration to infants. The present nutritional composition is preferably for enteral administration, more preferably for oral administration.

Preferably the nutritional composition used according to the present invention present is not a probiotic composition or a composition comprising probiotics. The lactic acid producing bacteria are preferably either rendered non-replicating or inactivated during the production and/or do not survive under conditions present in the human upper gastro-intestinal tract.

The present nutritional composition is preferably an infant formula, follow on formula, toddler milk or toddler formula, or growing up milk intended for young children. The present nutritional composition can be advantageously applied as a complete nutrition for infants. Preferably the present nutritional composition is an infant formula. An infant formula is defined as a formula for use in infants and can for example be a starter formula, intended for infants of 0 to 6 or 0 to 4 months of age. A follow on formula is intended for infants of 4 or 6 months to 12 months of age. At this age infants start weaning on other food. A toddler or growing up milk or formula is intended for children of 12 to 36 months of age. The present composition preferably comprises a lipid component, protein component and carbohydrate component and is preferably administered in liquid form. The present nutritional composition may also be in the form of a dry food, preferably in the form of a powder which is accompanied with instructions as to mix

said dry food, preferably powder, with a suitable liquid, preferably water. The nutritional composition used according to the invention preferably comprises other fractions, such as vitamins, minerals, trace elements and other micronutrients in order to make it a complete nutritional composition. Preferably infant formulas comprise vitamins, minerals, trace elements and other micronutrients according to international directives.

The present nutritional composition preferably comprises lipid, protein and digestible carbohydrate wherein the lipid provides 5 to 50% of the total calories, the protein provides 5 to 50% of the total calories, and the digestible carbohydrate provides 15 to 90% of the total calories. Preferably, in the present nutritional composition the lipid provides 35 to 50% of the total calories, the protein provides 7.0 to 12.5% of the total calories, and the digestible carbohydrate provides 40 to 55% of the total calories. For calculation of the % of total calories for the protein, the total of energy provided by proteins, peptides and amino acids needs to be taken into account. Preferably the lipid provides 3 to 7 g lipid per 100 kcal, preferably 4 to 6 g per 100 kcal, the protein provides 1.6 to 4 g per 100 kcal, preferably 1.7 to 2.5 g per 100 kcal and the digestible carbohydrate provides 5 to 20 g per 100 kcal, preferably 8 to 15 g per 100 kcal of the nutritional composition. Preferably the present nutritional composition comprises lipid providing 4 to 6 g per 100 kcal, protein providing 1.6 to 2.0 g per 100 kcal, more preferably 1.7 to 1.9 g per 100 kcal and digestible carbohydrate providing 8 to 15 g per 100 kcal of the nutritional composition. In one embodiment, the lipid provides 3 to 7 g lipid per 100 kcal, preferably 4 to 6 g per 100 kcal, the protein provides 1.6 to 2.1 g per 100 kcal, preferably 1.6 to 2.0 g per 100 kcal and the digestible carbohydrate provides 5 to 20 g per 100 kcal, preferably 8 to 15 g per 100 kcal of the nutritional composition and wherein preferably the digestible carbohydrate component comprises at least 60 wt% lactose based on total digestible carbohydrate, more preferably at least 75 wt%, even more preferably at least 90 wt% lactose based on total digestible carbohydrate. The amount of total calories is determined by the sum of calories derived from protein, lipids, digestible carbohydrates and non-digestible oligosaccharide.

The present nutritional composition preferably comprises a digestible carbohydrate component. Preferred digestible carbohydrate components are lactose, glucose, sucrose, fructose, galactose, maltose, starch and maltodextrin. Lactose is the main digestible carbohydrate present in human milk. The present nutritional composition preferably comprises lactose. As the present nutritional composition comprises a fermented composition that is obtained by fermentation by

lactic acid producing bacteria, the amount of lactose is reduced compared to its source due to the fermentation whereby lactose is converted into lactate and/or lactic acid. Therefore in the preparation of the present nutritional composition lactose is preferably added. Preferably the present nutritional composition does not comprise high amounts of carbohydrates other than lactose. Compared to digestible carbohydrates such as maltodextrin, sucrose, glucose, maltose and other digestible carbohydrates with a high glycemic index, lactose has a lower glycemic index and is therefore preferred. The present nutritional composition preferably comprises digestible carbohydrate, wherein at least 35 wt%, more preferably at least 50 wt%, more preferably at least 60 wt%, more preferably at least 75 wt%, even more preferably at least 90 wt% , most preferably at least 95 wt% of the digestible carbohydrate is lactose. Based on dry weight the present nutritional composition preferably comprises at least 25 wt% lactose, preferably at least 40 wt%, more preferably at least 50 wt% lactose.

The present nutritional composition preferably comprises at least one lipid selected from the group consisting of animal lipid (excluding human lipids) and vegetable lipids. Preferably the present composition comprises a combination of vegetable lipids and at least one oil selected from the group consisting of fish oil, animal oil, algae oil, fungal oil, and bacterial oil. The lipid of the present nutritional composition preferably provides 3 to 7 g per 100 kcal of the nutritional composition, preferably the lipid provides 4 to 6 g per 100 kcal. When in liquid form, e.g. as a ready-to-feed liquid, the nutritional composition preferably comprises 2.1 to 6.5 g lipid per 100 ml, more preferably 3.0 to 4.0 g per 100 ml. Based on dry weight the present nutritional composition preferably comprises 12.5 to 40 wt% lipid, more preferably 19 to 30 wt%. Preferably the lipid comprises the essential fatty acids alpha-linolenic acid (ALA), linoleic acid (LA) and/or long chain polyunsaturated fatty acids (LC-PUFA). The LC-PUFA, LA and/or ALA may be provided as free fatty acids, in triglyceride form, in diglyceride form, in monoglyceride form, in phospholipid form, or as a mixture of one of more of the above. Preferably the present nutritional composition comprises at least one, preferably at least two lipid sources selected from the group consisting of rape seed oil (such as colza oil, low erucic acid rape seed oil and canola oil), high oleic sunflower oil, high oleic safflower oil, olive oil, marine oils, microbial oils, coconut oil, palm kernel oil. The present nutritional composition is not human milk.

The present nutritional composition preferably comprises protein. The protein used in the nutritional composition is preferably selected from the group consisting of non-human animal

proteins, preferably milk proteins, vegetable proteins, such as preferably soy protein and/or rice protein, and mixtures thereof. The present nutritional composition preferably contains casein, and/or whey protein, more preferably bovine whey proteins and/or bovine casein. Thus in one embodiment the protein in the present nutritional composition comprises protein selected from the group consisting of whey protein and casein, preferably whey protein and casein, preferably the whey protein and/or casein is from cow's milk. Preferably the protein comprises less than 5 wt% based on total protein of free amino acids, dipeptides, tripeptides or hydrolyzed protein. The present nutritional composition preferably comprises casein and whey proteins in a weight ratio casein : whey protein of 10 : 90 to 90 : 10, more preferably 20 : 80 to 80 : 20, even more preferably 35 : 65 to 55 : 45.

The wt% protein based on dry weight of the present nutritional composition is calculated according to the Kjeldahl-method by measuring total nitrogen and using a conversion factor of 6.38 in case of casein, or a conversion factor of 6.25 for other proteins than casein. The term 'protein' or 'protein component' as used in the present invention refers to the sum of proteins, peptides and free amino acids.

The present nutritional composition preferably comprises protein providing 1.6 to 4.0 g protein per 100 kcal of the nutritional composition, preferably providing 1.6 to 3.5 g, even more preferably 1.75 to 2.5 g per 100 kcal of the nutritional composition. In one embodiment, the present nutritional composition comprises protein providing 1.6 to 2.1 g protein per 100 kcal of the nutritional composition, preferably providing 1.6 to 2.0 g, more preferably 1.75 to 2.1 g, even more preferably 1.75 to 2.0 g per 100 kcal of the nutritional composition. In one embodiment, the present nutritional composition comprises protein in an amount of less than 2.0 g per 100 kcal, preferably providing 1.6 to 1.9 g, even more preferably 1.75 to 1.85 g per 100 kcal of the nutritional composition. A too low protein content based on total calories will result is less adequate growth and development in infants and young children. A too high amount will puta metabolic bude, e.g. on the kidneys of infants and young children. When in liquid form, e.g. as a ready-to-feed liquid, the nutritional composition preferably comprises 0.5 to 6.0 g, more preferably 1.0 to 3.0 g, even more preferably 1.0 to 1.5 g protein per 100 ml, most preferably 1.0 to 1.3 g protein per 100 ml. Based on dry weight the present nutritional composition preferably comprises 5 to 20 wt% protein, preferably at least 8 wt% protein based on dry weight of the total nutritional composition, more preferably 8 to 14 wt%, even more preferably 8 to 9.5 wt% protein based on dry weight of the total nutritional composition.

In order to meet the caloric requirements of an infant or toddler, the nutritional composition preferably comprises 45 to 200 kcal/100 ml liquid. For infants the nutritional composition has more preferably 60 to 90 kcal/100 ml liquid, even more preferably 65 to 75 kcal/100 ml liquid. This caloric density ensures an optimal ratio between water and calorie consumption. For toddlers, human subjects with an age between 12 and 36 months, the nutritional composition more preferably has a caloric density between 45 and 65, even more preferably between 50 and 60 kcal/100 ml. The osmolarity of the present composition is preferably between 150 and 420 mOsmol/l, more preferably 260 to 320 mOsmol/l. The low osmolarity aims to further reduce the gastrointestinal stress.

When the nutritional composition is in a ready to feed, liquid form, the preferred volume administered on a daily basis is in the range of about 80 to 2500 ml, more preferably about 200 to 1200 ml per day. Preferably, the number of feedings per day is between 1 and 10, preferably between 3 and 8. In one embodiment the nutritional composition is administered daily for a period of at least 2 days, preferably for a period of at least 4 weeks, preferably for a period of at least 8 weeks, more preferably for a period of at 25 least 12 weeks, in a liquid form wherein the total volume administered daily is between 200 ml and 1200 ml and wherein the number of feedings per day is between 1 and 10.

The present nutritional composition, when in liquid form, preferably has a viscosity between 1 and 60 mPa.s, preferably between 1 and 20 mPa.s, more preferably between 1 and 10 mPa.s, most preferably between 1 and 6 mPa.s. The low viscosity ensures a proper administration of the liquid, e.g. a proper passage through the whole of a nipple. Also this viscosity closely resembles the viscosity of human milk. Furthermore, a low viscosity results in a normal gastric emptying and a better energy intake, which is essential for infants which need the energy for optimal growth and development. The present nutritional composition alternatively is in powder form, suitable for reconstitution with water to a ready to drink liquid. The present nutritional composition is preferably prepared by admixing a powdered composition with water. Normally infant formula is prepared in such a way. The present invention thus also relates to a packaged power composition wherein said package is provided with instructions to admix the powder with a suitable amount of liquid, thereby resulting in a liquid composition with a viscosity between 1 and 60 mPa.s. The viscosity of the liquid is determined using a

Physica Rheometer MCR 300 (Physica Messtechnik GmbH, Ostfilden, Germany) at a shear rate of 95 s^{-1} at 20 °C.

Application

In the context of the present invention, 'prevention' of a disease or certain disorder also means 'reduction of the risk' of a disease or certain disorder and also means 'treatment of a person at risk' of said disease or said certain disorder.

The methods according to the present invention comprising administering the present nutritional composition also refer to administering an effective amount of the nutritional composition to an individual in need of such treatment.

In this age of -omics-based technologies increasingly sophisticated tools have become available to study the gut microbiota at different molecular levels. In the last decade, the most widely employed tools to investigate the gut microbiota are based on sequencing (part of) bacterial 16S rRNA gene sequences and identifying which bacterial lineages are present. However, gut microbiota profiling by DNA based methodologies does not provide a direct view of the functional level of the gut ecosystem.

Metabolomics is the systematic study of the unique chemical fingerprints that specific cellular processes leave behind, the study of their small-molecule metabolite profiles. The metabolome represents the collection of all metabolites in a biological cell, tissue, organ or organism, which are the end products of cellular processes. The metabolome refers to the complete set of small-molecule chemicals found within a biological sample. The small molecule chemicals found in a given metabolome may include both endogenous metabolites that are naturally produced by an organism (such as amino acids, organic acids, nucleic acids, fatty acids, amines, sugars, vitamins, co-factors, pigments, antibiotics, etc.) as well as exogenous chemicals. To qualify as a metabolite, or to be considered to be part of the metabolome, a small molecule must typically have a molecular weight <1500 Da. Metabolomics could contribute considerably to the functional picture of the gut microbiota. After all, metabolites portray a more final phenotype that are the results of all the genetically encoded functions in an ecosystem, as they represent the final sum of all activated genes, epigenetic expression modifications and other transcriptional regulations, posttranslational protein modifications, and environmental factors (both biotic and abiotic).

The significantly different metabolites represented numerous functional categories. This untargeted data set illustrates that infant gut ecosystem functioning is highly dependent on and reactive to the diet.

The characteristics of the faecal samples in this study reflected that the infants consuming the formula with non-digestible oligosaccharides, preferably consuming partly fermented formula with non-digestible oligosaccharides, have a more breastfed like microbiota composition and more breastfed like physiological conditions in the gut as compared to the control group. Moreover, the more in-depth untargeted analyses showed that the gut microbiota functioning in infants fed the inventive, preferably partly fermented, formula deviated less from the breastfed reference group than in infants fed control formula, suggesting that the formula with non-digestible oligosaccharides, preferably the partly fermented formula with non-digestible oligosaccharides, drives the gut ecosystem towards a more breastfed-like situation. Superpathways relating to amino acid, lipid, xenobiotics, carbohydrates, nucleotides, cofactors and vitamins, energy and peptide were found to be affected. The largest effects in change in number of metabolites were shown in pathways relating to amino acids, lipids, and when looking at relative changes (number of pathway metabolites changed/total pathway metabolites tested) also high differences were observed in pathways relating to energy, nucleotides and cofactors and vitamins.

In their study of metabolite profiles, the inventors have found that that upon consumption of the nutritional composition of the present invention the intestinal metabolome of infants is more similar to the intestinal metabolome of breastfed infants. The intestinal metabolome was more similar to breastfed infants' intestinal metabolome when compared to the intestinal metabolome of infants fed formula without non-digestible oligosaccharides and when compared to the intestinal metabolome of infants fed non-fermented formula without non-digestible oligosaccharides.

Hence in a preferred embodiment according to the methods or uses of the present invention, promoting the development of an intestinal microbiota-function refers an intestinal metabolome that is more similar to the intestinal metabolome of human subjects at the same age fed with human milk when compared to the intestinal metabolome of human subjects with the same age fed a nutritional composition not comprising non-digestible oligosaccharides.

Also in a preferred embodiment according to the methods or uses of the present invention, promoting the development of an intestinal microbiota-function refers an intestinal metabolome that is more similar to the intestinal metabolome of human subjects at the same age fed with human milk when compared to the intestinal metabolome of human subjects with the same age fed a nutritional composition not being at least partly fermented by lactic acid producing bacteria and comprising 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight and not comprising non-digestible oligosaccharides.

In one aspect, the present invention concerns a method for establishing a metabolome in human subjects with an age of 36 months or below that is more similar to the intestinal metabolome of human subjects at the same age fed with human milk, comprising administering a nutritional composition that comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide, when compared to the intestinal metabolome of human subjects with the same age fed a nutritional composition not comprising non-digestible oligosaccharides.

In one embodiment, the method according to the above aspect invention can be seen as a non-medical method for establishing a metabolome.

The invention can also be worded as the use of non-digestible oligosaccharide for the manufacture of a nutritional composition that comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide, for use in establishing a metabolome in human subjects with an age of 36 months or below that is more similar to the intestinal metabolome of human subjects at the same age fed with human milk when compared to the intestinal metabolome of human subjects with the same age fed a nutritional composition not comprising non-digestible oligosaccharides.

The invention can also be worded as a nutritional composition that comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide, for use in establishing a metabolome in human subjects with an age of 36 months or below that is more similar to the intestinal metabolome of human subjects at the same age fed with human milk when compared to the intestinal metabolome of human subjects with the same age fed a nutritional composition not comprising non-digestible oligosaccharides.

In one embodiment, the present invention concerns a method for establishing a metabolome in human subjects with an age of 36 months or below that is more similar to the intestinal metabolome of human subjects at the same age fed with human milk, comprising administering a nutritional composition that is at least partly fermented by lactic acid producing bacteria wherein the nutritional composition comprises 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight and wherein the nutritional composition comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide, when compared to the intestinal metabolome of human subjects with the same age fed a nutritional composition not being at least partly fermented by lactic acid producing bacteria and comprising 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight and not comprising non-digestible oligosaccharides.

The invention can also be worded as the use of a fermented composition and non-digestible oligosaccharide for the manufacture of a nutritional composition that is at least partly fermented by lactic acid producing bacteria wherein the nutritional composition comprises 0.02 to 1.5 wt% based on dry weight of the sum of lactic acid and lactate and wherein the nutritional composition comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide, for use in establishing a metabolome in human subjects with an age of 36 months or below that is more similar to the intestinal metabolome of human subjects at the same age fed with human milk when compared to the intestinal metabolome of human subjects with the same age fed a nutritional composition not being at least partly fermented by lactic acid producing bacteria and comprising 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight and not comprising non-digestible oligosaccharides.

The invention can also be worded as a nutritional composition that is at least partly fermented by lactic acid producing bacteria wherein the nutritional composition comprises 0.02 to 1.5 wt% based on dry weight of the sum of lactic acid and lactate and wherein the nutritional composition comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide, for use in establishing a metabolome in human subjects with an age of 36 months or below that is more similar to the intestinal metabolome of human subjects at the same age fed with human milk when compared to the intestinal metabolome of human subjects with the same age fed a nutritional composition not being at least partly fermented by lactic acid producing bacteria and comprising 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight and not comprising non-digestible oligosaccharides.

Preferably the intestinal metabolic profile or metabolome observed in infants consuming the nutritional composition according to the present invention has less than 45 % of metabolites that are significantly different in level, when compared to that observed for breastfed infants, more preferably less than 40 %, even more preferably less than 35 % of the metabolites are at a significantly different level. Preferably the intestinal metabolic profile or metabolome observed in infants consuming the nutritional composition according to the present invention has more than 10 % of metabolites that are significantly different in level, when compared to control formula fed infants, more preferably more than 15%, even more preferably more than 20% of the metabolites are at a significantly different level.

In a preferred embodiment according to the methods or uses of the present invention, the intestinal metabolome has less than 45 % of metabolites that are significantly different in level, when compared to the intestinal metabolome of human subjects at the same age fed with human milk, more preferably less than 40 %, even more preferably less than 35 % of the metabolites are at a significantly different level, and has more than 10 % of metabolites that are significantly different in level, when compared to the intestinal metabolome of human subjects with the same age fed a nutritional composition not comprising non-digestible oligosaccharides, more preferably more than 15%, even more preferably more than 20% of the metabolites are at a significantly different level.

In a preferred embodiment according to the methods or uses of the present invention, the intestinal metabolic profile or metabolome observed in infants consuming the nutritional composition according to the present invention has at least 5 % less metabolites that are significantly different in level related to the intestinal metabolome of human subjects at the same age fed with human milk, when compared to the metabolites that are significantly different in level in human subjects with the same age fed a nutritional composition not comprising non-digestible oligosaccharides related to the intestinal metabolome of human subjects at the same age fed with human milk, more preferably the intestinal metabolome has at least 10 % less metabolites that are significantly different in level.

In one aspect, the present invention concerns a method for establishing a metabolome in human subjects with an age of 36 months or below that has at least 5 % less metabolites that are significantly different in level related to the intestinal metabolome of human subjects at the

same age fed with human milk, comprising administering a nutritional composition that comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide, when compared to the metabolites that are significantly different in level in human subjects with the same age fed a nutritional composition not comprising non-digestible oligosaccharides related to the intestinal metabolome of human subjects at the same age fed with human milk, more preferably the intestinal metabolome has at least 10 % less metabolites that are significantly different in level.

The invention can also be worded as the use of and non-digestible oligosaccharide for the manufacture of a nutritional composition that comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide, for use in establishing a metabolome in human subjects with an age of 36 months or below that has at least 5 % less metabolites that are significantly different in level related to the intestinal metabolome of human subjects at the same age fed with human milk, when compared to the metabolites that are significantly different in level in human subjects with the same age fed a nutritional composition not comprising non-digestible oligosaccharides related to the intestinal metabolome of human subjects at the same age fed with human milk, more preferably the intestinal metabolome has at least 10 % less metabolites that are significantly different in level.

In one aspect, the present invention can also be worded as a nutritional composition that comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide, for use in establishing a metabolome in human subjects with an age of 36 months or below that has at least 5 % less metabolites that are significantly different in level related to the intestinal metabolome of human subjects at the same age fed with human milk, when compared to the metabolites that are significantly different in level in human subjects with the same age fed a nutritional composition not comprising non-digestible oligosaccharides related to the intestinal metabolome of human subjects at the same age fed with human milk, more preferably the intestinal metabolome has at least 10 % less metabolites that are significantly different in level.

In one aspect, the present invention concerns a method for establishing a metabolome in human subjects with an age of 36 months or below that has less than 45 % of metabolites that are significantly different in level, when compared to the intestinal metabolome of human subjects at the same age fed with human milk, more preferably less than 40 %, even more preferably less than 35 % of the metabolites are at a significantly different level, comprising administering

a nutritional composition that comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide.

The invention can also be worded as the use of non-digestible oligosaccharide for the manufacture of a nutritional composition that comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide, for use in establishing a metabolome in human subjects with an age of 36 months or below that has less than 45 % of metabolites that are significantly different in level, when compared to the intestinal metabolome of human subjects at the same age fed with human milk, more preferably less than 40 %, even more preferably less than 35 % of the metabolites are at a significantly different level.

The invention can also be worded as a nutritional composition that comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide, for use in establishing a metabolome in human subjects with an age of 36 months or below that has less than 45 % of metabolites that are significantly different in level, when compared to the intestinal metabolome of human subjects at the same age fed with human milk, more preferably less than 40 %, even more preferably less than 35 % of the metabolites are at a significantly different level.

In one aspect, the present invention concerns a method for establishing a metabolome in human subjects with an age of 36 months or below that has more than 10% of metabolites that are significantly different in level, when compared to the intestinal metabolome of human subjects with the same age fed a nutritional composition not comprising non-digestible oligosaccharides, more preferably more than 15%, even more preferably more than 20% of the metabolites are at a significantly different level.

The invention can also be worded as the use of non-digestible oligosaccharide for the manufacture of a nutritional composition that comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide, for use in establishing a metabolome in human subjects with an age of 36 months or below that has more than 10 % of metabolites that are significantly different in level, when compared to the intestinal metabolome of human subjects with the same age fed a nutritional composition not comprising non-digestible oligosaccharides, more preferably more than 15%, even more preferably more than 20% of the metabolites are at a significantly different level.

The invention can also be worded as a nutritional composition that comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide for use in establishing a metabolome in human subjects with an age of 36 months or below that has more than 10 % of metabolites that are significantly different in level, when compared to the intestinal metabolome of human subjects with the same age fed a nutritional composition not comprising non-digestible oligosaccharides, more preferably more than 15%, even more preferably more than 20% of the metabolites are at a significantly different level.

In a preferred embodiment of establishing a metabolome according to the present invention, a metabolome is established that has less than 45 % of metabolites that are significantly different in level, when compared to the intestinal metabolome of human subjects at the same age fed with human milk, more preferably less than 40 %, even more preferably less than 35 % of the metabolites are at a significantly different level, and has more than 10 % of metabolites that are significantly different in level, when compared to the intestinal metabolome of human subjects with the same age fed a nutritional composition not comprising non-digestible oligosaccharides, more preferably more than 15%, even more preferably more than 20% of the metabolites are at a significantly different level.

In a preferred embodiment according to the methods or uses of the present invention, the intestinal metabolome has less than 45 % of metabolites that are significantly different in level, when compared to the intestinal metabolome of human subjects at the same age fed with human milk, more preferably less than 40 %, even more preferably less than 35 % of the metabolites are at a significantly different level, and has more than 10 % of metabolites that are significantly different in level, when compared to the intestinal metabolome of human subjects with the same age fed a nutritional composition not being at least partly fermented by lactic acid producing bacteria and comprising 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight and not comprising non-digestible oligosaccharides, more preferably more than 15%, even more preferably more than 20% of the metabolites are at a significantly different level.

In a preferred embodiment according to the methods or uses of the present invention, the intestinal metabolic profile or metabolome observed in infants consuming the nutritional composition according to the present invention has at least 5 % less metabolites that are significantly different in level related to the intestinal metabolome of human subjects at the same age fed with human milk, when compared to the metabolites that are significantly

different in level in human subjects with the same age fed a nutritional composition not being at least partly fermented by lactic acid producing bacteria and comprising 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight and not comprising non-digestible oligosaccharides related to the intestinal metabolome of human subjects at the same age fed with human milk, more preferably the intestinal metabolome has at least 10 % less metabolites that are significantly different in level.

In one aspect, the present invention concerns a method for establishing a metabolome in human subjects with an age of 36 months or below that has at least 5 % less metabolites that are significantly different in level related to the intestinal metabolome of human subjects at the same age fed with human milk, comprising administering a nutritional composition that is at least partly fermented by lactic acid producing bacteria wherein the nutritional composition comprises 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight and wherein the nutritional composition comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide, when compared to the metabolites that are significantly different in level in human subjects with the same age fed a nutritional composition not being at least partly fermented by lactic acid producing bacteria and comprising 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight and not comprising non-digestible oligosaccharides related to the intestinal metabolome of human subjects at the same age fed with human milk, more preferably the intestinal metabolome has at least 10 % less metabolites that are significantly different in level.

The invention can also be worded as the use of a fermented composition and non-digestible oligosaccharide for the manufacture of a nutritional composition that is at least partly fermented by lactic acid producing bacteria wherein the nutritional composition comprises 0.02 to 1.5 wt% based on dry weight of the sum of lactic acid and lactate and wherein the nutritional composition comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide, for use in establishing a metabolome in human subjects with an age of 36 months or below that has at least 5 % less metabolites that are significantly different in level related to the intestinal metabolome of human subjects at the same age fed with human milk, when compared to the metabolites that are significantly different in level in human subjects with the same age fed a nutritional composition not being at least partly fermented by lactic acid producing bacteria and comprising 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight and not comprising non-digestible oligosaccharides related to the intestinal metabolome of human

subjects at the same age fed with human milk, more preferably the intestinal metabolome has at least 10 % less metabolites that are significantly different in level.

In one aspect, the present invention can also be worded as a nutritional composition that is at least partly fermented by lactic acid producing bacteria wherein the nutritional composition comprises 0.02 to 1.5 wt% based on dry weight of the sum of lactic acid and lactate and wherein the nutritional composition comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide, for use in establishing a metabolome in human subjects with an age of 36 months or below that has at least 5 % less metabolites that are significantly different in level related to the intestinal metabolome of human subjects at the same age fed with human milk, when compared to the metabolites that are significantly different in level in human subjects with the same age fed a nutritional composition not being at least partly fermented by lactic acid producing bacteria and comprising 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight and not comprising non-digestible oligosaccharides related to the intestinal metabolome of human subjects at the same age fed with human milk, more preferably the intestinal metabolome has at least 10 % less metabolites that are significantly different in level.

In one aspect, the present invention concerns a method for establishing a metabolome in human subjects with an age of 36 months or below that has less than 45 % of metabolites that are significantly different in level, when compared to the intestinal metabolome of human subjects at the same age fed with human milk, more preferably less than 40 %, even more preferably less than 35 % of the metabolites are at a significantly different level, comprising administering a nutritional composition that is at least partly fermented by lactic acid producing bacteria wherein the nutritional composition comprises 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight and wherein the nutritional composition comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide.

The invention can also be worded as the use of a fermented composition and non-digestible oligosaccharide for the manufacture of a nutritional composition that is at least partly fermented by lactic acid producing bacteria wherein the nutritional composition comprises 0.02 to 1.5 wt% based on dry weight of the sum of lactic acid and lactate and wherein the nutritional composition comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide, for use in establishing a metabolome in human subjects with an age of 36 months or below that has less than 45 % of metabolites that are significantly different in level, when compared to the intestinal

metabolome of human subjects at the same age fed with human milk, more preferably less than 40 %, even more preferably less than 35 % of the metabolites are at a significantly different level.

The invention can also be worded as a nutritional composition that is at least partly fermented by lactic acid producing bacteria wherein the nutritional composition comprises 0.02 to 1.5 wt% based on dry weight of the sum of lactic acid and lactate and wherein the nutritional composition comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide, for use in establishing a metabolome in human subjects with an age of 36 months or below that has less than 45 % of metabolites that are significantly different in level, when compared to the intestinal metabolome of human subjects at the same age fed with human milk, more preferably less than 40 %, even more preferably less than 35 % of the metabolites are at a significantly different level.

In one aspect, the present invention concerns a method for establishing a metabolome in human subjects with an age of 36 months or below that has more than 10% of metabolites that are significantly different in level, when compared to the intestinal metabolome of human subjects with the same age fed a nutritional composition not being at least partly fermented by lactic acid producing bacteria and comprising 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight and not comprising non-digestible oligosaccharides, more preferably more than 15%, even more preferably more than 20% of the metabolites are at a significantly different level.

The invention can also be worded as the use of a fermented composition and non-digestible oligosaccharide for the manufacture of a nutritional composition that is at least partly fermented by lactic acid producing bacteria wherein the nutritional composition comprises 0.02 to 1.5 wt% based on dry weight of the sum of lactic acid and lactate and wherein the nutritional composition comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide, for use in establishing a metabolome in human subjects with an age of 36 months or below that has more than 10 % of metabolites that are significantly different in level, when compared to the intestinal metabolome of human subjects with the same age fed a nutritional composition not being at least partly fermented by lactic acid producing bacteria and comprising 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight and not comprising non-digestible

oligosaccharides, more preferably more than 15%, even more preferably more than 20% of the metabolites are at a significantly different level.

The invention can also be worded as a nutritional composition that is at least partly fermented by lactic acid producing bacteria wherein the nutritional composition comprises 0.02 to 1.5 wt% based on dry weight of the sum of lactic acid and lactate and wherein the nutritional composition comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide for use in establishing a metabolome in human subjects with an age of 36 months or below that has more than 10 % of metabolites that are significantly different in level, when compared to the intestinal metabolome of human subjects with the same age fed a nutritional composition not being at least partly fermented by lactic acid producing bacteria and comprising 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight and not comprising non-digestible oligosaccharides, more preferably more than 15%, even more preferably more than 20% of the metabolites are at a significantly different level.

In a preferred embodiment of establishing a metabolome according to the present invention, a metabolome is established that has less than 45 % of metabolites that are significantly different in level, when compared to the intestinal metabolome of human subjects at the same age fed with human milk, more preferably less than 40 %, even more preferably less than 35 % of the metabolites are at a significantly different level, and has more than 10 % of metabolites that are significantly different in level, when compared to the intestinal metabolome of human subjects with the same age fed a nutritional composition not being at least partly fermented by lactic acid producing bacteria and comprising 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight and not comprising non-digestible oligosaccharides, more preferably more than 15%, even more preferably more than 20% of the metabolites are at a significantly different level.

The % of metabolites that differ in level is expressed based on total metabolites tested. The nutritional composition according to the invention is a nutritional composition that comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide, preferably the nutritional composition according to the invention is a nutritional composition that is at least partly fermented by lactic acid producing bacteria wherein the nutritional composition comprises 0.02 to 1.5 wt% based on dry weight of the sum of lactic acid and lactate and wherein the nutritional composition comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide.

Control formula are formula that do not comprise non-digestible oligosaccharides. Preferably, control formula are formula that do not comprise fermented composition and do not comprise non-digestible oligosaccharides.

In a preferred embodiment, the intestinal metabolic profile or metabolome observed in infants consuming the nutritional composition according to the present invention has less than 350 metabolites that are significantly different in level, when compared to that observed for infants fed with human milk, more preferably less than 300 metabolites are at a significantly different level. In a preferred embodiment, the intestinal metabolic profile or metabolome observed in infants consuming the nutritional composition according to the present invention has more than 150 metabolites that are significantly different in level, when compared to control formula fed infants, more preferably more than 200 metabolites are at a significantly different level.

In a preferred embodiment, the intestinal metabolic profile or metabolome observed in infants consuming the nutritional composition according to the present invention has at least 75 more metabolites, preferably at least 100, more preferably at least 125, that are not statistically different related to infants fed with human milk, compared to the number of metabolites that is not statistically different in control formula fed infants related to infants fed with human milk.

In a further preferred embodiment of the methods and uses according to the present invention, the invention further is for use in promoting the development in human subjects with an age of 36 months or below of an intestinal microbiota that is in composition closer to the intestinal microbiota of human subjects at the same age fed with human milk when compared to the intestinal microbiota of human subjects with the same age fed a nutritional composition not comprising non-digestible oligosaccharides. Preferably the promoting the development of an intestinal microbiota refers to an intestinal microbiota that has a lower alpha-diversity, preferably as determined by Chao-1 index, compared to the intestinal microbiota of the human subject fed a nutritional composition not comprising non-digestible oligosaccharides. Also preferably the promoting the development of an intestinal microbiota refers to an intestinal microbiota that has a lower abundance of *Blautia* and/or *Erysipelotrichales* and/or an increased abundance of *Lactobacillus*, preferably refers to an intestinal microbiota that has a lower abundance of *Blautia* and/or *Erysipelotrichales*, more preferably refers to an intestinal microbiota that has a lower abundance of Blautia, compared to the intestinal microbiota of the human subject fed a nutritional composition not comprising non-digestible oligosaccharides.

In a further preferred embodiment of the methods and uses according to the present invention, the invention further is for use in promoting the development in human subjects with an age of 36 months or below of an intestinal microbiota that is in composition closer to the intestinal microbiota of human subjects at the same age fed with human milk when compared to the intestinal microbiota of human subjects with the same age fed a nutritional composition not being at least partly fermented by lactic acid producing bacteria and comprising 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight and not comprising non-digestible oligosaccharides. Preferably the promoting the development of an intestinal microbiota refers to an intestinal microbiota that has a lower alpha-diversity, preferably as determined by Chao-1 index, compared to the intestinal microbiota of the human subject fed a nutritional composition not being at least partly fermented by lactic acid producing bacteria and comprising 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight and not comprising non-digestible oligosaccharides. Also preferably the promoting the development of an intestinal microbiota refers to an intestinal microbiota that has a lower abundance of *Blautia* and/or *Erysipelotrichales* and/or an increased abundance of *Lactobacillus*, preferably refers to an intestinal microbiota that has a lower abundance of *Blautia* and/or *Erysipelotrichales*, more preferably refers to an intestinal microbiota that has a lower abundance of Blautia, compared to the intestinal microbiota of the human subject fed a nutritional composition not being at least partly fermented by lactic acid producing bacteria and comprising 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight and not comprising non-digestible oligosaccharides.

The inventors found effects on the faecal metabolome, and it is believed that the results obtained from faecal samples are representative for the situation of the intestine, in particular the large intestine or colon. In the context of the present invention, 'intestinal metabolome' or 'intestinal microbiota-function' equally refers to 'large intestinal metabolome' or 'large intestinal microbiota-function' or 'colonic metabolome' or 'colonic microbiota-function' or 'faecal metabolome' or 'faecal microbiota function'.

For bile acids, it was found that a difference in secondary bile acids was observed in the intestine. Secondary bile acids are generated from primary bile acids via enzymes of the intestinal bacteria and therefore reflect the functioning of the intestinal microbiota. It was found after the intervention periods that the pattern of secondary bile acids in the experimental group

was more similar to the pattern in the breastfed reference group than the control group. In general, the secondary bile acids were lower in the breastfed group and in the experimental group when compared to the control group. This was the case of 14 of the 23 secondary bile acids measured, and including 5 out of 6 more abundant secondary bile acids 1,2-dehydrocholate, 3-hydrocholate, 7-ketodeoxycholate, 7-ketolithocholate, and hyocholate. Of the 6 more abundant secondary bile acids only taurocholenate sulfate was higher in the breastfed group compared to control and experimental group. For the remaining 9 minor or very minor present secondary bile acids no or very low difference was observed, except for glycocholenate sulfate and taurolithocholate 3 sulfate that were both the lowest in the experimental group. These effects were in particular observed for 3b-hydroxy-5-cholenoic acid, 6-oxolithocholate, 7-ketolithocholate, 7-ketodeoxycholate, glycocholenate sulfate, and ursodeoxycholate. In addition also effects were found on the primary bile acids, that are not a direct result of microbial bioconversion, which levels were in general lower in the experimental group, and closer to the breast fed reference group, than in the control group, in particular glycochenodeoxycholate and glycocholate.

In a preferred embodiment of the methods and uses according to the present invention the promoting the development of an intestinal microbiota-function refers to the profile of bile acids, preferably of secondary bile acids.

In a preferred embodiment of the methods and uses according to the present invention the promoting the development of an intestinal microbiota-function refers to the level of bile acids, preferably the level of secondary bile acids, that is reduced compared to the level of bile acids of the human subject fed a nutritional composition not comprising non-digestible oligosaccharides.

In a preferred embodiment of the methods and uses according to the present invention the promoting the development of an intestinal microbiota-function refers to the level of bile acids, preferably the level of secondary bile acids, that is reduced compared to the level of bile acids of the human subject fed a nutritional composition not being at least partly fermented by lactic acid producing bacteria and comprising 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight and not comprising non-digestible oligosaccharides.

Interestingly, also the level of intestinal gamma-aminobutyrate (GABA) was found to be affected. The level of GABA was at 8 weeks and 17 weeks significantly higher in the experimental group compared to the control group. Likewise also the levels were significantly higher in the breastfed group compared to the control group. On the other hand the difference between the experimental group and breastfed group was not statistically different. Intestinal GABA is considered beneficial and to have a beneficial effect on visceral sensitivity and pain perception. GABA is thought to beneficially effect the enteral and central nervous system.

In a preferred embodiment of the methods and uses according to the present invention the promoting the development of an intestinal microbiota-function refers to the level of gamma–aminobutyrate (GABA).

In a preferred embodiment of the methods and uses according to the present invention the promoting the development of an intestinal microbiota-functionrefers to the level of gamma–aminobutyrate (GABA) that is increased compared to the level of GABA of the human subject fed a nutritional composition not comprising non-digestible oligosaccharides.

In a preferred embodiment of the methods and uses according to the present invention the promoting the development of an intestinal microbiota-functionrefers to the level of gamma–aminobutyrate (GABA) that is increased compared to the level of GABA of the human subject fed a nutritional composition not being at least partly fermented by lactic acid producing bacteria and comprising 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight and not comprising non-digestible oligosaccharides.

In the context of the present invention, synonyms for promoting the development of the intestinal microbiota-function are developing, improving, inducing, maintaining, supporting or driving the intestinal microbiota-function.

The effects described herein, i.e. the promoting, developing, improving, inducing, maintaining or driving the intestinal microbiota-function towards an intestinal microbiota-function more similar to the intestinal microbiota-function found in breastfed infants, are observed when compared to the intestinal microbiota-function of infants having been administered a nutritional composition not comprising a fermented composition and the non-digestible oligosaccharide,

preferably not comprising the combination of fermented composition and the non-digestible oligosaccharide.

In one embodiment, the present nutritional composition is used for improving the intestinal microbiota-function in a human subject with an age of 0 to 36 months. In one embodiment the present nutritional composition is used for improving the intestinal microbiota-function in a human subject of 0 to 18 months, even more preferably an infant with an age of 12 months of age or below, even more preferably an infant with an age of 0 to 6 months, most preferably an infant of 0 to 4 months. In one embodiment the present nutritional composition is used for improving the intestinal microbiota-function in a toddler of 12 to 36 months, most preferably a toddler with an age of 18 to 30, or 24 months. Preferably the present nutritional composition is further used for providing nutrition to said human subject. Preferably the nutritional composition is administered for at least 1 week, more preferably for at least 4 weeks, more preferably for at least 8 weeks, even more preferably for at least 4 months.

In a preferred embodiment, the methods or uses according to the present invention are for use in vaginally delivered infants. In a preferred embodiment, the methods or uses according to the present invention are for use in term infants, preferably for healthy term infants. In a preferred embodiment, the methods or uses according to the present invention are for use in healthy vaginally delivered infants. In a preferred embodiment, the methods or uses according to the present invention are for use in healthy infants born by Caesarean section.

In one embodiment the methods or uses according to the present invention are for use in human subjects with an age of 36 months or below that have a fragile or unbalanced intestinal microbiota or have intestinal microbial dysbiosis or human subjects with an age of 36 months or below that are at risk of having a fragile or unbalanced intestinal microbiota or intestinal microbial dysbiosis, preferably human subjects with an age of 36 months or below selected from the group consisting of preterm infants, infants born small for gestational age, infants with low birth weight, infants or toddlers treated or having been treated by antibiotics, infants born by Caesarean section, or infants or toddlers suffering or having suffered from an intestinal inflammation or intestinal infection or infants form mothers having been treated with antibiotics peri-natally. Microbial dysbiosis includes and preferably is dysbacteriosis. Preferably the microbial dysbiosis or dysbacteriosis is the dysbiosis in the colon.

In one embodiment the nutritional composition of the present invention is for use in providing a healthy intestinal function and/or for use in preventing and/or treating intestinal microbiota dysbiosis in human subjects with an age of 36 months or below.

In one embodiment of the methods or uses according to the present invention, the final nutritional composition comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide an optionally comprises a fermented composition as defined herein.

In this document and in its claims, the verb "to comprise" and its conjugations is used in its non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded. In addition, reference to an element by the indefinite article "a" or "an" does not exclude the possibility that more than one of the element is present, unless the context clearly requires that there be one and only one of the elements. The indefinite article "a" or "an" thus usually means "at least one. Wt% means weight percentage.

DESCRIPTION OF THE FIGURES

Figure 1 shows numeric information on metabolites that are significantly different in level per visit and when comparing different diets groups.

Figure 2 show the distribution of significantly changing metabolites per arm per visit, per metabolic superpathway; A: number of metabolites; B: percentage of metabolites based on total number of metabolites analysed; upper panels: breastfed versus control group; middle panels: breastfed versus experimental group; lower panels: experimental versus control group.

EXAMPLES

Example 1: Effect of partly fermented infant formula with non-digestible oligosaccharides or control formula on intestinal microbiota function compared with a breastfed reference group

Growth and safety of an experimental formula (formula 1) versus control formula (formula 2) was investigated in an explorative clinical study, using a 3-4 months intervention in healthy, term infants. In a randomized, controlled, multi-centre, double-blinded, prospective clinical trial, infants were enrolled before 28 days of age and assigned to receive one of two formulae until 17 weeks of age.

Experimental infant formula 1 is an infant formula containing 0.8g/100ml non-digestible oligosaccharides of scGOS (source Vivinal® GOS) and lcFOS (source RaftilinHP®) in a 9:1

wt ratio. Of this infant formula 30% based on dry weight was derived from Lactofidus™, a commercially available infant formula marketed under brand name Gallia. Lactofidus™ is a fermented milk derived composition and is produced by fermenting with *S. thermophilus* and comprises *B. breve*. A mild heat treatment is employed. The infant formula 1 comprised about 0.33 wt% (lactic acid + lactate) based on dry weight, of which at least 95% is L-lactic acid + L-lactate. The level of colony forming units of lactic acid producing bacteria, *S. thermophilus*, in infant formula 1 was about $2x10^4$ cfu/g dry weight and was derived from the fermented composition Lactofidus™.

Control infant formula 2 is a commercially available non-fermented infant formula without scGOS/lcFOS. The composition of the two formulae was similar in energy and macronutrient composition (per 100 ml: 66 kcal, 1.2 g protein (bovine whey protein/casein in 1/1 weight ratio), 7.7 g digestible carbohydrate (of which 7.6 g lactose), 3.4 g fat (mainly vegetable fat). The two infant formula further comprised vitamins, minerals, trace elements and other micronutrients according to international directive 2006/141/EC for infant formula.

As a reference, a group of infants was included being exclusively breastfed until 17 weeks of age. The Intention-To-Treat (ITT) population consisted of all subjects randomised to infant formula (n=199), in addition 100 subjects were included in the breastfed reference group. The ITT population consisted of 94 subjects in the experimental group, 105 subjects in the control group.

Faecal samples were collected at of randomization or the day thereafter (baseline), at 8 weeks of age and at 16-17 weeks of age no later than one day after the last intake of study product. The faecal parameters were analysed in a subgroup of infants, which were selected on: natural birth (vaginal delivery), no use of probiotics, thickeners, antibiotics or other medication that could influence the microbiota from birth until the end of study participation, no laxatives three days or less prior to faecal sampling. This subgroup consisting of 30 subjects from each of the three study arms - a total of 90 subjects, resulting in a total of 270 stool samples. As the subjects potentially were already consuming their respective study product for one day or – even more relevant – might have consumed other commercially available infant formula containing fermented formula, or prebiotics, or probiotics, already there can be influences compared to the breastfed reference group.

DNA extraction from stools samples was performed with QIAmp DNA Stool Mini Kit (Qiagen) according to the manufacturer's protocol except for the addition of two bead-beating steps. To 0.2 – 0.3 g of faecal sample 300 mg of 0.1 mm glass beads together with 1.4 mL of ASL (lysis) buffer and on this suspension the first bead-beating step was applied for 3x 30 sec (FastPrep-24 instrument program 5.5). After addition of the InhibitEx tablet the second bead-beating step was applied for 3x 30 sec (FastPrep-24 instrument program 5.5) to homogenize the sample. Following each bead-beating step samples were cooled for 5 min on ice. Extracted DNA purity was checked using the NanoDrop™ spectrophotometer (Thermo Fisher Scientific Inc.), whereas DNA quality and concentration was measured using the Quant-iTTM 193 dsDNA BR Assay kit (Invitrogen™). DNA aliquots were stored at -80°C until use.

From the purified faecal DNA extracts the V3–V5 regions of the bacterial 16S rRNA gene were amplified, using primers 357F and 926Rb. A 454 FLX Sequencer (454 Life Sciences, Branford, CT, USA) was used to pyrosequence the obtained 16S rRNA gene amplicons, as described previously.

The Quantitative Insights Into Microbial Ecology (QIIME) pipeline version 1.8.0 was used to analyse the sequence data. Quality control filters were set to discard sequences with a length below 200 bases, with a length above 1000 bases, with a mean sequence quality score of less than 25, with any ambiguous bases, or contained homopolymer stretches of more than 6 bases. Chimeric sequences were filtered with QIIME's own ChimeraSlayer. On the filtered sequences de novo Operational Taxonomic Unit (OTU) picking was applied with the USEARCH algorithm, which grouped sequences with more than 97% identity. Rarefaction was applied to the OTUs by QIIME to ensure identical number of reads per sample in order to perform alpha-diversity calculations using the following metrics: Chao1 and Observed species. Subsequently, the Ribosomal Database Project Classifier (RDP) was applied to assign taxonomy to the representative sequence (i.e. the most abundant sequence) of each OTU by alignment to the SILVA ribosomal RNA database (release version 1.0.8).

Statistics: For the targeted physiological and microbial parameters, a Wilcoxon Rank Sum test was used to calculate p-values for the difference between Experimental and Control on each time point. If the percentage of values of a given parameter were detected in 70% or more of the samples then the values below the quantification limit were replaced by (detection limit + quantification limit)/2, whereas the values below the detection limit were replaced by detection

limit / sqrt of 2. In case the percentage of measurement that was below limit of quantification in either group, then the parameter converted to binary (1 indicating presence, 0 indicating absence or below detection limit). For all binary parameters, the Chi-square test (fisher Exact if expected cell counts < 5) was utilized for inference making.

For the 16S rRNA gene amplicon sequencing results, the relative abundances of each taxon is primarily subjected to the Two-part statistics test (Wagner et al., 2011). In this test first the proportions of zeros in the two groups are compared, and then the medians of the non-zero data in the two groups are compared. The two parts are combined and one p-value is obtained for each taxon at each visit. Two-part statistics test is not reliable when the smallest group has at least 10 observations (i.e., non-zero value) (Wagner et al., 2011), therefore the data are analyzed according to the following steps: Two-part statistics is performed if both groups have >=10 non-zero values; If either group has <10 non-zero values the data is treated as binary and a Chi-square test is performed unless 50% of the cells have expected counts < 5, in which case a Barnard test is performed. On the resulting p-values, correction for multiple testing is applied by assessing the positive false discovery rate (pFDR) (Benjamini & Hochberg, 1995). The bootstrap method described by Storey, Taylor, & Siegmund (2004) is used to estimate π_0 and subsequently calculate *q*-values a measure of each feature's significance. For the metabolomics results, the normalized and re-scaled signals were subjected only to the Two-part statistics test (Wagner et al., 2011), and subsequently q-values were calculated as well. Results of the sequencing were considered to be statistically significant when the p-value<0.05 and the q-value<0.05. Results of the metabolomics were considered to be statistically significant when the p-value<0.05 and the q-value<0.1.

Frozen faecal aliquots were shipped under dry ice to a commercial laboratory (Metabolon, Durham, NC) for metabolite analysis. Procedures for metabolic profiling have been described previously (Chow, J. et al., Journal of Proteome Research, 2014. 13(5): p. 2534-2542) for the three platforms used in combination for the analysis, including GC/MS and two LC/MS systems, one optimized for positive ionization and one optimized for negative ionization. Data were collected over multiple platform run days and were adjusted by scaling to the median values for each group-balanced run-day block for each individual compound. This minimizes any inter-day instrument gain or drift, but does not interfere with intra-day sample variability. Data were not otherwise adjusted or normalized.

Results:

Targeted microbiota quantification by qPCR showed that in comparison to the control the samples from the experimental group at 8 and 17 weeks of age showed statistically significant increased amounts of Bifidobacterium and a lower amount of the Clostridium difficile and Clostridium perfringens group, while at baseline these measurements showed no significant differences between the treatment groups. No significant effects on the total amount of bacteria was observed.

Untargeted 16S rRNA gene amplicon sequencing showed that with the experimental formula various bacterial taxa (4-11 genera depending on the time point) did change consistently when comparing the experimental formula with the control formula after 4 months of intervention. Already at 2 months intervention an intermediate effect was observed. At the end of intervention, the levels of these differential bacterial groups in the experimental arm appeared to be more in line with the levels detected in the breastfed reference arm. See Table 1.

In particular at week 17, the relative abundance of members of *Clostridium*, *Blautia*, and Erysipelotrichales was significantly decreased in the experimental group and more comparable to the breastfed reference group. Bifidobacteria had increased. Also effects on Lactobacilli (decreased in control group), where statistically significant differences were observed at week 8, were found. Also it was found that the control group deviated more from the experimental group and breastfed reference group in having higher *Clostridiales*, *Blautia* and *Erysipelotrichales*, and lower *Bifidobacteria*.

The overall microbiota profile diversity can be summarized into one diversity index per sample. Such a diversity index, better known as alpha-diversity, can be calculated in various ways. The Chao-1 index (an estimate on species richness based on abundance data) was and remained low for the breastfed reference group (at 4 months median Chao 1 index was 91.37, Q1-Q3 66.71-119.2; mean 90.17, 95% Confidence Interval (CI) range 79.48-100.9) while it increased in time in the control group (at 4 months median 117.9, Q1-Q3 96.46-128.1; mean 114.3, 95% CI range 104.7-124). For the experimental group the Chao-1 index remained low and was more similar to the breastfed reference group (at 4 months median 96.5, Q1-Q3 86.17-114.3; mean 105.2, 95% CI range 93.78-116.6). In line with this observation the median number of observed species, expressed as OTUs (operational taxonomic units) in the experimental group at 4

months was lower and more similar to the breastfed reference group and the median was higher in the control group.

These results are indicative that the intestinal microbiota of the infants that consumed the party fermented formula with non-digestible oligosaccharides have a more breastfed like microbiota composition in the gut as compared to the control group.

Table 1: Genera with $p < 0.05$ and $q < 0.05$ (Two-part statistics) for the difference between Experimental and Control.

	P-value / Q-value	*Median (Q1-Q3)*			*Zeros / Non-zeros (% Zeros / % Non-zeros)*	
Taxon		*Experimental*	*Control*	*Breas-fed*	*Experimental*	*Control*
8 weeks						
Bifidobacterium	0.007 [w] / 0.042	0.903 (0.811 - 0.970)	0.811 (0.507 - 0.870)	0.657 (0.452 - 0.912)	0/28 (0.0/100.0)	0/30 (0.0/100.0)
Lactobacillus	0.004 [c] / 0.042	0.015 (0.001 - 0.089)	0.003 (0.001 - 0.004)	0.002 (1.59E-04 - 0.050)	10/18 (35.7/64.3)	22/8 (73.3/26.7)
Streptococcus	0.010 [w] / 0.047	0.008 (0.001 - 0.012)	0.021 (0.005 - 0.061)	0.008 (0.001 - 0.039)	0/28 (0.0/100.0)	0/30 (0.0/100.0)
Clostridium sensu stricto 1	0.005/ 0.042	0 (0 - 0.001)	0.001 (1.85E-04 - 0.002)	0.020 (0.004 - 0.182)	16/12 (57.1/42.9)	4/26 (13.3/86.7)
Uncultured Peptostreptococcaceae	0.004 [c]/ 0.042	0.001 (1.29E-04 - 0.003)	0.002 (0.001 - 0.005)	0.005 (8.96E-05 - 0.009)	20/8 (71.4/28.6)	10/20 (33.3/66.7)
17 weeks						
Bifidobacterium	0.001 [w] / 0.009	0.868 (0.681 - 0.917)	0.643 (0.316 - 0.753)	0.651 (0.491 - 0.769)	0/27 (0.0/100.0)	0/30 (0.0/100.0)
S24-7 (Bacteroidales order)	0.007 [c] / 0.033	2.45E-04 (1.9E-04 - 0.001)	0.001 (1.94E-04 - 0.002)	0.002 (0.001 - 0.004)	22/5 (81.5/18.5)	14/16 (46.7/53.3)
Other Firmicutes	<0.001 [c] / 0.009	1.01E-04 (8.7E-05 - 1.15E-04)	2.44E-04 (1.48E-04 - 0.001)	3.27E-04 (2.07E-04 - 4.47E-04)	25/2 (92.6/7.4)	16/14 (53.3/46.7)

Other Clostridiales	<0.001/ <0.001	4.2E-04 (2.22E-04 - 0.001)	0.004 (0.001 - 0.014)	0.001 (1.45E-04 - 0.006)	15/12 (55.6/44.4)	2/28 (6.7/93.3)
Blautia	<0.001[C] / <0.001	0.004 (0.001 - 0.018)	0.005 (0.001 - 0.038)	0.003 (4.26E-04 - 0.023)	20/7 (74.1/25.9)	6/24 (20.0/80.0)
Clostridium	0.006/ 0.031	4.9E-04 (1.9E-04 - 0.002)	0.002 (0.001 - 0.009)	0.003 (4.09E-04 - 0.008)	10/17 (37.0/63.0)	3/27 (10.0/90.0)
Incertae_sedis (Clostridiales)	0.001/ 0.009	0.004 (0.002 - 0.013)	0.042 (0.004 - 0.103)	0.008 (0.001 - 0.035)	8/19 (29.6/70.4)	1/29 (3.3/96.7)
Uncultured Clostridiales	<0.001/ <0.001	3.87E-04 (1.9E-04 - 0.002)	0.013 (0.003 - 0.019)	0.001 (3.43E-04 - 0.004)	8/19 (29.6/70.4)	1/29 (3.3/96.7)
Incertae_sedis (Erysipelotrichales)	0.001/ 0.009	0.001 (1.69E-04 - 0.003)	0.006 (0.001 - 0.017)	0.002 (0.001 - 0.034)	16/11 (59.3/40.7)	5/25 (16.7/83.3)
Uncultured B38	0.005/ 0.028	3.44E-04 (1.8E-04 - 0.001)	0.001 (0.001 - 0.004)	0.001 (3.14E-04 - 0.003)	6/21 (22.2/77.8)	1/29 (3.3/96.7)
Unassigned	0.001 [W] / 0.009	0.002 (0.001 - 0.004)	0.005 (0.003 - 0.007)	0.002 (0.001 - 0.006)	0/27 (0.0/100.0)	0/30 (0.0/100.0)

Next the untargeted metabolomics on the faecal samples was performed and it resulted in the identification of in total 786 unique metabolites (470-625 unique metabolites per sample). Upon analysis the metabolomics data with Principal Component Analysis (PCA) a separation is observed between the two formula fed infants and breastfed infants at baseline. During and after the trial the separation remained the same for the experimental group, but it increased in time for the control group (See Figure 1). The observed separations in the metabolomics PCA plots are larger than in PCA plots generated on the 16S rRNA gene amplicon sequencing data (data not shown) and, moreover, the first two principle components explain for more variation in the metabolomics PCAs. These results suggest that metabolomics provides a higher resolution of biological data and that the metabolite profiles are more sensitive in displaying the differential functionality of the faecal microbiota, and hence also the effect of dietary intervention on intestinal metabolomics

The number of metabolites that were significantly different between the between breastfed group vs control group and experimental group and control group, but at the same time not signifivcantly different diff between breastfed group vs experimental group was 58 at 8 weeks and 91 at 17 weeks.

The detected metabolites showed that there are significant differences at baseline between the breastfed infants and either the control or experimental group, 268 (34.7%) or 250 (32.6%) respectively, whereas between the two treatment arms no major difference was observed (i.e. only 16 (2.1%) metabolites were significantly different; Figure 1). This can be explained by the fact that the breastfed reference group upon inclusion was already completely breastfed, whereas the infants that were enrolled into the groups fed on the two formula were already (in part) formula fed. Interestingly, the number of significantly different metabolites between the breastfed group and control group increased in time in the control arm up to 404 (51.9%) metabolites, while the number of differential metabolites between the experimental and breastfed reference arm remain more or less constant, i.e. 261 (34.3%) metabolites at study end and this effect was already observed to a large extent at the intermediate time point of 8 weeks. At week 8 87, and at week 17 116 metabolites had levels that on the one hand were significantly different ($p < 0.05$ and q value < 0.1) when comparing the breastfed reference group with the control group, and when comparing the control group with the experimental group, while on the other hand the difference between the breastfed reference group and experimental group was not significantly different.

This suggests that during the consumption of the control formula the microbiota functioning increasingly deviates from the breastfed reference group in time. For the faecal microbiota functioning in infants on experimental formula the baseline difference did not increase in the 3-4-month time period measured here. When comparing the two treatment arms with 16S rRNA gene amplicon sequencing, more detailed differences in the microbiota composition were revealed that showed that the levels of the differential bacterial genera at 17 weeks of age were mainly detected in the experimental arm at the levels observed in breastfed reference arm. These changes appear to follow at a slower pace in comparison to the targeted physiological parameters as measured by metabolomics, which are already at week 8 significantly different (figure 1). Many of the genera that were reduced in Experimental are known adult commensal bacteria and are absent/reduced as in the breastfed reference infants.

The significantly different metabolites represented numerous functional categories. This untargeted data set illustrates that infant gut ecosystem functioning is highly dependent on and reactive to the diet.

The characteristics of the faecal samples in this study reflected that the infants consuming the partly fermented formula have a more breastfed like microbiota composition and more breastfed like physiological conditions in the gut as compared to the control group. Moreover, the more in depth untargeted analyses showed that the gut microbiota functioning in infants on partly fermented formula deviates less from the breastfed reference group than in infants on control formula, suggesting that the partly fermented formula drives the gut ecosystem towards a more breastfed-like situation. Superpathways relating to amino acid, lipid, xenobiotics, carbohydrates, nucleotides, cofactors and vitamins, energy and peptide were involved (see figure 2). The largest effects in change in number of metabolites were shown in pathways relating to amino acid and lipids and when looking at relative changes also high differences were observed in pathways relating to energy, nucleotides and cofactors and vitamins.

Zooming in the bile acids, it was found that a difference in secondary bile acids was observed in the intestine. Secondary bile acids are generated from primary bile acids via enzymes of the intestinal bacteria and therefore reflect the functioning of the intestinal microbiota. It was found after the intervention periods that the pattern of secondary bile acids in the experimental group was more similar to the pattern in the breastfed reference group than the control group. In

general, the secondary bile acids were lower in the breastfed group and in the experimental group when compared to the control group. This was the case of 14 of the 23 secondary bile acids measured, and including 5 out of 6 more abundant secondary bile acids 1,2-dehydrocholate, 3-hydrocholate, 7-ketodeoxycholate, 7-ketolithocholate, and hyocholate, in particular 7-ketolithocholate. Taurocholenate sulfate was higher in the breastfed group compared to control and experimental group. For the remaining 9 minor or very minor present secondary bile acids no or very low difference was observed, except for glycocholenate sulfate and taurolithocholate 3 sulfate that were both the lowest in the experimental group. For 3b-hydroxy-5-cholenoic acid, 6-oxolithocholate, 7-ketolithocholate, 7-ketodeoxycholate, glycocholenate sulfate, and ursodeoxycholate the changes were significant. In addition also effects were found on the primary bile acids, that are not a direct result of microbial bioconversion, which levels were in general lower in the experimental group, and closer to the breastfed reference group, than in the control group, in particular glycochenodeoxycholate and glycocholate.

Interestingly, also the level of intestinal gamma-aminobutyrate (GABA) was found to be affected. The level of GABA was at 8 weeks and 17 weeks significantly higher in the experimental group compared to the control group. Likewise also the levels were significantly higher in the breastfed group compared to the control group. On the other hand, the difference between the experimental group and breastfed group was not statistically different. Intestinal GABA is considered beneficial and to have a beneficial effect on visceral sensitivity and pain perception. GABA is thought to beneficially affect the enteral and central nervous system.

These results are indicative for promoting the development of an intestinal microbiota upon administration of a nutritional formula that is partly fermented and comprises non-digestible oligosaccharide, that is in function closer to the intestinal microbiota-function upon feeding with human milk when compared to administration of a control formula.

Example 2: Consumption of formula with non-digestible oligosaccharides improves the intestinal microbiota

In another randomized, multi-centre, double-blinded, prospective clinical trial, infants were enrolled before 28 days of age and assigned to receive until 17 weeks of age one of three formulas:

Test group 1: Infant formula 1 comprising per 100 ml: 66 kcal, 1.35 g protein (bovine whey protein/casein in 1/1 weight ratio), 8.2 g digestible carbohydrate (of which 5.6 g lactose, and 2.1 g maltodextrin), 3.0 g fat (mainly vegetable fat), 0.8 g non-digestible oligosaccharides comprising scGOS (source Vivinal® GOS) and lcFOS (source RaftilinHP®) in a 9:1 wt ratio. Of this infant formula about 50 % based on dry weight is derived from Lactofidus™. The infant formula comprised about 0.55 wt% lactic acid + lactate based on dry weight, of which at least 95% is L(+)- lactic acid/lactate. The composition further comprised vitamins, minerals, trace elements and other micronutrients according to international directive 2006/141/EC for infant formula.

Test group 2: Infant formula 2, similar as formula 1 of which about 15% based on dry weight was derived from Lactofidus™. The infant formula comprised about 0.17 wt% lactic acid + lactate based on dry weight, of which at least 95% is L(+)- lactic acid/lactate.

Test group 3: Infant formula 3, similar to infant formula 1, but without the non-digestible oligosaccharides scGOS and lcFOS.

Test group 4: Infant formula 4, a non-fermented infant formula with 0.8 g non-digestible oligosaccharides comprising scGOS (source Vivinal® GOS) and lcFOS (source RaftilinHP®) in a 9:1 wt ratio, but not comprising Lactofidus™ and for the remainder similar in composition as infant formula 1.

Stool samples were collected at baseline and after 17 weeks of intervention for microbiological analysis, in a way similar as described in example 1 with the exception of the QIIME version (1.6.0), and the statistical analysis on the 16S rRNA gene amplicon sequencing results were subjected to Wilcoxon Rank Sum tests to calculate p-values for the difference between the groups on each time point, combined with pFDR estimation (q-value calculation) to control for false discoveries due to multiple testing. Only samples of a subgroup of vaginally born subjects (30 subjects of each group, resulting in 240 stool samples) were analysed that had a complete set of stool samples (both visits) with sufficient amount of stool for all analyses. In addition, samples from infants that used any systemic antibiotics any time after birth or that used thickeners added to formula during the study were excluded.

In the selected set of faecal samples the impact of the used infant formulas was assessed on microbiota. After the intervention (at 17 weeks) the measured faecal microbial parameters of the infants from test group 3, with no fermented composition but with GOS/FOS, showed an increased amount of Bifidobacterium, and low occurrence of pathogens as measured by

Clostridium difficile levels. A difference was observed in at least 5 taxa, relating to Lactobacillus, Blautia, Clostridiales, Peptostreptococcae and Erysipelotrichales, and the results are shown in Table 2.

Table 2: Abundance of taxes with significant q values <=0.1 – at 17 weeks

	Test group 4 NDO	Test group 2 15%FERM + NDO	Test group 1 50%FERM+ NDO	Test group 3 50%FERM
Lactobacillus				
Q1	0.000001	0.000001	0.0005742	0.000001
Median	0.0001681	0.0003313	0.006128	0.000123
Q3	0,002566	0.01197	0.0241	0.003478
Blautia				
Q1	0	0	0	0
Median	0.0003703	0	0	0.0001343
Q3	0.005597	0.001246	0.0001669	0.07478
Erysipelotrichales Incertae sedis				
Q1	0	0	0	0
Median	0	0	0	0.0008127
Q3	0.003203	0.0004028	0.0001957	0.007706

An increased amount of *Lactobacilli* was found in group 1 compared to the groups 3 and 4. A decreased amount of *Blautia* and *Erysipelotrichales* was found in groups 1 and 2 compared to the groups 3 and 4. In all cases the difference between group 1 and 3 was statistically significant with $p < 0.05$.

The overall microbiota profile diversity can be summarized into one diversity index per sample. For each sample the Chao-1 Index was calculated at a depth of 1496 sequences per sample (deepest measurement possible with no missing values). At 4 months the Chao-1 estimate for group 1 had a lower value, so a indicative of a lower diversity (median 70.68, Q1-Q3 52.5-145.9; mean 73.11, 95 % CI interval 60.34 – 85.89) than group 3 without non-digestible oligosaccharides (median 104.2, Q1-Q3 77.69-139.8; mean 104.8, 95 % CI interval 88.97-120.7) and also lower than group 4 without fermented composition (median 97.32, Q1-Q3

68.99-120.5; mean 96.04, 95 % CI 82.71-109.4). The difference between group 1 and group 3 was statistically significant (p=0.005).

Metabolomics:

In a way similar to example 1 the difference in number of metabolites was determined between the groups consuming formula 1, 3 and 4 (group 2 was not analysed). At 17 weeks the presence of NDO had the biggest influence on the difference in number and percentage of metabolites. The difference in metabolites between the group consuming formula 4 and 3 was 41.4 % (393 metabolites) and the difference between the group consuming formula 3 and 1 was 34 % (323 metabolites).

An effect on the amino acid and peptide superpathway was obeserved due to the presence of NDO, and an additional effect of the presence of fermented fomula next to NDO was observed for other superpathways. When looking at the number of metabolites this was the case for the superpathways relating to lipids and when looking at the percentage of metabolites this was the case for superpathways relating to lipids, nucleotides, vitamins and cofactors, energy, xenobiotics and carbohydrates.

Also for the primary and secondary bile acids, the difference between formula 4 and 1 was smaller than the difference between formula 3 and 1, but of the 9 primary bile acids tested glycochenodeoxychelate 3-sulfate and cholate sulfate were the lowest with formula 1. For the 25 secondary bile acids identified the levels were low for formula 1 and 4 compared to formula 3, and 3-hydrocholate, 6-oxylithocholate, dehydrolithocholate, taurocholenate sulfate and 3b-hydroxy-5-cholenoic acid were lowest in group consuming formula 1.

Zooming in on specific metabolites, the highest level in group 1 was observed for 4 hydroxyphenylpyruvate, which was also higher in the breast fed goup of example 1. The levels of tryptophan, cysteine sulfinic acid, homocitruline, stearoyl ethanolamide, sphingosine, uridine, 5-methyluridine, beta-alanine, oxalate and D-urobilin were lowest in group 1, whereas they were also lowest in the breast fed reference group of example 1.

When combining the results of the clinical trial examples 1 and 2, the control group and the breastfed reference group are present. It can therefore be deduced that an improved effect on the intestinal microbiota function is observed in infants fed a formula with non-digestible

oligosaccharides which is more similar to the breastfed reference group, when compared to formula without non-digestible oligosaccharides. Further improved effects are observed when the nutritional composition is partly fermented. The improved effects are observed when compared to formula without fermented composition and without non-digestible oligosaccharides.

These results are indicative of an effect of non-digestible oligosacchardes, in particular GOS and/or FOS, more in particular GOS and FOS, on the intestinal metabolome or functioning of the intestinal microbiota, making it more close to the intestinal metabolome or functioning of the intestinal microbiota of breastfed infants when compared to infants fed a formula without non-digestible oligsaccharides. This effect is further improved when the infants consumed a formula that additionally is at least partly fermented by lactic acid producing bacteria.

CLAIMS

1. A nutritional composition that comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide selected from the group consisting of fructo-oligosaccharide, non-digestible dextrin, galacto-oligosaccharide, xylo-oligosaccharide, arabino-oligosaccharide, arabinogalacto-oligosaccharide, gluco-oligosaccharide, gentio-oligosaccharide, glucomanno-oligosaccharide, galactomanno-oligosaccharide, mannan-oligosaccharide, isomalto-oligosaccharide, nigero-oligosaccharide, glucomanno-oligosaccharide, chito-oligosaccharide, soy oligosaccharide, uronic acid oligosaccharide, sialyloligosaccharide, and fuco-oligosaccharide, and mixtures thereof, for use in promoting the development in human subjects with an age of 36 months or below of an intestinal microbiota that is in function closer to the intestinal microbiota-function of human subjects at the same age fed with human milk when compared to the intestinal microbiota-function of human subjects with the same age fed a nutritional composition not comprising non-digestible oligosaccharides.

2. The nutritional composition for use according to claim 1 wherein the promoting the development of an intestinal microbiota-function refers an intestinal metabolome that is more similar to the intestinal metabolome of human subjects at the same age fed with human milk when compared to the intestinal metabolome of human subjects with the same age fed a nutritional composition not comprising non-digestible oligosaccharides.

3. A nutritional composition that comprises 2.5 to 15 wt% based on dry weight of non-digestible oligosaccharide selected from the group consisting of fructo-oligosaccharide, non-digestible dextrin, galacto-oligosaccharide, xylo-oligosaccharide, arabino-oligosaccharide, arabinogalacto-oligosaccharide, gluco-oligosaccharide, gentio-oligosaccharide, glucomanno-oligosaccharide, galactomanno-oligosaccharide, mannan-oligosaccharide, isomalto-oligosaccharide, nigero-oligosaccharide, glucomanno-oligosaccharide, chito-oligosaccharide, soy oligosaccharide, uronic acid oligosaccharide, sialyloligosaccharide, and fuco-oligosaccharide, and mixtures thereof, for use in establishing a metabolome in human subjects with an age of 36 months or below that is more similar to the intestinal metabolome of human subjects at the same age fed with human milk when compared to the intestinal metabolome of human subjects with the same age fed a nutritional composition not comprising non-digestible oligosaccharides

4. The nutritional composition for use according to claim 2 or 3 wherein the intestinal metabolome has less than 45 % of metabolites that are significantly different in level, when compared to the intestinal metabolome of human subjects at the same age fed with human milk, and has more than 10 % of metabolites that are significantly different in level, when compared to the intestinal metabolome of human subjects with the same age fed a nutritional composition not comprising non-digestible oligosaccharides.

5. The nutritional composition for use according to any one of claims 2-4 wherein the intestinal metabolome has at least 5 % less metabolites that are significantly different in level related to the intestinal metabolome of human subjects at the same age fed with human milk, when compared to the metabolites that are significantly different in level in human subjects with the same age fed a nutritional composition not comprising non-digestible oligosaccharides related to the intestinal metabolome of human subjects at the same age fed with human milk infants fed a control formula, more preferably the intestinal metabolome has at least 10 % less metabolites that are significantly different in level.

6. The nutritional composition for use according to claim 2, wherein the intestinal metabolome has at least 75 more metabolites, preferably at least 100, more preferably at least 125, that are not statistically different related to human subjects at the same age fed with human milk, compared to the number of metabolites that is not statistically different in human subjects at the same age fed a nutritional composition not comprising non-digestible oligosaccharides.

7. The nutritional composition for use according to any one of the preceding claims, which further is for use in promoting the development in human subjects with an age of 36 months or below of an intestinal microbiota that is in composition closer to the intestinal microbiota of human subjects at the same age fed with human milk when compared to the intestinal microbiota of human subjects with the same age fed a nutritional composition not comprising non-digestible oligosaccharides.

8. The nutritional composition for use according to any one of the preceding claims, wherein the promoting the development of an intestinal microbiota-function refers to the profile of bile acids, preferably of secondary bile acids.

9. The nutritional composition for use according to any one of the preceding claims, wherein the promoting the development of an intestinal microbiota-function refers to the level of bile acids, preferably the level of secondary bile acids, that is reduced compared to the level of bile acids of the human subject fed a nutritional composition not comprising non-digestible oligosaccharides.

10. The nutritional composition for use according to any one of claims 1-7 and 9, wherein the promoting the development of an intestinal microbiota-function refers to the level of gamma–aminobutyrate (GABA) that is increased compared to the level of GABA of the human subject fed a nutritional composition not comprising non-digestible oligosaccharides.

11. The nutritional composition for use according to any one of the preceding claims that is at least partly fermented by lactic acid producing bacteria wherein the nutritional composition comprises 0.02 to 1.5 wt% based on dry weight of the sum of lactic acid and lactate.

12. The nutritional composition for use according to any one of the preceding claims wherein the promoting the development of an intestinal microbiota-function is when compared to the intestinal microbiota-function of human subjects with the same age fed a nutritional composition not being at least partly fermented by lactic acid producing bacteria and comprising 0.02 to 1.5 wt% of the sum of lactic acid and lactate based on dry weight.

13. The nutritional composition for use according to any one of the preceding claims wherein the human subject is an infant below 12 months, more preferably below 6 months.

14. The nutritional composition for use according to any one of the preceding claims wherein the human subject has a fragile or unbalanced intestinal microbiota or dysbiosis of intestinal microbiota or is at risk of having a fragile or unbalanced intestinal microbiota or dysbiosis of intestinal microbiota.

15. The nutritional composition for use according to any one of claims 11-14, wherein at least 90 wt% of the sum of lactic acid and lactate is L(+)-lactic acid and/or L(+)-lactate.

16. The nutritional composition for use according to any one of claims 11-15, wherein the fermented composition is fermented by *Bifidobacterium* and/or *Streptococcus*.

17. The nutritional composition for use according to any one of claims 11-16, wherein the lactic acid producing bacteria are inactivated to a level below 10^6 cfu/g dry weight of the nutritional composition.

18. The nutritional composition for use according to any one of claims 11-17, wherein the amount of fermented composition is 10 to 90 wt% based on the nutritional composition.

19. The nutritional composition for use according to any one of the preceding claims wherein the non-digestible oligosaccharide comprises galacto-oligosaccharide and/or fructo-oligosaccharide.

20. The nutritional composition for use according to any one of the preceding claims, which is an infant formula, a follow on formula, a toddler milk or toddler formula, or a growing up milk intended for young children, preferably an infant formula.

Fig. 1

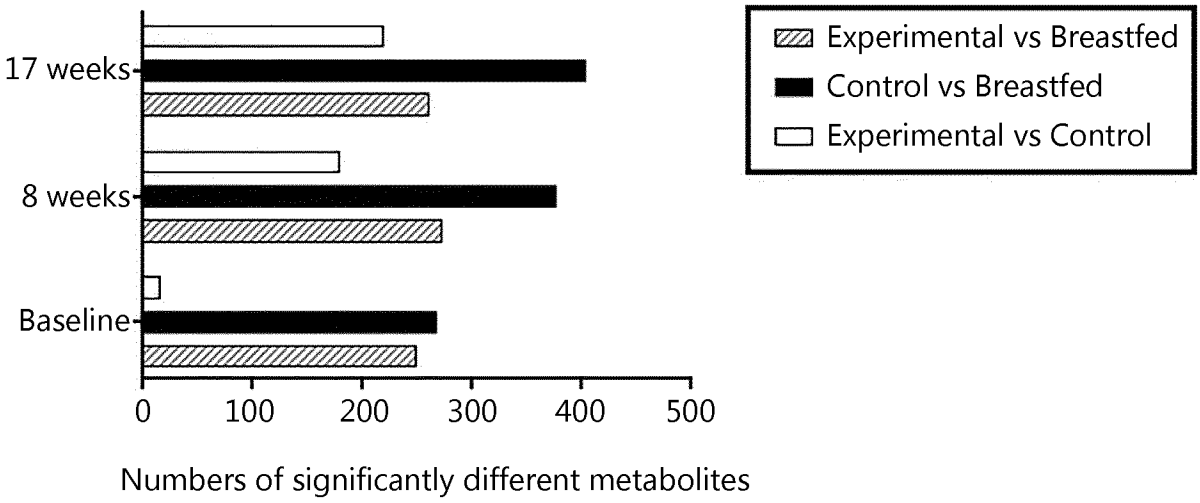

17 weeks
8 weeks
Baseline
0
100
200
300
400
500
Experimental vs Breastfed
Control vs Breastfed
Experimental vs Control
Numbers of significantly different metabolites

Fig. 2A

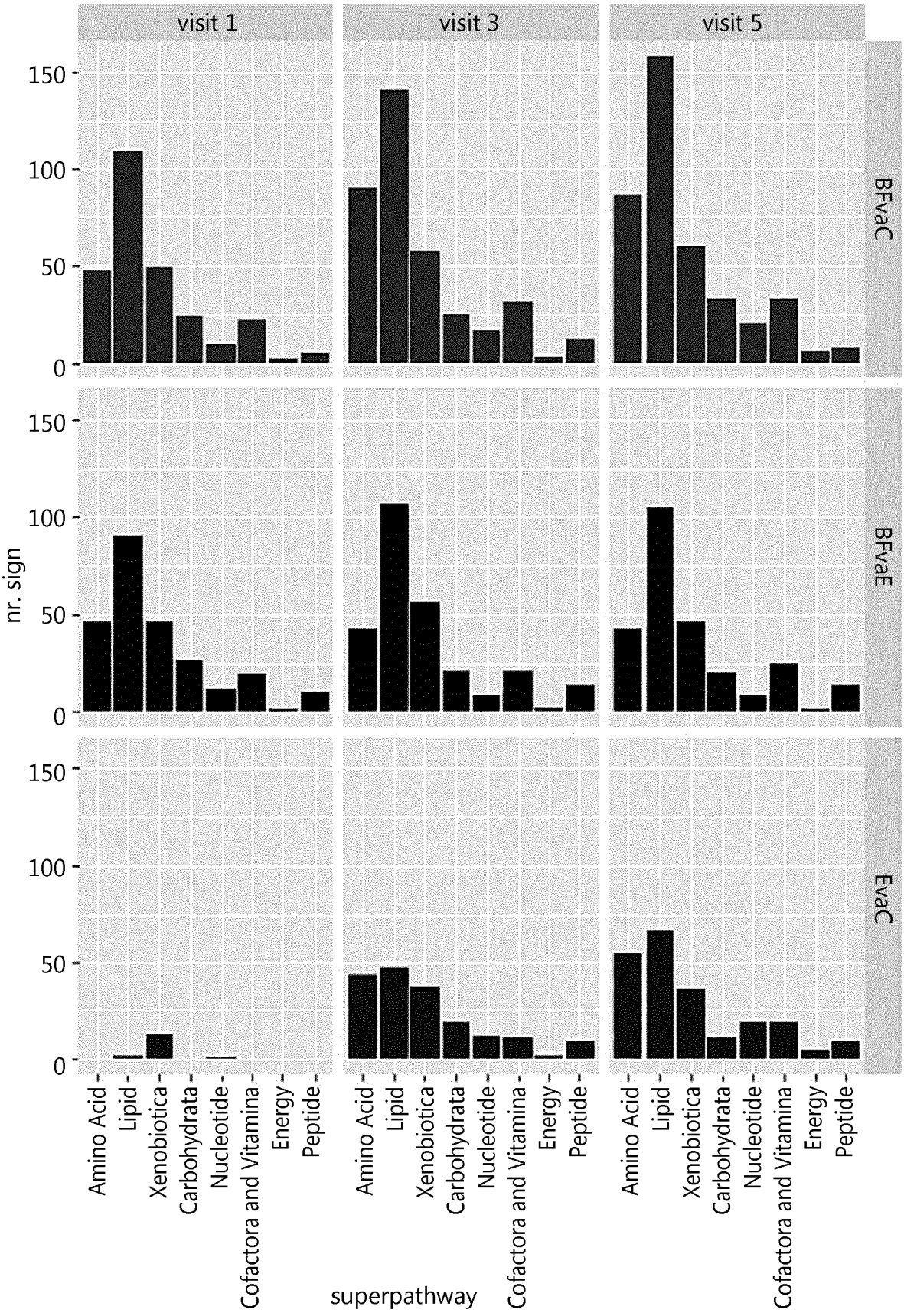
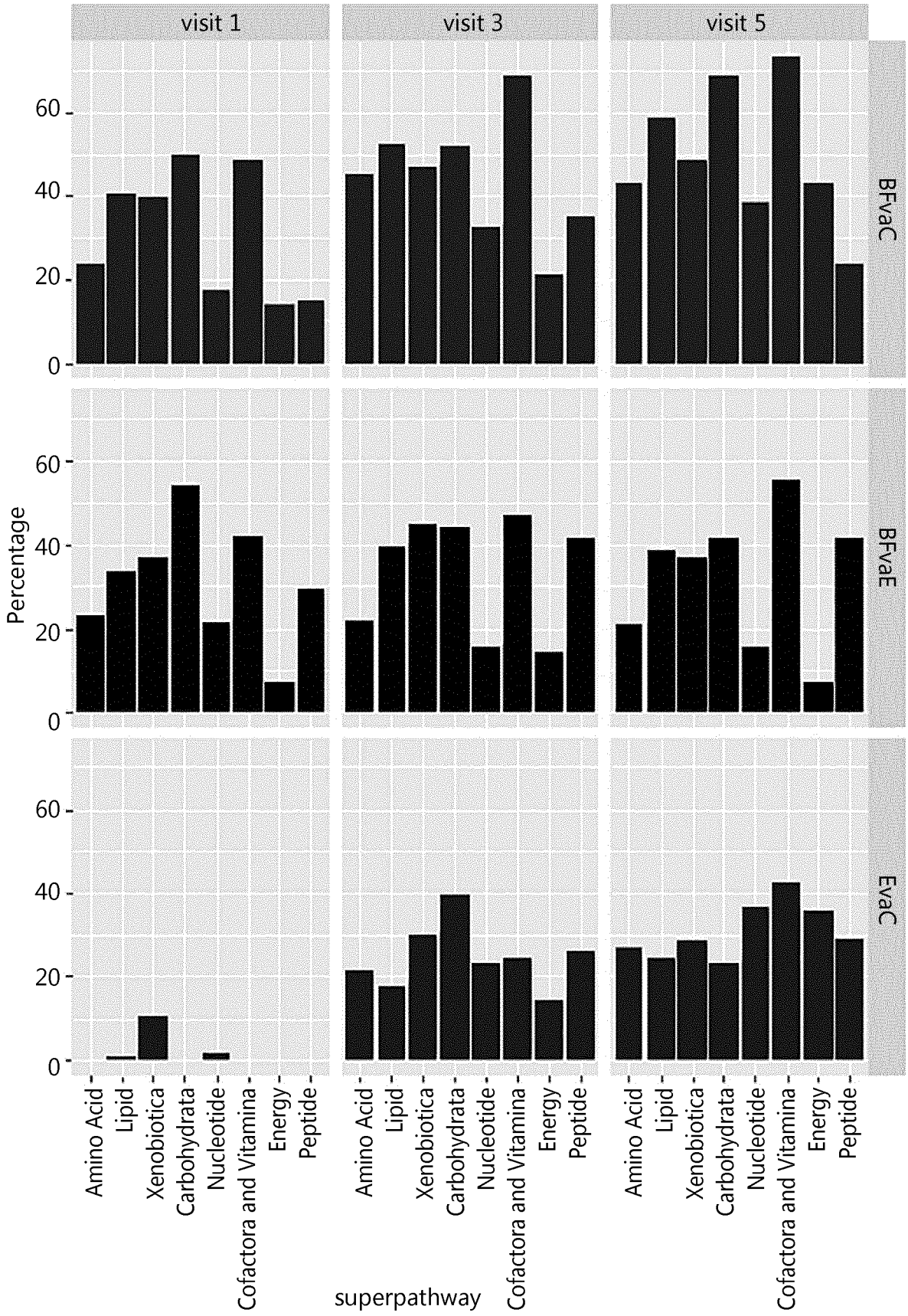

visit 1
visit 3
visit 5
BFvaC
BFvaE
EvaC
nr. sign
0
50
100
150
Amino Acid
Lipid
Xenobiotica
Carbohydrata
Nucleotide
Cofactora and Vitamina
Energy
Peptide
superpathway

Fig. 2B


visit 1
visit 3
visit 5
BFvaC
BFvaE
EvaC
Percentage
0
20
40
60
Amino Acid
Lipid
Xenobiotica
Carbohydrata
Nucleotide
Cofactora and Vitamina
Energy
Peptide
superpathway

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/053237

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/7016 A61K31/702 A61K31/715 A61K35/744 A23L33/00
A23L33/135 A23L33/21
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FRÉDÉRIC HUET ET AL: "Partly Fermented Infant Formulae With Specific Oligosaccharides Support Adequate Infant Growth and Are Well-Tolerated", JOURNAL OF PEDIATRIC GASTROENTEROLOGY AND NUTRITION, vol. 63, no. 4, 23 September 2016 (2016-09-23), pages e43-e53, XP055464610, US ISSN: 0277-2116, DOI: 10.1097/MPG.0000000000001360	1-18
Y	abstract page e43, right-hand column, paragraph 1-2 page e44, left-hand column, paragraphs 2, 4, 5 page e44, right-hand column, paragraph 3 - page e45, left-hand column, paragraph 1; table 1	10
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
9 April 2019	23/04/2019

Name and mailing address of the ISA/	Authorized officer
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	page e48, right-hand column, paragraph 2 - page e50, left-hand column, paragraph 3 page e50, right-hand column, paragraph 2 - page e52, left-hand column, paragraph 2	

X	US 2017/156389 A1 (LUDWIG THOMAS [NL] ET AL) 8 June 2017 (2017-06-08)	1-18
Y	paragraphs [0012], [0014], [0115], [0119] - [0122], [0159], [0160]; examples 2-4	10

X	US 2011/150851 A1 (SCHMITT JOACHIM [DE] ET AL) 23 June 2011 (2011-06-23)	1-18
Y	paragraphs [0001], [0014] - [0019]; examples 2, 3	10

X	US 2016/206667 A1 (KNOL JAN [NL] ET AL) 21 July 2016 (2016-07-21)	1-18
Y	paragraphs [0003], [0008] - [0010], [0052], [0067]; figure 1; examples 1, 3	10

X	US 2011/034407 A1 (NIEUWENHUIZEN WILLEM FERDINAND [NL] ET AL) 10 February 2011 (2011-02-10)	1-18
Y	paragraphs [0001], [0006] - [0012], [0021] - [0029], [0047] - [0059]; examples 1, 3	10

X	KNOL JAN ET AL: "Colon microflora in infants fed formula with galacto- and fructo-oligosaccharides: More like breast-fed infants", JOURNAL OF PEDIATRIC GASTROENTEROLOGY AND NUTRITION, LIPPINCOTT WILLIAMS WILKINS, INC, US, vol. 40, no. 1, 1 January 2005 (2005-01-01), pages 36-42, XP002668009, ISSN: 0277-2116, DOI: 10.1097/00005176-200501000-00007	1-18
Y	abstract page 37, left-hand column, paragraph 3 - right-hand column, paragraph 1; table 1 page 40, left-hand column, paragraph 2 - page 41, right-hand column, paragraph 2	10

X	WO 2018/024870 A1 (NESTEC SA [CH]) 8 February 2018 (2018-02-08)	1-18
Y	page 1, lines 6-10 page 6, line 30 - page 7, line 18 claims 1, 14-21; example 3	10

Y	US 2015/320778 A1 (CHOW JOMAY [US] ET AL) 12 November 2015 (2015-11-12) paragraphs [0039] - [0041]; example 51	10

	-/--	

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOMAY CHOW ET AL: "Fecal Metabolomics of Healthy Breast-Fed versus Formula-Fed Infants before and during In Vitro Batch Culture Fermentation", JOURNAL OF PROTEOME RESEARCH., vol. 13, no. 5, 2 May 2014 (2014-05-02), pages 2534-2542, XP055275560, US ISSN: 1535-3893, DOI: 10.1021/pr500011w abstract figure 3; tables 2-3 page 2541, left-hand column, paragraph 4 - right-hand column, paragraph 1 -----	1-18
A	AUDREY GRANIER ET AL: "Fermentation products: immunological effects on human and animal models", PEDIATRIC RESEARCH, vol. 74, no. 2, 13 May 2013 (2013-05-13), pages 238-244, XP055464611, US ISSN: 0031-3998, DOI: 10.1038/pr.2013.76 abstract page 240, left-hand column, paragraph 5 - right-hand column, paragraph 1 -----	1-18

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2019/053237

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2017156389 A1	08-06-2017	AU 2013274964 A1	18-12-2014
		BR 112014030434 A2	27-06-2017
		CN 104717890 A	17-06-2015
		CN 109105879 A	01-01-2019
		DK 2827724 T3	03-10-2016
		EP 2827724 A1	28-01-2015
		EP 3132829 A1	22-02-2017
		ES 2594855 T3	23-12-2016
		HU E031289 T2	28-07-2017
		LT 2827724 T	26-09-2016
		NZ 631404 A	29-07-2016
		PH 12014502642 A1	26-01-2015
		PL 2827724 T3	31-01-2017
		PT 2827724 T	07-09-2016
		RU 2015100867 A	10-08-2016
		RU 2017143399 A	14-02-2019
		US 2015132365 A1	14-05-2015
		US 2017156389 A1	08-06-2017
		WO 2013187755 A1	19-12-2013
		WO 2013187764 A1	19-12-2013
US 2011150851 A1	23-06-2011	AR 072435 A1	25-08-2010
		CN 102131399 A	20-07-2011
		EP 2306843 A1	13-04-2011
		US 2011150851 A1	23-06-2011
		WO 2010002241 A1	07-01-2010
		WO 2010002244 A1	07-01-2010
US 2016206667 A1	21-07-2016	AT 526836 T	15-10-2011
		AU 2006217160 A1	31-08-2006
		BR PI0607743 A2	16-03-2010
		CA 2598961 A1	31-08-2006
		CN 101128129 A	20-02-2008
		CN 104223094 A	24-12-2014
		DK 1855550 T3	16-01-2012
		DK 2351492 T3	01-12-2014
		EP 1855550 A2	21-11-2007
		EP 2351492 A1	03-08-2011
		EP 2805625 A2	26-11-2014
		ES 2373971 T3	10-02-2012
		ES 2524344 T3	05-12-2014
		ES 2702631 T3	04-03-2019
		PT 1855550 E	16-01-2012
		PT 2351492 E	02-12-2014
		RU 2011110056 A	27-09-2012
		US 2006194241 A1	31-08-2006
		US 2009004164 A1	01-01-2009
		US 2016206667 A1	21-07-2016
		WO 2006091103 A2	31-08-2006
		ZA 200706875 B	26-11-2008
US 2011034407 A1	10-02-2011	AR 071550 A1	30-06-2010
		AR 077520 A1	07-09-2011
		CN 101945589 A	12-01-2011
		CN 101951794 A	19-01-2011
		EP 2229063 A1	22-09-2010
		EP 2234505 A1	06-10-2010
		EP 2589302 A1	08-05-2013

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2019/053237

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
		ES 2402687 T3	07-05-2013
		ES 2611203 T3	05-05-2017
		PL 2589302 T3	31-05-2017
		RU 2010130468 A	27-01-2012
		RU 2012155333 A	27-06-2014
		US 2011009359 A1	13-01-2011
		US 2011034407 A1	10-02-2011
		WO 2009082214 A1	02-07-2009
		WO 2009082216 A1	02-07-2009
WO 2018024870 A1	08-02-2018	AU 2017307455 A1	29-11-2018
		CN 109562117 A	02-04-2019
		WO 2018024870 A1	08-02-2018
US 2015320778 A1	12-11-2015	BR 112015014420 A2	11-07-2017
		CA 2893217 A1	26-06-2014
		CN 104822279 A	05-08-2015
		EP 2934189 A1	28-10-2015
		HK 1217082 A1	23-12-2016
		PH 12015501309 A1	24-08-2015
		SG 11201504579Q A	30-07-2015
		TW 201438719 A	16-10-2014
		US 2015320778 A1	12-11-2015
		WO 2014100126 A1	26-06-2014