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(54) **Title**: SYNBIOTIC COMPOSITION FOR TREATMENT OF INFECTIONS IN ALLERGIC PATIENTS

(57) **Abstract**: The invention concerns the use of prebiotics and probitics for the treatment of infections in allergic patients.

Synbiotic composition for treatment of infections in allergic patients

FIELD OF THE INVENTION

The present invention relates to nutritional compositions comprising synbiotics for use in the treatment or prevention of infections in allergic patients.

BACKGROUND OF THE INVENTION

Synbiotic combinations of probiotic lactic acid bacteria and prebiotic indigestible fibers have been tested in models for inflammation and in human studies. Although reductions in inflammatory responses have been shown in several treatment protocols, results are conflicting and not consistent depending on the model or patient group used. Allergy has long been related to improved hygiene in the developed world. Based on this hygiene hypothesis a large number of studies have been done where it was tried to treat allergy, or improve the allergic symptoms, e.g. atopic dermatitis. The allergic patients were treated with probiotic bacteria or with dietary fibers or both, but the results allergy on prevention were inconsistent.

For example in Allergy (2011) 66:170–177 van der Aa et al. reported effects on asthma symptoms but the number of respiratory infections (lower and upper) during the intervention period did not differ between the synbiotic and the placebo group. The treatment product used in this study was an infant formula with galactooligosaccharides and inulin as prebiotics and B.breve as probiotic.

Currently, probiotics or prebiotics are not commonly used for treating infections in allergic patients.

Kukkonen et al., J Allergy Clin Immunol (2007) 119: 192-198 described a study using synbiotics that consisted of 4 probiotic strains and prebiotic galactooligosaccharides. The synbiotics were given in a double-blinded manner to pregnant mothers and to their healthy infants from birth to the age of 6 months. It is not reported if the infants were allergic. Kukkonen finds indications for an inverse association between modification of the indigenous gut microbiota and the prevalence of eczema, especially when IgE associated. This preventive study did not use a nutritional

composition with synbiotics but used capsules to be eaten by the mother or to be mixed with liquids for the infant. It is also not disclosed what the effect would be of the synbiotics when given to allergic infants.

WO 2010/033768 discloses compositions including infant formula comprising probiotics for reducing inflammation. The inflammation may be caused by allergy, chronic inflammatory disease, etc. An inflammation is an immune reaction that can result from an infection. Inflammation is in general an immune response of the body against a harmful stimulus such as pathogenic micro organisms, chemicals, damaged tissues. The treatment or prevention of infections is thus different from treating an inflammation and the document does not disclose the treatment or prevention of infections in allergic patients, but only the treatment of inflammation.

Böhme et al. in Acta Derm Venereol. (2002) 82(2):98-103 describe that during the first 2 years of life there is a significant association between atopic dermatitis and respiratory infections manifested in an increased rate of acute otitis media, pneumonia and use of antibiotics. It is known that these infections often exacerbate the allergic manifestations. There is thus a real need to limit the microbial infection rate in allergic patients.

SUMMARY OF THE INVENTION

The inventors have surprisingly found for the first time that a synbiotic, i.e. a combination of a probiotic lactic acid bacterium and an indigestible fiber significantly reduced the microbial infection rate in allergic patients when given in a hypoallergenic formula, see example. In addition a statistical significant decrease in antibiotic use was found in the treatment group receiving the synbiotic composition.

Advantageously the present synbiotic composition provides the treatment of the infection and not the inflammation that can result from an infection. A beneficial consequence is that the inflammation can be prevented and therefore there is no need any more for treating the inflammation in a later stage, for example by administering analgesia or COX enzyme inhibitors like ibuprofen.

DETAILED DESCRIPTION OF THE INVENTION

The present invention thus concerns a method for the treatment or prevention of infection in an allergic subject, said method comprising administering a composition comprising i) a protein source consisting essentially of free amino acids, ii) at least one soluble indigestible fiber selected from the group consisting of fructooligosaccharides, pectin degradation products, non-milk derived fucosyloligosaccharides and polydextrose, and iii) at least one lactic acid bacterium selected from the group consisting of *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobacterium lactis* and *Lactobacillus rhamnosus*, to said allergic subject.

In other words the invention concerns the use of i) a protein source, ii) a prebiotic and iii) a probiotic for the manufacture of a nutritional composition for the treatment or prevention of infection in an allergic subject, wherein i) the protein source consists essentially of free amino acids, ii) the prebiotic comprises at least one soluble indigestible fiber selected from the group consisting of fructooligosaccharides, pectin degradation products, non-milk derived fucosyloligosaccharides and polydextrose, and iii) the probiotic comprises at least one lactic acid bacterium selected from the group consisting of *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobacterium lactis* and *Lactobacillus rhamnosus*.

The invention can also be worded as a composition comprising i) a protein source consisting essentially of free amino acids, ii) at least one soluble indigestible fiber selected from the group consisting of fructooligosaccharides, pectin degradation products, non-milk derived fucosyloligosaccharides and polydextrose, and iii) at least one lactic acid bacterium selected from the group consisting of *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobacterium lactis* and *Lactobacillus rhamnosus*, for use in the treatment or prevention of infection in an allergic subject.

Lactic acid bacteria

The fecal flora of breast fed infants is dominated by bifidobacteria, due to the presence of oligosaccharides in human milk. These act as bifidogenic factors, stimulating the proliferation of these species in the infant gut. Bifidobacteria are among the earliest colonizers of the human

gastrointestinal tract and their presence in large numbers in the intestines of breast-fed infants has been associated with improved health. Atopic infants have been shown to have an altered gut microflora with increased clostridia and decreased bifidobacteria. The bifidobacteria microflora of atopic infants has been shown to be more adult like with decreased strains of *B. bifidium* and *B. breve* and increased *B. adolescentis*.

The composition for use according to the present invention comprises at least one lactic acid bacterium selected from the group consisting of *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobacterium lactis*, and *Lactobacillus rhamnosus*. Typically, these lactic acid bacteria are commercially available from producers of lactic acid bacteria, but they can also be directly isolated from faeces, identified, characterised and produced.

The composition for use according to the present invention comprises at least $1.0x10^9$ living lactic acid bacteria (colony forming units; CFU) per liter, preferably between $1.0x10^9$ and $1x10^{11}$ CFU per liter. Preferably the composition for use according to the present invention comprises at least $1.0x10^7$ living lactic acid bacteria (colony forming units; CFU) per gram dry weight, preferably between $1.0x10^7$ and $1x10^9$ CFU per gram dry weight.

It is important for the synbiotic effect of the lactic acid bacteria and the prebiotic fiber that the concentration of the two ingredients is well balanced. Therefore preferably the concentration of the lactic acid bacteria is at least $1.0x10^8$ CFU lactic acid bacteria per gram prebiotic fiber, even more preferably between $2.0x10^8$ and $2.0x10^{10}$ CFU lactic acid bacteria per gram prebiotic fiber, more preferably between $1.0x10^9$ and $1.0x10^{10}$ CFU lactic acid bacteria per gram prebiotic fiber, most preferably between $1.0x10^9$ and $5.0x10^9$ CFU lactic acid bacteria per gram prebiotic fiber.

In one embodiment according to the method or use according to the present invention, the composition is administered in an amount that provides at least $1.0x10^7$ CFU lactic acid bacteria per day, preferably in an amount that provides from at least $2.0x10^7$ to at most $2.0x10^{11}$ CFU lactic acid bacteria per day, in an amount that provides from at least $4.0x10^7$ to at most $1.2x10^{11}$ CFU lactic acid bacteria per day, even more preferably in an amount that provides from at least $1.0x10^8$ to at most $6.0x10^{10}$ CFU lactic acid bacteria per day.

Lactobacillus rhamnosus, in particular *Lactobacillus rhamnosus GG*, also referred to as *Lactobacillus GG* or LGG, is one of the best studied species in humans and is also found in high amounts in the gut of infants. In a preferred embodiment the at least one lactic acid bacterium is selected from the group consisting of *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobacterium lactis* and *Lactobacillus rhamnosus GG*. LGG is commercially available and can be obtained from Valio Ltd.

Bifidobacterium is a genus of Gram-positive, non-motile, often branched anaerobic bacteria. Bifidobacteria are ubiquitous, endosymbiotic inhabitants of the gastrointestinal tract, vagina and mouth of mammals and other animals. Some bifidobacteria are used as probiotics. In a preferred embodiment, the lactic acid bacterium in the composition for use according to the present invention is *Bifidobacterium breve*, or in one embodiment consist of *Bifidobacterium breve*. According to a preferred embodiment, the composition for use according to the present invention comprises at least one *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, YIT4065, FERM BP-6223 and CNCM I-2219. Most preferably, the *B. breve* is selected from the group consisting of *B. breve* M-16V and *B. breve* CNCM I-2219, most preferably M-16V. *B. breve* I-2219 was published in WO 2004/093899 and was deposited at the Collection Nationale de Cultures de Microorganisms, Institute Pasteur, Paris, France on 31 May 1999 by Compagnie Gervais Danone. *B. breve* M-16V was deposited as BCCM/LMG23729 and is commercially available from Morinaga Milk Industry Co., Ltd.

Preferably the bacteria are alive, however, non-living lactic acid bacteria can also have beneficial effects on the immune system. Without being bound by theory it is hypothesized that dead lactic acid bacteria can be used in the treatment or prevention of infection in allergic patients. In a preferred embodiment at least part of the lactic acid bacteria present in the composition are dead or at least not capable to multiply.

Indigestible fiber

Soluble indigestible fiber is a term known in the art and refers to non digestible carbohydrate that can be used by the probiotic bacteria as a source of energy (fermentation) in the intestinal tract. Most of the formation and proliferation of the probiotic bacteria will take place in the colon. Without being bound by theory the inventors believe that administering live probiotic bacteria enteraly results in a relatively high concentration of these microorganisms in the small intestines where the fermentation can start resulting in fermentation products that are beneficial for the stimulation of the immune system resulting in a lower infection rate.

Thus, a soluble indigestible fiber can be defined as a non-digestible carbohydrate that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon. Preferred soluble indigestible fibers in the composition for use according to the present invention are not milk derived. Preferred soluble indigestible fibers in the composition for use according to the present invention include fructooligosaccharides, polydextrose and non-milk derived fucosyloligosaccharides, such as fucosyllactoses, fucosylated lactosamine-lactoses, and the like, and sialylated oligosaccharides characterized by one or more residues of N-acetylneuraminic acid, such as 3'- and 6'-sialyllactose (SL) and sialyl-lacto-N-tetraose.

The term “oligosaccharide” as used in the present invention preferably refers to a saccharide with an average degree of polymerization (DP) of 2 to 100, more preferably an average DP of 2 to 60. It is understood that in the context of this invention an oligosaccharide with a DP in a certain range may include a mixture of saccharides with different average DP’s, for example, if an oligosaccharide with a DP of 2 to 100 is included in the present composition, this may include compositions which contain oligosaccharides with an average DP between 2 and 5, an average DP between 50 and 70 and an average DP between 7 and 60.

Other preferred soluble indigestible fibers include oligosaccharides containing residues of uronic acid such as pectin degradation products.

In one embodiment the soluble indigestible fibers in the composition for use according to the present invention comprise pectin degradation product. Preferably the pectin degradation product is obtainable or obtained by enzymatic digestion of pectin with pectin lyase, pectate lyase, endopolygalacturonase and/or pectinase. Pectin is commonly used as thickener in many nutritional products. However, for the purpose of this invention the viscosity preferably is not substantially increased by the addition of the soluble indigestible fibers. Therefore the pectin degradation product preferably has an average degree of polymerization less than 70, preferably less than 50. Preferably the pectin degradation product has an average DP of higher than 3. More preferably the DP of the pectin degradation product is between 3 and 20. Pectin degradation product is fermented by Bifidobacteria and Lactobacilli.

In one embodiment the soluble indigestible fibers in the composition for use according to the present invention comprise polydextrose. Polydextrose is a soluble indigestible fiber favorably fermented by Bifidobacteria and Lactobacilli. It has the additional advantage of delivering only 1 kcal per gram of fiber, compared to 2 kcal per g for fructooligosaccharides and pectin degradation product. It is widely used and can be commercially obtained for example under the trade names LITESSE, STA-LITE, and TRIMCAL.

In a preferred embodiment the soluble indigestible fibers in the composition for use according to the present invention comprise fructooligosaccharides. The term “fructooligosaccharide” as used herein refers to a soluble indigestible fiber comprising a chain of at least 2 β-linked fructose units. A fructooligosaccharide can comprise a terminal glucose unit. In a preferred embodiment, the average degree of polymerisation of the fructooligosaccharides in the composition for use according to the present invention is in the range of 2 to 60, preferably the degree of polymerisation of the fructooligosaccharides is in the range from 2 to 60.

Preferably the soluble indigestible fibers in the composition for use according to the present invention is a combination of short chain fructooligosaccharides (scFOS) and long chain fructooligosaccharides (lcFOS). Long chain fructooligosaccharides is also referred to as inulin. Preferably the ratio scFOS : lcFOS is in the range of 95/5 to 10/90, even more preferably in the range of 95/5 to 40/60. In the context of this invention, scFOS has an average DP between 2 and

6. In the context of this invention lcFOS means any fructooligosaccharide composition with an average DP larger or equal to 7. A suitable source of scFOS is RAFTILOSE® (Orafti). RARTILINE® HP (Orafti) is a particularly preferred source of lcFOS and has an average DP > 20. Products commonly marketed as inulin comprise scFOS and lcFOS has in general an average DP larger than 7.

The composition for use according to the present invention preferably comprises more scFOS than lcFOS. Preferably the ratio scFOS : lcFOS is at least 1, preferably between 2 and 12, even more preferably between 3 and 10, most preferably the ratio scFOS : lcFOS is about 9. Both scFOS and lcFOS stimulate the growth of Bifidobacteria and Lactobacilli. It has been found that scFOS stimulates the growth already at the beginning of the colon, while the lcFOS stimulates the growth of the bacteria at the distal part of the colon.

The soluble indigestible fiber is preferably present in the composition for use according to the invention in an amount to provide a dose of 0.1-7 g/day more preferably 0.2 to 6 g/day, even more preferably 0.5 to 3 g/day. In one embodiment according to the method or use according to the present invention, the composition is administered in an amount that provides 0.1-7 g soluble indigestible fiber per day more preferably 0.2 to 6 g soluble indigestible fiber day, even more preferably 0.5 to 3 g soluble indigestible fiber day.

The soluble indigestible fiber is preferably present in a concentration of at least about 15 mg per gram dry weight of the composition, or at least 3 gram per liter composition. More preferably the concentration of soluble indigestible fiber in the composition for use according to the present invention is from 15 to 75 mg per g dry weight of the composition, and even more preferably from 35 to 60 mg per g dry weight of the composition.

Galactooligosaccharides (GOS) commonly used as prebiotic fiber in nutritional composition, including infant formula, is not suitable for the purpose of the present invention. GOS is derived from milk lactose, and is normally polluted with small amounts of milk protein. This milk protein, although present in small amounts, can still trigger immune reactions in the allergic

patient. Thus in one embodiment, the composition for use according to the present invention does not comprise galactooligosaccharides

Protein source

Allergy patients normally have an overreacting immune response against protein allergens. In particular food allergy is caused by many food related proteins. Cow's milk proteins are the most common allergens in infancy, followed by chicken egg proteins. In order to be absolutely sure that no protein is present in the composition for use according to the invention, the protein source exclusively consists of free amino acids.

The present invention advantageously concerns the use of a composition wherein the protein source provides 7 to 20% of the total calories of the composition, preferably the protein source provides 8 to 17% of the total calories, even more preferably the protein source provides 9 to 15% of the total calories of the composition.

Alternatively, in the composition for use according to the present invention, the content of the protein source is between 10 and 20 wt% free amino acids based on dry weight of the total composition, preferably between 11 and 18 wt%, and even more preferably between 12 and 16 wt% free amino acids based on dry weight of the total composition.

In one embodiment, the composition for the use according to the present invention is an infant formula. Therefore in one embodiment, the protein source comprises all essential amino acids. The optimal amino acid profile for infant formula is know in the art. A preferred embodiment of an amino acid composition is given in table 2.

Fat

The composition for use according to the present invention preferably comprises fat. The term 'fat' as used in the present invention includes all fat sources commonly used in nutritional products and may comprise a source of triglycerides, diglycerides, monoglycerides or free fatty acids. In particular when the composition for use according to the invention is for the treatment of infants, the composition preferably comprises long-chain polyunsaturated fatty acids (LCPUFA). In a preferred embodiment the composition for use according to the present invention comprises eicosapentaenoic acid (EPA), arachidonic acid (ARA) or docosahexaenoic

acid (DHA), preferably the composition comprises ARA or DHA or both, more preferably the composition comprises ARA and DHA. In a preferred embodiment, the fat provides 30 to 50% of the total calories of the composition.

In a preferred embodiment according to the present invention the composition comprises at least 0.05 g ARA and/or at least 0.05 g DHA per liter composition, or even more preferably from at least 60 mg to at most 420 mg ARA per liter final composition and/or from at least 60 mg to at most 420 mg DHA per liter final composition or even more preferably from at least 80 mg to at most 240 mg ARA per liter final composition and/or from at least 80 mg to at most 240 mg DHA per liter final composition. In one embodiment the composition for use according to the present invention comprises at least 0.35 mg ARA per g dry weight of the composition and/or at least 0.35 mg DHA per g dry weight of the composition. Preferably the composition comprises from at least 0.4 mg to at most 10 mg ARA per g dry weight of the composition and/or from at least 0.4 mg to at most 10 mg DHA per g dry weight of the composition, preferably from at least 0.5 mg to at most 6 mg ARA per g dry weight of the composition and/or from at least 0.5 mg to at most 6 mg DHA per g dry weight of the composition, more preferably from at least 0.6 mg to at most 3 mg ARA per g dry weight of the composition and/or from at least 0.6 mg to at most 3 mg DHA per g dry weight of the composition.

Subjects

The present method or use is for allergic subjects. Allergic subjects not only include subjects that have been diagnosed to have an allergy, but also subjects that have an increased risk of developing an allergy such as infants of parents having an allergy. The present method or use is specifically intended for allergic infants and/or allergic toddlers. Infants have an age of 0-12 months, toddlers have an age of 12-36 months, even more preferably for infants. Thus in one embodiment according to the present invention, the allergic subject is an allergic infant and/or toddler.

Application and compositions

The present method or use is for the treatment or prevention, preferably the prevention of infections in subjects with an allergy.

The composition according to the present use is preferably enterally administered, more preferably orally. The present composition is preferably a nutritional formula, preferably an infant formula. The present composition can advantageously be applied as a complete nutrition for infants. The present composition preferably comprises lipid, protein, and carbohydrate and is preferably administered in liquid form. The present invention includes dry compositions, e.g. powders, which are accompanied with instructions as to admix said dry compositions, in particular nutritional formula, with a suitable liquid, e.g. water.

In a preferred embodiment in the composition for the use according to the present invention, the soluble indigestible fiber comprises fructooligosaccharide and the lactic acid bacterium is *Bifidobacterium breve*.

In one embodiment in the composition for use according to the present invention the soluble indigestible fiber comprises a mixture of short chain fructooligosaccharide with an average degree of polymerization from 2 to 6 and long chain fructooligosaccharide with an average degree of polymerization of at least 7, and the weight ratio short chain fructooligosaccharide : long chain fructooligosaccharide is at least 1, preferably the weight ratio scFOS : lcFOS between 2 and 12, even more preferably between 3 and 10, most preferably the weight ratio scFOS : lcFOS is about 9.

In a preferred embodiment the composition for use according to the present invention is a nutritional composition comprising an allergen free protein source, essentially consisting of free amino acids, and Bifidobacteria, preferably Bifidobacterium breve, and a source of non digestible carbohydrates comprising fructooligosaccharides with an average DP of 2-60.

In yet a further preferred embodiment the composition for use according to the present invention is a nutritional composition, preferably an infant formula, comprising a protein source, a fat source, soluble indigestible fiber, and live lactic acid bacteria, wherein the protein source essentially consist of free amino acids and provides from 7 to 20% of the total calories of the nutritional composition, the fat source comprises at least arachidonic acid (AA) and

docosahexaenoic acid (DHA), energy percent, the soluble indigestible fiber comprises fructooligosaccharides with an average DP of 2-60 in a concentration from 15 to 75 mg per g dry weight of the nutritional composition and the live lactic acid bacteria are selected from the group consisting of *Bifidobacteria* and *Lactobacillus rhamnosus*, preferably selected from the group consisting of *Bifidobacterium breve* and *Lactobacillus rhamnosus LGG*, preferably the lactic acid bacteria comprise *Bifidobacterium breve*.

In a preferred embodiment in the composition for use according to the present invention the protein source provides from 10 to 20% of the total calories of the composition, the concentration of soluble indigestible fiber is from 15 to 75 mg per g dry weight of the composition and the concentration of lactic acid bacteria, preferably *Bifidobacterium breve*, is $2.0x10^8$ and $2.0x10^{10}$ CFU lactic acid bacteria, preferably *Bifidobacterium breve*, per gram soluble indigestible fiber, more preferably between $1.0x10^9$ and $1.0x10^{10}$ CFU lactic acid bacteria, preferably *Bifidobacterium breve*, per gram soluble indigestible fiber, most preferably between $1.0x10^9$ and $5.0x10^9$ CFU lactic acid bacteria, preferably *Bifidobacterium breve*, per gram soluble indigestible fiber. Preferably the soluble indigestible fiber is a mixture of short chain fructooligosaccharide with an average degree of polymerization from 2 to 6 and long chain fructooligosaccharide with an average degree of polymerization of at least 7, and the weight ratio short chain fructooligosaccharide : long chain fructooligosaccharide is at least 1. Preferably the weight ratio scFOS : lcFOS between 2 and 12, even more preferably between 3 and 10, most preferably the weight ratio scFOS : lcFOS is about 9. Preferably the composition further comprises fat providing 30 to 50% of the total calories of the composition, and the composition comprises DHA or ARA or both in a concentration of at least 0.35 mg per gram dry weight of the composition, preferably from at least 0.4 mg to at most 10 mg ARA per g dry weight of the composition and/or from at least 0.4 mg to at most 10 mg DHA per g dry weight of the composition, preferably from at least 0.5 mg to at most 6 mg ARA per g dry weight of the composition and/or from at least 0.5 mg to at most 6 mg DHA per g dry weight of the composition, more preferably from at least 0.6 mg to at most 3 mg ARA per g dry weight of the composition and/or from at least 0.6 mg to at most 3 mg DHA per g dry weight of the composition.

EXAMPLES

Example 1. Clinical study showing the anti-inflammatory effects of a synbiotic prebiotic fiber mix with Bifidobacterium breve in a population of allergic patients.

Pre- and probiotics (synbiotics) were investigated for the potential beneficial effects on human health. This study describes the functional effects of an amino-acid based formula (AAF) with synbiotics in infants with cow's milk allergy (CMA).

Methods

In a prospective, randomized, double-blind controlled study, full term infants with IgE and/or non-IgE mediated CMA randomly received a commercially available AAF (NEO; n=56) or an AAF with synbiotics (NEO-SYN; n=54) for 16 weeks. Primarily, infant growth and tolerance of the formula was monitored. Secondarily, dermatological (including severity of atopic manifestations by SCORAD) and respiratory allergic characteristics and stool characteristics were either recorded in subject diaries and/or evaluated by a physician. The NEO-SYN group were exclusively fed with a commercially available AAF supplemented with a milk protein free *Bifidobacterium breve* and a prebiotic fiber mix comprising short chain fructooligosaccharides (scFOS, with an average degree of polymerization below 6) and lcFOS (with an average degree of polymerisation above 7) in a weight ratio scFOS : lcFOS of approximately 9:1 in a concentration of about 45 mg scFOS + lcFOS per gram dry weight of the composition. The *B. breve* strain used was the commercially available strain M-16V of Morinaga. *B. breve* was used in a concentration of $1.9x10^9$ colony forming units (CFU) per gram prebiotic fiber.

Results

Average age of infants at inclusion was 4.58±2.45 months. Overall NEO-SYN and NEO were equally well tolerated and both supported normal growth. Both formulas reduced allergic symptoms, and no significant differences between the groups were observed; The NEO-SYN group was reported to have less subjects suffering from infections (p=0.008) and less subjects receiving medication for functional gastrointestinal (GI) disorders (p=0.029) when compared with the NEO group. In addition the NEO-SYN group had a lower number of infants with antibiotics usage (p=0.049), especially amoxicillin (p=0.004), compared with the NEO group. The results are summarised in table 1.

Table 1: Reported infections and antibiotic use in window of 16 weeks

	NEO	NEO-SYN	*p*-value
Infections (reported)	17.9%	1.9%	0.008
Antibiotic use			
- overall	33.9%	16.7%	0.049
- amoxicillin	32.1%	9.3%	0.004

Conclusion

This study shows that an AAF with synbiotics is equally well tolerated, supports normal growth and has similar efficacy to manage CMA symptoms compared to an AAF without synbiotics. Addition of synbiotics improves resistance against infections and reduces specific medication usage in infants receiving AAF.

Example 2.Composition for use according to the invention

	UNIT	per 100g	per 100ml*
energy:	kcal	483	67
Protein (see table 2):	g	13	1.8
% of total energy		10.8	10.8
Carbohydrate:	g	52.5	7.3
Sugars	g	4.7	0.65
% of total energy		43.5	43.5
Fat:	g	24.5	3.4
% of total energy		45.7	45.7
Saturates	g	8.9	1.2
Monounsaturates g		9.6	1.3
Polyunsaturates	g	4.8	0.67
DHA	mg	110	15
ARA	mg	110	15

Prebiotic fibre: g 4.9 0.68

Ratio scFOS/lcFOS about 9:1

Lactic acid bacteria: B.breve M-16V - $1.9x10^9$ (CFU) per gram prebiotic fiber

*14.7 g powder is dissolved in 100 ml water

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Table 2: Composition of protein source

COMPONENT	UNIT	Per 100 g composition
Amino Acids		
L-Alanine	g	0,6
L-Arginine	g	1,0
L-Aspartic acid	g	1,0
L-Cystine	g	0,4
L-Glutamine	g	1,3
Glycine	g	0,9
L-Histidine	g	0,6
L-Isoleucine	g	0,9
L-Leucine	g	1,6
L-Lysine	g	1,1
L-Methionine	g	0,2
L-Phenylalanine	g	0,7
L-Proline	g	1,1
L-Serine	g	0,7
L-Threonine	g	0,8
L-Tryptophan	g	0,3
L-Tyrosine	g	0,7
L-Valine	g	1,0
L-Carnitine	g	0,01

CLAIMS

1. Use of i) a protein source, ii) a prebiotic and iii) a probiotic for the manufacture of a nutritional composition for the treatment or prevention of infection in an allergic subject, wherein i) the protein source consists essentially of free amino acids, ii) the prebiotic comprises at least one soluble indigestible fiber selected from the group consisting of fructooligosaccharides, pectin degradation products, non-milk derived fucosyloligosaccharides and polydextrose, and iii) the probiotic comprises at least one lactic acid bacterium selected from the group consisting of *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobacterium lactis* and *Lactobacillus rhamnosus*.

2. The use according to claim 1, wherein the soluble indigestible fiber comprises fructooligosaccharide and the lactic acid bacterium is Bifidobacterium breve.

3. The use according to claim 1 or 2, wherein the soluble indigestible fiber comprises a mixture of short chain fructooligosaccharide with an average degree of polymerisation from 2 to 6 and long chain fructooligosaccharide with an average degree of polymerisation of at least 7, and the weight ratio short chain fructooligosaccharide : long chain fructooligosaccharide is at least 1.

4. The use according to any one of claims 1 - 3, wherein the protein source provides from 7 to 20% of the total calories of the composition.

5. The use according to any one of claims 1 – 4, wherein the composition further comprises DHA or ARA or both.

6. The use according to any one of claims 1 – 5, wherein the protein source provides from 10 to 20% of the total calories of the composition, the concentration of soluble indigestible fiber is from 15 to 75 mg per g dry weight of the composition and the concentration of lactic

acid bacteria is $2.0x10^8$ and $2.0x10^{10}$ CFU lactic acid bacteria per gram soluble indigestible fiber.

7. The use according to claim 6, wherein the soluble indigestible fiber is a mixture of short chain fructooligosaccharide with an average degree of polymerization from 2 to 6 and long chain fructooligosaccharide with an average degree of polymerization of at least 7, and the weight ratio short chain fructooligosaccharide : long chain fructooligosaccharide is at least 1.

8. The use according to claim 6 or 7, wherein the composition further comprises fat providing 30 to 50% of the total calories of the composition, and the composition comprises DHA or ARA or both in a concentration of at least 0.35 mg per gram dry weight of the composition.

9. The use according to any one of claims 1 – 8, wherein the allergic subject is an infant.

10. The use according to any one of claims 1 – 9, wherein the composition is an infant formula.

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2013/050423

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/00 A61K31/702 A61K35/74 A23L1/305 A61P31/00
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 A61P

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, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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

[X] See patent family annex.

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 August 2013	16/08/2013

Name and mailing address of the ISA/	Authorized officer
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