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(54) **Nutritional compositions comprising human milk oligosaccharides and uses thereof.**

(57) The invention relates to nutritional compositions comprising oligosaccharides and to the uses thereof. In particular, it relates to compositions comprising complex human milk oligosaccharides (HMO) and use thereof as infant food or dietary supplement. Provided is a nutritional composition comprising (i) at least one α 1,2-fucosylated HMO comprising at least four monosaccharide units and (ii) at least one HMO-protective compound selected from the group consisting of prebiotic oligosaccharides, maltodextrin and lactoferrin.

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Title: Nutritional compositions comprising human milk oligosaccharides and uses thereof.

The invention relates to nutritional compositions comprising oligosaccharides and to the uses thereof. In particular, it relates to compositions comprising complex human milk oligosaccharides (HMO) and use thereof as infant food or dietary supplement.

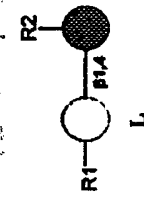
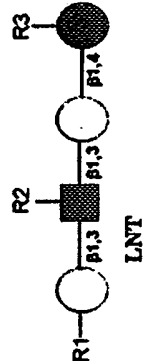
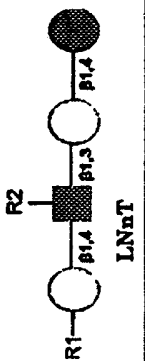
5 Exclusive breast feeding during the first six months after birth is officially recommended by the WHO and efforts are made to support and promote breast feeding amongst mothers worldwide. Important components of breast milk are human milk oligosaccharides (HMOs), present to 3-19 g/L. They are composed of a core of galactose, glucose, N-acetyl-glucosamine and
10 decorated with fucose and sialic acid to different extents. Numerous studies have pointed out the biological importance of HMOs, e.g. their role in inhibiting the adhesion of pathogenic bacteria to the epithelial surface or the establishment of gut microbiota. The HMO-composition of breast milk cannot be generalized though, as it is genetically determined. The mother's Secretor-
15 and Lewis type determine the fucosylation pattern and thus the set of HMOs present in breast milk. Breast milks from Le(a-b+)-secretors, Le(a+b-)-non-secretors and Le(a-b-)-secretors/-non-secretors can be distinguished and have been thoroughly studied in view of their structural composition as well as their development during different stages of lactation. Differences in HMO-profiles
20 may have an influence on the biological functioning of breast milk.

HMOs are synthesized in the mammary glands and can be further decorated by glycosyltransferases with fucose and sialic acid. Oligosaccharides in human milk can thus be divided into a neutral and an acidic fraction, with acidic oligosaccharides being present in a tenfold lower concentration than
25 neutral oligosaccharides. The structural composition of HMOs has been extensively studied, leading to a well-defined picture on the glycobiology of oligosaccharides in human milk.

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Table 1 gives an overview on the structural composition of fucosylated and sialylated HMOs up to a core structure of six sugar units, as reviewed by Urashima et al. (*Comprehensive glycoscience. From chemistry to system biology; vol.4*;

5 Kamerling, J.P., Boons, G.J., Lee, Y.C., Suzuki, A., Taniguchi, N., Voragen, A.G.J., Eds; Elsevier: Oxford, UK, 2007; pp 695-722)

core-HMO structures	$\alpha(1\rightarrow2)\beta$	$\alpha(1\rightarrow3)\beta$	$\alpha(1\rightarrow4)\beta$	$\alpha(1\rightarrow6)\beta$	$\alpha(2\rightarrow3)\beta$	$\alpha(2\rightarrow6)\beta$	name	$\frac{\alpha(1\rightarrow2)\beta}{\text{Lactose}}$	$\frac{\alpha(1\rightarrow3)\beta}{\text{Lactose}}$	$\frac{\alpha(1\rightarrow4)\beta}{\text{Lactose}}$
 L	R1	-	-	-	-	-	L	57	58	55
	-	-	-	-	-	-	2'FL	3.13	-	4.57
	-	-	R2	-	-	-	3'FL	0.42	1.79	0.15
	R1	-	R2	-	-	-	DF-L	0.41	-	0.17
	-	-	-	-	R1	-	3'SL	0.27	0.24	0.31
	-	-	-	-	-	R1	6'SL	1.22	1.14	1.31
 LNT	-	-	-	-	-	-	LNT	0.88	1.19	0.85
	R1	-	-	-	-	-	LNFP I	1.58	-	3.18
	-	-	R2	-	-	-	LNFP II	0.22	1.25	-
	-	-	R3	-	-	-	LNFP V	nd	nd	nd
	R1	-	R2	-	-	-	LNDFH I	1.28	-	-
	-	-	R3	-	-	-	LNDFH II	0.20	0.45	-
	-	-	-	-	R1	-	S-LNTa	0.04	0.04	0.06
	-	-	-	-	-	R2	S-LNTb	0.08	0.11	0.08
 LNnT	-	-	-	-	R1	R2	DS-LNT	0.34	0.42	0.41
	-	-	R2	-	-	-	LNnT	0.23	0.08	0.23
	-	-	-	-	-	R1	LNFP III	0.38	0.38	0.31
	-	-	-	-	-	-	S-LNTc	0.29	0.21	0.31

The presence and abundance of HMOs in breast milk is genetically determined. See Thurl et al. *Glycoconjugate J.*, **1997**, *14*, 795-799; Oriol et al. *Glycobiology*, **1999**, *9*, 323-334.; Oriol et al. *Vox Sang.*, **1986**, *51*, 161-171.; Erney et al. *J. Pediatr. Gastr. Nutr.*, **2000**, *30*, 181-192. The (α 1,2)-

5 fucosyltransferase (FUT2) and (α 1,3/4)-fucosyltransferases (FUT3, 4, 5, 6, 7, 9), which are responsible for the fucosylation of the HMOs in the mammary glands, correspond to the fucosyltransferases responsible for fucosylation of other body glycoproteins (e.g. on erythrocytes and mucins). The expression of FUT2 and FUT3 depends on the maternal Secretor- and Lewis type,

10 respectively. Accordingly, human milk can be classified into four groups:

- 1) Human milk from Le(a-b+)-secretors contains (α 1,2)-, (α 1,3)- and (α 1,4)-fucosylated oligosaccharides, which accounts for 70% - 80% of the European population.
- 2) Human milk from Le(a+b-)-non-secretors contains (α 1,3)- and (α 1,4)-

15 fucosylated oligosaccharides, but lacks (α 1,2)-fucosylated oligosaccharides. This is found for approximately 20% of the European population.

- 3) Le(a-b)-secretor-milk contains (α 1,2)- and (α 1,3)-fucosylated oligosaccharides and does not contain (α 1,4)-fucosylated oligosaccharides. The occurrence of the Le(a-b)-secretor status is only approximately 10%, as was determined for the French population.
- 5 4) Le(a-b)-non-secretor-milk is composed of (α 1,3)-fucosylated oligosaccharides, but lacks (α 1,2)- as well as (α 1,4)-fucosylated oligosaccharides. The occurrence of this milk type was estimated as rather low (1%, as was determined for the French population).
- 10 The concentration of HMOs in breast milk of Le(a-b+)- Le(a+b)- and Le(a-b)-mothers is indicated in Table 1.

Breast-feeding is connected to a lowered risk of inflammatory bowel diseases and gastrointestinal, respiratory and urinary infections in the postnatal

15 period. The health-beneficial characteristics of breast milk are mainly connected to the complex HMO-structures, which were also proposed to be involved in the development of the immune system (Klein et al. In *Short and long term effects of breast feeding on child health*; Koletzko, B., Michaelsen, K.F., Hernell, O., Eds; Kluwer Academic / Plenum Publ: New York, 2000; pp

20 251-259). Even an influence on brain development has been proposed (Wang et al. *Am. J. Clin. Nutr.*, 2003, 78, 1024-1029) Direct pathogen inhibition and bifidogenicity are well-studied examples of health-related structure-function relationships of HMOs.

The intestinal mucosa is the largest surface of the human body and it is

25 among the most heavily glycosylated tissues. The mucosa of the intestine is covered with complex glycans, including glycoproteins, glycolipids, mucins, and others. The principal function of these glycans is thought to be the mediation of communication with the extracellular environment, including cell-cell communication, molecular discrimination, barrier functions, and diverse

30 signalling actions. To overcome this barrier, the first step of bacterial and viral infection is to recognize and bind specific cell surface glycans of the intestinal

mucosa, where sialylated and fucosylated oligosaccharides are the primary targets. Because many milk oligosaccharides contain structural units that are homologous to these carbohydrate structures, it has been suggested that they act as soluble receptor analogs that inhibit the adhesion of pathogens, thus preventing infection. In fact, HMO are synthesized by the same glycosyl- and fucosyltransferases, enzymes responsible for the formation of glycans present on different cell types. Fucosylated and sialylated milk oligosaccharides inhibit the binding of pathogenic bacteria by blocking bacteria from attaching to target oligosaccharides on the intestinal mucosal surface. Milk oligosaccharides, especially those containing alpha1-2-linked fucose, have adhesion-inhibiting activity for both Gram-negative and Gram-positive bacteria. HMOs can act as receptor analogs for preventing the adhesion of pathogenic bacteria to the mucosal surface. This ligand-receptor mechanism is based on the structural characteristics of HMOs, which are complementary to the structures of the carbohydrate epitopes in the mucosa. *In vitro* experiments showed that sialylated HMOs prevent the adhesion of S-fimbriated enteropathogenic *E.coli* and Influenza A virus. Special attention has to be paid to (alpha1,2)-fucosylated HMOs, which showed inhibition of campylobacter, norovirus and toxin-producing *E-coli in vitro* and a lower incidence of diarrhea in breast-fed infants *in vivo*. Babies who consumed milk rich in (alpha1,2)-fucosylated HMOs showed less incidence for diarrhea than babies, who received milk with low (alpha1,2)-fucosylated HMO-levels. There is still insufficient knowledge in order to judge different types of breast milks on their functional value.

The microbiota of breast-fed infants is described as "simple", with a considerable contribution of Bifidobacteria. Bifidobacteria are known to inhibit the growth of pathogens by short chain fatty acid production and to stimulate the immune response, cholesterol assimilation and the synthesis of vitamins. Infant-specific *Bifidobacterium* species exhibit cellular transporter systems for intact HMOs or exhibit extracellular enzymes necessary to degrade HMOs,

such as sialidase, (α 1,2)-/-(α 1,3/4)-fucosidase and lacto-N-biosidase-phosphorylase.

Prebiotic oligosaccharide-supplementation to infant formula aims at simulating the health-promoting effect of HMOs. Due to the complexity of HMOs, their synthesis or extraction cannot be performed on a production scale to date. In view of the high galactose content in human milk and the lactose-based structural composition of HMOs, galactooligosaccharides (GOS) are frequently used for the supplementation of infant formula. In addition, long-chain fructooligosaccharides (FOS) are nowadays added to infant formula in order to simulate the higher molecular weight oligosaccharide fraction of human milk.

In vitro- and *in vivo* studies with prebiotic GOS and FOS demonstrated a specific stimulation of health-beneficial gut bacteria, although structurally different oligosaccharide mixtures were used in the respective studies. Correspondingly, the presence and expression of saccharidases (β -galactosidases and β -fructofuranosidases) from health-beneficial bacteria and their mode of action has been described. Similar to HMOs, anti-adhesive functions towards an enteropathogenic *E.coli*-strain were found for GOS *in vitro*. For the application in infant formula, a mixture of GOS : FOS (9:1) has been used (Boehm et al., *Arch. Dis. Child.*, 2002, 86, 178-181). This proportioning emulates the molecular size distribution of HMOs in breast milk. Furthermore, a synergistic effect of GOS and FOS on the stimulation of health-beneficial colonic bacteria was proposed. The FOS added to the infant formula have a size of DP > 10. Feeding infant formula supplemented with a combination of GOS and FOS resulted in the stimulation of bifidobacterial growth and was accompanied by increased stool frequency, soft stools and decreased fecal pH. The health-promoting effects observed were dose-dependent, with 0.8 g/dL supplementation being considered as optimal and approved as safe by the EFSA authority.

The supplementation of infant formula with GOS and/or FOS neglects the presence of acidic oligosaccharides in human milk. These may contribute to the gastrointestinal defense of pathogenic bacteria and to the systemic effects

proposed for HMOs. So far, synthetically produced, sialylated oligosaccharides are not suitable for the application in food. Instead, acidic oligosaccharides from pectin (AOS) are being considered for their potential use in infant formula. Pectin, which is a plant cell wall polysaccharide contains acidic
5 polygalacturonan-chains. AOS are obtained by enzymatic hydrolysis and are renowned for their curative functioning for diarrhea, which may be related to the anti-adhesive effects found for these oligosaccharides towards *E. coli*.

The use of non-digestible oligosaccharides in infant foods is for example
10 disclosed in WO2007/067053, WO2005/039597, WO01/642255, US 6,576,251, WO 99/11773, WO2005/055944, WO2007/105945, EP1629850 and WO2011/008086.

The ample amount of available literature underscores the ongoing efforts made
15 to further improve nutritional oligosaccharide compositions, especially with the aim to enhance infant health and development.

The present inventors surprisingly found that a combination of complex HMOs with less complex oligosaccharides leads to unexpected health effects. More in
20 particular, it was observed that the admixture of specific additives, such as prebiotic oligosaccharides, maltodextrin or lactoferrin, delays gastrointestinal metabolism of α 1,2-fucosylated HMOs. Herewith, the efficacy of these beneficial milk saccharides is enhanced. Interestingly, delayed gastrointestinal metabolism of α 1,2-fucosylated HMOs stimulated the body weight gain in
25 preterm infants. Furthermore, the presence of HMOs was found to enhance the prebiotic action of admixed prebiotics. These findings open up new applications of HMOs and known prebiotics. For example, the protective effect of the additive allows for the supplementation with lower concentrations of (synthetic) complex HMOs while still achieving an effect similar to breast
30 milk.

Accordingly, in one embodiment the invention provides a nutritional composition comprising (i) at least one α 1,2-fucosylated human milk oligosaccharide (HMO) comprising at least four monosaccharide-units and (ii) at least one protective compound selected from the group consisting of

prebiotics, maltodextrin and lactoferrin. Typically, prebiotics are carbohydrates (such as oligosaccharides), but the definition may include non-carbohydrates. As used herein, the term prebiotic refers to a non-digestible food ingredient that beneficially affects the host, by stimulating the growth and/or activity of bacteria in the digestive system e.g. by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon. Prebiotics are well known in the art. Prebiotics suitable for use in infant formula are preferred. Preferably, the prebiotic is a (mixture of) oligosaccharides which are essentially not digested in the upper intestinal tract and thus reach the colon intact to stimulate the growth of beneficial bacteria in the colon, providing several health benefits. The prebiotic definition does not emphasize a specific bacterial group. Generally, however, it is assumed that a prebiotic should increase the number and/or activity of bifidobacteria and lactic acid bacteria. The importance of the bifidobacteria and the lactic acid bacteria is that these groups of bacteria have several beneficial effects on the host, especially in terms of improving digestion (including enhancing mineral absorption) and the effectiveness and intrinsic strength of the immune system.

Exemplary prebiotic oligosaccharides for use in a composition of the invention include galacto-oligosaccharides (GOS), fructose-oligosaccharides (FOS) and GOS/FOS mixtures. GOS prepared by trans-galactosylation, also referred to as trans- galacto-oligosaccharides or TOS, is particularly preferred. Preferably, GOS has a polymerization degree (DP) of 2 to 10. Suitable GOS preparations are commercially available. For example, Vivinal GOS made by the company FrieslandCampina DOMO is a prebiotic ingredient rich in trans-galacto-oligosaccharides. Suitable FOS preparations are those having a polymerization degree (DP) of 2 to 60, preferably 10 to 60. Mixtures of FOS and GOS, preferably in a 1:9 weight ratio, are also envisaged.

Another additive found to have beneficial effects in combination with a α 1,2-fucosylated HMO is maltodextrin. Maltodextrin is an oligosaccharide

mixture that is used as a food additive. It is produced from starch by partial hydrolysis and digested and absorbed in the small gastrointestinal tract, although it is not clear whether if digestion is complete in the immature gastrointestinal tract of an (pre)term infant. [As shown herein below, a mixed
 5 diet of HMOs and maltodextrin yielded increased counts of beneficial lactobacilli in preterm infants as compared to maltodextrin-based infant formula alone.

As yet another example, the protective compound is a non-carbohydrate component like lactoferrin. Lactoferrin (LF), also known as lactotransferrin
 10 (LTF), is a multifunctional protein of the transferrin family. Lactoferrin is a globular glycoprotein with a molecular mass of about 80 kDa. Besides its antibacterial, antifungal and antiviral effects, whey-derived lactoferrin was shown to support the growth of bifidobacteria and lactobacillus, thus also indicating prebiotic properties (Harper WJ. Biological properties of whey
 15 components: a review. Chicago, IL: The American Dairy Products Institute; 2000).

A nutritional composition of the invention is not disclosed or suggested in the art. WO2011/008086 only relates to simple fucosylated oligosaccharide 2'FL and 3'FL, and is silent about more complex HMOs comprising more than
 20 three monosaccharide units. US 6,576,251 discloses carbohydrate mixtures comprising a complex fucosylated structure and GOS, yet fails to teach a preference for α -1,2-fucosyl-containing HMOs.

Suitable α 1,2-fucosylated HMOs for use in a nutritional composition of the invention include those found in human breast milk such as
 25 difucosyllactose (DF-L), lacto-N-fucopentaose I (LNFP I), lacto-N-difucohexaose I (LNDFH I), fucosyllacto-N-hexaose I (F-LNH I), difucosyllacto-N-hexaose I (DF-LNH I), trifucosyllacto-N-hexaose I (TF-LNH I), fucosyl-lacto-N-neohexaose I or II (F-LNnH I/ II) and trifucosyl-para-lacto-N-hexaose I (TF-*para*-LNH I). The more predominant HMOs in milk such as LNFP I, LNDFH I
 30 and DF-LNH I (see Table 1, last column) are of particular interest.

In one embodiment, the α 1,2-fucosylated HMO has four monosaccharide units, like DF-L. In another embodiment, the structure has five or more monosaccharide units, like LNFP I. In another embodiment, the α 1,2-fucosylated HMO has six or more monosaccharide units like LNDFH I. In yet another embodiment, the HMO has seven monosaccharide units, such as F-LNH I or F-LNnH I or II. In still a further embodiment, the HMO has eight or more monosaccharide units, for example DF-LNH I. An exemplary structure consisting of nine monosaccharide units is TF-para-LNH I.

In one aspect, the HMO is a monofucosylated compound, preferably selected from the group consisting of LNFP I, F-LNH I and F-LNnH I/II. In another aspect, the HMO is a difucosylated compound, preferably selected from the group consisting of DF-L, LNDFH I and DF-LNH I. In yet another aspect, the HMO is a trifucosylated compound, preferably TF-LNH or TF-para-LNH I.

As is clear from Table 1, the HMO can be based on different core structures, for example lactose, LNT, LNnT, LNH, LNnH, para-LNH or para-LNnH. Lacto-N-biose is considered as bifidogenic component of breast milk. Therefore, the α 1,2-fucosylated complex HMO preferably comprises at least one lacto-N-biose unit, preferably as present in lacto-N-tetraose (LNT). Preferred LNT-containing α 1,2-fucosylated HMOs are LNFP I and LNDFH I. Interestingly, LNDFH I was also found to be associated with enhanced body weight gain in preterm infants. Also preferred is F-LNH I.

An α -1,2-fucosylated HMO comprising at least four monosaccharide-units can be obtained from various sources. Commercial suppliers include Dextra, Reading, UK. In one embodiment, it is prepared (semi)-synthetically. HMOs can be derived using any number of sources and methods known to those of skill in the art. For example, HMOs can be purified from human milk using methods known in the art. One such method for extraction of oligosaccharides from pooled mother's milk entails the centrifugation of milk at 5,000*g for 30 minutes at 4°C and fat removal. Ethanol may then be added

to precipitate proteins. After centrifugation to sediment precipitated protein, the resulting solvent is collected and dried by rotary evaporation. The resulting material may be adjusted to the appropriate pH of 6.8 with phosphate buffer and [beta]-galactosidase is added. After incubation, the solution is extracted with chloroform-methanol, and the aqueous layer is collected. Monosaccharides and disaccharides are removed by selective adsorption of HMOs using solid phase extraction with graphitized nonporous carbon cartridges. The retained oligosaccharides can be eluted with water-acetonitrile (60:40) with 0.01% trifluoroacetic acid. Individual HMOs can be further separated using methods known in the art such as capillary electrophoresis, HPLC (e.g., high-performance anion-exchange chromatography with pulsed amperometric detection; HPAEC-PAD), and thin layer chromatography. See, e.g., U.S. Patent Application No. 2009/0098240.

Alternately, enzymatic methods can be used to synthesize HMOs. See for example EP1637611 and WO2010/070104. In general, any oligosaccharide biosynthetic enzyme or catabolic enzyme (with the reaction running in reverse) that converts a substrate into any of the HMO structures (or their intermediates) may be used to prepare the HMOs. Another approach to the synthesis of the HMOs entails the chemical or enzymatic synthesis of or isolation of oligosaccharide backbones containing Lacto-N-biose, or Lacto-N-tretrose from non-human mammalian milk sources (e.g., cows, sheep, buffalo, goat, etc.) and enzymatically adding Lacto-N-biose, Fucose and Sialic Acid units as necessary to arrive at HMO structures. Examples of such oligosaccharide modifying enzymes include sialidases, sialate O-Acetyl esterases, N-Acetylneuraminate lyases, N-acetyl-beta-hexosaminidase, beta-galactosidases, N-acetylmannosamine-6-phosphate 2-epimerases, alpha-L-fucosidases, and fucose dissimilation pathway proteins, among others, which may be used to catalyze a biosynthetic reaction under the appropriate conditions. Alternatively, conventional chemical methods may be used for the

de novo organic synthesis of or conversion of pre-existing oligosaccharides into HMO structures. These techniques for synthesizing HMOs are described in more detail in U.S. Patent Application No. 2009/0098240.

In one embodiment, HMOs for use in the present invention, or precursors thereof, are obtained from non-human mammalian milk. Fucose- α 1, 2-lactose may be produced in large scale quantities by recombinant *E. coli* which utilize enzymes FKP (fucokinase pyrophosphorylase) and WbsJ (α 1, 2-fucosyltransferase) to enzymatically synthesize fucose- α 1, 2-lactose. FKP is a bifunctional enzyme which catalyzes the reaction between fucose, ATP, and GTP to produce the expensive and hard to obtain GDP-fucose, meanwhile WbsJ is a known α 1, 2-FucT which demonstrates promiscuous acceptor substrate specificity. This method is cost effective by regenerating the sugar nucleotides required for the synthesis and require only inexpensive, commercially available, fucose and lactose.

Whey streams have the potential to be commercially viable sources of complex oligosaccharides that have the structural resemblance and diversity of the bioactive oligosaccharides in human milk.

In a specific aspect, a nutritional composition of the invention solely contains α 1,2-fucosylated HMO's obtained from human milk. For example, it is human breast milk obtained from a Le(a-b+) or Le(a-b-)-secretor-type donor subject and supplemented with at least one HMO-protective compound selected from the group consisting of prebiotic oligosaccharides, maltodextrin and lactoferrin.

The relative mixing ratio between (i) α 1,2-fucosyl HMO and (ii) protective compound(s) can vary, depending on type of HMO and/or protective compound. For example, the ratio (i) to (ii) is between about 10:1 and 1:200 by weight, for example between 10:1 and 1:200. In one embodiment, the protective compound is present in excess of the HMO. For instance, 4 g/L GOS or maltodextrin was found to delay the metabolism of 1.2 g/L α 1,2-fucosyl HMO. In another embodiment, the protective compound is present in about equal amounts as

the HMO. It is also possible to achieve protection with lower amounts of protective compounds. For example, 1 g/L lactoferrin was found to significantly protect >1 g/L α 1,2-fucosyl HMO.

The absolute concentration of α 1,2-fucosyl HMO and protective compound can vary likewise. In one embodiment, the composition comprises 0.02 to 10 wt% α -1,2-fucosylated HMO having at least four monosaccharide-units and/or 0.25 wt% to 15wt% protective compound based on the dry weight of the composition. In one embodiment, the α 1,2-fucosylated HMO concentration mimics the concentration found in breast milk. The total concentration of α -1,2-fucosylated HMOs in breast milk is about 6-9 g/L.

Typical compositions comprising GOS and/or FOS as protective compound contain 0.4 to 0.8 g/dL of GOS and/or FOS in total. Maltodextrin is also suitably used as protective compound at 0.4 to 0.8 g/dL. Lactoferrin can be used at 0.05 to 0.2 g/dL, preferably about 0.1 g/dL. The skilled person will be able to determine, e.g. by HMO profiling of feces, whether a given type and amount of protective compound exerts a desired effect.

A nutritional composition may comprise one or more further nutritionally useful additives. In one embodiment, the additive(s) is a compound found in human milk, preferably a HMO, more preferably a HMO selected from the group consisting of sialyllactose, fucosyllactose, di-sialylated oligosaccharides, lacto-N-neo-tetraose (LNnT), lacto-N-tetraose (LNT), lacto-N-fucopentaose (LNFP)-isomers, lacto-N-difucohexaose (LNDFH)-isomers, fucosyllacto-N-hexaose (F-LNH)-isomers, difucosyllacto-N-hexaose (DF-LNH)-isomers and trifucosyllacto-N-hexaose (TF-LNH)-isomers. The sialyllactose may originate from bovine milk.

In one embodiment, the nutritional composition further comprises a protein source, a lipid source and a carbohydrate source, preferably in amounts suitable to provide nutrition to an infant. Many different sources and types of carbohydrates, lipids, proteins, minerals and vitamins are known and can be used in the nutritional formulas of the present disclosure, provided that such

nutrients are compatible with the added ingredients in the selected formula, are safe for their intended use, and do not otherwise unduly impair product performance.

Carbohydrates suitable for use in the nutritional formulas of the present disclosure can be simple, complex, or variations or combinations thereof. Non-limiting examples of suitable carbohydrates include hydrolyzed, intact, naturally and/or chemically modified cornstarch, glucose polymers, sucrose, corn syrup, corn syrup solids, rice or potato derived carbohydrate, glucose, fructose, lactose, high fructose corn syrup and combinations thereof. Preferably carbohydrate sources contribute between 35 and 65% of the total energy of the formula.

Non-limiting examples of lipids suitable for use in the nutritional formulas include coconut oil, soy oil, corn oil, olive oil, safflower oil, high oleic safflower oil, MCT oil (medium chain triglycerides), sunflower oil, high oleic sunflower oil, palm and palm kernel oils, palm olein, canola oil, marine oils, cottonseed oils, long-chain polyunsaturated fatty acids such as arachidonic acid (ARA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA), and combinations thereof.

The lipid source is preferably a lipid or fat which is suitable for use in infant formulas. Preferred fat sources include palm olein, high oleic sunflower oil and high oleic safflower oil. In total, the fat content is preferably such as to contribute between 30 to 55% of the total energy of the composition. The fat source preferably has a ratio of n-6 to n-3 fatty acids of about 5:1 to about 15:1; for example about 8:1 to about 10:1.

In addition to these food grade oils, structured lipids may be incorporated into the nutritional formulas if desired. Structured lipids are known in the art, descriptions of which can be found in INFORM, Vol. 8, no. 10, page 1004, Structured lipids allow fat tailoring (October 1997); and US 4,871,768. Structured lipids are predominantly triacylglycerols containing

mixtures of medium and long chain fatty acids on the same glycerol nucleus. Structured lipids are also described in US 6,160,007.

Non-limiting examples of proteins suitable for use in the nutritional
5 formulas include extensively hydrolyzed, partially hydrolyzed or non-
hydrolyzed proteins or protein sources, and can be derived from any known or
otherwise suitable source such as milk (e.g., casein, whey), animal (e.g., meat,
fish), cereal (e.g., rice, corn), vegetable (e.g., soy), or combinations thereof. It
may be desirable to supply partially hydrolyzed proteins (degree of hydrolysis
10 between 2 and 20%), for example for infants believed to be at risk of developing
cows' milk allergy. If hydrolyzed proteins are required, the hydrolysis process
may be carried out as desired and as is known in the art. For example, a whey
protein hydrolysate may be prepared by enzymatically hydrolyzing the whey
fraction in one or more steps. The proteins for use herein can also include, or be
15 entirely or partially replaced by, free amino acids known for use in nutritional
formulas, non-limiting examples of which include tryptophan, glutamine,
tyrosine, methionine, cysteine, arginine, and combinations thereof. Other (non-
protein) amino acids typically added to nutritional formulas include carnitine
and taurine. In some cases, the D-forms of the amino acids are considered as
20 nutritionally equivalent to the L-forms, and isomer mixtures are used to lower
cost (for example, D,L-methionine).

The nutritional composition of the present disclosure may further comprise
any of a variety of vitamins in addition to the components described above.
25 Non-limiting examples of vitamins include vitamin A, vitamin D, vitamin E,
vitamin K, thiamine, riboflavin, pyridoxine, vitamin B 12, niacin, folic acid,
pantothenic acid, biotin, vitamin C, choline, chromium, carnitine, inositol,
salts and derivatives thereof, and combinations thereof. The nutritional
composition may further comprise any of a variety of minerals, non-limiting
30 examples of which include calcium, phosphorus, magnesium, iron, zinc,

manganese, copper, iodine, sodium, potassium, chloride, and combinations thereof.

The infant formula embodiments of the present disclosure preferably comprise
5 nutrients in accordance with the relevant infant formula guidelines for the
targeted consumer or user population. The composition can be a children's
food, liquid, semi-liquid or solid, especially for children between 0 and 7 years,
or between 0 and 3 years. The composition of the invention can also be
targeted to adolescent and adults suffering from particular physio-pathological
10 conditions, especially those in need and/or having compromised gastro-
intestinal systems and/or compromised immune / defense systems.

The nutritional composition may further comprise other optional
components that may modify the physical, chemical, aesthetic or processing
characteristics of the formulas or serve as pharmaceutical or additional
15 nutritional components when used in the targeted population. Many such
optional ingredients are known or other suitable for use in food and nutritional
products, including infant formulas, and may also be used in the nutritional
formulas of the present disclosure, provided that such optional materials are
compatible with the essential materials described herein, are safe for their
20 intended use, and do not otherwise unduly impair product performance.

Non-limiting examples of such optional ingredients include preservatives, anti-
oxidants, emulsifying agents, buffers, colorants, flavors, nucleotides, and
nucleosides, probiotics, additional prebiotics, and related derivatives,
thickening agents and stabilizers, and so forth.

25 The probiotic can be present in the composition in an amount equivalent
to between 10^3 and 10^{12} cfu/g of dry composition. Preferably the probiotic is
present in an amount equivalent to between 10^7 to 10^{12} cfu/ g of dry
composition. The bacteria may be used live, inactivated or dead or even be
present as fragments such as DNA or cell wall materials. In other words, the
30 quantity of bacteria which the formula contains is expressed in terms of the

equivalent colony forming units of bacteria irrespective of whether they are, all or partly, live, inactivated, dead or fragmented

The probiotic bacterial strain may be any lactic acid bacteria or Bifidobacteria with established probiotic characteristics. The probiotic of the invention may be any probiotic bacterium or probiotic microorganism ("probiotics"), especially of human origin, in particular probiotics that have been or can be originated from, found in, extracted or isolated from milk upon excretion, preferably in human breast milk. Suitable probiotic lactic acid bacteria include *Lactobacillus rhamnosus* ATCC 53103 obtainable inter alia from Valio Oy of Finland under the trademark LGG, *Lactobacillus rhamnosus* CGMCC 1.3724, *Lactobacillus reuteri* ATCC 55730 and *Lactobacillus reuteri* DSM 17938 obtainable from Biogaia, *Lactobacillus fermentum* VRI 003 and *Lactobacillus paracasei* CNCM 1-21 16, *Lactobacillus johnsonii* CNCM I-1225, *Lactobacillus helveticus* CNCM I-4095, *Bifidobacterium breve* CNCM I-3865, *Bifidobacterium longum* CNCM 1-2618

Suitable probiotic Bifidobacteria strains include *Bifidobacterium longum* ATCC BAA-999 sold by Morinaga Milk Industry Co. Ltd. of Japan under the trade mark BB536, the strain of *Bifidobacterium breve* sold by Danisco under the trade mark Bb-03, the strain of *Bifidobacterium breve* sold by Morinaga under the trade mark M-16V and the strain of *Bifidobacterium breve* sold by Institut Rosell (Lallemand) under the trade mark R0070. A particularly preferred Bifidobacterium strain is *Bifidobacterium lactis* CNCM I-3446 which may be obtained from the Christian Hansen Company of Denmark under the trade mark Bb12. A mixture of suitable probiotic lactic acid bacteria and Bifidobacteria may be used.

The nutritional composition of the present invention may be prepared as any product form suitable for use in humans, including liquid or powdered complete nutritionals, liquid or powdered supplements (such as a supplement that can be mixed with a drink), reconstitutable powders, ready-to-feed liquids,

bars, and dilutable liquid concentrates, which product forms are all well known in the nutritional formula arts.

The nutritional composition may have any caloric density suitable for the targeted or intended population, or provide such a density upon reconstitution of a powder embodiment or upon dilution of a liquid concentrate embodiment. Most common caloric densities for the infant formula embodiments of the present disclosure are generally at least about 660 kcal/liter, more typically from about 675-680 kcal/liter to about 820 kcal/liter, even more typically from about 675-680 kcal/liter to about 800-810 kcal/liter. Generally, the 720-810 kcal/liter formulas are more commonly used in pre-term or low birth weight infants, and the 675-680 to 700 kcal/liter formulas are more often used in term infants. Non-infant and adult nutritional formulas may have any caloric density suitable for the targeted or intended population.

The nutritional composition of the present disclosure may be packaged and sealed in single or multi-use containers, and then stored under ambient conditions for up to about 36 months or longer, more typically from about 12 to about 24 months. For multi-use containers, these packages can be opened and then covered for repeated use by the ultimate user, provided that the covered package is then stored under ambient conditions (e.g., avoid extreme temperatures) and the contents used within about one month or so.

A nutritional composition of the invention finds various uses in human nutrition and (preventive) therapy. In one embodiment, it is used for providing nutrition to an infant, preferably a preterm infant. In humans, preterm birth typically refers to the birth of a baby of less than 37 weeks gestational age. The cause for preterm birth is in many situations elusive and unknown; many factors appear to be associated with the development of preterm birth, making the reduction of preterm birth a challenging proposition. Preterm infants usually show physical signs of prematurity in reverse proportion to the gestational age. As a result, they are at risk for numerous medical problems affecting different organ systems. Respiratory problems are common,

specifically the respiratory distress syndrome (RDS or IRDS). Gastrointestinal and metabolic issues can arise from hypoglycemia, feeding difficulties, rickets of prematurity, hypocalcemia, inguinal hernia, and necrotizing enterocolitis (NEC). Typical infections in preterms include sepsis, pneumonia, and urinary tract infection.

The presence of anti-pathogenic α 1,2-fucosylated HMO in a composition of the invention makes it particularly effective for use in a method for the prophylaxis and/or treatment of symptoms which are connected with the association and/or adhesion of a pathogenic substance to the epithelia. The method may comprise administering to a subject in need thereof the composition in an amount of at least 100 mg/kg body weight per day, preferably at least 500 mg/kg body weight per day.

Also provided is the use of a synthetic infant formula comprising at least one HMO-protective compound selected from the group consisting of prebiotic oligosaccharides, maltodextrin and lactoferrin, as a dietary supplement for an infant receiving breast milk. For example, the synthetic formula is used in amount to make up between about 5 and 40% of the total diet of the infant based on the total volume of breast milk and infant formula. This approach is advantageously used for the nutrition of a preterm infant, e.g. in a hospital setting.

A further aspect related to the use of a synthetic infant formula comprising at least one compound selected from the group consisting of prebiotic oligosaccharides, maltodextrin and lactoferrin, to enhance a health-promoting effect of human milk oligosaccharide (HMO), preferably α 1,2-fucosylated HMO. As said, the protective effect of prebiotics, maltodextrin or lactoferrin prolongs the presence of HMOs in the gut, thereby promoting e.g. their beneficial effects. Beneficial or health-promoting effects include body weight gain (of special importance for preterm infants), increased resistance against pathogenic bacteria and stimulation or establishment of beneficial gut flora.

Typically, establishing beneficial gut flora comprises populating the gut with bifidobacteria or lactobacilli or both.

Enhanced growth of bifidobacteria and lactobacilli typically occurs at the expense of other groups of potentially harmful bacteria such as clostridia,

5 Enterobacteriaceae, and others. Therefore, a composition comprising of the invention can selectively feed the saccharolytic bacteria (bifidobacteria and lactobacilli), allowing them to dominate the gut and compete with potentially harmful bacteria by creating an acidic environment that is less favorable to pathogens. In one specific embodiment, an infant formula comprising GOS
10 (and/or FOS) and α 1,2-fucosylated HMO is used to enhance populating the gut with Bifidobacteria. For example, the synthetic infant formula comprises GOS and/or FOS, preferably in an amount of 0.4 to 0.8 g/dL. In another embodiment, an infant formula comprising maltodextrin, preferably in an amount of 0.4 to 0.8 g/dL, and α 1,2-fucosylated HMO is used to enhance
15 populating the gut with Lactobacilli.

The invention also provides the use of specific fucosylated HMOs, preferably LNT-based HMOs, more preferably LNDFH I and/or LNFP II, to promote body weight gain in an infant, preferably a preterm infant.

20

LEGENDS TO THE FIGURES

Figure 1. CE-LIF electropherograms of oligosaccharides from feces of preterm babies who got (A) >60% breast feeding and infant formula supplemented with
25 GOS (A6) or lactoferrin (A7, A10). (B) >60% breast feeding and maltodextrin-containing infant formula. (C/D) 100% breast feeding.

Figure 2. Relative quantification of LNT and fucosylated LNT-based HMOs (LNFP II; LNFP I[co-migrates with LNFP III]; LNDFH I) from feces of breast-
30 and mixed-fed preterm babies. Quantification is based on CE-LIF data.

Figure 3. Comparison of fecal microbiota analysed by I-Chip in the feces of (A) preterm babies who got 100% infant formula supplemented with GOS and preterm babies who got >60% breast feeding and infant formula supplemented with GOS. (B) preterm babies who got 100% infant formula supplemented with lactoferrin and preterm babies who got >60% breast feeding and infant formula supplemented with lactoferrin. (C) preterm babies who got 100% infant formula containing maltodextrin and preterm babies who got >60% breast feeding and infant formula containing maltodextrin.

Figure 4. Correlation between fecal excretion of LNFP II and body weight gain of preterm babies.

Figure 5. Correlation between fecal excretion of LNDFH I and body weight gain of preterm babies.

EXPERIMENTAL SECTION

The gastrointestinal fate of the complex HMOs remains vague. In the 1980s and early 1990s, several studies have been performed in order to investigate fecal oligosaccharides from breast-fed babies. Blood group A active oligosaccharides were found in the feces of a single blood group A breast-fed baby and a gastrointestinal metabolization of the feeding-related HMOs was supposed. On the other hand, no HMO metabolization products were found for a single blood group B breast-fed baby. In a third study, no indication for gastrointestinal HMO-metabolization was found as HMO-profiles similar to the respective breast milks were observed. In other studies HMOs and blood group characteristic oligosaccharides were detected in urine of breast-fed babies and lactating women, pointing out their gastrointestinal absorbance and importance on a systemic level. Clearly, further research is needed in order to understand the gastrointestinal fate of complex HMOs and their possible conjugation with blood group antigenic structures. The application of novel analytical methods, which provide low detection limits and require limited preparative sample work in combination with a short analysis time,

may open new possibilities in this context. It may help to establish an advanced scientific underpinning of the feeding-guidelines set up for neonates. Capillary electrophoresis with laser induced fluorescence detection (CE-LIF), combined with mass spectrometry, has been shown to be a suitable tool for the
 5 analysis of HMOs in breast milk and baby feces.

Materials and Methods

Set-up study and sample collection

Twenty-seven fecal samples of approximately two months old preterm infants
 10 (born after 27-35 weeks of gestation and a birth weight between 770 g and 2285 g) were selected from a clinical study on the investigation of the effect of the supplementation of preterm infant formula with prebiotic GOS or lactoferrin on the performance of preterm babies. The study was performed at the level III neonatal intensive care unit of the Isala clinics, Zwolle, The
 15 Netherlands. Enteral feeding of the babies was started after birth as soon as possible and full enteral feeding was established 5-19 days after birth. Fecal samples were taken 6 weeks after full enteral feeding was established and samples were frozen at -20°C until analysis.

The babies received either exclusively expressed human milk from their
 20 own mother (n = 5) or preterm infant formula (n = 6) [supplemented with 0.4 g/dL GOS (Frisolac prematuur, FrieslandCampina DOMO, Zwolle, The Netherlands), or without GOS (Frisolac prematuur, GOS replaced by maltodextrin (0.4 g/dL), or supplemented with 0.1 g/dL lactoferrin]. Sixteen babies got mixed feeding (breast milk and formula). Breast milk meals
 25 contributed to > 80%, > 60% or < 55% of the total number of feedings, which the babies received during the study period (Table 2). The babies were randomly assigned to one of the three formula groups. The study was performed double-blind and was approved by the medical ethical review board of the hospital. Written informed consent was obtained from all the parents.
 30 Except for baby A2, the blood group of the babies was known. Amongst the twenty-seven babies studied were two twin pairs and one triplet pair, but they were not further specified in this study.

5 **Table 2: Overview baby codes**

100% HMO	A1, A4, A9, A11, A13
Mixed formula GOS+HMO(>60%)	A6
Mixed formula lactoferrin+HMO(>60%)	A7, A10
Mixed formula maltodextrin+HMO(>60%)	A3, A5, A8, A12
100% formula GOS	B1, B2
100% formula lactoferrin	B4
100% formula maltodextrin (control)	B3, B9, B11

Analysis of fecal oligosaccharide profiles (CE-LIF/CE-LIF-MS)

- 10 For CE-LIF analysis, the carbohydrates present in the fecal extracts were derivatized with the fluorescent 9-aminopyrene-1,4,6-trisulfonate (APTS) overnight at room temperature as reported elsewhere.^{6,7} One nanomole xylose was added as internal standard and mobility marker. CE-LIF was performed on a ProteomeLab PA 800 characterization system (Beckman Coulter, Fullerton, CA), equipped with a laser induced fluorescence detector (LIF) (excitation: 488 nm, emission: 520 nm) (Beckman Coulter) and a polyvinyl alcohol-coated capillary (50 μ m x 50.2 cm (Beckman Coulter), detector after 40 cm), kept at 25 °C. Samples were loaded hydrodynamically (4 s at 0.5 psi, representing approx. 14 nL sample solution) on the capillary. Separation was performed in the reversed polarity mode (30 kV, 20 min) in a 25 mM acetate buffer containing 0.4% polyethylene oxide provided in the ProteomeLab Carbohydrate Labeling and Analysis Kit (Beckman Coulter). Due to the low pK_a of the sialic acid residues (pK_a 2.6) and in order to provide the same

buffer-pH as for CE-MS analysis, the separation buffer was adjusted to pH 2.4 by adding 1.2% (v/v) formic acid.⁸ Peaks were integrated manually using Chromeleon software 6.8 (Dionex, Sunnyvale, CA).

5 CE-LIF-ESI-MSⁿ experiments were performed on a P/ACETM System MDQ (Beckman Coulter) according to Albrecht et al.⁸ For the fluorescent detection (excitation: 488 nm, emission: 520 nm), a Picometrics ZetaLIF discovery system was used (Picometrics, Toulouse, France). Separation in 0.3% (v/v) formic acid (pH 2.4) was performed on a fused silica capillary (50 μ m x 85 cm
10 (Beckman Coulter), capillary window fitted with an ellipsoid for LIF detection after 60 cm) in reversed polarity mode (20 kV, 15 °C, 40 min). Samples were injected at 10 psi for 2 s. LIF signals were sent to Beckmann 32Karat software via a SSXL4002 converter (Agilent Technologies, Santa Clara, CA). The ESI-MS (LTQ ion trap, Thermo Fisher Scientific Inc., Waltham, MA) was operated
15 in the negative mode using a spray voltage of 1.9 kV and an MS-capillary temperature of 190 °C. The end of the CE capillary was installed in front of the ESI source by leading it through a T-part designed in our laboratory⁷ and provided the coaxial addition of a sheath liquid (50/50 isopropanol/water) at 2 μ L/min. Mass spectra were acquired from m/z 300 to 2000. MSⁿ was performed
20 in the data dependent mode using a window of 1 m/z and collision energy of 35%. For increasing the S/N ratio, ions of m/z 311, 314 and 329 were excluded from detection in MSⁿ experiments (mass exclusion list). MSⁿ data were interpreted using Xcalibur software 2.0.7 (Thermo).

25 Analysis of fecal microbiota (I-chip):

The composition of the microbiota was analyzed using the Intestinal-Chip (I-chip) as described before (Maathuis et al. (10) and Rose et al.(2010)) for which probe sequences of intestinal micro-organisms were selected via a literature survey. The I-Chip contains roughly 350 probes, some for phylogenetic groups
30 (e.g. Actinomycetes), some for group-level detection (e.g. all Bifidobacterium

species) and some for detection of individual species (e.g. *Bifidobacterium longum*). Some species and groups were covered by more than one probe and the hybridization to these multiple probes correlated very well. Taxonomic selection was confirmed and expanded based on massive parallel sequencing of 16S rDNA. For each species represented on the microarray a unique short oligonucleotide sequence from within the 16S rDNA was selected. Some species are represented by multiple (unique) sequences. Criteria for sequence selection, apart from being unique, included length and melting temperature. Short oligonucleotide sequences (approx. 20 nt) were used for which a one nucleotide mismatch already resulted in an absence (or very strong decrease) of signal after hybridization. For I-Chip analysis, DNA was isolated from luminal samples at the start and at the end of the long-term TIM-2 experiments. In short, DNA was isolated from 200 mg of faecal material using a commercial DNA isolation kit (Agowa(r), Germany) following the manufacturer's instructions. This method is described in detail elsewhere (10; Rose et al., 2010). Subsequently the DNA was labeled and hybridized to arrays. After washing, the arrays were scanned and analyzed. Imagen 5.6 software (BioDiscovery, Marina del Rey, CA) was used for data analysis. Signals were quantified by calculating the mean of all pixel values of each spot and calculating the local background around each spot. For each spot a signal to background ratio was calculated. For further analyses spots which had a minimal number of observations more than two times above its local background were selected.

25 Abbreviations HMOs

BGA: blood group A; DF-L: di-fucosyllactose; DF-LNH: difucosyl-lacto-N-hexaose; DS-LNT: disialyl-lacto-N-tetraose; FL: fucosyllactose; F-LNH: fucosyl-lacto-N-hexaose; GOS: galactooligosaccharides; HMO: human milk oligosaccharides; Lf: lactoferrin; LNFP: lacto-N-fucopentaose (*I, II, III, Y: differen^t isomers*); LNDFH: lacto-N-difucosa-hexaose; LNT: lacto-N-tetraose;

SL: sialyllactose; S-LNT: sialyl-lacto-N-tetraose (*different isomers present*); TF-LNH: trifucosyl-lacto-N-hexaose.

5 Results

EXAMPLE 1: Prebiotics delay the gastrointestinal metabolization of HMOs

10 Figures 1A and 1B show the profiles of feces of seven mixed-fed babies (2 months old). For five babies (=71%) it was observed that the HMO-profile is still present. For two babies (= 29%) both receiving control-formula: A12: besides SL, no HMOs present; A3: exceptional HMO-profile present (because of the abundant presence of LNT).

15

As a comparison, feces of five 100% HMO-fed babies (2 months old) were analyzed. For three babies, an advanced gastrointestinal metabolization was observed. See Figure 1C. Baby A1: only acidic HMOs are present; Baby A11: only blood-group A characteristic oligosaccharides (m/z 377 and m/z 498) are present; Baby A13: no HMOs present. Figure 1D shows that for the other two
20 100% HMO-fed babies, the HMO-profiles are still present.

These data show that the admixture of prebiotics (lactoferrin, GOS or maltodextrin) has a protective effect on the metabolism of complex HMOs.

25

EXAMPLE 2: Presence of fucosylated, lacto-N-biose containing HMOs in different sample groups

The simplest HMO-structure which contains lacto-N-biose is LNT. Lacto-N-biose is considered as bifidogenic component of breast milk¹. Additionally, fucosylated (especially α 1,2-fucosylated) HMOs are recognized for their health-beneficial effects via pathogen-inhibition:

- 5 - receptor-analogs for campylobacter (can cause diarrhea)²
- receptor-analogs for caliciviruses (=norovirus; diarrhea)³
- breast-fed babies who were infected with stable toxin from *E.coli* showed less diarrheal symptoms⁴
- Fucosylated oligosaccharides of human milk protect suckling mice from heat-stabile enterotoxin of *Escherichia coli* ⁵

Thus, delayed metabolism and therefore the prolonged presence of fucosylated LNT-based HMOs in the gastrointestinal lumen is of special interest.

Unexpectedly, fucosylated HMOs were found to be present especially in the feces of mixed-fed babies. See Figure 2, showing the relative abundance of LNT and fucosylated LNT-based HMOs (LNFP II; LNFP I[co-migrates with LNFP III]; LNDFH I) in baby feces of different feeding groups. Panels A and B (LNT); panels C and D (LNFP II); panels E and F (LNFP I and III); panels G and H (LNDFH I).

EXAMPLE 3: Effect of mixed feeding on fecal microbiota

A comparison was made between the fecal microbiota in babies receiving 100% prebiotic (GOS, lactoferrin or maltodextrin) feeding versus babies receiving a mixture of prebiotic and >60% HMO. Figure 3 summarizes the results obtained. Panel A: prebiotic is GOS. Panel B: prebiotic is lactoferrin. Panel C: prebiotic is maltodextrin.

From Figure 3A it can be concluded that a mixed feeding which contains GOS has a beneficial effect for the gastrointestinal growth of Bifidobacteria species.

An increased presence of all Bifidobacteria-subgroups was found compared to the babies who got exclusively formula containing GOS. Mixed feeding also increased the presence of the groups Eubacterium and Arthrobacter. To a small extent, higher values of Butyrivibrio and Campylobacter were found for the mixed-fed group as compared to the prebiotic only group.

Figure 3B shows that a mixed feeding which contains lactoferrin yields a lower presence of Bifidobacteria than babies who got exclusively lactoferrin-supplemented formula. To a lower extent, the mixed-fed group showed a decreased presence of Streptococci, Clostridium, Lactobacillus, Arthrobacter, Streptococcus

Figure 3C shows that mixed feeding with a control infant formula comprising maltodextrin has a marked stimulatory effect on the presence of Lactobacilli. Babies who got exclusively control formula showed a lower presence of these lactobacilli-groups in their feces. To a lower extent, the mixed-fed group showed an increase for *Bacteroides prevotella* group and *Butyrivibrio* group.

EXAMPLE 4: Effect of HMOs on body weight gain

The correlation between delayed metabolism of complex 1,2-fucosylated HMOs on body weight gain in preterm infants was investigated. From Figure 4 it can be concluded that there is a positive association between fecal LNFP II and body weight of a preterm child after 6 weeks treatment when the child is 8 weeks old. LNFPII excretion is increased in the mix-fed group.

Figure 5 shows that there is also a positive correlation between body weight gain and the fecal concentration of LNDFH I. This could be explained by an increased excretion of Campylobacter and Ruminococcus in the feces.

EXAMPLE 5: Infant formulas

Tables 3 and 4 show the composition of exemplary nutritional formulas according to the invention, e.g. infant formulas for the age group between 0-6 months, for supporting the delayed metabolization of HMO.

Table 3: Composition of the formulas (per 100 ml)

	Formula A: di- fucosyllactose + GOS	Formula B: di- fucosyllactose + GOS/FOS	Formula C: di- fucosyllactose + lactoferrin
Energy, kcal	67	67	67
Protein (g)	1.4	1.4	1.4
Carbohydrates (g)	7.6	7.6	7.9
Fat (g)	3.5	3.5	3.5
Difucosyllactose (g)	0.1	0.1	0.1
Vivinal GOS (g)	0.4	0.36	
FOS (g)		0.04	
Lactoferrin (g)			0.1

Vitamins and minerals according to legislation.

Table 4: Composition of the formulas (per 100 ml)

	Formula D: lacto-N- fucopentaose I + GOS	Formula E: lacto-N- fucopentaose I + GOS + lactoferrin
Energy, kcal	67	67
Protein (g)	1.4	1.4
Carbohydrates (g)	7.6	7.6
Fat (g)	3.5	3.5
lacto-N- fucopentaose I (g)	0.05	0.05
Vivinal GOS (g)	0.6	
Lactoferrin (g)		0.6 0.1

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Aangepaste Conclusies

- 5 1. Toepassing van een synthetische kindervoeding omvattende ten minste één verbinding gekozen uit de groep bestaande uit prebiotische oligosacchariden, maltodextrine en lactoferrine, om het gezondheidsbevorderende effect van een humane melk oligosaccharide (HMO) te versterken, waarbij genoemde HMO
- 10 een α 1,2-gefucosyleerde HMO omvattende ten minste vier monosaccharide eenheden is.
2. Toepassing volgens conclusie 1, waarin het genoemde gezondheidsbevorderende effect gekozen is uit een toename in lichaamsgewicht, verhoogde resistentie tegen pathogene
- 15 bacteriën en stimulering van nuttige darmflora, bij voorkeur stimulering van bifidobacteriën en/of lactobacilli.
3. Toepassing volgens een der voorgaande conclusies, waarin genoemde kindervoeding galacto-oligosacchariden (GOS) of fructose-oligosacchariden (FOS) bevat, of een combinatie van
- 20 FOS en GOS, bij voorkeur in een hoeveelheid van 0.4 tot en met 0.8 g/dL.
4. Toepassing volgens conclusie 3, waarin genoemde GOS trans-galacto-oligosacchariden (TOS) zijn.
5. Toepassing volgens conclusie 3 of 4, waarin genoemde
- 25 kindervoeding GOS en FOS in een 9:1 gewichtsverhouding bevat.
6. Toepassing volgens een der conclusies 1-5, waarin de synthetische kindervoeding maltodextrine omvat, bij voorkeur in een hoeveelheid van 0.4 tot en met 0.8 g/dL.
- 30 7. Toepassing volgens een der conclusies 1-6, waarin de synthetische kindervoeding lactoferrine omvat, bij voorkeur in een hoeveelheid van 0.05 tot en met 0.2 g/dL.

8. Toepassing volgens een der voorgaande conclusies, voor het versterken van het gezondheidsbevorderende effect van een α 1,2-gefucosyleerde HMO gekozen uit de groep bestaande uit difucosyllactose (DF-L), lacto-N-fucopentaose I (LNFP I), lacto-N-difucohexaose I (LNDFH I), fucosyllacto-N-hexaose I (F-LNH I), difucosyllacto-N-hexaose I (DF-LNH I), trifucosyllacto-N-hexaose I (TF-LNH I), fucosyllacto-N-neohexaose I of II (F-LNnH I / II) en trifucosyl-para-lacto-N-hexaose I (TF-*para*-LNH I).
9. Toepassing volgens conclusie 8, waarin genoemde α 1,2-gefucosyleerde HMO ten minste één lacto-N-biose eenheid bevat, bij voorkeur LNFP I en/of LNDFH I.
10. Toepassing volgens een der voorgaande conclusies, waarin genoemde α 1,2-gefucosyleerde HMO LNDFH I, LNFP I of F-LNH I is.
11. Toepassing volgens een der voorgaande conclusies, waarin het genoemde ten minste één α 1,2-gefucosyleerde HMO omvattende ten minste vier monosaccharide-eenheden op (semi)-synthetische wijze is bereid.

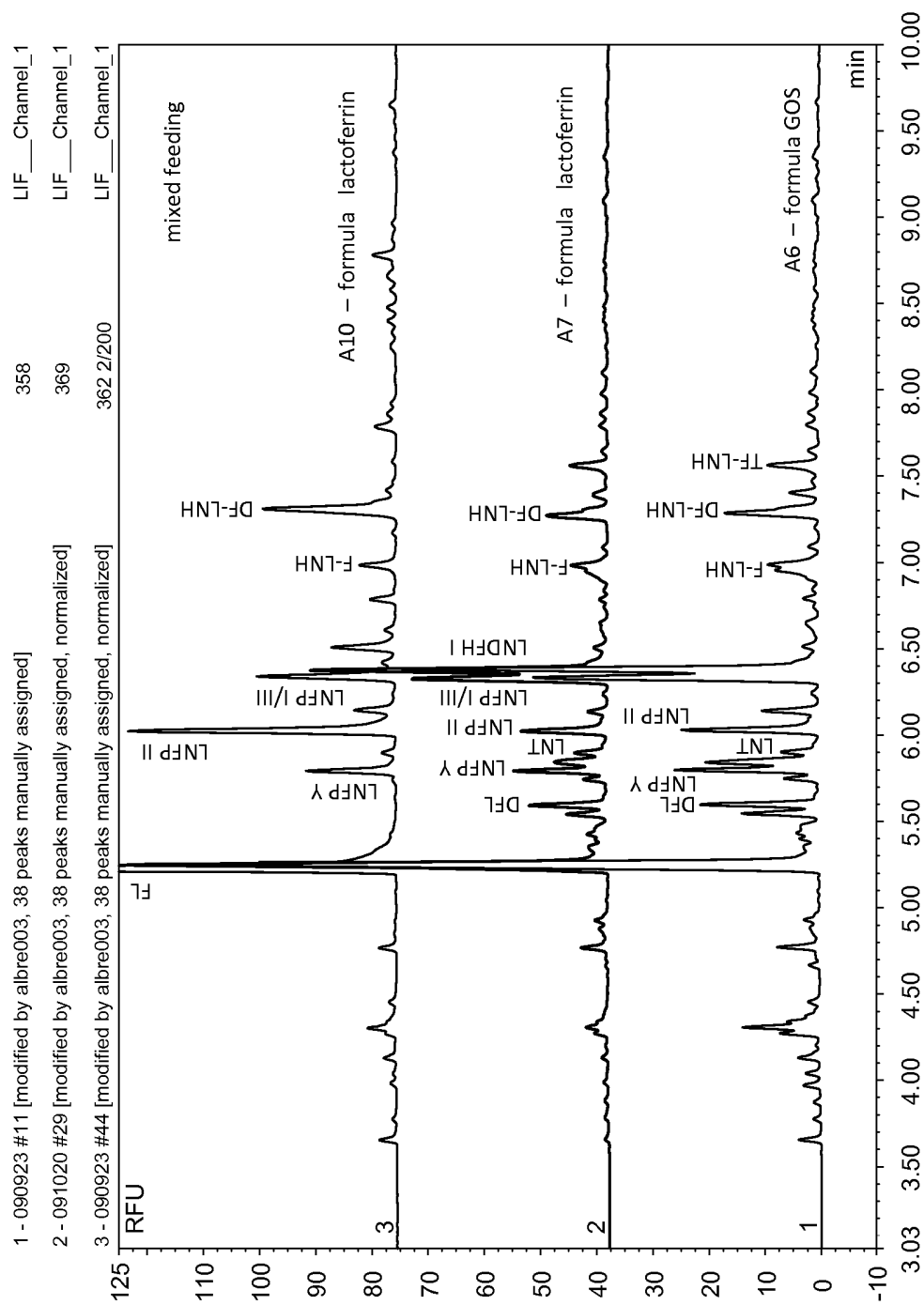


Fig. 1A

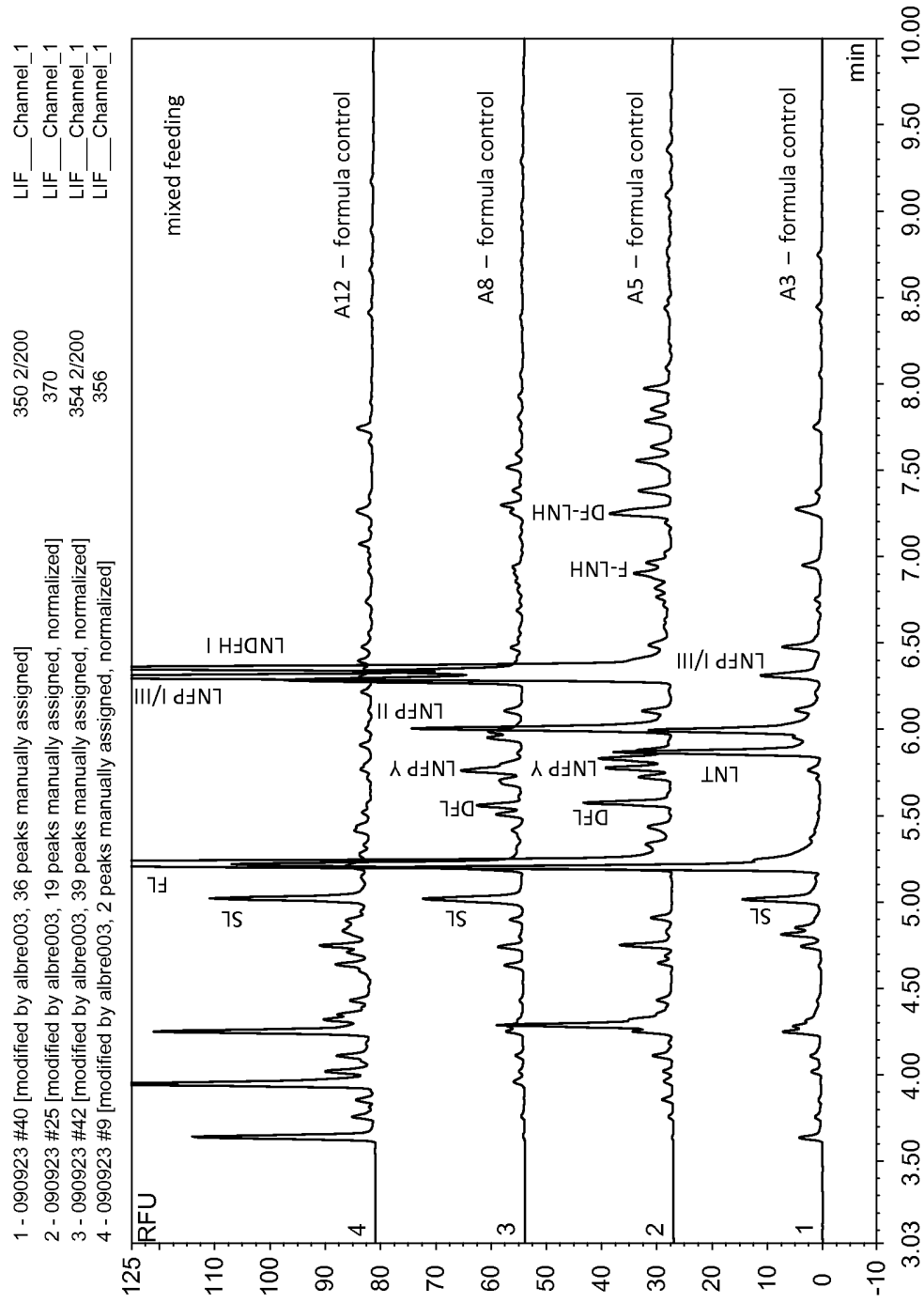


Fig. 1B

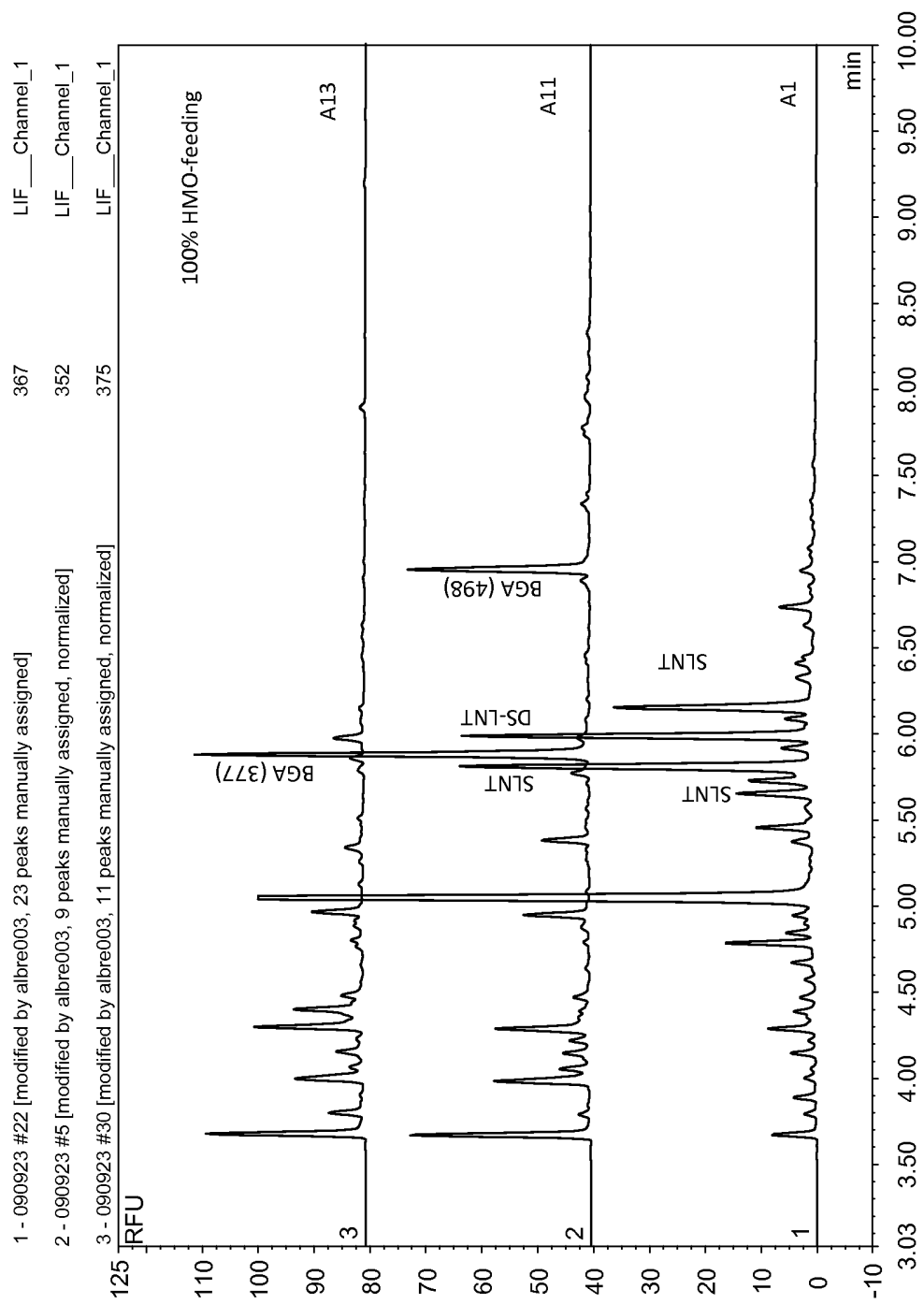


Fig. 1C

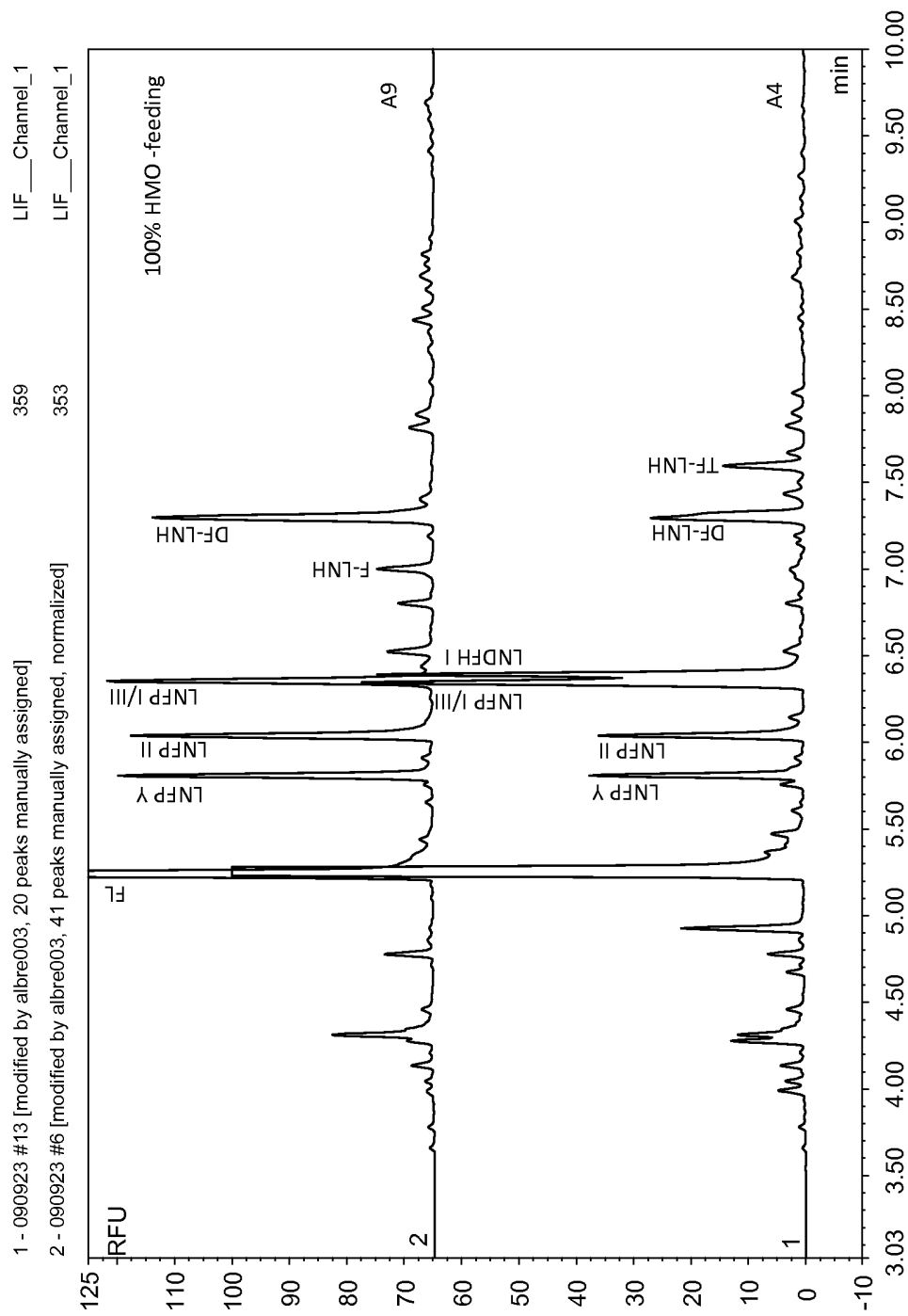


Fig. 1D

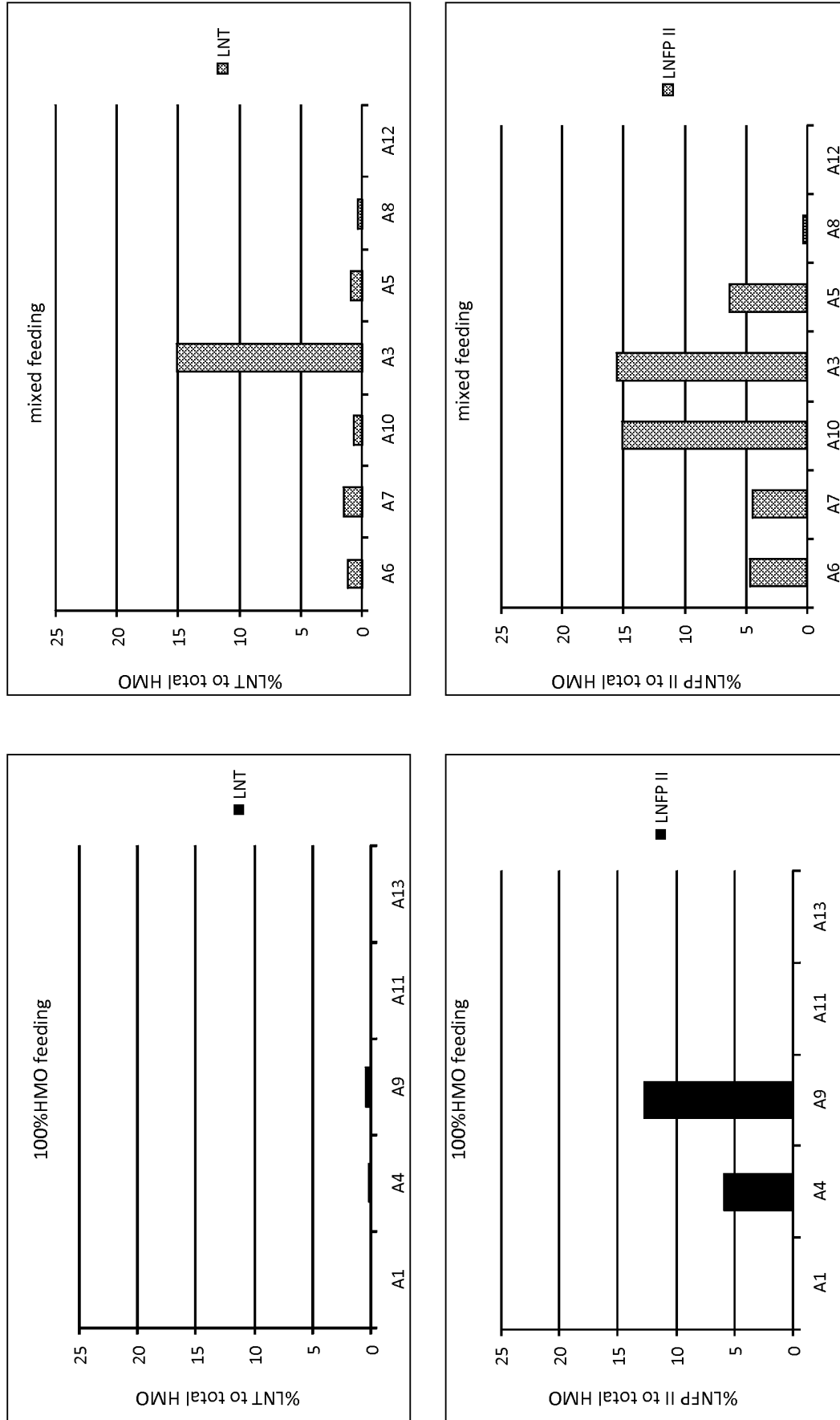


Fig. 2

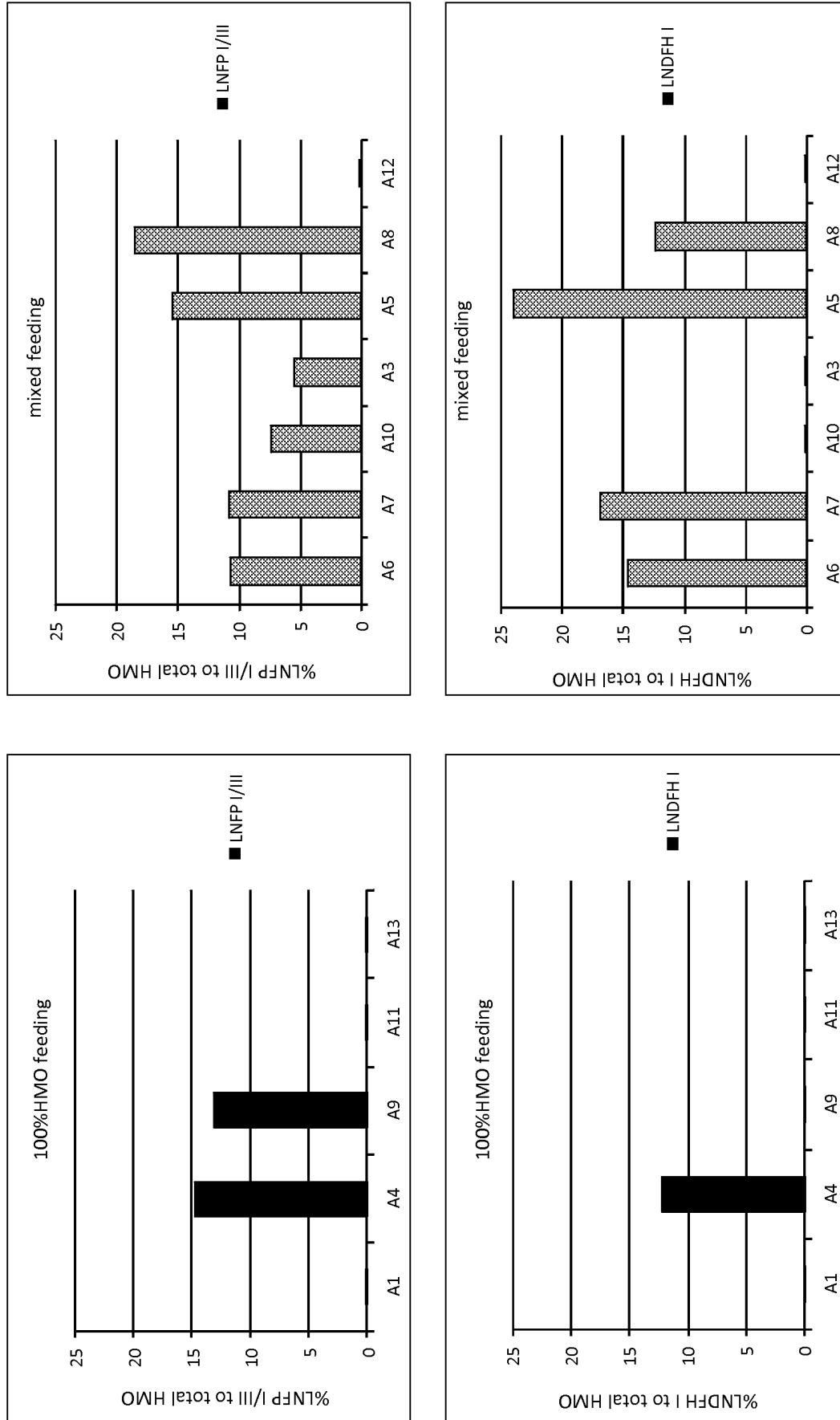


Fig. 2, cont'd

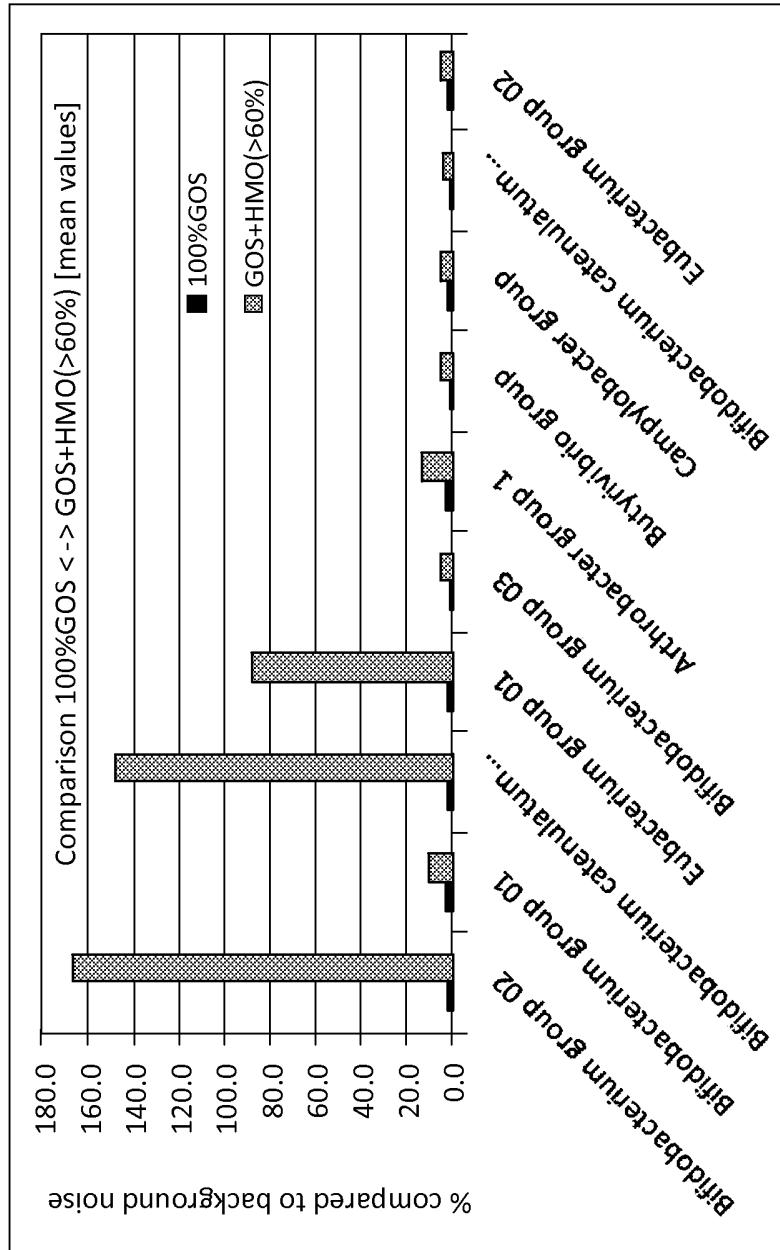


Fig. 3A

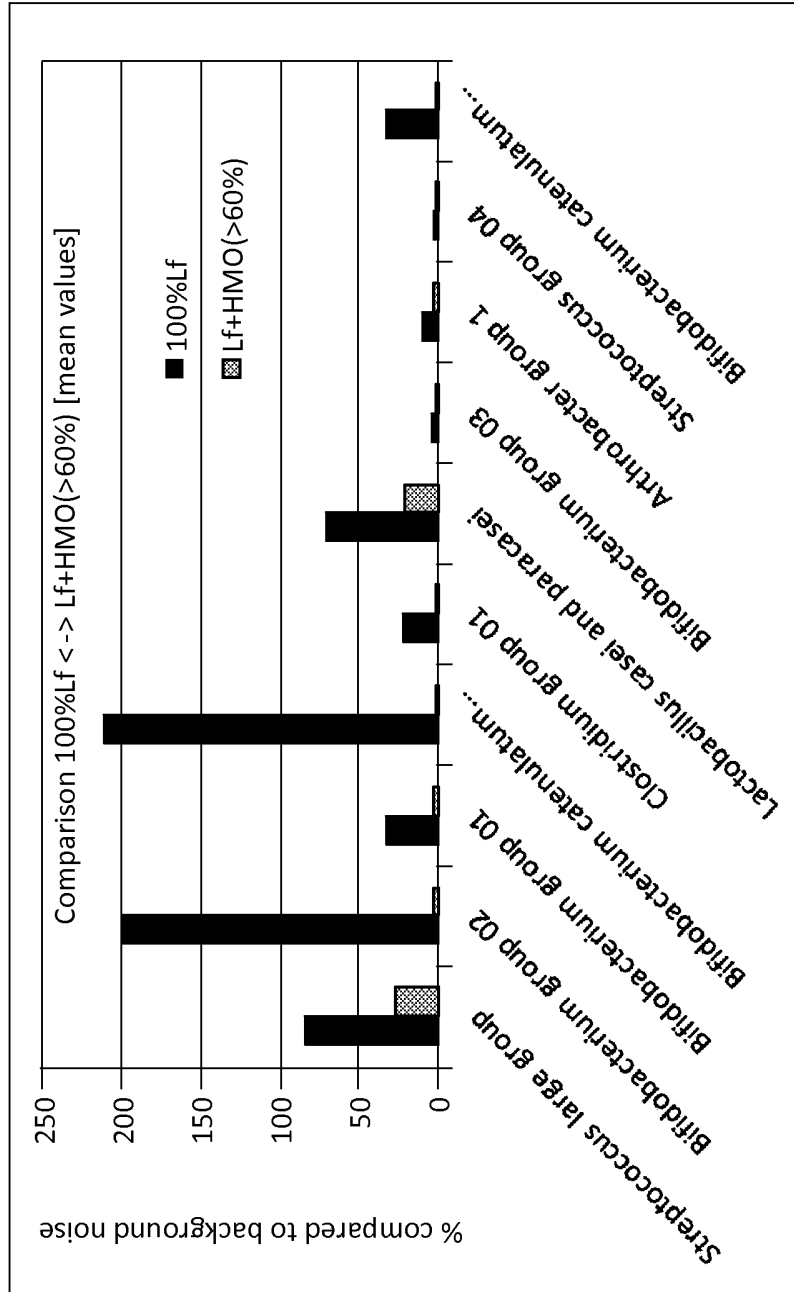


Fig. 3B

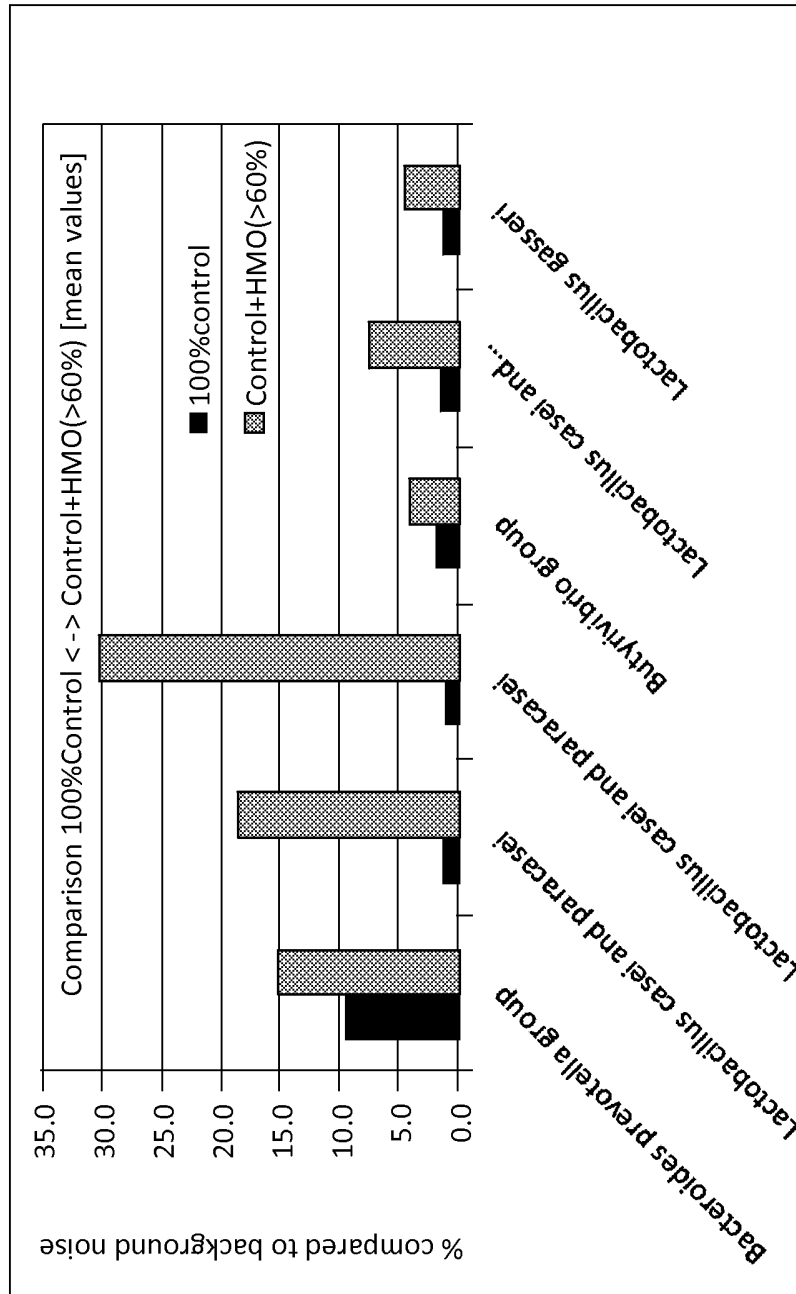


Fig. 3C

10/11

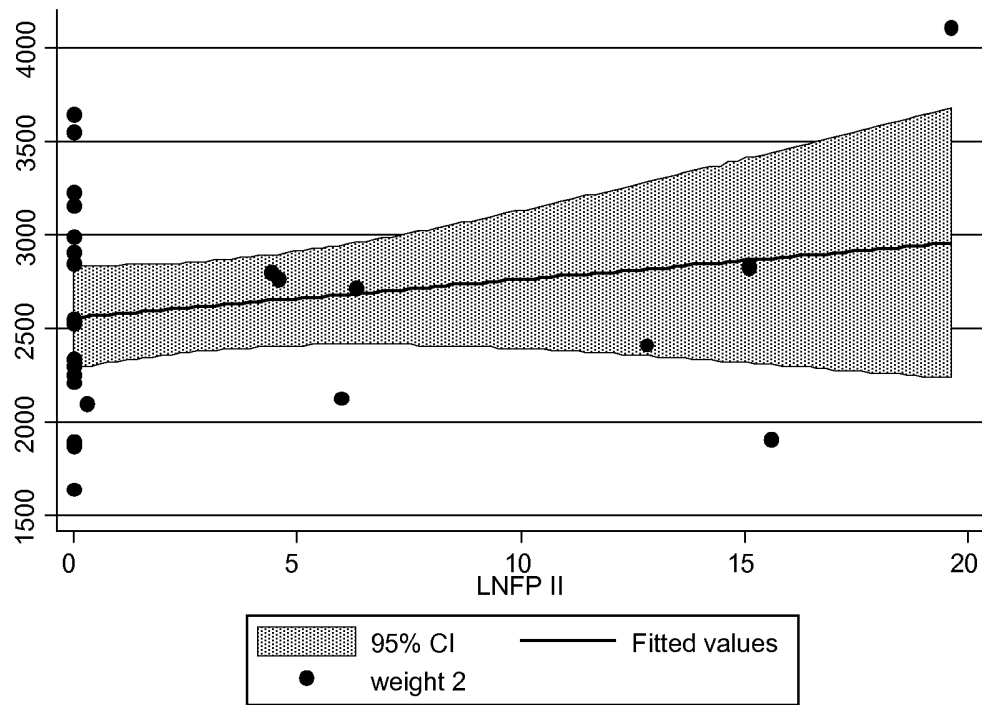


Fig. 4

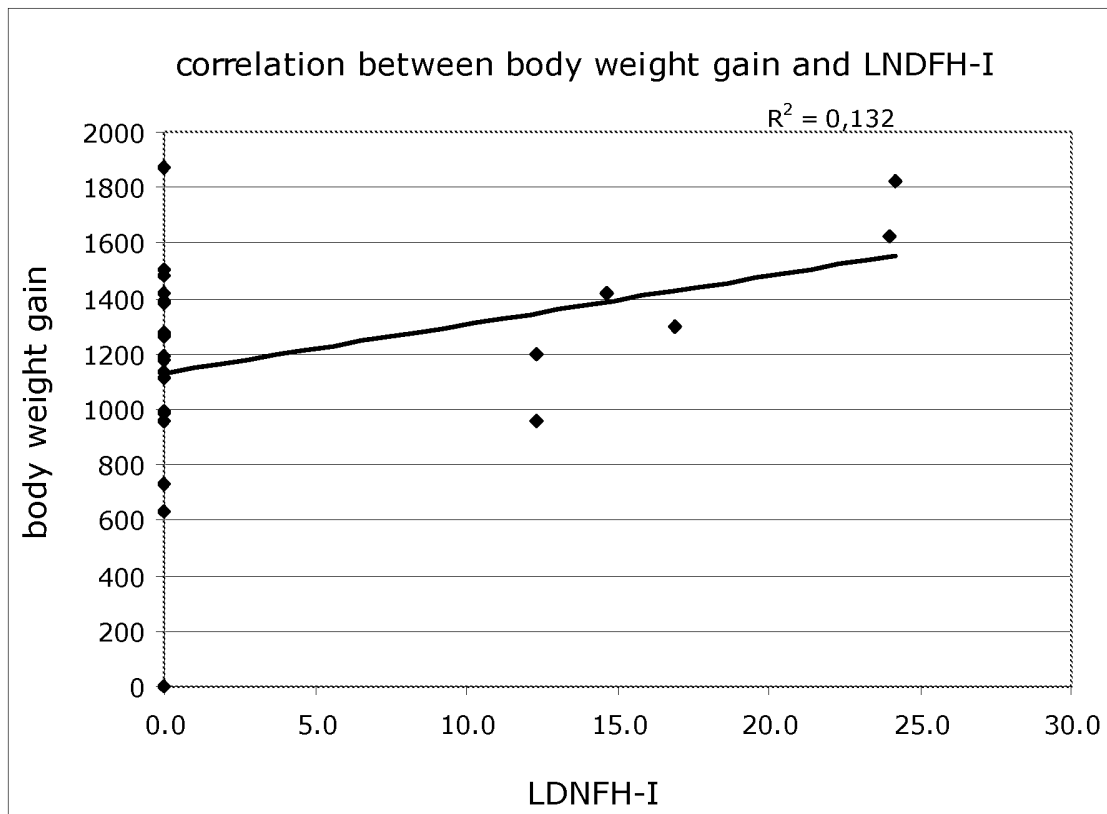


Fig. 5

SAMENWERKINGSVERDRAG (PCT)

RAPPORT BETREFFENDE NIEUWHEIDSONDERZOEK VAN INTERNATIONAAL TYPE

IDENTIFICATIE VAN DE NATIONALE AANVRAGE		KENMERK VAN DE AANVRAGER OF VAN DE GEMACHTIGDE	
		P95519NL00	
Nederlands aanvraag nr.		Indieningsdatum	
2007268		16-08-2011	
		Ingeroepen voorrangsdatum	
Aanvrager (Naam)			
Friesland Brands B.V., et al			
Datum van het verzoek voor een onderzoek van internationaal type		Door de Instantie voor Internationaal Onderzoek aan het verzoek voor een onderzoek van internationaal type toegekend nr.	
19-11-2011		SN57205	
I. CLASSIFICATIE VAN HET ONDERWERP (bij toepassing van verschillende classificaties, alle classificatiesymbolen opgeven)			
Volgens de internationale classificatie (IPC)			
A23L1/29			
II. ONDERZOCHE GEBIEDEN VAN DE TECHNIEK			
Onderzochte minimumdocumentatie			
Classificatiesysteem		Classificatiesymbolen	
IPC		A23L	
Onderzochte andere documentatie dan de minimum documentatie, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen			
III.	☐	GEEN ONDERZOEK MOGELIJK VOOR BEPAALDE CONCLUSIES (opmerkingen op aanvullingsblad)	
IV.	☒	GEBREK AAN EENHEID VAN UITVINDING (opmerkingen op aanvullingsblad)	

**ONDERZOEKSRAPPORT BETREFFENDE HET
RESULTAAT VAN HET ONDERZOEK NAAR DE STAND
VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE**

Nummer van het verzoek om een onderzoek naar
de stand van de techniek
NL 2007268

A. CLASSIFICATIE VAN HET ONDERWERP

INV. A23L1/29
ADD.

Volgens de Internationale Classificatie van octrooien (IPC) of zowel volgens de nationale classificatie als volgens de IPC.

B. ONDERZOCHE GEBIEDEN VAN DE TECHNIEK

Onderzochte minimum documentatie (classificatie gevolgd door classificatiesymbolen)
A23L

Onderzochte andere documentatie dan de minimum documentatie, voor dergelijke documenten, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen

Tijdens het onderzoek geraadpleegde elektronische gegevensbestanden (naam van de gegevensbestanden en, waar uitvoerbaar, gebruikte trefwoorden)

EP0-Internal, WPI Data, BIOSIS, FSTA, EMBASE

C. VAN BELANG GEACHTE DOCUMENTEN

Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
X	EENHEID VAN UITVINDING ONTBREEKT zie aanvullingsblad B ----- WO 2010/134800 A1 (NUTRICIA NV [NL]; VAN DER BEEK ELINE MARLEEN [NL]; BOEHM GUENTHER [DE]) 25 november 2010 (2010-11-25) * bladzijde 8, regel 11 - bladzijde 9, regel 13 *	1-11, 13-21, 24-26
X	US 2008/274230 A1 (JOHNS PAUL W [US] ET AL) 6 november 2008 (2008-11-06) * alinea [0049] - alinea [0052] * * alinea [0071] * * alinea [0120] - alinea [0121] * ----- -/-	1-11, 13-21, 24-26

☒ Verdere documenten worden vermeld in het vervolg van vak C.

☒ Leden van dezelfde octrooifamilie zijn vermeld in een bijlage

° Speciale categorieën van aangehaalde documenten

"A" niet tot de categorie X of Y behorende literatuur die de stand van de techniek beschrijft

"D" in de octrooiaanvraag vermeld

"E" eerdere octrooi(aanvraag), gepubliceerd op of na de indieningsdatum, waarin dezelfde uitvinding wordt beschreven

"L" om andere redenen vermelde literatuur

"O" niet-schriftelijke stand van de techniek

"P" tussen de voorrangsdatum en de indieningsdatum gepubliceerde literatuur

"T" na de indieningsdatum of de voorrangsdatum gepubliceerde literatuur die niet bezwarend is voor de octrooiaanvraag, maar wordt vermeld ter verheldering van de theorie of het principe dat ten grondslag ligt aan de uitvinding

"X" de conclusie wordt als niet nieuw of niet inventief beschouwd ten opzichte van deze literatuur

"Y" de conclusie wordt als niet inventief beschouwd ten opzichte van de combinatie van deze literatuur met andere geciteerde literatuur van dezelfde categorie, waarbij de combinatie voor de vakman voor de hand liggend wordt geacht

"Z" lid van dezelfde octrooifamilie of overeenkomstige octrooipublicatie

Datum waarop het onderzoek naar de stand van de techniek van internationaal type werd voltooid

15 mei 2012

Verzenddatum van het rapport van het onderzoek naar de stand van de techniek van internationaal type

Naam en adres van de instantie

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

De bevoegde ambtenaar

Vermeulen, Stéphane

**ONDERZOEKSRAPPORT BETREFFENDE HET
RESULTAAT VAN HET ONDERZOEK NAAR DE STAND
VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE**

Nummer van het verzoek om een onderzoek naar
de stand van de techniek
NL 2007268

C.(Vervolg). VAN BELANG GEACHTE DOCUMENTEN		
Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
X	US 6 294 206 B1 (BARRETT-REIS BRIDGET [US] ET AL) 25 september 2001 (2001-09-25) * kolom 5, regel 42 - regel 50 * * kolom 9, regel 12 - regel 20 * -----	1-11, 13-21, 24-26
X	US 2006/233915 A1 (PUSKI GABOR [US] ET AL) 19 oktober 2006 (2006-10-19) * alinea [0058] - alinea [0060] * -----	1-11, 13-21, 24-26
X	WESTERBEEK ELISABETH A M ET AL: "The effect of enteral supplementation of a prebiotic mixture of non-human milk galacto-, fructo- and acidic oligosaccharides on intestinal permeability in preterm infants", BRITISH JOURNAL OF NUTRITION, deel 105, nr. 2, januari 2011 (2011-01), bladzijden 268-274, XP002675984, * Experimental methods; bladzijde 269; tabel 1 * -----	1-11, 13-21, 24-26
X	ROSEGGER H: "[Maltodextrin in a 13% solution as a supplement in the first 4 days of life in breast-fed mature newborn infants. Effect on drinking behavior, weight curve, blood picture, blood glucose and bilirubin].", WIENER KLINISCHE WOCHENSCHRIFT 16 MAY 1986 LNKD- PUBMED:3727591, deel 98, nr. 10, 16 mei 1986 (1986-05-16), bladzijden 310-315, XP009159222, ISSN: 0043-5325 * bladzijde 311 * -----	1-11, 13-21, 24-26
X	US 6 576 251 B1 (STAHL BERND [DE] ET AL) 10 juni 2003 (2003-06-10) * kolom 1, regel 44 - kolom 2, regel 19 * * kolom 2, regel 45 - regel 50 * * voorbeelden 1-6 * -----	1-19
X	US 2005/004070 A1 (STAHL BERND [DE] ET AL) 6 januari 2005 (2005-01-06) * alinea [0015] * * alinea [0031] - alinea [0032] * * voorbeelden 1,2,6 * -----	1-19
X	US 2011/177044 A1 (THOMAS DEBRA L [US] ET AL) 21 juli 2011 (2011-07-21) * alinea [0039] - alinea [0043]; voorbeelden 11,12 * -----	1-19
	-/--	

**ONDERZOEKSRAPPORT BETREFFENDE HET
RESULTAAT VAN HET ONDERZOEK NAAR DE STAND
VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE**

Nummer van het verzoek om een onderzoek naar
de stand van de techniek
NL 2007268

C.(Vervolg). VAN BELANG GEACHTE DOCUMENTEN		
Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
X	US 2009/098240 A1 (MILLS DAVID A [US] ET AL) 16 april 2009 (2009-04-16) * alinea [0008] - alinea [0011] * * alinea [0013] - alinea [0016] * -----	1,9-12, 14-19
X	WO 2011/008086 A1 (NUTRICIA NV [NL]; STAHL BERND [DE]; NAUTA ALMA JILDOU [NL]; GARSSSEN JO) 20 januari 2011 (2011-01-20) * bladzijde 3, regel 14 - regel 26 * * bladzijde 4, regel 20 - regel 27 * * bladzijde 9, regel 21 - regel 25 * -----	22-24
E	WO 2011/136648 A1 (NUTRICIA NV [NL]; STAHL BERND [DE]) 3 november 2011 (2011-11-03) * bladzijde 6, regel 4 - bladzijde 7, regel 19 * * bladzijde 9, regel 15 - regel 22 * -----	1-26

GEBREK AAN EENHEID VAN UITVINDING

Octrooiaanvraag Nr.:

SN 57205
NL 2007268

AANVULLINGSBLAD B

De Instantie belast met het uitvoeren van het onderzoek naar de stand van de techniek heeft vastgesteld dat deze aanvraag meerdere uitvindingen bevat, te weten:

1. conclusies: 1-26

Infant nutritional compositions and uses thereof comprising a combination of (i) a 1,2-fucosylated HMO and (ii) a HMO-protective compound.

2. conclusie: 27

Use of LNDFH I/II for promoting body weight gain in an infant

Het vooronderzoek werd tot het eerste onderwerp beperkt.

The present set of claims lacks unity as it comprises two inventions which are not so linked as to form a single general inventive concept. The above mentioned groups of claims do not have common or corresponding special technical features and they deal with two different technical problems, not being linked to form a single general inventive concept (Claims 1-26: protection of 1,2-fucosylated HMO by combination with a HMO protective compound. Claim 27: Promotion of body weight gain in infants by use of LNDFH I/II).

**ONDERZOEKSRAPPORT BETREFFENDE HET
RESULTAAT VAN HET ONDERZOEK NAAR DE STAND
VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE**

Informatie over leden van dezelfde octrooifamilie

Nummer van het verzoek om een onderzoek naar
de stand van de techniek

NL 2007268

In het rapport genoemd octrooi geschrift	Datum van publicatie	Overeenkomend(e) geschrift(en)	Datum van publicatie
WO 2010134800	A1	25-11-2010	CN 102438466 A 02-05-2012
			EP 2432333 A1 28-03-2012
			WO 2010134800 A1 25-11-2010
			WO 2010134810 A1 25-11-2010
US 2008274230	A1	06-11-2008	AU 2004279621 A1 21-04-2005
			CA 2542612 A1 21-04-2005
			EP 1689772 A2 16-08-2006
			JP 2008500271 A 10-01-2008
			US 2008274230 A1 06-11-2008
			WO 2005035557 A2 21-04-2005
US 6294206	B1	25-09-2001	AR 023414 A1 04-09-2002
			AT 298200 T 15-07-2005
			AU 770601 B2 26-02-2004
			AU 4212500 A 14-11-2000
			BR 0007084 A 10-09-2002
			CA 2369007 A1 19-10-2000
			CN 1353577 A 12-06-2002
			CO 5170426 A1 27-06-2002
			DE 60020945 D1 28-07-2005
			EP 1168929 A2 09-01-2002
			ES 2243252 T3 01-12-2005
			HK 1045082 A1 21-04-2006
			IL 145395 A 31-05-2010
			JP 4755762 B2 24-08-2011
			JP 2002540806 A 03-12-2002
			JP 2011172569 A 08-09-2011
			MX PA01010013 A 06-05-2002
			NZ 514164 A 28-03-2003
			TW 1256881 B 21-06-2006
			US 6294206 B1 25-09-2001
			WO 0060949 A2 19-10-2000
US 2006233915	A1	19-10-2006	US 2006233915 A1 19-10-2006
			WO 2006112998 A1 26-10-2006
US 6576251	B1	10-06-2003	AT 246455 T 15-08-2003
			AU 6210698 A 07-08-1998
			CA 2278479 A1 23-07-1998
			DE 19701382 A1 23-07-1998
			DK 957692 T3 01-12-2003
			EP 0957692 A1 24-11-1999
			ES 2205445 T3 01-05-2004
			US 6576251 B1 10-06-2003
			WO 9831241 A1 23-07-1998
US 2005004070	A1	06-01-2005	AT 395066 T 15-05-2008
			DE 10147100 A1 17-04-2003
			EP 1429789 A2 23-06-2004
			EP 1970065 A1 17-09-2008
			ES 2306811 T3 16-11-2008
			US 2005004070 A1 06-01-2005
			WO 03028738 A2 10-04-2003
US 2011177044	A1	21-07-2011	US 2011177044 A1 21-07-2011
			WO 2011090926 A1 28-07-2011

**ONDERZOEKSRAPPORT BETREFFENDE HET
RESULTAAT VAN HET ONDERZOEK NAAR DE STAND
VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE**

Informatie over leden van dezelfde octrooifamilie

Nummer van het verzoek om een onderzoek naar
de stand van de techniek

NL 2007268

In het rapport genoemd octrooigescrift	Datum van publicatie	Overeenkomend(e) geschrift(en)	Datum van publicatie
US 2009098240	A1	16-04-2009	GEEN
WO 2011008086	A1	20-01-2011	EP 2453902 A1 23-05-2012 WO 2011008086 A1 20-01-2011
WO 2011136648	A1	03-11-2011	GEEN



Agentschap NL
Ministerie van Economische Zaken,
Landbouw en Innovatie

WRITTEN OPINION

File No. SN57205	Filing date (day/month/year) 16.08.2011	Priority date (day/month/year)	Application No. NL2007268
International Patent Classification (IPC) INV. A23L1/29			
Applicant Friesland Brands B.V., et al			

This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☒ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the application
- ☐ Box No. VIII Certain observations on the application

	Examiner Vermeulen, Stéphane
--	---------------------------------

WRITTEN OPINION

Application number
NL2007268

Box No. I Basis of this opinion

1. This opinion has been established on the basis of the latest set of claims filed before the start of the search.
2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material:
 - ☐ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material:
 - ☐ on paper
 - ☐ in electronic form
 - c. time of filing/furnishing:
 - ☐ contained in the application as filed.
 - ☐ filed together with the application in electronic form.
 - ☐ furnished subsequently for the purposes of search.
3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

WRITTEN OPINION

Application number

NL2007268

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step, or to be industrially applicable have not been examined in respect of

☐ the entire application

☒ claims Nos. 27

because:

☐ the said application, or the said claims Nos. relate to the following subject matter which does not require a search (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):

☒ no search report has been established for the whole application or for said claims Nos. 27

☐ a meaningful opinion could not be formed as the sequence listing was either not available, or was not furnished in the international format (WIPO ST25).

☐ a meaningful opinion could not be formed without the tables related to the sequence listings; or such tables were not available in electronic form.

☐ See Supplemental Box for further details.

Box No. IV Lack of unity of invention

1. The requirement of unity of invention is not complied with for the following reasons:

see separate sheet

2. This report has been established in respect of the following parts of the application:

☐ all parts.

☒ the parts relating to claims Nos. (see Search Report)

WRITTEN OPINION

Application number

NL2007268

Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty	Yes: Claims	
	No: Claims	1-26
Inventive step	Yes: Claims	
	No: Claims	1-26
Industrial applicability	Yes: Claims	1-26
	No: Claims	

2. Citations and explanations

see separate sheet

Box No. VI Certain documents cited

☒ Certain published documents

see the Search Report

☐ Non-written disclosures

Re Item I

Basis of the report

Reference is made to the following documents:

- D1 WO 2010/134800 A1 (NUTRICIA NV [NL]; VAN DER BEEK ELINE MARLEEN [NL]; BOEHM GUENTHER [DE]) 25 november 2010
- D2 US 2008/274230 A1 (JOHNS PAUL W [US] ET AL) 6 november 2008
- D3 US 6 294 206 B1 (BARRETT-REIS B [US] ET AL) 25 september 2001
- D4 US 2006/233915 A1 (PUSKI GABOR [US] ET AL) 19 oktober 2006
- D5 WESTERBEEK ELISABETH A M ET AL: "The effect of enteral supplementation of a prebiotic mixture of non-human milk galacto-, fructo- and acidic oligosaccharides on intestinal permeability in preterm infants", BRITISH JOURNAL OF NUTRITION, deel 105, nr. 2, januari 2011, bladzijden 268-274
- D6 ROSEGGER H: "[Maltodextrin in a 13% solution as a supplement in the first 4 days of life in breast-fed mature newborn infants. Effect on drinking behavior, weight curve, blood picture, blood glucose and bilirubin].", WIENER KLINISCHE WOCHENSCHRIFT, deel 98, nr. 10, 16 mei 1986, bladzijden 310-315
- D7 US 6 576 251 B1 (STAHL BERND [DE] ET AL) 10 juni 2003
- D8 US 2005/004070 A1 (STAHL BERND [DE] ET AL) 6 januari 2005
- D9 US 2011/177044 A1 (THOMAS DEBRA L [US] ET AL) 21 juli 2011
- D10 US 2009/098240 A1 (MILLS DAVID A [US] ET AL) 16 april 2009
- D11 WO 2011/008086 A1 (NUTRICIA NV [NL]; STAHL BERND [DE]; NAUTA ALMA JILDOU [NL]; GARSSSEN JO) 20 januari 2011
- D12 WO 2011/136648 A1 (NUTRICIA NV [NL]; STAHL BERND [DE]) 3 november 2011

Re Item IV

Lack of unity of invention

The present set of claims lacks unity as it comprises two inventions which are not so linked as to form a single general inventive concept. Claims 1-26 and claim 27 do not have common or corresponding special technical features and they deal with two different technical problems, not being linked to form a single general inventive concept:

Claims 1-26: protection of 1,2-fucosylated HMO by combination with a HMO protective compound

Claim 27: promotion of body weight gain in infants by using LNDFH I/II

A partial search has been carried for the first invention only (claims 1-26). Accordingly, the present opinion regarding novelty, inventive step and industrial applicability relates to the first invention.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The nutritional compositions according to independent claims 1 and 18 lack novelty over D1-D5 and D7-D10. Reference is made to the relevant passages cited in the search report for the respective documents. D1-D5 disclose the admixture with human milk of at least one compound selected from prebiotic oligosaccharide, maltodextrin and lactoferrin for use in infant feeding. Since human milk inherently comprises the defined alpha-1,2-fucosylated oligosaccharides the resulting compositions of D1-D5 anticipate the presently claimed compositions. D7-D10 disclose synthetic compositions combining the claimed alpha-1,2-fucosylated oligosaccharides with prebiotic oligosaccharides and/or maltodextrin. The therapeutic use of said compositions as defined in the independent claim 19 is furthermore anticipated by D7 and D8.

The use according to independent claim 20 lacks novelty over D1-D6, since these documents disclose the feeding of at least one compound selected from prebiotic oligosaccharide, maltodextrin and lactoferrin to an infant receiving breast milk. Reference is made to the relevant passages cited in the search report for the respective documents.

The use according to independent claim 22 lacks novelty over D11, since this document discloses to combine a prebiotic oligosaccharide with a human milk oligosaccharide (HMO) in order to achieve a synergetic (=increased) health-promoting effect. Reference is made to the relevant passages cited in the search report for said document. The synergy reported in D11 is limited to 2-fucosyllactose. According to the present application an effect can be seen for 1,2-fucosylated oligosaccharides with higher monomer counts. However this is not reflected by the wording of present claim 22.

In the light of D1-D11 also the dependent claims 2-17, 21 and 23-26 do not appear to contain any additional features which would render the claimed subject-matter novel and/or inventive. The specific embodiments are readily anticipated by the prior art or represent obvious alternatives which cannot clearly be linked to an unexpected effect and/or technical problem solved. As regards the composition claims it is furthermore noted that human milk per se anticipates a combination of a 1,2-fucosylated oligosaccharide and further prebiotic oligosaccharides or lactoferrin, since these are all natural compounds present in human milk.

The subject-matter of claims 1-26 is considered applicable in the food industry.

Re Item VI

Certain documents cited

Document D12, published after the effective date of filing of the present application, contains subject-matter which might be relevant for novelty assessment. Reference is made to the passages cited in the search report.