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(54) Title: USE OF 6'-SIALYL LACTOSE IN INFANT NUTRITION

(57) Abstract: Nutritional composition for providing nutrition to infants are provided, wherein adapted absolute amounts of 6'-SL and relative amounts of 6'-SL to 3'-SL are applied. The ratio of 6'-SL to 3'-SL is higher than two, preferably between 2.5 and 5.5. The amount of 6'-SL is at least 1 g/l, preferably between 1 and 3 g/l.

Use of 6'-sialyl lactose in infant nutrition**FIELD OF THE INVENTION**

The present invention is in the field of infant nutrition.

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BACKGROUND OF THE INVENTION

Sialyl oligosaccharides are present in milk of mammals and have been found to be of biological significance. They are known to possess prebiotic activity, and bifidogenic effects have been described. Also sialyl oligosaccharides are known to have an anti-adhesive effect, thereby playing an important role in inhibiting or preventing intestinal infections by pathogens and/or toxins. It is known that the concentration of the sialyl oligosaccharides in for instance cow's milk is very low whereas in human milk concentrations are considerably higher. Because most infant formulas are manufactured with bovine milk components, it follows that formula-fed infants are subject to reduced sialyl oligosaccharide intake compared to breast-fed infants. Prominent members of the sialyl oligosaccharides are 3'-sialyllactose (3'-SL) and 6'-sialyllactose (6'-SL).

Martín-Sosa et al (2003) J Dairy Sci 86:52-59 carried out a comparative study of sialyl oligosaccharide contents of bovine and human milk during various stages of lactation. Asakuma et al. (2007) Biosci Biotechnol Biochem, 71(6):1447-1451 studied the changes in concentration of sialyl oligosaccharides in human colostrum during the first three days of lactation. It was found that the concentration of 3'-sialyllactose was significantly higher on day 1 than on day 2 and 3, and on average the concentration of 3'-SL over the first three days post partum was about 297 mg/l. The level of 6'-sialyllactose was higher on day 3 than on day 1 and on average the concentration of 6'-SL over the first three days post partum was 370 mg/l.

WO 2009/059996 concerns nutritional compositions for use in preventing secondary infections following a viral infection, comprising sialylated oligosaccharides and in particular 3'-SL and 6'-SL.

In view of the aim to make infant and baby foods resemble human milk as much as possible, it has already been recognized in the art to enrich such foods with sialic acid containing oligosaccharides and preferably with sialyllactose. With this in mind, for example in WO 2009/113861 a process is described for the isolation of in particular

sialyllactose from milk in order to be able to enrich infant nutrition with sialyllactose. No distinction between 3'-SL and 6'-SL is made.

SUMMARY OF THE INVENTION

5 The present inventors surprisingly found that in order to match the quantities of 6'-sialyllactose (6'-SL) that are actually present in human milk, a much higher amount of 6'-SL needs to be formulated in infant formula than nowadays is done.

Further the present inventors also found that the relative amount of 3'-sialyllactose (3'-SL) to 6'-SL as actually is present in human milk is shifted to a relatively lower
10 contribution of 3'-SL compared to the relative amounts or ratio of the two as is nowadays applied in infant formula.

Thus, in summary, the present inventors found that in order to resemble human milk as close as possible and to preferably arrive at a more optimal prebiotic activity, and bifidogenic effect and/or anti-adhesive effect of sialyl oligosaccharides, preferably of
15 6'-SL and 3'-SL, infant formula need to be formulated differently than currently is done, in particular with respect to the amounts of sialyl oligosaccharides, preferably of 6'-SL and 3'-SL. In doing so, an improved intestinal healthy will be achieved and preferably an improvement in inhibiting or preventing intestinal infections by pathogens and/or toxins is achieved.

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DETAILED DESCRIPTION

The present invention thus concerns a method of providing nutrition to an infant, said method comprising administering a composition comprising at least 1 g/l 6'-sialyllactose (6'-SL) and wherein the weight ratio 6'-SL : 3'-SL is higher than 2.

25 The present invention can also be worded as concerning the use of a composition comprising 6'-sialyllactose (6'-SL) in the manufacture of a nutritional composition for providing nutrition to an infant, said nutritional composition comprising at least 1 g/l 6'-SL and wherein the weight ratio 6'-SL : 3'-SL is higher than 2.

The invention can also be worded as a composition comprising at least 1 g/l 6'-sialyllactose (6'-SL) and wherein the weight ratio 6'-SL : 3'-SL is higher than 2 for use
30 in providing nutrition to an infant.

The invention also concerns a nutritional composition, preferably an infant formula, that comprises lipid that provides 35 to 50% of the total calories, protein that provides 7.5 to 12.5% of the total calories, and digestible carbohydrate that provides 40 to 55% of the total calories and further comprising at least 1 g/l 6'-SL and wherein the ratio 6'-SL : 3'-SL is higher than 2.

6'-SL

The present nutritional composition preferably comprises at least 1 g/l 6'-SL, more preferably at least 1.1 g/l. This concentration is more reminiscent of human milk commonly applied in infant milk formula and is therefore to be preferred. The present nutritional composition preferably comprises less than 3 g/l 6'-SL, more preferably less than 2.5 g/l, more preferably less than 2 g/l, more preferably less than 1.5 g/l 6'-SL, more preferably between 1.1 and 1.5 g/l, more preferably between 1.1 and 1.4 g/l, more preferably between 1.2 and 1.4 g/l.

The present nutritional composition further preferably comprises 3'-SL and the weight ratio 6'-SL : 3'-SL is higher than 2. Preferably the weight ratio 6'-SL : 3'-SL is higher than 2.5, preferably higher than 3, preferably higher than 3.5. Preferably the weight ratio 6'-SL : 3'-SL is lower than 6. Preferably lower than 5.5, preferably lower than 5. Preferably the weight ratio 6'-SL : 3'-SL is between 2.5 and 5.5, preferably between 3 and 5.

The present composition in one embodiment comprises at least 0.7 wt% 6'-SL based on total dry weight of the composition, preferably between 0.7 wt% and 2.1 wt%, preferably between 0.8 and 1.5 wt%, preferably between 0.85 and 1.0 wt% based on total dry weight of the composition.

The present nutritional composition can be prepared by a process for manufacturing an infant milk formula comprising a) adding non digestible oligosaccharides derived from cow's milk, b) adding 6'-SL.

6'-SL is commercially available and can be purchased from Sigma-Aldrich. Also 6'-SL can be synthesized by metabolically engineered *E. coli* expressing a multifunctional sialyltransferase from the *Photobacterium* sp. JT-ISH-224 as described by Drouillard et al in a submission to Carbohydrate Research which at present is in press. The corrected proof is available at

http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6TFF-4YG1M15-

1&_user=3112367&_coverDate=02%2F24%2F2010&_rdoc=1&_fmt=high&_orig=search&_sort=d&_docanchor=&view=c&_searchStrId=1311365313&_rerunOrigin=scholar.google&_acct=C000059787&_version=1&_urlVersion=0&_userid=3112367&md5=ec8dccad77c1e06a5b102da46d8403b6.

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Non-digestible oligosaccharides other than 6'-sialyllactose and 3'-sialyllactose

The nutritional composition preferably comprises non-digestible oligosaccharides (NDO) other than 6'-SL and 3'-SL. Preferably the NDO other than 26'-SL and 3'-SL stimulate the growth of bifidobacteria and/or lactobacilli, more preferably
 10 bifidobacteria. An increased content of bifidobacteria and/or lactobacilli stimulate the formation of a healthy intestinal microbiota. The NDO are preferably not or only partially digested in the intestine by the action of acids or digestive enzymes present in the human upper digestive tract, in particular in the small intestine and stomach, and are fermented by the human intestinal microbiota. For example, sucrose, lactose,
 15 maltose and the common maltodextrins are considered digestible.

Preferably the present composition comprises non-digestible oligosaccharides with a DP in the range of 2 to 250, more preferably 2 to 60. The non-digestible oligosaccharide is preferably at least one, more preferably at least two, preferably at
 20 least three selected from the group consisting of fructo-oligosaccharides, galacto-oligosaccharides, xylo-oligosaccharides, arabino-oligosaccharides, arabinogalacto-oligosaccharides, gluco-oligosaccharides, chito-oligosaccharides, glucomanno-oligosaccharides, galactomanno-oligosaccharides, mannan-oligosaccharides, sialic acid comprising oligosaccharides, and uronic acid oligosaccharides. The group of fructo-
 25 oligosaccharides includes inulins, the group of galacto-oligosaccharides includes transgalacto-oligosaccharides or beta-galacto-oligosaccharides, the group of gluco-oligosaccharides includes cyclodextrins, gentio- and nigero-oligosaccharides and non-digestible polydextrose, the group of galactomanno-oligosaccharides includes partially hydrolyzed guar gum, and the group of uronic acid oligosaccharides includes
 30 galacturonic acid oligosaccharides and pectin degradation products.

More preferably the present composition comprises at least one, more preferably at least two, most preferably three selected from the group consisting of fructo-

oligosaccharides, beta-galacto-oligosaccharides and uronic acid oligosaccharides. More preferably the composition comprises beta-galacto-oligosaccharides.

In a preferred embodiment the composition comprises a mixture of inulin and short chain fructo-oligosaccharides. In a preferred embodiment the composition comprises a mixture of galacto-oligosaccharides and fructo-oligosaccharides selected from the group consisting of short chain fructo-oligosaccharides and inulin, more preferably inulin. A mixture of at least two different non-digestible oligosaccharides advantageously stimulates the beneficial bacteria of the intestinal microbiota to a greater extent. Preferably the weight ratio in a mixture of the two different non-digestible oligosaccharides, preferably galacto-oligosaccharides and fructo-oligosaccharide, is between 25 and 0.05, more preferably between 20 and 1. Galacto-oligosaccharides, preferably beta-galacto-oligosaccharides, are more capable of stimulating bifidobacteria. Preferably the present composition comprises galacto-oligosaccharides, preferably beta-galacto-oligosaccharides, with a degree of polymerization (DP) of 2 to 10 and/or fructo-oligosaccharides with a DP of 2 to 60.

The galacto-oligosaccharides preferably are beta-galacto-oligosaccharides. In a particularly preferred embodiment the present composition comprises beta-galacto-oligosaccharides ([galactose]_n-glucose; wherein n is an integer ranging from 2 to 60, i.e. 2, 3, 4, 5, 6, ..., 59, 60; preferably n is selected from 2, 3, 4, 5, 6, 7, 8, 9, and 10), wherein the galactose units are in majority linked together via a beta linkage. Beta-galacto-oligosaccharides are also referred to as trans-galacto-oligosaccharides (TOS). Beta-galacto-oligosaccharides are for example sold under the trademark Vivinal^(TM) (Borculo Domo Ingredients, Netherlands). Another suitable source is Bi2Munno (Classado). Preferably the TOS comprises at least 80 % beta-1,4 and beta-1,6 linkages based on total linkages, more preferably at least 90 %.

Fructo-oligosaccharide is a NDO comprising a chain of beta-linked fructose units with a DP or average DP of 2 to 250, more preferably 2 to 100, even more preferably 10 to 60. Fructo-oligosaccharide includes inulin, levan and/or a mixed type of polyfructan. An especially preferred fructo-oligosaccharide is inulin. Fructo-oligosaccharide

suitable for use in the compositions is also commercially available, e.g. Raftiline®HP (Orafti). Preferably the fructo-oligosaccharide has an average DP above 20.

Uronic acid oligosaccharides are preferably obtained from pectin degradation products.

- 5 Hence the present composition preferably comprises a pectin degradation product with a DP of 2 to 100. Preferably the pectin degradation product is prepared from apple pectin, beet pectin and/or citrus pectin. Preferably the uronic acid oligosaccharide is a galacturonic acid oligosaccharide. Preferably the composition comprises FL and one of the group selected from galacto-oligosaccharide and uronic acid oligosaccharide.

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Besides 6'-SL and 3'-SL, most preferably the composition comprises beta-galacto-oligosaccharide, fructo-oligosaccharide and a uronic acid oligosaccharide. It was found that such a combination acts synergistically with 6'-SL and 3'-SL. The weight ratio beta-galacto-oligosaccharide : fructo-oligosaccharide : uronic acid oligosaccharide is

15 preferably (20 to 2) : 1 : (1 to 20), more preferably (20 to 2) : 1 : (1 to 10), even more preferably (20 to 2) : 1 : (1 to 3), even more preferably (12 to 7) : 1 : (1 to 2). Most preferably the weight ratio is about 9 : 1 : 1.1..

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Preferably, the nutritional composition comprises 100 mg to 4 g non-digestible oligosaccharides, including 6'-SL and 3'-SL, per 100 ml, more preferably 500 mg to 3 g, even more preferably 800 mg to 2 g non-digestible oligosaccharides per 100 ml. Based on dry weight, the composition preferably comprises 0.5 wt% to 25 wt% non-digestible oligosaccharides including 6'-SL and 3'-SL, more preferably 1 wt% to 15 wt%, even more preferably 5 wt% to 10 wt%. A lower amount of non-digestible

25 oligosaccharides will be less effective in stimulating the beneficial bacteria in the microbiota, whereas a too high amount will result in side-effects of bloating and abdominal discomfort.

Nutritional composition

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The nutritional composition of the present invention is not human milk. The present nutritional composition is preferably enterally administered, more preferably orally.

The present nutritional composition is preferably an infant formula. The present nutritional composition can be advantageously applied as a complete nutrition for infants. The present composition preferably comprises a lipid component, protein component and carbohydrate component and is preferably administered in liquid form.

- 5 The present invention includes dry food, preferably a powder, which is accompanied with instructions as to admix said dry food mixture with a suitable liquid, preferably with water.

- The present nutritional composition preferably comprises lipid, protein and digestible carbohydrate, wherein the lipid component provides 5 to 50% of the total calories, the protein component provides 5 to 50% of the total calories, and the digestible carbohydrate component provides 15 to 85% of the total calories. Advantageously, the lipid component provides 20 to 50% of the total calories, the protein component provides 5 to 30% of the total calories, and the digestible carbohydrate component provides 30 to 70% of the total calories. Preferably, the lipid component provides 35 to 50% of the total calories, the protein component provides 7.5 to 12.5% of the total calories, and the digestible carbohydrate component provides 40 to 55% of the total calories. For calculation of the % of total calories for the protein component, the total of energy provided by the proteins, peptides and amino acids needs to be taken into account.
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- The nutritional composition preferably comprises at least one lipid selected from the group consisting of animal lipid, excluding human lipids, and vegetable lipids. Preferably the present composition comprises a combination of vegetable lipids and at least one oil selected from the group consisting of fish oil, animal oil, algae oil, fungal oil, and bacterial oil. The present composition preferably comprises long chain polyunsaturated fatty acids (LC-PUFA). LC-PUFA are fatty acids or fatty acyl chains with a length of 20 to 24 carbon atoms, preferably 20 or 22 carbon atoms comprising two or more unsaturated bonds. More preferably the present composition comprises eicosapentaenoic acid (EPA, n-3), docosahexaenoic acid (DHA, n-3) and/or arachidonic acid (ARA, n-6).
- 25
30

Preferably the present composition comprises at least 0.1 wt.%, preferably at least 0.25 wt.%, more preferably at least 0.6 wt.%, even more preferably at least 0.75 wt.% LC-PUFA with 20 and 22 carbon atoms based on total lipid content.

- 5 The content of LC-PUFA, particularly the LC-PUFA with 20 and 22 carbon atoms, preferably does not exceed 6 wt%, more preferably does not exceed 3 wt.% of the total lipid content as it is desirable to mimic human milk as closely as possible. The LC-PUFA may be provided as free fatty acids, in triglyceride form, in diglyceride form, in monoglyceride form, in phospholipid form, or as a mixture of one or more of the
- 10 above. The present composition preferably comprises between 5 and 75 wt.% polyunsaturated fatty acids based on total fat, preferably between 10 and 50 wt.%.

- The protein in the nutritional composition is preferably selected from the group consisting of non-human animal proteins (preferably milk proteins), vegetable proteins
- 15 (preferably soy protein, pea protein and/or rice protein), hydrolysates thereof, free amino acids and mixtures thereof. The nutritional composition preferably contains casein, whey, hydrolyzed casein and/or hydrolyzed whey protein. Preferably the protein comprises intact proteins, more preferably intact bovine whey proteins and/or intact bovine casein proteins.

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The nutritional composition preferably contains digestible carbohydrates selected from the group consisting of sucrose, lactose, glucose, fructose, corn syrup solids, starch and maltodextrins, more preferably lactose.

- 25 Preferably the liquid food does not have an excessive caloric density, however still provides sufficient calories to feed the infant. Hence, the liquid food preferably has a caloric density between 0.1 and 2.5 kcal/ml, even more preferably a caloric density of between 0.5 and 1.5 kcal/ml, most preferably between 0.6 and 0.8 kcal/ml.
- 30 Preferably the nutritional composition comprises nucleotides and/or nucleosides, more preferably nucleotides. Preferably, the composition comprises cytidine 5'-monophosphate, uridine 5'-monophosphate, adenosine 5'-monophosphate, guanosine 5'-monophosphate, and/or inosine 5'-monophosphate, more preferably cytidine 5'-

monophosphate, uridine 5'-monophosphate, adenosine 5'-monophosphate, guanosine 5'-monophosphate, and inosine 5'-monophosphate. Preferably the composition comprises 5 to 100, more preferably 5 to 50 mg, most preferably 10 to 50 mg nucleotides and/or nucleosides per 100 gram dry weight of the composition.

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Infant

The present method is advantageously applied to a human infant of 0-36 months, more preferably to a human infant of 0-18 months, more preferably to a human infant of 0-12 months, even more preferably to a human infant of 0-6 months. To decrease the risk of infection by pathogens to a minimum it is advantageous to formulate the infant formula with the amounts of 6'-SL and ratio of 6'-SL to 3'-SL according to the present invention. Preferably a Stage 1 and a Stage 2 infant formula are formulated with the amounts of 6'-SL and ratio of 6'-SL to 3'-SL according to the present invention. Preferably a Stage 1 infant formula is formulated with the amounts of 6'-SL and ratio of 6'-SL to 3'-SL according to the present invention. A Stage 1 formula is for providing nutrition to an infant of preferably less than 6 months, preferably for providing nutrition to an infant of preferably less than 4 months.

EXAMPLES

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Example 1 Determination of 6'-SL in mother's milk

Materials and Methods

Serologic tests

Lewis blood groups of the women were determined within 3 days post partum on the day of blood sampling by a haemagglutination tube test. Haemagglutination was examined using corresponding erythrocyte suspensions (3%-5% erythrocytes suspended in 0,9% NaCl) and monoclonal anti-Le^a and anti-Le^b antibodies (Immucor, Rödermark, Germany and BAG, Lich, Germany). Incubation was performed at room temperature for 15 min. Due to discrepancies between serologic tests and chromatographic profiles, some haemagglutination tests were repeated 18 - 25 months post partum (Thurl et al. 1998 Milchwissenschaft 53:127-129). The women were not pregnant at that time.

Collection of samples

All mothers had given written consent for participating in this study the design of which had been approved by the ethical committee of the university hospital of Dresden, Germany. The 30 caucasian women were living in the region of Dresden, they were between 20 and 35 years old and had given birth to healthy infants who were exclusively breast-fed during the study period. As a whole 175 breast milk samples were taken predominantly at following time intervals including seven major days: day 3, 2 - 5 days postpartum; day 8, d6 - d9; day 15, d13 - d18; day 22, d20 - d26; day 30, d28 - d33; day 60, d57 - d65; day 90, d88 - d96. In cases when mothers collected more than one milk sample corresponding to the above mentioned time intervals, all samples were analysed with oligosaccharide concentrations expressed as arithmetic mean values. In a preexamination with six Caucasian women from the region of Frankfurt/Main, Germany, we could not find any significant oligosaccharide variation during two 24 hour periods at days 7 and 60 post partum (data not shown). Nevertheless, in order to exclude even small diurnal effects, the sampling time in this study was fixed to the morning feed. The milk samples generally were collected during the morning hours at 6-10 am by applying the mid-feed sampling technique, previously shown to be an adequate sampling technique for carbohydrate analysis. About 5-10 ml aliquots were expressed manually in the middle of feeding into plastic containers. Milk samples were immediately frozen and stored at -20°C until analysis.

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Chromatographic analysis of oligosaccharides

Sample preparation including gel permeation chromatographic purification, as well as HPAEC-analyses were performed as already described (Thurl et al. 1996 Anal Biochem. 235:202-206). Briefly, human milk samples were heated for 30 min at 70°C . One millilitre of human milk was added with 0,1 ml of an aqueous solution containing the internal standards stachyose and galacturonic acid. The samples were subsequently centrifuged and ultrafiltrated (Millipore Centrifree, 30 kDa cutoff). The protein and lipid reduced samples were fractionated into lactose, neutral oligosaccharides and acidic oligosaccharides by gel permeation chromatography using Toyopearl HW 40 (S) columns (1,6 x 80 cm TosoHaas, Stuttgart, Germany). The carbohydrate fractions were eluted with water (flow rate of 1 ml/min) and monitored by refractive index detection. The lactose fraction was discarded; the neutral and acidic fractions were analysed by HPAEC-PED. The elution conditions for acidic oligosaccharides were 0-8 min, 100

mM NaOH / 20 mM NaOAc; 8-30 min, 100 mM NaOH / 20-80 mM NaOAc; 30-55 min, 100 mM NaOH / 80-200 mM NaOAc; 55-60 min, 100 mM NaOH / 200 mM NaOAc.

In order to monitor possible artificial hydrolytic degradation of especially susceptible sialyloligosaccharides, free N-acetyl neuraminic acid (Neu5Ac) was quantified along with acidic oligosaccharides. Neu5Ac concentrations were relatively constant (average concentration of 0,019 g/L in secretor mothers with Lewis blood group Le(a-b+)). The amounts of free NeuAc were of the same magnitude as already reported and correspond to approximately 2% and 4% of oligosaccharide bound NeuAc at the beginning of lactation and after three months, respectively. Therefore significant degradation of acidic oligosaccharides due to the action of sialidases or to heat treatment could be excluded.

Statistical analyses

Data that were analyzed by statistical methods either consisted of concentrations of individual oligosaccharides or of sums of various carbohydrates. Apart from total neutral and total acidic oligosaccharides, the following total core structures and fucosylated carbohydrates were summed up, respectively: core Lac = Lac + 3-FL + 2'-FL + LDFT + 3'-SL + 6'-SL; core LNT = LNT + LNFP I + LNFP II + LNDFH I + LNDFH II + LSTa + LSTb + DSLNT; core LNnT = LNnT + LNFP III + LSTc; core LNH = LNH + 2'-F-LNH + 3'-F-LNH + 2',3'-DF-LNH; Fuc α 1-2Gal = 2'-FL + LDFT + LNFP I + LNDFH I + 2'-F-LNH + 2',3'-DF-LNH; Fuc α 1-4GlcNAc = LNFP II + LNDFH I + LNDFH II; Fuc α 1-3Glc = 3-FL + LDFT + LNDFH II; Fuc α 1-3GlcNAc = LNFP III + 3'-F-LNH + 2',3'-DF-LNH.

The data set is two-factorially organized, in three milk groups and seven lactation times, respectively. Besides, the data set is very unbalanced due to different sample numbers. In milk group 1 (secretor mothers with Lewis blood group Le(a-b+)) the 109 samples in total are allocated to the times in a range from 10 to 21 samples, whereas in group 2 (non-secretor mothers with Lewis blood group Le(a+b-); 28 samples) a lactation time is represented by 3 – 5 samples and in group 3 (secretor mothers with Lewis blood group Le(a-b-); 17 samples) by 2 – 3 samples. Therefore several methods for analyzing the means of oligosaccharide concentrations were applied. In the case of group 1, an one-factorial analysis of variance (ANOVA) followed by a Student-

Newman-Keuls-test to compare the mean values of lactation times was used. Comparing the averages of the three milk groups a two-factorial ANOVA with Type III sum of squares was applied and then least-squares means were calculated. So, the group averages are unbiased and completely comparable together. Differences between averages were tested with the Tukey-Kramer-method on a significance level of 5%. In both variance models the differences between the sample concentrations of the participating women per group and time yield the experimental error. Generally, oligosaccharide concentrations of milks from women of the same milk group at a given lactation period were highly variable. Due to these large interindividual variations, in same cases no significant differences between means ($P > 5\%$) were found despite seemingly clear differences.

Trends of oligosaccharide concentrations during lactation were modelled by regression analysis. Simple linear regression as well as polynomial regression of 2nd and 3rd degree were fitted and tested on significant coefficients of regression. The model was accepted if the coefficients of regression were significant ($P < 5\%$). All analyses of regression were carried out with the individual values, although in the figures due to a better clarity only the means of lactation times are drawn. All computations were done using the SAS System (SAS Institute Inc. 2002-2003 SAS/STAT release 9.1 SAS Institute Inc., Cary, NC, Usa).

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Results

Carbohydrate fractions

Six major nonfucosylated acidic oligosaccharides, 3'-SL, 6'-SL, LSTa, LSTb, LSTc, DSLNT, as shown in Table 1, could be determined chromatographically. The sum of these carbohydrates approximately represents the acidic oligosaccharide fraction of human milk. Because of the importance of the Lewis blood group system these sugar also were examined separately according to the three milk groups. Milk samples of all three milk types exhibited the above mentioned six acidic oligosaccharides. The quantities of the acidic oligosaccharide fractions did not differ significantly among the three milk groups. In addition, all milk groups exhibited a similar roughly threefold decrease of the acidic sugar concentrations during the study period.

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Table 1. Structures of the acidic milk oligosaccharides determined in this study

Trivial name (abbreviated)	Structure
3'-SL	NeuAc α (2-3)Gal β (1-4)Glc
6'-SL	NeuAc α (2-6)Gal β (1-4)Glc
LSTa	NeuAc α (2-3)Gal β (1-3)GlcNAc β (1-3)Gal β (1-4)Glc
LSTb	Gal β (1-3)GlcNAc β (1-3)Gal β (1-4)Glc NeuAc α (2-6) /
LSTc	NeuAc α (2-6)Gal β (1-4)GlcNAc β (1-3)Gal β (1-4)Glc
DSLNT	NeuAc α (2-3)Gal β (1-3)GlcNAc β (1-3)Gal β (1-4)Glc NeuAc α (2-6) /

3'-SL, 3'-Sialyllactose; 6'-SL, 6'-Sialyllactose; LSTa-c, Sialyllacto-N-tetraoses a-c; DSLNT, Disialyllacto-N-tetraose.

Acidic sialylated oligosaccharides

- 5 Mean concentrations of the six acidic oligosaccharides determined in this study are shown in Table 2. 6'-SL quantitatively was by far the most important acidic carbohydrate in all milks. Medium amounts of 3'-SL, LSTc and DSLNT were detected, whereas LSTa and LSTb did not occur at concentration levels above 0,1 g/L. Average concentrations of individual acidic sugars, though statistically different in some cases,
- 10 did not vary to a great extent among the three milk groups, thus confirming the results of the whole carbohydrate fraction.
- Analogously, time effects, as shown in Table 2 with milk group one, were similar to tendencies in the other milk groups (data not shown). Concentrations of 6'-SL peaked in transitional milk at day 8, and declined at least threefold until 90 days after birth.
- 15 LSTc, also containing a Neu5Ac α 2-6Gal-linkage, decreased in a similar manner approximately fivefold during the study period. 3'-SL was expressed in mature milk at a relatively constant level after a significant decrease in the first phase of lactation. Concentrations of LSTa, a minor acidic sugar bearing a Neu5Ac α 2-3Gal-linkage, decreased after one week postpartum, so that it could not be detected in several milk
- 20 samples after one month. In contrast, concentrations of LSTb exhibiting a Neu5Ac α 2-6GlcNAc-linkage increased during the first month and remained relatively constant thereafter. DSLNT, the only disialylated carbohydrate analyzed and a kind of hybrid structure between LSTa and LSTb, exhibited a maximum time curve.

Table 2. Concentrations (g/l) of acidic oligosaccharides¹⁾

Group	Days	n	3'-SL	LSTa	DSLNT	LSTb	6'-SL	LSTc
1	3	21	0,35a	0,06b	0,29b	0,05c	1,31b	0,48a
1	8	19	0,30ab	0,09a	0,38a	0,06bc	1,77a	0,53a
1	15	17	0,27ab	0,05cb	0,44a	0,07abc	1,57ab	0,31b
1	22	16	0,26ab	0,03cd	0,41a	0,09ab	1,42b	0,25b
1	30	14	0,27ab	0,03cd	0,41a	0,10a	1,35b	0,24b
1	60	12	0,23b	0,01d	0,23b	0,08abc	0,63c	0,11c
1	90	10	0,24b	0,01d	0,21b	0,08abc	0,49c	0,09c
1	Av	109	0,27ab	0,04a	0,34b	0,08b	1,22a	0,29a
2	Av	28	0,24b	0,04a	0,42a	0,11a	1,14a	0,21b
3	Av	17	0,31a	0,06a	0,41ab	0,08b	1,31a	0,31a

5 ¹⁾ Means with the same letter are not significantly different; Student-Newman-Keuls-test for comparisons within group 1, Tukey-Kramer-test for comparisons of least-squares means (as consequence of unbalanced data) of groups 1-3.

Discussion

Acidic oligosaccharides

- 10 The six acidic oligosaccharides determined in this study did not exhibit important differences among the three milk groups. This is not unexpected since these sugars lacked fucose moieties. Concentrations of 6'-SL, the predominant acidic sugar in our study, exceeded the amounts reported from other groups at least twofold, whereas the concentrations of DSLNT were relatively low (Coppa et al., 1999 Acta Paediatr. Suppl.
- 15 430:89-94; Martin-Sosa et al., 2003 J Dairy Sci. 86:52-59; Asakuma et al., 2007 Biosci Biotechnol Biochem. 71:1447-1451; Bao et al., 2007 Anal Biochem. 370:206-214). The amounts of 3'-SL, LST a, LSTb and LSTc were in a similar range as the amounts detected by Asakuma et al., lower than the concentrations reported from Coppa et al. and higher than the values found by Bao et al. Although quantitative results from
- 20 different studies including our report revealed large differences, a significant decrease

of the acidic sugar fraction as a whole and of most individual sugars was generally found during the first months of lactation.

6'-SL as well as LSTc declined in a similar manner, a fact that supports the hypothesis that a single sialyltransferase, possibly ST6GalI, exclusively accepting type 2 structures is involved in the biosynthesis of these carbohydrates. The more pronounced decrease of LSTc can be explained with a decrease of core LNnT, the precursor of LSTc, whereas the amounts lactose, precursor of 6'-SL, remained constant after one week postpartum. 3'-SL decreasing to a small extent during the study period could be synthesized by ST3Gal IV or also by ST3Gal VI, two α 2,3-sialyltransferases acting preferentially on type 2 structures and we found that LSTa, a minor acidic sugar, declined very much and could not or only partially detected after 2 and 3 months. We hypothesize that ST3Gal III, an α 2,3-sialyltransferase acting preferentially on type 1 structures, is involved in the biosynthesis of LSTa as well as of DSLNT. LSTb was the only acidic carbohydrate that increased within the first month postpartum confirming previous results. A so-called ST6GlcNAc could transfer sialic acid moieties to subterminal GlcNAc yielding LSTb as well as DSLNT, an oligosaccharide exhibiting both α 2,6-linked and α 2,3-linked neuraminic acid.

Example 2 Infant formula

Infant formula comprising per 100 ml (13.9 dry weight):

1.4 g protein (whey and casein)

7.3 g digestible carbohydrates (including lactose)

3.6 g fat (vegetable fat, fish oil)

1.03 g non-digestible oligosaccharides of which 120 mg 6'-SL, 30 mg 3'-SL, 80 mg 2'-fucosyllactose, 720 mg beta-galacto-oligosaccharides, and 80 mg fructo-oligosaccharides

Further are included: choline, myo-inositol, taurine, minerals, trace elements, and vitamins as known in the art.

CLAIMS

1. Use of a composition comprising 6'-sialyllactose (6'-SL) in the manufacture of a nutritional composition for providing nutrition to an infant, said nutritional composition comprising at least 1 g/l 6'-SL and wherein the ratio 6'-SL : 3'-SL is higher than 2
2. The use according to claim 1 wherein the concentration of 6'-SL is at least 1.1. g/l.
3. The use according to claim 1 or 2, wherein the ratio 6'-SL : 3'-SL is higher than 3
4. The use according to any one of the preceding claims, wherein the ratio 6'-SL : 3'-SL is higher than 4.
5. The use according to any one of the preceding claims, wherein the nutritional composition further comprises non-digestible oligosaccharides selected from fructooligosaccharides and galactooligosaccharides.
6. The use according to any one of the preceding claims, wherein the nutritional composition further comprises uronic acid oligosaccharides selected from the group consisting of galacturonic acid oligosaccharides and pectin degradation products.
7. The use according to any of any one of the preceding claims, wherein the composition comprises lipid that provides 35 to 50% of the total calories, protein that provides 7.5 to 12.5% of the total calories, and digestible carbohydrate that provides 40 to 55% of the total calories.
8. The use according to any of any one of the preceding claims wherein the nutritional composition is for providing nutrition to an infant of 0-6 months.
9. Nutritional composition that comprises lipid that provides 35 to 50% of the total calories, protein that provides 7.5 to 12.5% of the total calories, and digestible carbohydrate that provides 40 to 55% of the total calories and further comprising between 1 and 3 g/l 6'-SL and wherein the ratio 6'-SL : 3'-SL is between 2.5 and 5.5.

10. The nutritional composition according to claim 9, further comprising beta-galactooligosaccharide, fructo-oligosaccharide and a uronic acid oligosaccharide.
11. The nutritional composition according to claim 8 or 9, which is a Stage 1 infant formula.

INTERNATIONAL SEARCH REPORT

International application No

PCT/NL2010/050239

A. CLASSIFICATION OF SUBJECT MATTER

INV. A23L1/29 A23L1/308 A61K31/7028 A61P37/04
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)

A23L A61K 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 practical, search terms used)

EPO-Internal, BIOSIS, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/002241 A1 (NUTRICIA NV [NL]; SCHMITT JOACHIM [DE]; LECROIX FRANCIS [FR]; JESENNE) 7 January 2010 (2010-01-07)	1-11
Y	claims 1-17 page 6, lines 10-25 page 11, line 25 - page 12, line 31	1-11
X	WO 2004/112509 A2 (NESTEC SA [CH]; GARCIA-RODENAS CLARA LUCIA [CH]; BERGONZELLI GABRIELA)	1-11
Y	29 December 2004 (2004-12-29) claims 2-5,7-14 page 7, line 30 - page 8, line 10 page 10, line 31 - page 11, line 10 ----- -/--	1-11

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

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

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"&" document member of the same patent family

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INTERNATIONAL SEARCH REPORT

International application No

PCT/NL2010/050239

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	claims 1-10 page 3, lines 19-26 page 7, lines 4-24 -----	1-11
X	WO 01/60346 A2 (AMERICAN HOME PROD [US]) 23 August 2001 (2001-08-23)	1-11
Y	claims 1-14 page 4, lines 27-37 example 1 -----	1-11
X	EP 2 143 341 A1 (NESTEC SA [CH]) 13 January 2010 (2010-01-13)	1-11
Y	claims 1-17 examples 1-2 -----	1-11
Y	BAO ET AL: "Simultaneous quantification of sialyloligosaccharides from human milk by capillary electrophoresis" ANALYTICAL BIOCHEMISTRY, ACADEMIC PRESS INC, NEW YORK LNKD- DOI:10.1016/J.AB.2007.07.004, vol. 370, no. 2, 1 October 2007 (2007-10-01), pages 206-214, XP022278428 ISSN: 0003-2697 the whole document -----	1-11
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/NL2010/050239

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