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(54) Title: NUTRITIONAL COMPOSITION FOR USE IN THE TREATMENT OF DIARRHEA, ITS PREPARATION AND METHOD OF TREATMENT

(57) Abstract: The invention concerns composition comprising a fucosylated human milk oligosaccharide, preferably 2'-fucosyllactose, for use in the treatment of virus induced diarrhea. The composition may be an infant formula. The use of a fucosylated human milk oligosaccharide for the preparation of a nutritional composition and the treatment of virus induced diarrhea in a human subject are also claimed.



NUTRITIONAL COMPOSITION FOR USE IN THE TREATMENT OF
DIARRHEA, ITS PREPARATION AND METHOD OF TREATMENT

FIELD OF THE INVENTION

The present invention relates to infant nutrition with non-digestible oligosaccharides, in particular to
5 the use thereof for treatment of virus induced diarrhea.

BACKGROUND OF THE INVENTION

Viral infections are common among people of all ages but often seem to be concentrated in infants
and children. Many viral infections result in fever and body aches or discomfort and are not that
10 serious and most children with viral infections get better without treatment. Particularly during
infancy, it is difficult for parents to tell whether their infant is ill with a potentially serious infection
and needs immediate medical care.

Rotavirus (RV) is the main causative agent of acute diarrheal disease in infants, which can lead to
15 dehydration or to more severe complications and even to death if left untreated. As the human RV
pathogenesis is still unclear and RV vaccines are not globally implemented, the modulation by
nutritional interventions with bioactive components is of interest. Recent studies have suggested
the possible role of HMOS in the protection against RV-associated diarrhea (Laucirica et al. J Nutr
2017; 147:1709–1714; Comstock et al. J Nutr Biochem Mol Genet Mech 2017; 147:1041–1047.

20 Human milk is the preferred food for infants. Human milk provides several bioactive factors that
benefit the relatively immature immune system of neonates early in life. These components have
been categorized into two different groups according to either their protective role or their ability to
promote maturation. In this sense, human milk oligosaccharides (HMOS) are considered to play a
25 part both in protection and maturation. However, it is not always possible or desirable to breast-
feed an infant. In such cases infant formulae or follow on formulae are a good alternative. These
formulae should have an optimal composition in order to mimic the beneficial effects of human milk
as close as possible.

30 The 162 structures of HMOS discovered to date and described in Urashima et al. (Trends in
Glycoscience and Glycotechnology, 2018, 30; 172, SE51-SE65) exhibit a lactose,
polylactosamine or lacto-N-biose core, which can be further bound to either fucose or sialic acids
or both. This structure confers on them a high chemical variability and protection from digestion.
Therefore, the majority of HMOS are neither absorbed nor metabolized in the proximal gut and
35 reach the distal gut undigested to exert prebiotic effects on certain bacterial populations, to reinforce
the intestinal barrier and to protect against enteropathogen infections. HMOS are present in human
breast milk in a relatively high proportion (5–20 g/L). The most abundant HMOS is 2'-fucosyllactose
(2'-FL), representing ~20% of the total oligosaccharides in human breast milk. *In vitro* and *in vivo*
studies with 2'-FL have evidenced its immunomodulatory effects, which include promoting anti-

inflammatory activities (He et al. Gut 2016; 65:33–46 and the inhibition of the colonization of pathogens (Ruiz-Palacios et al. J Biol Chem 2003; 278:14112–20).

WO 2016/139333 concerns nutritional compositions comprising a fucosylated oligosaccharide such as 2'-fucosyllactose and a particular N-acetylated oligosaccharide such as lacto-N-neotetraose (LNnT) for use against gastrointestinal infections and inflammations in infants or young children.

SUMMARY OF THE INVENTION

The inventors found that in a neonatal rat model diarrhea that was induced by rotavirus could be ameliorated by the presence of 2'-fucosyllactose. In particular amelioration of diarrhea in terms of severity and duration of diarrhea was observed. In an animal model involving suckling rats it was found that early nutritional intervention by administering 2'-fucosyllactose significantly reduced the maximum severity of diarrhea by 15% and significantly reduced the average severity of diarrhea by 22%. Moreover, the duration of diarrhea was significantly reduced by 44% upon administering 2'-fucosyllactose. In addition, when analysing the weight of the feces, it was observed that after infection with virus fecal weight increased and that upon administration of 2'-fucosyllactose this increase of fecal weight was statistically significant reduced.

Hence the inventors have found that the supplementation of 2'-FL during RV infection induced a clear protective role, significantly reducing the severity and duration of the diarrhea and the fecal weight.

DETAILED DESCRIPTION OF THE INVENTION

The present invention thus concerns a method for the treatment of virus induced diarrhea in a human subject by administering a nutritional composition that comprises a fucosylated human milk oligosaccharide, preferably 2'-fucosyllactose.

In other words, the invention concerns a nutritional composition comprising a fucosylated human milk oligosaccharide, preferably 2'-fucosyllactose, for use in the treatment of virus induced diarrhea in a human subject.

In a preferred embodiment, the virus induced diarrhea is rotavirus induced diarrhea.

For some jurisdiction, the invention can also be worded as the use of a fucosylated human milk oligosaccharide, preferably 2'-fucosyllactose, for the preparation of a nutritional composition for the treatment of virus induced diarrhea in a human subject.

The amount of the fucosylated HMO, preferably 2'-fucosyllactose is preferably an effective amount to treat virus induced diarrhea in a human subject and/or an effective amount to reduce the severity

of the virus induced diarrhea and/or an effective amount to shorten the duration of the virus induced diarrhea.

Fucosylated human milk oligosaccharides and 2'-fucosyllactose

5 Prebiotics are typically indigestible sugar-type compounds such as non-digestible oligosaccharides (NDOs). These compounds pass through the first part of the gastrointestinal tract substantially without being digested. In the intestine these compounds are fermented by the microbiota releasing, amongst others, short chain fatty acids which are adsorbed by the human body.

10 There are many sources of NDO's, amongst which is human breast milk. Usually these oligosaccharides are denoted as human milk oligosaccharides (HMOS). Typical NDOs used in infant foods are GOS and FOS.

Human milk is the preferred food for infants and is also denoted as the golden standards. Human
15 milk contains a particularly high level of oligosaccharides of roughly 10 to 15 g/L, which is typically much more than the level of NDO in the milk from domestic animals. Also, compared to the NDOs in the milk of domestic animals, HMOS are structurally different. Human NDO is very complex and consists of a heterogenic group of many different compounds with diverse sugar composition. Because of their complex and polymorphic structure, large-scale synthesis is complicated. It is
20 therefore not yet technically and economically feasible to prepare compositions, such as infant formulas, with NDO composition identical to human milk. In the method or use according to the present invention, a fucosylated non-digestible human milk oligosaccharide is used.

Fucosyllactose (FL) is a fucosylated non-digestible oligosaccharide present in human milk. It is not
25 present in bovine milk. It consists of three monose units, fucose, galactose and glucose linked together. Lactose is a galactose unit linked to a glucose unit via a beta 1,4 linkage. A fucose unit is linked to a galactose unit of a lactose molecule via an alpha 1,2 linkage (2'-fucosyllactose, 2'-FL) or via an alpha 1,3 linkage to the glucose unit of a lactose (3-Fucosyllactose, 3-FL). 2'FL is the most abundant NDO in human milk. The HMOS used in the current invention is 2'-FL.

30 2'-FL, (β -L-Fuc-(1 $\rightarrow$ 2)- β -D-Gal-(1 $\rightarrow$ 4)-D-Glc) is commercially available for instance from Sigma-Aldrich. Alternatively, it can be isolated from human milk, for example as described in Andersson & Donald, 1981, J Chromatogr. 211:170-1744, or produced by genetically modified micro-organisms, for example as described in Albermann et al, 2001, Carbohydrate Res. 334:97-103.

35 Preferably, a composition according to the invention comprises 1 mg to 3 g 2'-FL per 100 ml, more preferably 10 mg to 2 g, even more preferably 20 mg to 100 mg 2'-FL per 100 ml. Based on dry weight, the present composition preferably comprises 0.007 wt.% to 20 wt.% 2'-FL, more preferably 0.07 wt.% to 10 wt.%, even more preferably 0.15 wt.% to 1 wt.%. A lower amount of fucosyllactose

will be less effective in ameliorating virus induced diarrhea, whereas a too high amount will result in unnecessary high costs of the product.

5 In one embodiment, the nutritional composition for use according to the present invention does not comprise lacto-N-neotetraose (LNnT). In one embodiment, the nutritional composition for use according to the present invention does not comprise a human milk oligosaccharide other than 2'-FL.

Non-digestible oligosaccharides other than human milk oligosaccharides

10 The nutritional composition preferably also comprises non-digestible oligosaccharides other than HMOS. Non-digestible oligosaccharides other than HMOS can have an effect on stool consistency and in particular an effect of loosening stool and as such have an effect that would lead to the apparent contrary finding that diarrhea would not be ameliorated. However, when correcting for common effects provided by non-digestible oligosaccharides other than HMO, in particular when
15 correcting for the intrinsic effect on fecal consistency of non-digestible oligosaccharides other than HMO, it was found that their inclusion had an additional role in significantly reducing the severity score and duration of the diarrhea.

The NDO are preferably not or only partially digested in the intestine by the action of acids or
20 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, maltose and the common maltodextrins are considered digestible.

Preferably the present composition comprises non-digestible oligosaccharides with a DP in the
25 range of 2 to 250, more preferably 2 to 60. The non-digestible oligosaccharide is preferably at least one, more preferably at least two selected from the group consisting of fructo-oligosaccharides, galacto-oligosaccharides, xylo-oligosaccharides, arabino-oligosaccharides, arabinogalacto-oligosaccharides, gluco-oligosaccharides, chito-oligosaccharides, glucomanno-oligosaccharides, galactomanno-oligosaccharides and mannan-oligosaccharides. The group of fructo-
30 oligosaccharides includes inulins and the group of galacto-oligosaccharides includes transgalacto-oligosaccharides or beta-galacto-oligosaccharides.

More preferably the present composition comprises fructo-oligosaccharides (FOS), and/or galacto-oligosaccharides (GOS), preferably the galacto-oligosaccharides comprise beta-galacto-
35 oligosaccharides and/or alpha galactooligosaccharides. More preferably the fructo-oligosaccharides are long chain fructo-oligosaccharides (lcFOS). More preferably the galacto-oligosaccharides are short chain galacto-oligosaccharides and the beta-galacto-oligosaccharides are short chain beta-galacto-oligosaccharides. Most preferably the composition comprises long chain fructo oligosaccharides and short chain galacto-oligosaccharides. The weight ratio of short

chain galacto-oligosaccharides and long chain fructo oligosaccharides lies preferably between 100:1 and 1:10, preferably between 20:1 and 1:1, preferably is about 9:1.

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 galacto-oligosaccharides comprise beta-1,3, beta-1,4 and/or beta-1,6 linkages. In a preferred embodiment, galacto-oligosaccharides comprise at least 80 % beta-1,4 and beta-1,6 linkages based on total linkages, more preferably at least 90 %. In another preferred embodiment, the galacto-oligosaccharides comprise at least 50 % beta-1,3 linkages based on total linkages, more preferably at least 60 % based on total linkages.

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.

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.

In a preferred embodiment, the composition comprises a combination of a fucosylated human milk oligosaccharide, preferably 2'-fucosyllactose, short chain galacto-oligosaccharides and long chain fructo oligosaccharides. In a preferred embodiment, the weight ratio of the sum of short chain galacto-oligosaccharides and long chain fructo oligosaccharides to 2'-fucosyllactose contained in

the nutritional composition lies preferably between 20:1 and 1:100, preferably between 10:1 and 1:50, more preferably between 4:1 and 1:20, most preferably between 1:1 and 1:15.

LC-PUFA

5 The composition may further comprise long chain polyunsaturated fatty acids (LC-PUFA). LC-PUFA are fatty acids wherein the acyl chain has a length of 20 to 24 carbon atoms (preferably 20 or 22 carbon atoms) and wherein the acyl chain comprises at least two unsaturated bonds between said carbon atoms in the acyl chain. More preferably the present composition comprises at least one LC-PUFA selected from the group consisting of eicosapentaenoic acid (EPA, 20:5 n3),
10 docosahexaenoic acid (DHA, 22:6 n3), arachidonic acid (ARA, 20:4 n6) and docosapentaenoic acid (DPA, 22:5 n3), preferably DHA, EPA and/or ARA. Such LC-PUFAs have a further beneficial effect on improving intestinal barrier integrity.

The preferred content of LC-PUFA in the present composition does not exceed 15 wt.% of total
15 fatty acids, preferably does not exceed 10 wt.%, even more preferably does not exceed 5 wt.%. Preferably the present composition comprises at least 0.2 wt.%, preferably at least 0.25 wt.%, more preferably at least 0.35 wt.%, even more preferably at least 0.5 wt.% LC-PUFA of total fatty acids, more preferably DHA. The present composition preferably comprises ARA and DHA, wherein the weight ratio ARA/DHA preferably is above 0.25, preferably above 0.5, more preferably 0.75 – 2,
20 even more preferably 0.75-1.25. The weight ratio is preferably below 20, more preferably between 0.5 and 5. The amount of DHA is preferably above 0.2 wt.%, more preferably above 0.3 wt.%, more preferably at least 0.35 wt.%, even more preferably 0.35 – 0.6 wt.% on total fatty acids.

Compositions

25 The present invention advantageously concerns a composition for the indicated use wherein the lipid provides 5 to 50% of the total calories, the protein provides 5 to 50% of the total calories, and the carbohydrate provides 15 to 90% of the total calories. Preferably, in the present composition the lipid provides 35 to 50% of the total calories, the protein provides 7.5 to 12.5% of the total calories, and the carbohydrate provides 40 to 55% of the total calories. For calculation of the % of
30 total calories for the protein component, the total of energy provided by the proteins, peptides and amino acids needs to be taken into account.

The present 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
35 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 comprising 2'-FL is not human milk.

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

proteins (preferably milk proteins, preferably proteins from cow's milk), vegetable proteins (preferably soy protein and/or rice protein), free amino acids and mixtures thereof. The present composition preferably contains casein, whey, hydrolysed casein and/or hydrolysed whey protein. Preferably the protein comprises intact proteins, more preferably intact bovine whey proteins and/or intact bovine casein proteins.

The present composition preferably comprises digestible carbohydrates. The present composition preferably comprises a digestible carbohydrate component, wherein at least 35 wt.%, more preferably at least 50 wt.%, more preferably at least 75 wt.%, even more preferably at least 90 wt.%, most preferably at least 95 wt.% is lactose. The present composition preferably comprises at least 25 grams lactose per 100 gram dry weight of the present composition, preferably at least 40 grams lactose/100 gram.

When in liquid for, the nutritional composition 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. The amount of nutritional composition administered per day is preferably between 50 and 2000 ml, more preferably between 200 and 1500, most preferably between 400 and 1000 ml.

In one embodiment the present invention concerns a supplement, suitable to fortify human milk, to fortify human milk fortified with a standard human milk fortifier or to fortify a standard preterm formula. In the context of this invention, a supplement does not comprise all macro- and micronutrients needed for preterm infants so as to achieve a growth similar to fetal growth coupled with satisfactory functional development. The term "preterm" herein is synonymous for prematurely born and means any human infant born before the 37th week of gestation.

Thus in one embodiment the nutritional composition according to the present invention or for use according to the present invention comprises protein, fat and/or digestible carbohydrates and is selected from the group consisting of an infant starter formula, an infant follow on formula, a toddler milk, a preterm formula, a post discharge formula and a human milk fortifier.

Application

According to the present invention, virus induced diarrhea is treated by administering a nutritional composition comprising a fucosylated human milk oligosaccharide, preferably 2'-fucosyllactose. In a preferred embodiment, the diarrhea that is treated is induced by rotavirus, also referred to as rotavirus induced diarrhea.

In a preferred embodiment, the treatment of virus induced diarrhea is reducing the severity of the virus induced diarrhea. In a further preferred embodiment, the treatment of virus induced diarrhea is shortening the duration of the virus induced diarrhea. Preferably, the treatment of virus induced

diarrhea is reducing of the severity of the virus induced diarrhea and shortening the duration of the virus induced diarrhea.

In a preferred embodiment, the administration or use of the fucosylated human milk oligosaccharide, preferably 2'-fucosyllactose, is to reduce the severity of the virus induced diarrhea. In a further preferred embodiment, the administration or use of the fucosylated human milk oligosaccharide, preferably 2'-fucosyllactose, is to shorten the duration of the virus induced diarrhea. Preferably, the administration or use of fucosylated human milk oligosaccharide, preferably 2'-fucosyllactose, is to reduce the severity of the virus induced diarrhea and to shorten the duration of the virus induced diarrhea.

The nutritional composition for use according to the present invention is for use in a human subject. Preferably the human subject is an infant or a toddler. An infant is a human subject with an age from 0-12 months. A toddler is a human subject with an age from 12-36 months. In a preferred embodiment, the human subject is a preterm or prematurely born infant. Especially this subgroup of infants may benefit from being administered the fucosylated human milk oligosaccharide, preferably 2'-fucosyllactose, or a combination of the fucosylated human milk oligosaccharide, preferably 2'-fucosyllactose, and long chain fructo oligosaccharides and short chain galacto-oligosaccharides since organs making up the intestinal tract of preterms are vulnerable and immature, as opposed to term-born infants and thus in need of shortened duration of diarrhea and/or less severe diarrhea as treatment would it occur.

Preferably, the human subject is administered the fucosylated human milk oligosaccharide, preferably 2'-fucosyllactose, or the nutritional composition comprising the fucosylated human milk oligosaccharide, preferably 2'-fucosyllactose, on a daily basis. Also, the administration of the nutritional composition comprising the fucosylated human milk oligosaccharide, preferably 2'-fucosyllactose, or the use thereof, preferably commences prior to the virus induced diarrhea occurs and spans the time period the subject can be diagnosed with diarrhea.

In a preferred embodiment, the human subject is vaginally-born, also known as naturally born.

In a preferred embodiment, the reduction in the severity of the virus induced diarrhea and/or the shortened duration of the virus induced diarrhea is accompanied by improved virus elimination by the human subject or reduced viral particle counts in the stool. It was found by the inventors that the addition of 2'-fucosyllactose, short chain galacto-oligosaccharides and long chain fructo-oligosaccharides reduced shedding of viral particles in the feces by the human subject. This is in particular advantageous in the prevention of virus transmittal or a second round of virus-induced diarrhea via self-contamination following the primary virus infection, like rotavirus, via the stool. Thus, in a preferred embodiment, the reduction in the severity of the virus induced diarrhea and/or

the shortened duration of the virus induced diarrhea is accompanied by reduced virus elimination by the human subject.

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

EXAMPLES

Example 1

Newborn rats were distributed into five groups of 24 animals each: the reference (REF) group, rotavirus-infected (RV) group, and 3 rotavirus-infected groups supplemented with: a) a mixture of scGOS and lcFOS (RV+GOS/FOS group); b) 2'-FL (RV+2'-FL group); and c) both scGOS/lcFOS and 2'-FL (RV+GOS/FOS+2'-FL group).

Suckling rats were orally administered once daily with the same normalized volume/body weight of all products (4.5 μ L/g/day), from the second to the sixteenth day of life, corresponding to the strict lactation period. The RV+GOS/FOS group was supplemented with 0.8 g of scGOS/lcFOS/100 g of body weight. The RV+2'-FL group was supplemented with 0.2 g of 2'-FL/100 g of body weight. The RV+GOS/FOS+2'-FL group received both products at the same doses as when given separately and maintaining the volume of administration (4.5 μ L/g/day). The REF and RV groups were administered with a matched volume of water.

The RV (simian SA-11) was obtained as previously described (Pérez-Cano et al. *Pediatr Res* 2007;62:658–63), and inoculated at day 5 of life (4×10^8 Tissue Culture Infectious Dose 50 [TCID₅₀]/rat) in all the experimental groups with the exception of the REF group, which received the same volume of phosphate-buffered solution (PBS) under the same conditions.

Body weight was recorded daily throughout the study to assess weight gain. Half (n=12) of each group of animals were sacrificed at day 8, to analyze variables associated with the peak of diarrhea, and the other half (n=12 per group) at day 16, to analyze the effects of the supplementations once the diarrhea was resolved. Moreover, the naso–anal and tail lengths were measured to determine the body/tail ratio, the body mass index (BMI), calculated as $\text{body weight}/\text{length}^2$ (g/cm²) and the Lee Index, calculated as $(\text{weight}^{0.33}/\text{length}) \times 1000$ (g^{0.33}/cm).

Fecal samples collection and clinical indices

Fecal sampling was performed once daily throughout the study (from day 4 to day 16 of life) by gently pressing and massaging the abdomen. Fecal samples were stored at -20 °C for the analysis of RV shedding. Severity of diarrhea was expressed by fecal weight and by scoring fecal samples from 1 to 4 (diarrhea index [DI]) based on color, texture and amount, as follows: normal feces (1); soft yellow-green feces (2); totally loose yellow-green feces (3); high amount of watery feces (4). Scores ≥ 2 indicate diarrheic feces, whereas scores < 2 indicate absence of diarrhea. The severity-area under the curve (S-AUC) during days 5–11, coinciding with the period in which animals displayed diarrhea, was calculated as a global value of severity. The maximum severity (MS) was defined as the highest score during the diarrhea period. The diarrhea period (DP) was calculated by counting the number of days in which the animals displayed $DI \geq 2$.

The DI, S-AUC, MS and DP were normalized (nDI, nS-AUC, nMS and nDP) in RV+GOS/FOS and RV+GOS/FOS+2'-FL groups, taking into account the basal values before and after the diarrhea period because of intrinsic fecal aspects of GOS/FOS supplementation, as previously described (Rigo-Adrover et al. Eur J Nutr 2017;56:1657–70).

Statistical analysis

The Statistical Package for the Social Sciences (SPSS v22.0) (IBM, Chicago, IL, USA) was used for statistical analysis. Data was tested for homogeneity of variance and normality distribution by the Levene's and Shapiro–Wilk tests, respectively. When data was homogeneous and had a normal behavior, conventional one-way ANOVA test followed by the post hoc Bonferroni was performed. Otherwise, the nonparametric Kruskal–Wallis test followed by the post hoc Mann–Whitney U (MWU) test were performed. Finally, the chi-square test was used to compare frequencies of diarrhea incidence. Significant differences were established when $p < 0.05$.

Results*Growth*

The RV infection did not produce any significant change in growth either at the peak of diarrhea (day 8) or at the end of the study (day 16), as shown by the results in body weight, body/tail ratio, BMI and Lee Index (Table 1). The group supplemented with scGOS/lcFOS had a slightly higher body weight at the end of the study (day 16, $p < 0.05$), and although none of these growth changes modified the BMI, some differences were seen in the body/tail length ratio and the Lee Index. All supplementations increased the body/tail length ratio compared to REF or RV at some time point. Moreover, the Lee Index was decreased exclusively at the peak of diarrhea compared to REF.

Table 1. Growth-associated variables

	REF	RV	RV+GOS/FOS	RV+2'-FL	RV+GOS/FOS+2'-FL
Body weight (g)					
day 8	13.52 ± 0.25	13.60 ± 0.19	14.08 ± 0.17	13.51 ± 0.19	13.67 ± 0.19
day 16	31.40 ± 0.89	31.91 ± 0.65	34.16 ± 0.55* [#]	32.79 ± 0.50	33.53 ± 0.40
Body/tail length ratio					
day 8	2.14 ± 0.07	2.15 ± 0.03	2.26 ± 0.03* [#]	2.32 ± 0.03* [#]	2.22 ± 0.03
day 16	1.77 ± 0.02	1.77 ± 0.02	1.92 ± 0.04* [#]	1.86 ± 0.05	1.84 ± 0.03*
BMI (g/cm²)					
day 8	0.30 ± 0.01	0.29 ± 0.00	0.29 ± 0.00	0.28 ± 0.01	0.28 ± 0.00
day 16	0.35 ± 0.01	0.36 ± 0.00	0.37 ± 0.01	0.37 ± 0.01	0.36 ± 0.00
Lee Index					
day 8	354.76 ± 3.42	348.80 ± 1.73	345.21 ± 1.34*	343.49 ± 2.46* [#]	344.52 ± 1.43*
day 16	334.44 ± 2.51	335.88 ± 2.41	337.12 ± 3.36	337.93 ± 3.48	332.44 ± 1.26

Results are expressed as mean ± S.E.M. (n=12); *p<0.05 compared to REF (by MWU test);

[#]p<0.05 compared to RV (by MWU test).

5 *Clinical variables for the assessment of severity and duration of diarrhea*

Rats inoculated with RV displayed diarrheic feces from days 6 to 11 of life, the highest score being at day 8. As shown in a previous study using the SA11 RV infection model, GOS/FOS induced changes in fecal consistency, thereby increasing the number of feces considered as diarrheic (DI ≥ 2) before, during and after the diarrheic process (Rigo-Adrover et al. Eur J Nutr 2017;56:1657–70).

- 10 Nevertheless, the supplementation with GOS/FOS and GOS/FOS+2'-FL showed a tendency to reduce the severity of diarrhea at the maximum affectation point. When normalizing the severity score values in the groups supplemented with GOS/FOS, a significant reduction (p<0.05) was found at days 7, 8 and 9. The supplementation with 2'-FL did not induce this basal fecal consistency effect and displayed a trend to reduce the severity of diarrhea.

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Other indicators of severity and duration of diarrhea were also calculated (Table 2). Regarding the maximum severity (MS) of diarrhea, the supplementation with 2'-FL and GOS/FOS+2'-FL reduced the score by up to 15% and 10%, respectively, compared to that of the RV group (p<0.05). After the normalization of this indicator (nMS), a milder score was then seen for the GOS/FOS groups, showing a 28–30% reduction (p<0.05). The calculation of the severity-area under the curve (S-AUC) displays the average severity for each group. A clear tendency to reduce the S-AUC with respect to the RV group was seen for the supplementation with 2'-FL, with a 22% decrease. Only after normalizing the area was the nS-AUC reduced in the animals supplemented with GOS/FOS and GOS/FOS+2'-FL (45% and 58%, respectively, p<0.05). Finally, the amelioration was also found in terms of reducing the diarrhea period (DP), which was reduced more than 50% due to the supplementations (p<0.05).

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Table 2. Analysis of other variables associated with severity, incidence and duration of diarrhea

	RV	RV+GOS/FOS	RV+2'-FL	RV+GOS/FOS+2'-FL
Severity				
MS	2.23 ± 0.07	2.09 ± 0.10	1.88 ± 0.09 [#]	2.01 ± 0.07 [#]
nMS	-	1.61 ± 0.10 [#]	-	1.55 ± 0.07 [#]
S-AUC	2.85 ± 0.17	3.56 ± 0.31	2.22 ± 0.35	2.94 ± 0.33
nS-AUC	-	1.57 ± 0.19 [#]	-	1.18 ± 0.21 [#]
Duration				
DP	1.40 ± 0.15	1.84 ± 0.19	0.79 ± 0.16 [#]	1.74 ± 0.20
nDP	-	0.32 ± 0.13 [#]	-	0.17 ± 0.08 [#]

Results are expressed as mean ± S.E.M. for severity and duration variables (n=12–24 depending on the number of fecal samples obtained each day); [#]p<0.05 compared to RV (by MWU test and Chi Square test)

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The fecal weight was also measured as an objective severity variable of the diarrheic process. Before RV inoculation (corresponding to days 2–5 of life), animals receiving GOS/FOS and GOS/FOS+2'-FL had a higher fecal weight compared to both the REF and RV groups, which is likely to be related to the previously direct changes reported in the fecal consistency. After infecting with the virus (corresponding to days 6–11 of life), the RV group showed an increased fecal weight (p<0.05). The effects of the supplementations with oligosaccharides were seen at days 6–8, reducing this increase of fecal weight, although only statistical differences in the supplementation with 2'-FL were attained (p<0.05). This lower fecal weight observed in the 2'-FL group, was maintained until the end of the diarrhea period (days 9–11). At the end of the diarrheic process (post-diarrhea period days 12–16 of life), fecal weights from RV and 2'-FL were similar to those from REF, while the supplementation with GOS/FOS and GOS/FOS+2'-FL continued to show higher values due to the non-pathogenic changes in the fecal consistency.

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Example 2: Infant formula for reducing the severity of rotavirus induced diarrhea or shortening rotavirus induced diarrhea.

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An infant formula according to the invention 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)

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0.6 g non-digestible oligosaccharides of which 60 mg 2'-fucosyllactose and 480 mg beta-galacto-oligosaccharides, and 60 mg fructo-oligosaccharides

Further are included: choline, myo-inositol, taurine, minerals, trace elements, and vitamins in accordance with guidelines.

CLAIMS

1. A nutritional composition comprising a fucosylated human milk oligosaccharide, preferably 2'-fucosyllactose, for use in the treatment of virus induced diarrhea in a human subject.
2. The nutritional composition for use according to claim 1, wherein the virus induced diarrhea is rotavirus induced diarrhea.
3. The nutritional composition for use according to claim 1 or 2, wherein the treatment is reducing the severity of the virus induced diarrhea.
4. The nutritional composition for use according to any one of the preceding claims, wherein the treatment is shortening the duration of the virus induced diarrhea.
5. The nutritional composition for use according to any one of the preceding claims, wherein the nutritional composition further comprises at least one, more preferably at least two non-digestible oligosaccharides with a DP in the range of 2 to 250, wherein the non-digestible oligosaccharides are selected from the group consisting of fructo-oligosaccharides, galacto-oligosaccharides, xylo-oligosaccharides, arabino-oligosaccharides, arabinogalacto-oligosaccharides, gluco-oligosaccharides, chito-oligosaccharides, glucomanno-oligosaccharides, galactomanno-oligosaccharides and mannan-oligosaccharides.
6. The nutritional composition for use according to claim 5, wherein the non-digestible oligosaccharides are selected from the group consisting of fructo-oligosaccharides and galacto-oligosaccharides.
7. The nutritional composition for use according to any one of the preceding claims, wherein the nutritional composition is an infant formula or a follow-on formula, preferably an infant formula.
8. Use of a fucosylated human milk oligosaccharide, preferably 2'-fucosyllactose, for the preparation of a nutritional composition for the treatment of virus induced diarrhea in a human subject.
9. A method for the treatment of virus induced diarrhea in a human subject by administering a nutritional composition that comprises a fucosylated human milk oligosaccharide, preferably 2'-fucosyllactose.

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2018/050478

A. CLASSIFICATION OF SUBJECT MATTER
INV. A23L33/00 A61K31/702
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

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	VASSILIS TRIANTIS ET AL: "Immunological Effects of Human Milk Oligosaccharides", FRONTIERS IN PEDIATRICS, vol. 6, 2 July 2018 (2018-07-02), XP055548553, DOI: 10.3389/fped.2018.00190 page 5; table 2 Effects on Intestinal Viruses; page 7, right-hand column	1,2,8,9
X	WO 2012/092153 A1 (ABBOTT LAB [US]; BUCK RACHAEL [US]; THOMAS DEBRA L [US]; SCHALLER JOSE) 5 July 2012 (2012-07-05) paragraph [0013] - paragraph [0014] paragraph [0030] paragraph [0128] paragraph [0144] - paragraph [0145] ----- -/-	1-9



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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"P" document published prior to the international filing date but later than the priority date claimed

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

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"&" document member of the same patent family

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INTERNATIONAL SEARCH REPORT

International application No
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