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(54) Title: USE OF PROBIOTICS AND FIBERS FOR DIARRHOEA

(57) Abstract: The present invention relates to nutritional compositions comprising *Lactobacillus rhamnosus*, inulin and soy polysaccharides for the treatment of diarrhoea.

## Use of probiotics and fibers for diarrhoea

### FIELD OF THE INVENTION

The invention is in the field of infant nutrition comprising probiotics and fibers for  
5 prevention and/or treatment of diarrhoea.

### BACKGROUND OF THE INVENTION

Diarrhoea is a serious public health problem, a significant contributor to malnutrition,  
and associated with one-fourth of all deaths in children less than 5 years, especially  
10 infants, in developing countries. Each year, diarrhoea causes more than 1 billion  
episodes of illness, with a global average of 3 episodes per child and nearly 5 million  
deaths worldwide.

Current recommendations state that management of acute diarrhoea includes  
replacement of fluid and electrolytes loss along with nutritional support, especially in  
15 infants. But this treatment does not shorten the duration of diarrhoea. Options to  
shorten the duration of diarrhoea include the use of antibiotics, anti-motility and anti-  
secretory drugs. But this is not always effective and is often followed by serious side  
effects. There is general consensus that pharmacologic agents are unnecessary in  
treatment of acute gastroenteritis in infants.

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Probiotics, especially lactobacillus species, have been used as prophylaxis as well as  
therapy to hasten the curing of diarrhoea.

EP 0904784 relates to a nutritional preparation for the prevention and treatment of  
disorders of the gastrointestinal tract, comprising a *Bifidobacterium*, *Enterococcus*  
25 *faecium* and a *Lactobacillus* strain, and preferably containing prebiotic compounds.  
The preparation can be in the form of a food supplement, a ready-to-use food  
composition, an infant formula or a tube feeding, optionally comprising fiber.

WO 2006/046871 relates to compositions comprising *L. rhamnosus* and optionally  
fibers for the treatment and/or prevention of sepsis, bacteraemia and/or endotoxaemia.

30 US 6,241,983 relates to compositions for promoting gastrointestinal health containing  
probiotics and dietary fiber. Preferred beneficial human intestinal microorganisms  
include lactobacilli and bifidobacteria.

WO 2006/110406 relates to compositions comprising a probiotic component and a  
sweetener component.

WO 2005/039319 relates to compositions comprising mixtures of non-digestible oligosaccharides and *Bifidobacterium breve*.

WO 2006/091103 relates to compositions comprising *Bifidobacterium breve*, two non-digestible oligosaccharides and optionally *Lactobacillus paracasei*.

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#### SUMMARY OF THE INVENTION

The inventors have found that a composition comprising *L. rhamnosus* in combination with inulin and soy polysaccharides (soy PS) has a beneficial effect on shortening of the duration of diarrhoea at least similar to that reported for *L. rhamnosus* alone, while  
10 at the same time restoring the microbiota by offering fermentable substrates to the colon, restoring mucosal atrophy caused by the diarrhoea, and restoring nutritional deficits. The combination of both *L. rhamnosus* and inulin and soy PS will give more advantages in reducing diarrhoea in infants, improved survival of live bacteria in food products with, as a consequence, prolonged shelf life, an increased number of  
15 ingested bacteria reaching the colon in a viable form, stimulation in the colon of the growth and implantation of both exogenous and endogenous bacteria, and activation of the metabolism of these bacteria, and exerting an action along the small intestine and the colon.

The presence of the insoluble soy polysaccharides advantageously sequesters water  
20 from the liquid stools. A composition comprising a combination of *L. rhamnosus*, inulin and soy polysaccharides advantageously combines the shortening of diarrhoea effects, such as stimulation of the immune system, improved survival of the probiotic strain, improvement of the microbiota, anti-pathogenic effects, improved mineral retention, improved water retention, improved intestinal wall repair and/or restoration  
25 of nutritional deficits. The effects exceed the effects of the single components.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method for treatment and/or prevention of diarrhoea in a human subject, in particular acute diarrhoea, said method comprising  
30 administering a composition comprising *Lactobacillus rhamnosus*, inulin and soy polysaccharides to said human subject. In other words the present invention relates to the use of a composition comprising *Lactobacillus rhamnosus*, inulin and soy polysaccharides for the manufacture of a nutritional composition for treatment and/or prevention of diarrhoea in a human subject. The present invention can also be worded

as a composition comprising *Lactobacillus rhamnosus*, inulin and soy polysaccharides for treatment and/or prevention of diarrhea, in particular acute diarrhoea, in a human subject.

5 *Lactobacillus rhamnosus*

The present composition comprises *Lactobacillus rhamnosus*. Of the lactobacilli species present in the human intestines this species is one of the most abundant and the strains belonging to this species have been found effective in prevention and/or treatment of diarrhoea, including acute diarrhoea in infants. The mechanism of  
10 prevention or treatment of diarrhoea is alleged to involve stimulation of the immune system, improvement of the microbiota and/or direct anti-pathogenic effects. Preferably the present *Lactobacillus rhamnosus* has at least 95, more preferably at least 97, even more preferably at least 99 % identity of the 16S rRNA sequence when compared to the type strain of *L. rhamnosus* ATCC 7469 (Stackebrandt & Goebel,  
15 1994, Int. J. Syst. Bacteriol. 44:846-849). *L. rhamnosus* exerts its anti-diarrhoeal effect in the small intestine as well as in the colon. *L. rhamnosus* is preferred over the use of bifidobacteria, since it exerts its action also in the small intestine, the location where the infections causing diarrhoea often takes place, whereas bifidobacteria are active in the large intestine only. So, using *L. rhamnosus* instead of bifidobacteria has  
20 the advantage that the diarrhoea is also contested in the small intestine. Of the lactobacilli species *L. rhamnosus* is preferred, since strains belonging to this species are the most abundant in the intestine, the most robust and the most effective against diarrhoea.

25 Preferred *L. rhamnosus* strains are those isolated from the faeces of healthy human milk-fed infants. Several *L. rhamnosus* strains are commercially available from producers of lactic acid bacteria, but they can also be directly isolated from faeces, identified, characterized and produced. According to a preferred embodiment, the present composition contains at least one *L. rhamnosus* selected from the group  
30 consisting of *L. rhamnosus* LCS-742 (Morinaga), *L. rhamnosus* LR-35 (Chr. Hansen), *L. rhamnosus* GG (Valio), *L. rhamnosus* HN001 (Howaru), *L. rhamnosus* R1039, R0011, R0049 (Lallemand), *L. rhamnosus* LBA 12.5U, LR-32 (Rhodia), *L. rhamnosus* 271 231 (Probi AB), *L. rhamnosus* KCl (DSM), *L. rhamnosus* LMG P-22799. More preferably, the *L. rhamnosus* is the strain *Lactobacillus* GG (ATCC

53103). More preferably the *L. rhamnosus* is the strain LMG P-22799. *L. rhamnosus* strain LMG P-22799 is a strain showing good technological properties, not unwanted antibiotic resistances, a high survival in the gastro-intestinal tract, good adherence to intestinal cells and good prevention of adhesion of intestinal pathogens to intestinal cells.

The present composition preferably comprises  $10^2$  to  $10^{12}$  colony forming units (cfu) of *L. rhamnosus* per gram dry weight of the present composition, preferably  $10^4$  to  $10^{11}$ , more preferably  $10^5$  to  $10^{10}$ , most preferably from  $10^5$  to  $5 \times 10^9$ . Per 100 ml ready to use composition the present composition preferably comprises  $10^3$  to  $10^{13}$  cfu of the *L. rhamnosus*, preferably  $10^4$  to  $10^{12}$ , more preferably  $10^5$  to  $10^{11}$ , most preferably from  $10^6$  to  $5 \times 10^{10}$  cfu. The dose of *L. rhamnosus* according to the present invention is preferably administered at a daily dose of  $10^2$  to  $10^{13}$ , more preferably from  $10^5$  to  $10^{11}$ , most preferably from  $10^7$  to  $5 \times 10^{10}$  cfu.

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#### Inulin

The present composition comprises inulin. Inulin is a water soluble fiber which can be fermented by the human microbiota. Inulin is herein defined as a non-digestible oligosaccharide comprising a chain of  $\beta$  linked fructose units with a degree of polymerization (DP) of 2 to 250, with over 90% of the fructose units linked with a beta (2 $\rightarrow$ 1) linkage. The fructose chain may or may not be terminated by a glucose unit. Inulin can thus be described as GF<sub>n</sub> or F<sub>n</sub> wherein G represents a glucosyl unit, F represents a fructosyl unit and n is the number of fructosyl units linked to each other, n being 2 or more. Preferably, the average DP of the inulin used in the present invention is 7 or more. The average DP is preferably at least 10, more preferably at least 20. A suitable inulin is for example the commercially available as Raftiline ST, RaftilineHP (Orafti) or Frutafit (Sensus). The use of inulin with an average DP above 7, more preferably 10, even more preferably 20 decreases the osmotic load in the intestine compared to inulin with a smaller average DP, thereby preventing deleterious laxative effects, which are especially unwelcome when suffering from diarrhoea.

The presence of inulin selectively stimulates the growth and/or activity of intestinal bacteria, especially of lactobacilli. This stimulation especially occurs in the colon. Thereby advantageously the pH is lowered and organic acids such as lactate and short

chain fatty acids are formed (including especially butyrate) which have antipathogenic effects (including pathogens causing diarrhoea), and serve as a fuel for the enterocytes (thereby restoring the intestinal wall), and which aid in mineral retention which is especially advantageous during diarrhoea. Additionally inulin stimulates the growth,  
5 survival and/or metabolic activity of the probiotic strain *L. rhamnosus* along the intestinal tract. This includes not only the large intestine, but also advantageously the small intestine.

The present nutritional composition preferably comprises at least 0.5 mg of inulin per  
10 g dry weight of the composition, preferably at least 1.5 mg, more preferably at least 5 mg, even more preferably at least 10 mg. Preferably, the present composition does not comprise more than 0.5 g inulin per g dry weight of the composition, more preferably not more than 0.35 g, even more preferably not more than 0.2 g, most preferably not more than 0.1 g. The present nutritional composition preferably comprises at least 5  
15 mg of inulin per 100 ml of the present liquid composition, preferably at least 15 mg, more preferably at least 50 mg per 100 ml, even more preferably at least 100 mg. Preferably, the present composition does not comprise more than 10 g inulin per 100 ml, more preferably not more than 5 g per 100 ml, even more preferably not more than 2 g per 100 ml, most preferably not more than 1 g per 100 ml. The present inulin  
20 is preferably administered in a daily dose of 0.1 to 10 g, more preferably 0.2 to 5 g, more preferably 0.5 to 3 g.

#### Soy polysaccharides

The present composition comprises soy polysaccharides. In the present invention the  
25 term soy polysaccharide (PS) and soy fibre can be used interchangeably and are defined as the non-starch, largely insoluble polysaccharides isolated from soy bean. When referring to soy polysaccharides in the present invention, polysaccharides are defined as saccharides with a DP above 10, more preferably above 20, more preferably above 50, even more preferably above 100. A suitable source for soy fibre  
30 is Fibrim (Solae). Soy polysaccharides contain both cellulosic and non-cellulosic dietary fiber. Typically commercially available sources of soy polysaccharides suitable for the present invention comprise, based on the official AOAC method, 72-78% total dietary fiber and the fiber component consists of about 94-95 % water

insoluble fiber (cellulose and hemicellulose) and about 5-6 % water soluble fiber (arabinogalactan like polysaccharides).

The presence of the insoluble soy polysaccharides advantageously sequesters water from the liquid stools. Furthermore, the presence of soy PS advantageously results in the formation of butyrate. Butyrate is a fuel for enterocytes and helps in repairing intestinal damage caused by the inflammatory reactions against the pathogens causing diarrhoea.

The present nutritional composition preferably comprises at least 0.8 mg of soy PS per g dry weight of the composition, preferably at least 2.5 mg, more preferably at least 8 mg, even more preferably at least 15 mg per g dry weight. Preferably, the present composition does not contain more than 0.8 g soy PS per g dry weight of the composition, more preferably not more than 0.6 g, even more preferably not more than 0.35 g, most preferably not more than 0.15 g per g dry weight. The present nutritional composition preferably comprises at least 8 mg of soy PS per 100 ml of the present liquid composition, preferably at least 25 mg, more preferably at least 80 mg per 100 ml, even more preferably at least 150 mg per 100 ml. Preferably, the present composition does not contain more than 15 g soy PS 100 ml, more preferably not more than 8 g per 100 ml, even more preferably not more than 3 g per 100 ml, most preferably not more than 1.5 g per 100 ml. The present soy PS is preferably administered in a daily dose of 0.15 to 15 g, more preferably 0.3 to 8 g, more preferably 0.8 to 5 g.

Preferably the weight ratio soy PS : inulin is between 5 : 0.2, more preferably between 3 : 0.5, even more preferably between 2 : 1. A balanced ratio between inulin and soy PS is advantageous since it ensures a proper ratio of soluble and insoluble fibers and their different beneficial effects on diarrhoea. For the sake of clarity it is noted that "weight ratio soy PS : inulin is between 5 : 0.2" means that the weight of soy PS divided by the weight of inulin gives a number that lies between 5 and 0.2.

A composition comprising a combination of *L. rhamnosus*, inulin and soy polysaccharides advantageously combines the shortening of diarrhoea effects, such as stimulation of the immune system, improved survival of the probiotic strain, improvement of the microbiota, anti-pathogenic effects, improved mineral retention, improved water retention, improved intestinal wall repair and/or restoration of

nutritional deficits, along the entire intestinal tract. The effects are assumed to exceed the effects of the single components. Especially the combination of *L. rhamnosus*, soy PS and inulin results in an increased formation of short chain fatty acids, including butyrate, and lactate, and an improved action in the small intestine. Preferably the composition comprises  $10^4$  to  $10^{12}$  colony forming units (cfu) of *L. rhamnosus* per gram soy PS plus inulin, preferably  $10^6$  to  $10^{11}$ , more preferably  $10^7$  to  $10^{10}$ , most preferably from  $10^8$  to  $5 \times 10^9$  cfu.

#### Micronutrients

10 The present composition preferably comprises zinc. Zinc deficiency is highly prevalent in children in developing countries. Zinc supplements given during diarrhoea reduce the duration and severity of treated episodes. During diarrhoea generally an overproduction of nitric oxide (NO) occurs, which induces secretion and cellular damage as a free radical. Addition of zinc may contribute to improving the physiologic status of the small intestine and potentially reduce the risks of recurrent diarrhoea episodes. Preferably the composition comprises at least 35  $\mu\text{g}$  zinc per g dry weight of the composition, more preferably at least 60  $\mu\text{g}$ . Preferably the composition does not comprise more than 250  $\mu\text{g}$  zinc per g dry weight of the composition.

The present composition preferably comprises iron to replace iron loss. Furthermore, iron reduces the duration of diarrhoea. Preferably the composition comprises at least 40  $\mu\text{g}$  per g dry weight, more preferably at least 80  $\mu\text{g}$ . Preferably the composition does not comprise more than 250  $\mu\text{g}$  iron per g dry weight of the composition.

Preferably the composition comprises both zinc and iron, since a simultaneous supplementation of zinc and iron was found to reduce the risk of severe diarrhoea. Preferably the weight ratio of iron to zinc is between 0.8 and 1.3. A balanced ratio of zinc and iron ensures a proper absorption of both zinc and iron.

#### Nutritional composition

The present composition is particularly suitable for providing the daily nutritional requirements to an infant with the age below 36 months, particularly an infant with the age below 24 months, even more preferably an infant with the age below 12 months. Hence, the present composition preferably comprises a lipid, protein and digestible carbohydrate component wherein the lipid component provides 20 to 55%

of the total calories, the protein component provides 5 to 15% of the total calories and the digestible carbohydrate component provides 30 to 75% of the total calories. Preferably the present composition comprises a lipid component providing 25 to 50 % of the total calories, a protein component providing 6 to 13% of the total calories and  
5 a digestible carbohydrate component providing 40 to 65% of the total calories.

When in liquid form, e.g. as a ready-to-feed liquid, the composition preferably comprises 1.0 to 6.5 g fat per 100 ml, more preferably 1.8 to 4.0 g per 100 ml. Based on dry weight the present composition preferably comprises 8 to 40 wt.% fat, more  
10 preferably 12 to 30 wt.%. The amount of saturated fatty acids is preferably below 58 wt.% based on total fatty acids, more preferably below 45 wt.%. The concentration of monounsaturated fatty acids preferably ranges from 17 to 60% based on weight of total fatty acids. The concentration of polyunsaturated fatty acids preferably ranges from 20 to 60% based on weight of total fatty acids. Preferably the composition  
15 comprises the n-6 polyunsaturated fatty acid linoleic acid (LA) and the n-3 polyunsaturated fatty acid  $\alpha$ -linolenic acid (ALA). Preferably the weight ratio LA/ALA is between 4 and 10 more preferable between 5 and 7.

Preferably the composition comprises long chain poly-unsaturated fatty acids, such as arachidonic acid, docosahexaenoic acid and/or eicosapentaenoic acid. Preferably n-3  
20 LC-PUFA are present, such as EPA and/or DHA. n3-LC-PUFA reduce intestinal inflammatory responses, which is especially advantageous in infants suffering from diarrhoea.

When in liquid form, e.g. as a ready-to-feed liquid, the composition preferably  
25 comprises 0.5 to 4 g protein per 100 ml, more preferably 1.0 to 3.0 g per 100 ml. Based on dry weight the present composition preferably comprises 5 to 30 wt.% protein, more preferably 10 to 20 wt.%. The present composition preferably comprises at least 50 wt.% protein derived from non-human milk based on total protein, more preferably at least 90 wt.%. Preferably the present composition  
30 comprises at least 50 wt.% cow milk derived protein based on total protein, more preferably at least 90 wt.%. The present composition preferably comprises casein and/or whey proteins. Preferably the weight ratio casein:whey protein is 0:100 to 100:0, more preferably 10:90 to 90:10, more preferably 20:80 to 80:20. The term

protein as used in the present invention refers to the sum of proteins, peptides and free amino acids. The composition may optionally comprise hydrolysed proteins and/or free amino acids. Preferably the composition does not comprise hydrolysed proteins and/or free amino acids, since this increases the osmotic load of the composition  
5 which is undesirable for human subjects suffering from diarrhoea.

When in liquid form, e.g. as a ready-to-feed liquid, the composition preferably comprises 2 to 30 g digestible carbohydrates per 100 ml, more preferably 5 to 15 g per 100 ml. Based on dry weight the present composition preferably comprises 40 to  
10 80 wt. % digestible carbohydrate, more preferably 50 to 70 wt.%. Preferably the composition comprises at least one digestible carbohydrate selected from the group consisting of lactose, maltodextrin, starch, saccharose, glucose, and maltose. Preferably the present composition comprises maltodextrin and/or starch. Using digestible carbohydrates with a higher degree of polymerization instead of mono- and  
15 disaccharides reduces the osmotic load, which is advantageous in human subjects suffering from diarrhoea.

The present composition preferably comprises rice starch. Rice-based oral rehydration solutions (ORS) have found to be more effective in decreasing stool output, reducing duration of diarrhoea, and improving intestinal absorption of fluid and electrolytes in  
20 children with acute infectious diarrhoea compared with glucose-based ORS. The benefits of rice-based ORS have previously been attributed to its lower osmotic load, faster absorption, and higher carbohydrate content. Another explanation for the reduction of stool output and the improvement in absorption with rice-based ORS may be the ability of rice to inhibit intestinal excretion.

25 The present composition preferably comprises pectin or pectin degradation products. Pectin and/or its degradation products advantageously inhibit the adhesion of pathogenic micro-organisms causing diarrhoea to the intestinal wall, thereby reducing diarrhoea.

Preferably the composition comprises galacto-oligosaccharides (GOS), more  
30 preferably  $\beta$ -linked GOS. These  $\beta$ -linked GOS beneficially reduce the adherence of diarrhea causing bacteria to intestinal cells. Preferably the composition comprises 10 to 200 mg  $\beta$ -linked GOS per g dry weight, more preferably 20 to 90 mg. A suitable source of  $\beta$ -linked GOS is Vivinal (Borcuro).

Preferably the composition comprises nucleotides. Dietary nucleotides advantageously improve the repair of the intestinal cells, damaged by the pathogens causing diarrhoea.

- 5 The present composition is preferably administered in liquid form. In order to meet the caloric requirements of the infant, the composition preferably comprises 50 to 200 kcal/100 ml liquid, more preferably 60 to 90 kcal/100 ml liquid, even more preferably 60 to 75 kcal/100 ml liquid. This caloric density ensures an optimal ratio between water and calorie consumption. The osmolarity of the present composition is  
10 preferably between 150 and 420 mOsmol/l, more preferably 260 to 320 mOsmol/l. This osmolarity is of utmost importance for infants suffering from diarrhoea and aids to reduce the gastrointestinal stress and to decrease diarrhoea.

- Preferably the composition is in a liquid form, with a viscosity below 35 cps as  
15 measured in a Brookfield viscometer at 20°C at a shear rate of 100 s<sup>-1</sup>. Suitably, the composition is in a powdered form, which can be reconstituted with water to form a liquid, or in a liquid concentrate form, which should be diluted with water. When the composition is a liquid form, the preferred volume administered on a daily basis is in the range of about 80 to 2500 ml, more preferably about 450 to 1000 ml per day.  
20 Suitably the composition is reconstituted with water to form a semi-liquid, spoonable composition.

- In a preferred embodiment, the composition is a rehydration solution comprising *L. rhamnosus*, inulin, soy PS, glucose and sodium, potassium, chloride with an  
25 osmolarity between 200 and 320 mOsmol/l, more preferably between 230 and 285 mOsmol/l, most preferably between 235 and 245 mOsmol/l.

#### Application

- The composition according to the present invention has been found to be particularly  
30 useful as an infant nutrition, in particular for prematurely born babies, term born babies, as well as infants which are in the weaning period to solid food. Hence the present invention provides a method for providing nutrition to a human infant, said method comprising administering to the infant the present composition. Preferably the

infant has an age between 0 and 36 months, even more preferably between 0 and 18 months, most preferably between 0 and 12 months. The present composition can be advantageously used in the manufacture of a medicament for use in the prevention and/or treatment of diarrhoea, in particular infectious diarrhoea, more particular rotavirus diarrhoea. Preferably the composition comprising *L. rhamnosus*, inulin and soy PS is used to treat acute diarrhoea.

In a preferred embodiment the combination of *L. rhamnosus*, inulin and soy PS is present in an oral rehydration solution further comprising glucose and electrolytes in a first rehydration step to restore water and electrolyte balance, while in the mean time immediately establishing the presence of *L. rhamnosus*, inulin and soy PS in the gastro-intestinal tract.

The present composition is preferably used after a first rehydration step of about 3 to 4 h with a glucose-electrolyte mixture to restore the water and electrolyte balance. The combination of *L. rhamnosus*, inulin, soy PS, together with a lipid component, a digestible carbohydrate component and a protein component quickly restores normal stool output and consistency, while at the same time providing nutrition for growth and restoration of especially the small intestinal wall. Preferably the present composition is used for 2-10, more preferably 2-5 days until the diarrhoea has resolved.

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

30

## EXAMPLES

Example 1: Nutritional supplementation with a mixture of *L. rhamnosus*, inulin and soy polysaccharides and micronutrients to reduce the duration of acute infantile diarrhoea

## Methods:

A randomized, double blind clinical trial was conducted to assess the efficacy of an infant formula containing *Lactobacillus rhamnosus* (probiotic) and dietary fiber (inulin and soy polysaccharides) for management of 58 Indonesian well-nourished male infants aged 3-12 months suffering from acute diarrhoea with moderate dehydration. After adequate oral rehydration, the patients were randomly assigned to receive either a low lactose infant formula containing a combination of *Lactobacillus rhamnosus* ( $5 \times 10^8$  per 100 ml), dietary fiber (0.15 g inulin/100 ml and 0.25 g soy polysaccharides/100 ml) and enhanced concentration of a number of particular micronutrients (1.1 mg iron/100 ml, 0.9 mg zinc/100ml, 0.05 mg copper/100 ml, 7  $\mu$ g manganese/100 ml, 1.0  $\mu$ g selenium/100 ml, 1.9 mg vitamin D3/100ml and 1.1  $\mu$ g vitamin E/100 ml) (study group) or a low lactose infant formula without *Lactobacillus rhamnosus* or dietary fiber (control group) and with standard micronutrient concentrations (0.5 mg iron/100 ml, 0.5 mg zinc/100ml, 0.04 mg copper/100 ml, 1.4 mg vitamin D3/100ml and 0.8  $\mu$ g vitamin E/100 ml) (control group). The following data were recorded daily for each infant: stool volume, stool frequency and consistency, and accurate body weight measurement. The duration of diarrhoea was defined as the number of hours after admission until excretion of the last liquid or semiliquid stool that is not followed by another abnormal stool within 24 hours.

## Results:

The patients in the two groups were comparable on admission with respect to age, anthropometric indicators and nutritional status, clinical features, medication history and laboratory values that could be associated with diarrhoeal morbidity. The mean (SD) age was 8.1 (2.6) months in the study group and 8.0 (2.7) months in the control group. On admission, the patients had moderate dehydration status. No differences were detected between study and control groups in the isolation of pathogens.

Rotavirus was the most prevalent etiological factor in 76% of the cases, and it was found equally in the study and control groups.

The duration of diarrhoea was significantly shorter in the study group than in the control group (1.63 versus 2.45 days;  $p < 0.05$ ; for the study and control group respectively), and this became obvious after the first day of treatment. The present study supports the evidence that an anti-diarrhoeal formula containing probiotics, prebiotics and micronutrients after oral rehydration has a beneficial effect in shortening the duration of infantile diarrhoea in developing countries. There was a trend towards lower stool weight in the study group compared to the control group. The study group tended to have lower stool output (in grams per kilogram body weight per day) after dietary treatment compared to the control group, already during the first and second day after rehydration in the hospital.

Example 2: Synergistic effect of soy PS, inulin and *Lactobacillus rhamnosus*.

15 Materials:

An *in vitro* semi-dynamic batch fermentation system was used, using toddler faeces.

As a source of Soy PS Fibrin (The Solae Company) was used. As a source of inulin RaftilinST (Orafti) was used, As a source of *L. rhamnosus* L GG (Valio) was used.

Fresh faeces was obtained from healthy toddlers with an age of 2 years.

20 Experimental medium was McBain & MacFarlane medium (Buffered peptone water 3.0 g/l, yeast extract 2.5 g/l, Tryptone 3.0 g/l, L-Cysteine-HCl 0.4 g/l, bile salts 0.05 g/l,  $K_2HPO_4 \cdot 3H_2O$  2.6 g/l,  $NaHCO_3$  0.2 g/l, NaCl 4.5 g/l,  $MgSO_4 \cdot 7H_2O$  0.5 g/l,  $CaCl_2$  0.228 g/l,  $FeSO_4$  0.005 g/l).

25 Methods:

Fresh faecal material was mixed with McBain & MacFarlane medium, which is representative for the intestinal environment, in a weight ratio of 1:5.

At  $t = 0$ , 6 ml of the faecal suspension was mixed with fibres and/or L GG and transferred into a dialysis tube in a 100 ml bottle filled with buffered dialysis medium (30  $K_2HPO_4 \cdot 3H_2O$  2.6 g/l,  $NaHCO_3$  0.2 g/l, NaCl 4.5 g/l,  $MgSO_4 \cdot 7H_2O$  0.5 g/l,  $CaCl_2$  0.228 g/l,  $FeSO_4 \cdot 7H_2O$  0.005 g/l, pH 6.3). The bottle was closed and incubated at 37 °C. The mixtures of fibres and L GG were as follows:

Mixture:	1	2	3	4	5	6
Soy PS	0	192mg	0	0	120mg	120mg
Inulin	0	0	192mg	0	72mg	72mg
L GG	0	0	0	1.2x10 <sup>9</sup> cfu	0	1.2x10 <sup>9</sup> cfu

5

Samples of 0.5 ml were taken from the dialysis tube and from the dialysis buffer with a hypodermic syringe after 48 h and stored at -18 °C. Experiments were performed in duplicate and all handlings were performed in an anaerobic cabinet.

10 Lactate was determined enzymatically, using a lactic acid detection kit with D- and L-lactate-dehydrogenase (Raisio Diagnostics Spa, Rome, Italy). Short chain fatty acids were detected with gas chromatography: SCFA were extracted in MilliQ. 2-ethylbutyrate was used as an internal standard. Samples were analyzed on a capillary column (Restek Stabilwax DA 15m x 0.53 x 1.0 µm; ZB-FFAB 15mx0.13x1.0µm) with FID detector. The mobile phase was helium.

15 Results and Conclusions:

The results are expressed as amounts of mmol lactate or SCFA formed per g of fibre and shown in Table 1.

20 Table 1 Organic acids (in mmol/g fibre) formed upon fermentation of different fibre mixtures.

Mixture	1	2	3	4	5	6
Lactate	0.45	1.19	6.22	0.19	1.30	1.88
SCFA	7.11	19.09	25.42	7.01	21.80	23.53
Acetate	3.18	10.65	17.59	3.21	11.81	13.56
Propionate	1.22	2.62	0.62	1.10	2.89	2.84
Butyrate	0.26	0.21	0.01	0.21	0.84	0.30

25 These results demonstrate that *L. rhamnosus* alone does not improve the intestinal fermentation. A combination of soy PS with inulin shows a higher lactate and SCFA formation than when using soy PS alone. The presence of *L. rhamnosus* further enhances the lactate and SCFA formation of the soy PS and inulin mixture. When using inulin alone, a high lactate and SCFA formation is observed. However, almost no butyrate is formed. Having inulin present in a mixture together with soy PS or soy

PS and *L. rhamnosus* advantageously improves the formation of butyrate. Therefore a mixture of soy PS, inulin and *L. rhamnosus* shows the best fermentation characteristics for children suffering from diarrhoea.

5 Example 3: Infant nutrition

Infant nutrition comprising:

- a vegetable fat component providing 27% of the total calories, with 40% polyunsaturated fatty acids based on total fatty acids and a wt. ratio LA/ALA of 5.5.
- 10 - a cow's milk protein component providing 12% of the total calories consisting of 20 wt.% whey protein and 80 wt.% casein, and
- a digestible carbohydrate component providing 61% of the total calories, comprising over 80 wt.% of maltodextrin and starch.
- *L. rhamnosus*:  $5 \cdot 10^8$  cfu per 100 ml.
- 15 - Inulin: 0.15 g per 100 ml
- Soy polysaccharides 0.25 per 100 ml

The label of the package of this infant nutrition indicates that the nutrition is to be used in case of diarrhoea.

20 Example 4:

Oral rehydration solution comprising per 100 ml:

2.0 g glucose, 0.2 g citrate, 138 mg sodium, 78 mg potassium, 177 mg chloride,  $1 \cdot 10^8$  cfu *L. rhamnosus*, 0.05 g inulin, 0.10 g soy PS.

## CLAIMS

1. Use of a composition comprising *Lactobacillus rhamnosus*, inulin and soy polysaccharides for the manufacture of a nutritional composition for treatment and/or prevention of diarrhoea in a human subject.  
5
2. Use according to claim 1 wherein the the weight ratio soy PS : inulin is between 5 to 0.2.
- 10 3. Use according to any of the preceding claims wherein the composition further comprises a fat component providing 20 to 55% of the total calories, a protein component providing 5 to 15% of the total calories and a digestible carbohydrate component providing 30 to 75% of the total calories.
- 15 4. Use according to any of the preceding claims wherein the human subject has an age below 36 months.
5. Use according to any of the preceding claims wherein the composition comprises  $10^4$  to  $10^{11}$  cfu *L. rhamnosus* per g dry weight of the composition.  
20
6. Use according to any of the preceding claims wherein the *L. rhamnosus* is *L. rhamnosus* strain LMG P-22799.
7. Use according to any of the preceding claims wherein the composition comprises  
25 1.5 to 350 mg inulin per g dry weight of the composition.
8. Use according to any of the preceding claims wherein the composition comprises 2.5 to 600 mg soy polysaccharides per g dry weight of the composition.
- 30 9. Use according to any of the preceding claims wherein the composition further comprises at least 35  $\mu\text{g}$  zinc and/or 40  $\mu\text{g}$  iron per g dry weight of the composition.

10 Use according to any of claims 3-9 wherein the digestible carbohydrate component comprises maltodextrin and/or starch and wherein the protein component comprises non-hydrolysed proteins.

5 11 Use according to any of the preceding for the treatment of acute diarrhoea.

12 Use according to any of the preceding claims for the treatment of rotavirus diarrhoea.

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/NL2008/050454

**A. CLASSIFICATION OF SUBJECT MATTER**  
 INV. A23L1/30      A23L1/308      A61K31/7024      A61K31/715      A61K35/74

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, WPI Data, FSTA, BIOSIS

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2005/039319 A (NUTRICIA NV [NL]; SPEELMANS GELSKE [NL]; KNOL JAN [NL]; HAARMAN MONIQU) 6 May 2005 (2005-05-06) page 2, lines 1,2 page 3, line 30 page 4, lines 17-20 page 7 page 8, lines 13-23 page 10, lines 31-37 page 11, lines 3,4 page 13, lines 3,4 table 2  <div style="text-align: center;">----- -/--</div>	1-12

Further documents are listed in the continuation of Box C.

See patent family annex.

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| <ul style="list-style-type: none"> <li>* Special categories of cited documents :</li> <li>*A* document defining the general state of the art which is not considered to be of particular relevance</li> <li>*E* earlier document but published on or after the international filing date</li> <li>*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</li> <li>*O* document referring to an oral disclosure, use, exhibition or other means</li> <li>*P* document published prior to the international filing date but later than the priority date claimed</li> </ul> | <ul style="list-style-type: none"> <li>*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</li> <li>*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</li> <li>*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</li> <li>*Z* document member of the same patent family</li> </ul> |
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Date of the actual completion of the international search  <b>12 August 2008</b>	Date of mailing of the international search report  <b>06/10/2008</b>
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer  <b>de La Tour, Camille</b>
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## INTERNATIONAL SEARCH REPORT

International application No

PCT/NL2008/050454

C(Continuation), DOCUMENTS CONSIDERED TO BE RELEVANT		
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A	<p>WO 2006/091103 A (NUTRICIA NV [NL]; SPEELMANS GELSKE [NL]; KNOL JAN [NL]; HAARMAN MONIQU) 31 August 2006 (2006-08-31) abstract page 1, lines 5,11 page 2, lines 4-6,14-25 page 3, lines 28-31 page 6, lines 3-14 page 9, lines 12-16,24-32 page 10 page 11, lines 16-19 page 12 page 13, lines 4-15 page 14, lines 3,4 example 3; tables 1,4</p>	1-12
A	<p>WO 2006/046871 A (NUTRICIA NV [NL]; SPEELMANS GELSKE [NL]; VRIESEMA ADRIANUS JOHANNES MA) 4 May 2006 (2006-05-04) page 1, lines 5-9 page 2, lines 25-29 page 3, lines 15-17 page 5, lines 8-28 page 7, lines 15-17 example 6 claim 1</p>	1-12
P,A	<p>US 2007/218164 A1 (STOJANOVIC MARKO [US]) 20 September 2007 (2007-09-20) paragraphs [0001], [0015], [0019], [0024], [0025], [0027], [0030], [0032], [0039], [0040], [0056], [0059], [0061], [0069], [0071], [0084]</p>	1-10
P,A	<p>US 2007/218170 A1 (KENDRICK RONALD [CA]) 20 September 2007 (2007-09-20) paragraphs [0013], [0014], [0035] - [0038], [0043], [0052], [0073], [0085]</p>	1-10
A	<p>EP 0 904 784 A (NUTRICIA NV [NL]) 31 March 1999 (1999-03-31) cited in the application paragraphs [0004], [0008], [0011], [0012], [0016], [0018], [0029] - [0031], [0038] - [0042], [0049], [0050], [0062], [0063], [0066], [0068] paragraph [0071]; claims 1,4</p>	

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# INTERNATIONAL SEARCH REPORT

International application No

PCT/NL2008/050454

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Information on patent family members

International application No

PCT/NL2008/050454

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