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(54) **METHODS AND LIPID COMPOSITIONS FOR PROMOTING DEVELOPMENT OF GUT FLORA**

VERFAHREN UND FETTZUSAMMENSETZUNGEN ZUR FÖRDERUNG DER ENTWICKLUNG DER DARMFLORA

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Description**FIELD OF THE INVENTION**

5 **[0001]** This invention relates to the field of gut flora and in particular to products for enhancement of gut flora development and thereby, *inter alia*, enhancement of the immune system in a subject.

BACKGROUND OF THE INVENTION

10 **[0002]** As described in the art, gut flora consists of microorganisms that live in the digestive tracts of animals, and constitutes the largest reservoir of human flora. The symbiosis between the gastrointestinal tract and the large number of bacteria contributes substantially to normal digestive function. Thus, the gut flora serves as an effective barrier against opportunistic and pathogenic micro-organisms, and this 'colonization resistance' is one of their most important functions.

15 **[0003]** The normal flora presents an exceedingly complex equilibrium between the microorganisms that normally reside in the gastrointestinal tract, playing an important role in nutrition, physiology, and the regulation of the host's immune system [Bourlioux, P., et al. *Am J Clin Nutr* 78(4): 675-83,2003].

20 **[0004]** The numbers and species profile of gut flora varies greatly according to the region of the gastrointestinal tract, with the colon as the most heavily populated area. The majority of bacteria are nonsporing anaerobes, of which the numerically dominant are *Bacteroides* spp. and *Bifidobacterium* spp., *Eubacterium* spp., *Clostridium* spp., *Lactobacillus* spp., *Fusobacterium* spp. and various Gram-positive cocci. Bacteria present in lower numbers include *Enterococcus* spp., *Enterobacteriaceae*, methanogens and dissimilatory sulphate-reducing bacteria.

25 **[0005]** The importance of gut microflora is well appreciated. Flora metabolism is involved in the production of vitamins, modulation of the immune system, regulating the development of the gut, enhancement of digestion and absorption, inhibition of harmful species and removal of carcinogens and toxins and producing hormones to direct the host to store fats and in preventing the development of allergies.

30 **[0006]** Gut flora has a continuous and dynamic effect on the host's gut and the systemic immune systems. The bacteria are key components in promoting the early development of the gut's mucosal immune system in terms of both its physical components and function and continue to play a role in its operation, later in life. The bacteria stimulate the lymphoid tissue associated with the gut mucosa to produce antibodies to pathogens. The immune system recognizes and fights harmful bacteria, but does not act against the helpful/beneficiary species alone, a tolerance developed in infancy.

35 **[0007]** With respect to immunity, recent findings have shown that gut flora plays a role in the intestinal expression of Toll-like receptors (TLRs), which are a class of proteins that play a key role in the innate immune system. TLRs cause parts of the immune system to repair injury caused by radiation, for example. TLRs also provide the intestinal ability to discriminate between the pathogenic and commensal bacteria.

40 **[0008]** The human gut is sterile at birth and microbial colonization begins during delivery. The first bacteria to settle in are able to affect the immune response, making it more favorable to their own survival and less so to competing species; thus the first bacteria to colonize the gut are important in determining the person's lifelong gut flora makeup. Microflora development is then dependent on the type of feeding regime given in early life.

45 **[0009]** Breast-fed infants have a predominance of *Bifidobacteria*. In breastfed infants, the flora is not only much richer in bifidobacteria but also includes far fewer species liable to be pathogenic [Bourlioux et al., *ibid.*]. In contrast, formula-fed infants have a more complex flora which resembles that of an adult, in that *Bacteroides*, *Clostridia*, *Bifidobacteria*, *Lactobacilli*, Gram positive cocci, coliforms and other species are all represented in fairly equal proportions [Yoshioka, H., et al. *Pediatrics* 72(3): 317-21, 1983]. However, at the time of weaning there is a shift from predominantly facultative aerobic species such as *Streptococci* and *Escherichia coli* to mostly obligate anaerobic species. An age-related effect can be observed. The composition of the flora evolves over time, depending on the diet that the infants receive, until it resembles the flora of adults, at around 2 years of age, when it is thought to become fairly stable [Cummings, J. et al. *Eur J Nutr* 43 Suppl 2: II118-II173, 2004].

50 **[0010]** The gastrointestinal tract of newborns is sterile, but it becomes colonized immediately after birth with organisms from the environment, mainly from the mother. During vaginal delivery, the contact with the vaginal and intestinal flora is an important source for the start of the infant's colonization [Orrhage K & Nord CE., *Acta Paediatr.* 88: Suppl (430): 47-57, 1999]. During Cesarean delivery, direct contact of the mouth of the newborn with the vaginal and intestinal microbiota is absent, and environmental bacteria play an important role for infants' intestinal colonization. Some authors have suggested that the composition of the very first human microbiota could have long lasting effects, up to months [Grönlund MM, et al. *J Pediatr Gastroenterol Nutr.* 28: 19-25,1999] or even years [Salminen S, et al., *Gut*, 53: 1388-9, 2004]. The composition of enteric microbiota in early days of life seems therefore to be a very important factor for achieving and maintaining good health in the years to come.

55 **[0011]** Thus, there is a continuous and growing need for the development of infant formulations that are can mimic the protective effects of human milk, providing for gut microflora composition as much as possible similar to that of

breastfed infants.

[0012] Most commonly, probiotics are provided (as dietary product) in order to affect the composition of gut flora. In addition, prebiotics may be used. While probiotics are defined according to the World Health Organization (WHO), as living organisms, which when administered in adequate amounts, confer a health benefit on the host [Morais, M. B. and Jacob, C. M., *J Pediatr (Rio J)* 82(5 Suppl): S189-97, 2006], prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth of one or limited number of bacterial species already resident in the colon having a potential to improve health [Parracho, H., et al. *Proc Nutr Soc* 66(3): 405-11, 2007]. As such, any dietary component that reaches the colon intact is a potential prebiotic [Cummings et al., *ibid.*]. A prebiotic-like effect occurs when there is an increase in the activity of healthy bacteria in the human intestine. The prebiotics stimulate the growth of healthy bacteria such as bifidobacteria and lactobacilli in the gut and increase resistance to invading pathogens. Most interest in the development of prebiotics is aimed at non-digestible oligosaccharides such as fructooligosaccharides (FOS), trans-galactosylated oligosaccharide (TOS), Isomalto-oligosaccharide (IMO), xylooligosaccharides (XOS), soy-oligosaccharides (SOS), galactooligosaccharides (GOS) and lactosucrose [Cummings et al., *ibid.*].

[0013] A relatively new development in the area of flora enrichment lies in the field of synbiotics. The term synbiotics includes incorporation of a useful probiotic into an appropriate dietary vehicle with a suitable prebiotic [Cummings et al., *ibid.*].

[0014] Several patent applications describe infant formulations, many of which are based on compositions combining source of proteins, source of carbohydrates, source of lipids as well as vitamins or minerals combined with source of microorganism (probiotic) and/or of prebiotic.

[0015] WO 2010/003790 describes a nutritional composition comprising free amino acids, carbohydrate source and a lipid source and can be peptide-free or protein-free. The lipid source comprises triacylglycerides enriched with palmitic acid residue at the sn-2 position of the glycerol backbone. The composition is used for treatment of allergic infants or infants with impaired intestinal absorption, for treating, preventing or alleviating such symptoms while improving calcium absorption in the intestinal tract and/or improving the fat absorption in the intestinal tract and/or softening the stool consistency.

[0016] WO 2001/41581 (EP 1237 419) describes an infant formula comprising combinations of at least one protein component, at least one prebiotic component, at least one lipid component comprising triglycerides in which palmitic acid residues make up more than 10% (w/w) of all fatty acid residues present in the triglycerides and at least 30% of the palmitic acid residues are bonded at the sn-2 position of the triglycerol backbone.

[0017] WO 2006/019300 describes an infant nutritional composition of protein, fat, carbohydrate, nucleotide component and a negatively charged non-protein component, which mimics the protective effects of human milk particularly against allergies and infections.

[0018] WO 2008/005862 and WO2008/005032 describe infant formula comprising of fat, protein, carbohydrate, vitamins and minerals as well as on an as-fed basis: gangliosides, phospholipids, lactoferrin and sialic acid. This formulation is intended for reducing the risk of diarrhea infants, as well as producing gut microflora profile similar to that of breastfed infants.

[0019] WO 2004/112507 describes a formula intended for both infants and young children, comprising a source of proteins, a source of carbohydrates, a source of lipids including at least one long chain polyunsaturated fatty acids (LC-PUFA) and probiotics. The formula is used for strengthening natural immune system defects and promoting a healthy mental development.

[0020] WO 2004/112509 describes a nutritional composition comprising of specific fats or non-digestible oligosaccharides and at least one microorganism for inducing a pattern of gut barrier maturation similar to that observed with breastfeeding and for further improving gut barrier maturation, ensuring an optimal barrier function in infants and/or maintaining gut barrier homeostasis.

[0021] WO2006/108824 describes an infant formula comprising a source of protein, a source of lipids, a source of carbohydrates and a probiotic. The formula is used to modulate the immune system of a neonatal infant to promote the development in the first few weeks of the life of an infant of a beneficial intestinal microbiota comparable to that found in breastfed babies as well as to promote the maturation of the immune system of a neonatal infant in the first few weeks of life.

SUMMARY OF THE INVENTION

[0022] It is disclosed in accordance with a first aspect, not pertaining to the invention, a method of promoting development of gut flora in a subject, specifically beneficial gut flora, comprising administering to the subject an edible lipid composition comprising a

[0023] WO 2009/016632 describes edible fat composition, comprising triglycerides with 15-55% palmitic acid moieties out of the total fatty acids, wherein the level of palmitic acid moieties at the sn-2 position of the glycerol backbone is at least 30% of total palmitic acid, for enhancing bone strength.

[0024] WO 2009/047754 describes lipid compositions, comprising triglycerides with 23.5% palmitic acid moieties out of the total fatty acids, wherein the level of palmitic acid moieties at the sn-2 position of the glycerol backbone is 43% of total palmitic acid, for the treatment of gastrointestinal disorders and the promotion of intestinal development and maturation.

[0025] Lucas, A. et al., describe randomized controlled trial of a synthetic triglyceride milk formula for preterm infants [Archives of Disease in Childhood, BMJ Publishing, London, Vol. 77, No. 3, pages F178-F184, November 1, 1997].

[0026] Kennedy, K. et al., describe double-blind, randomized trial of a synthetic triacylglycerol in formula-fed term infants. Effects on stool biochemistry, stool characteristics and bone mineralization are specifically disclosed [The American Journal of Clinical Nutrition, American Society for Nutrition, US, [Online], Vol. 70, No. 5, pages 920-927, January 1, 1999].

[0027] Spurgeon et al., describe an investigation of the general, reproductive and postnatal developmental toxicity of the human milk fat equivalent Betapol™ [Food and Chemical Toxicology, Vol. 41, No. 10, pages 1355-1366, October 1, 2003].

[0028] Carnielli Virgilio, P et al., describe structural position and amount of palmitic acid in infant formulas. Effects on fat, fatty acid and mineral balance are specifically disclosed [Biosciences Information Service, Philadelphia, PA, US; 1996]. vegetable-derived fat source, wherein the fat source comprises triglycerides with 15-55% palmitic acid moieties out of the total fatty acids, and wherein the level of palmitic acid moieties at the sn-2 position of the glycerol backbone is at least 30% of total palmitic acid.

[0029] In a further aspect, not pertaining to the invention, it is disclosed the use of an edible lipid composition comprising a vegetable-derived fat source, wherein the fat source is a triglyceride fat source comprising triglycerides with 15-55% palmitic acid moieties out of the total fatty acids, and wherein the level of palmitic acid moieties at the sn-2 position of the glycerol backbone is at least 30% of total palmitic acid, for promoting development of gut flora in a subject, or for use in preparing a formulation for promoting development of gut flora in a subject, specifically beneficial gut flora.

[0030] The present invention provides an edible enzymatically prepared vegetable-derived fat source as claimed in any one of claims 1-12 for promoting development of gut flora in a subject, specifically beneficial gut flora, wherein the fat source is a triglyceride fat source comprising triglycerides with 15-55% palmitic acid moieties out of the total fatty acids, and wherein the level of palmitic acid moieties at the sn-2 position of the glycerol backbone is at least 30% of total palmitic acid.

[0031] In another aspect, not pertaining to the invention, it is disclosed a method of reducing the frequency and duration of crying periods in a subject, particularly an infant, comprising administering to the subject a lipid composition comprising a vegetable-derived fat source, wherein the fat source is a triglyceride fat source comprising triglycerides with 15-55% palmitic acid moieties out of the total fatty acids, and wherein the level of palmitic acid moieties at the sn-2 position of the glycerol backbone is at least 30% of total palmitic acid.

[0032] In a further aspect, not pertaining to the invention, it is disclosed the use of an edible lipid composition comprising a vegetable-derived fat source, wherein the fat source is a triglyceride fat source comprising triglycerides with 15-55% palmitic acid moieties out of the total fatty acids, and wherein the level of palmitic acid moieties at the sn-2 position of the glycerol backbone is at least 30% of total palmitic acid, for reducing the frequency and duration of crying periods in a subject, or for use in preparing a formulation for reducing the frequency and duration of crying periods in a subject.

[0033] In yet a further aspect, it is disclosed an edible vegetable-derived fat source for use in reducing the frequency and duration of crying periods in a subject, wherein the fat source is a triglyceride fat source comprising triglycerides with 15-55% palmitic acid moieties out of the total fatty acids, and wherein the level of palmitic acid moieties at the sn-2 position of the glycerol backbone is at least 30% of total palmitic acid.

[0034] In another aspect, not pertaining to the invention, it is disclosed a food article, wherein the food article comprises the vegetable-derived lipid composition (fat source) in accordance with the invention, as described above and below in connection with the aspects of promoting development of gut flora in a subject, specifically beneficial gut flora.

[0035] In another aspect, not pertaining to the invention, it is disclosed a food article, wherein the food article comprises the vegetable-derived lipid composition (fat source) in accordance with the invention, as described above and below, for use in reducing the frequency and duration of crying periods in a subject, specifically an infant.

[0036] In yet a further aspect, not pertaining to the invention, it is disclosed a commercial package comprising:

- a) an edible vegetable-derived fat source which upon enteral administration to a subject promotes development of gut flora in a subject, specifically beneficial gut flora;
- b) optionally at least one of edible physiologically acceptable protein, carbohydrate, vitamin, mineral and active or non-active additives;
- c) optionally at least one edible physiologically acceptable carrier or diluent for carrying the constituent/s defined in a) and b);
- d) means and receptacles for admixing the constituents defined in a), b) and/or c); and
- e) instructions for use.

[0037] In yet a further aspect, not pertaining to the invention, it is disclosed a commercial package comprising:

- a) an edible vegetable-derived fat source which upon enteral administration to a subject reduces the frequency and duration of crying periods in a subject, specifically an infant;
- b) optionally at least one of edible physiologically acceptable protein, carbohydrate, vitamin, mineral and active or non-active additives;
- c) optionally at least one edible physiologically acceptable carrier or diluent for carrying the constituent/s defined in a) and b);
- d) means and receptacles for admixing the constituents defined in a), b) and/or c); and
- e) instructions for use.

[0038] In some embodiments, the lipid composition defined herein provides one or more of at least the following beneficial effects:

- it has an effect on colonization of at least one pathogenic bacteria in the gut of the subject, the effect being selected from the group consisting of inhibiting, preventing and reducing colonization of the at least one pathogenic bacteria;
- it has an effect on colonization of at least one of bifidobacteria and/or lactobacilli bacteria in the gut of the subject, the effect being selected from the group consisting of enhancing, increasing and promoting colonization of the at least one of bifidobacteria and/or lactobacilli bacteria;- it has a beneficiary effect on the immune system;
- it has an effect on the development of gut flora in the gut of a subject, the effect may comprise one or more of (i) promoting development of gut flora comprising predominantly bifidobacteria and lactobacilli; (ii) increasing the abundance of bifidobacteria and lactobacilli; and (iii) reducing colonization of the at least one pathogenic bacteria;
- it has an effect on pH level in the gut of the subject, wherein the effect comprises decrease of the pH level in the gut (as determined, e.g. from a stool sample from the subject); and
- it has an effect on the crying period of the subject e.g., infant, wherein the effect may comprise reducing the number of crying periods (spells) and/or reducing the frequency, duration and/or intensity of the infant crying.

[0039] The effect on the immune system may be, but is not limited to an effect comprising one or more of (i) treatment at least one disorder of the immune system of the subject, more specifically where the at least one disorder of the immune system may result from gut flora imbalance in the subject; (ii) strengthening of the immune system of the subject; (iii) prevention (and/or reduction of incidence) of the development of immune disorders; and (iv) improving the response of the subject to vaccination.

[0040] With regard to crying, in specific embodiments the reduction effect is not related to the subjects stool characteristics.

[0041] In some embodiments, the at least one disorder of the immune system is selected from inflammation, allergy, atopy, feeding intolerance and infection and the lipid composition is effective to treat the disorder.

[0042] It is noted that in the various aspects and embodiments of the invention the subject may be a healthy subject. In such embodiments the effect of the composition according to the invention may be preventive.

BRIEF DESCRIPTION OF THE DRAWINGS

[0043] In order to understand the invention and to see how it may be carried out in practice, embodiments will now be described, by way of non-limiting example only, with reference to the accompanying drawings, in which:

Fig. 1 illustrates the effect of the invention on the beneficial bacteria count in the gut flora of the tested subjects.

DETAILED DESCRIPTION OF THE INVENTION

[0044] While the invention is described in the following detailed description with reference to a lipid composition according to the invention for promoting beneficial gut flora development in a subject, it is to be understood that also encompassed within the present disclosure is the lipid composition of the invention for promoting beneficial gut flora development in a subject as well as the remaining aspects of the invention as disclosed herein above and below.

[0045] The inventors of the instant invention have shown that infants who were fed with the lipid composition according to the invention e.g., infant formula comprising suitable structured lipid, as described herein, e.g. InFat™ as a non-limiting example, demonstrated intestinal flora profile that was similar to that of infants that were breastfed (as detailed herein below in Examples 3 and 4). Further, it was surprisingly found by the inventors that infants fed with a diet rich in palmitic acid at the sn-2 position, as in the lipid compositions employed by the present invention, experienced reduction in the number of pathogenic bacteria in the gut (as illustrated herein below in Tables 10 and 12) while the number of beneficial

bacteria in the gut increased (as illustrated in Figure 1 and in Tables 9 and 11 herein below). Furthermore, the inventors surprisingly found that infants fed with the lipid composition according to the invention showed statistically significant less crying, in intensity and duration of crying and also frequency of crying spells/periods (as illustrated for example herein below in Example 5 and in Table 13).

5 [0046] Thus, firstly, the present invention provides a lipid composition therefor, for promoting, in a subject such gut flora which is beneficial to the subject e.g., gut flora characterized by abundance of bacteria which contribute to and/or have positive effects on the digestive function.

10 [0047] It is disclosed, not pertaining to the invention, a method of promoting development of beneficial gut flora in a subject, the method comprises administering to the subject a lipid composition comprising a fat source, more specifically a vegetable oil fat source, wherein the fat source is a triglyceride, more specifically vegetable-derived triglyceride fat source comprising triglycerides with about 15 to about 55% (%w/w throughout the text, unless otherwise indicated) palmitic acid moieties out of the total fatty acids, and wherein the level of palmitic acid moieties at the sn-2 position of the glycerol backbone is at least about 30% of total palmitic acid.

15 [0048] It is disclosed, not pertaining to the invention, a method of reducing the frequency and duration of crying periods in a subject, e.g., an infant, comprising administering to the subject a lipid composition comprising a vegetable-derived fat source, wherein the fat source is a triglyceride fat source comprising triglycerides with 15-55% palmitic acid moieties out of the total fatty acids, and wherein the level of palmitic acid moieties at the sn-2 position of the glycerol backbone is at least 30% of total palmitic acid.

20 [0049] The present invention provides an edible fat source as claimed in any one of claims 1-12, more specifically vegetable-derived fat source, for promoting development of gut flora in a subject, wherein said fat source is a triglyceride fat source, more specifically vegetable-derived fat source, comprising triglycerides, more specifically vegetable-derived or vegetable-derived structured triglycerides with about 15 to about 55% palmitic acid moieties out of the total fatty acids, and wherein the level of palmitic acid moieties at the sn-2 position of the glycerol backbone is at least about 30% of total palmitic acid.

25 [0050] It is noted that as used herein, the term "palmitic acid *ratio*" means the level of palmitic acid moieties at the sn-2 position of the glycerol backbone as % of total palmitic acid in the triglyceride composition (oil). This "palmitic acid *ratio*" is also referred to herein as "*ratio*", and is specifically as defined and exemplified below.

30 [0051] In yet a further aspect, the present invention provides an edible enzymatically prepared vegetable-derived fat source according to any one of claims 1-12, wherein the fat source is a triglyceride fat source comprising triglycerides with about 15 to about 55% palmitic acid moieties out of the total fatty acids, and wherein the level of palmitic acid moieties at the sn-2 position of the glycerol backbone is at least about 30% of total palmitic acid, for promoting development of beneficial gut flora in a subject.

35 [0052] In the context of the invention, the term "***promoting development of gut flora***" is used to denote any one of enhancing, inducing, stimulating and similar effects on the construction and or generation of gut (intestine) flora in a subject. The gut flora in the context of the invention refers to bacterial gut population within at least a portion of the intestinal tract. In some embodiments the abundance of bifidobacteria and/or lactobacilli in the gut flora is increased. In some further embodiments the bacterial gut population is predominantly enriched with bifidobacteria and/or lactobacilli, meaning that bifidobacteria and/or lactobacilli are more abundant. Thus, in some embodiments the lipid composition in accordance with the invention increases the abundance of bifidobacteria and/or lactobacilli in the gut flora of the treated subject. The term "***promoting development of gut flora***" is also referred to herein as "***promoting development of beneficial gut flora***". In all embodiments and aspects of the invention the beneficial gut flora may be essentially equivalent and/or comparable to gut flora of breastfed subject.

40 [0053] In some embodiments the lipid composition is effective to promote development of gut flora comprising predominantly bifidobacteria and lactobacilli.

45 [0054] In some embodiments, the lipid composition is effective to maintain normal gut flora profile.

[0055] In yet further embodiments, the lipid composition is effective to induce the onset of gut flora thus providing an advantage e.g., when administered after birth, for example from day one, or beginning later after birth.

50 [0056] Further, in the context of the invention, it is to be understood that by promoting the development of gut flora, a favorable effect also may take place against colonization of pathogenic bacteria. Thus, the term "***promoting development of gut flora***" also denotes reducing, inhibiting and/or eliminating the colonization of pathogenic bacteria in the gut. Such pathogenic bacteria that may be affected by the presence of favorable gut flora (as compared to standard reference as discussed below) include, without being limited thereto, coliform organisms, enterobacteria, clostridia, staphylococcus, veillonella, proteus, P. aeruginosa, clostridium and different types of streptococci.

55 [0057] Further, the term "***promoting development of gut flora***" is to be understood as encompassing an effect on the gut pH level, i.e. a reduction of pH level in the gut. It is appreciated that in healthy breastfed infants the pH in the gut is typically between about 5.5 and 6.5. The pH of the gut may be determined based on stool samples obtained from the treated subject.

[0058] Yet further, the term "***promoting development of gut flora***" is to be understood as encompassing a beneficial

effect on the immune system of subject, whereby at least one or more of the following is achieved: (i) treating at least one disorder of the immune system of the subject, the at least one disorder of the immune system being as a result of gut flora imbalance in the subject; (ii) strengthening the immune system of the subject. Gut flora imbalance may be exhibited by low level of flora as well as by an imbalance in the flora population etc. as compared to the flora of a healthy breastfed infant. The disorder may be a chronic or acute disorder, and it may be a disorder involved with a reduced or weakened (immune deficiency) or, on the other hand, elevated function of the immune system (hyper-immune system). Such disorder may be selected from the group consisting of inflammation, atopy (e.g. allergy, asthma, eczema, rhinitis and atopic dermatitis), feeding intolerance and infection without being limited thereto. When referring to strengthening of the immune system it is to be understood as including induction, stimulation, enhancement and the like of a weakened immune system as well as of a healthy immune system.

[0059] In the context of the present invention the term "**treatment**" or "**treating**" and the like are used herein to refer to obtaining a desired pharmacological and physiological effect on the subject, including prophylactic in terms of "**preventing**" or partially preventing an undesired condition or symptoms from developing and/or therapeutic in terms of "**curing**" partial or complete curing of an already existing undesired condition. The term "**treating**" is used within the context of this application as treatment of subjects who are healthy and/or suffer from a disorder, disease, or impaired physiological/medical condition.

[0060] In some embodiments, the lipid composition is effective to promote beneficial gut flora development to obtain a gut flora profile that is essentially equivalent and/or comparable to pre-determined or known normal gut flora profile in a healthy breastfed infant. The normal gut flora profile is determined based on a pre-determined level from a group of healthy breastfed infants. A level that is essentially equivalent/comparable to that of a pre-determined normal profile includes deviations from the normal level of about 5%, at times, about 10% and even up to about 15% from the pre-determined level.

[0061] In some embodiments the disclosed method, not pertaining to the invention, is utilized for developing a gut flora profile that is essentially equivalent/comparable to that of a breastfed infant.

[0062] As mentioned above, in all of the various aspects and embodiments of the invention, the fat source is derived from a vegetable source.

[0063] In the various aspects and embodiments of the invention, the lipid composition may have an effect on colonization of at least one pathogenic bacteria in the gut of the subject, wherein the effect is selected from the group consisting of inhibiting, preventing and reducing colonization of the at least one pathogenic bacteria.

[0064] In the various aspects and embodiments of the invention, the lipid composition may have a beneficiary effect on the immune system of the subject. In some embodiments, the subject suffers from at least one disorder of the immune system resulting from or associated with gut flora imbalance.

[0065] In the various aspects and embodiments of the invention, the at least one disorder in the immune system is selected from inflammation, atopy, allergy, feeding intolerance and infection and the lipid composition is effective to treat or prevent or reduce the severity of the disorder. In some embodiments the atopy is selected from the group consisting of allergy, asthma, eczema, rhinitis and atopic dermatitis.

[0066] In the various aspects and embodiments of the invention, the lipid composition may promote development of gut flora abundant with bifidobacteria and/or lactobacilli. In some embodiments the promoted gut flora comprises predominantly bifidobacteria and/or lactobacillus.

[0067] In the various aspects and embodiments of the invention, the lipid composition may enhance colonization (increase the abundance) of the at least one beneficial bacterium in the gut of the subject. More specifically the bacteria may be selected from the group consisting of bifidobacteria and lactobacilli.

[0068] In the various aspects and embodiments of the invention, the lipid composition may inhibit colonization of the at least one pathogenic bacteria in the gut of the subject. In some embodiments the pathogenic bacteria is selected from the group consisting of coliform organisms, enterobacteria, clostridia, veillonella, proteus, P. aeruginosa, clostridium, staphylococcus and streptococci. In some embodiments the pathogenic bacteria are clostridium and/or staphylococcus.

[0069] The subject in accordance with a specific embodiment of the invention is a human child. Further, the term "**child**" denotes infants (from day of birth, newborn, to about 12 months i.e., about 1 year) as well as toddlers (from about one year up to about the age of 3). The infant may be pre-term infant and term infant, as well as an infant born by regular delivery, cesarean surgery (Caesarean section) as well as any other modes of delivery. The term "**newborn**" includes pre-mature infants, post-mature infants and full term newborns.

[0070] In some embodiments the disclosed method, not pertaining to the invention, comprises providing the lipid composition to the infant for a period of time from day one to weeks following birth.

[0071] In some embodiments, the child is one being diagnosed (by standard techniques) of having or susceptible of developing at least one of the following:

- an imbalanced level e.g. low level or an imbalance in the profile of gut flora population, particularly as compared to gut flora profile of breastfed infants;

- a disorder in the immune system that is associated with imbalance in level or imbalance in the profile of gut flora population;
- a disorder related or caused by a disorder of the immune system that is associated with an imbalance in the gut flora level (e.g. low flora level) or an imbalance in the profile of gut flora population. The disorder may be, without being limited thereto, inflammation, atopy (e.g. allergy, asthma, eczema, rhinitis and atopic dermatitis), feeding intolerance and infection.

[0072] Subject populations at risk for the aforementioned disorders include but are not limited to children born prematurely, infants born by Caesarean section, vegetarians, naturalists, subjects taking medicines e.g., antibiotics which may affect their gut flora, subjects with limited or deficient nutrition, subjects subjected to cancer therapy e.g., chemotherapy and/or radiation which may affect their gut flora.

[0073] Thus, in the various aspects and embodiments of the invention, the subject may be a child. The child may be an infant or a toddler. In some embodiments the infant is one delivered by a Caesarean section. In some embodiments the infant is a newborn that may be pre-term infant and term infant. In some embodiments the subject is prone to or at risk of developing an imbalanced profile of the gut flora population compared to breastfed infants. In some embodiments the subject is at risk of developing a disorder associated with imbalance in the profile of the gut flora population compared to breastfed infants. In some embodiments the subject is at risk of developing an imbalance in the profile of the gut flora population. In some embodiments the subject is formula fed and therefore at risk of developing an imbalance in the profile of the gut flora population compared to breastfed infants.

[0074] In the various aspects and embodiments of the invention, the lipid composition may be effective in promoting beneficial gut flora development, to obtain a gut flora profile that is essentially equivalent to pre-determined or known normal gut flora profile of a healthy breastfed infant.

[0075] In all aspects of the invention and embodiments the lipid composition is effective in developing a beneficial gut flora profile that is essentially equivalent or comparable to that of a breastfed infant.

[0076] In the various aspects and embodiments of the invention, the lipid composition may be provided to the infant for a period of time from day one to weeks, months, etc. following birth.

[0077] The triglycerides according to the invention may comprise saturated and/or mono-unsaturated and/or poly-unsaturated fatty acids residues.

[0078] In the various aspects and embodiments of the invention, the fatty acid residues at the sn-2 position of the glycerol backbone may be a saturated fatty acid residue, including C₈ to C₂₄, and in some particular embodiments C₁₄-C₁₈ fatty acid residues.

[0079] The saturated fatty acid may be any one of butyric acid (butanoic acid, C4:0), caproic acid (hexanoic acid, C6:0), caprylic acid (octanoic acid, C8:0), capric acid (decanoic acid, C10:0), lauric acid (dodecanoic acid, C12:0), myristic acid (tetradecanoic acid, C14:0), palmitic acid (hexadecanoic acid, C16:0), stearic acid (octadecanoic acid, C18:0), arachidic acid (eicosanoic acid, C20:0) and behenic acid (docosanoic acid C22:0).

[0080] In some specific embodiments, the saturated fatty acid residue is predominantly a palmitic acid residue.

[0081] In the various aspects and embodiments of the invention, in the vegetable-derived fat source according to the invention at least about 30%, at times, at least about 33%, at times, at least about 38%, and even, at times, at least about 40% of the total palmitic acid residues are present at the sn-2 position of the glycerol backbone.

[0082] In the various aspects and embodiments of the invention, in the vegetable-derived fat source according to the invention at least about 50%, at times, at least about 70% of the total fatty acid moieties at the sn-1 and sn-3 positions of the glycerol backbone are unsaturated.

[0083] The unsaturated fatty acid may be any one of oleic acid (C18:1), linoleic acid (C 18:2), α -linolenic acid (C18:3) and gadoleic acid (C20:1).

[0084] In the various aspects and embodiments of the invention, in the vegetable-derived fat source according to the invention at least about 35%, at times, at least about 40% of the unsaturated fatty acid moieties at the sn-1 and sn-3 positions are oleic acid moieties.

[0085] In the various aspects and embodiments of the invention, in the vegetable-derived fat source according to the invention at least about 4%, at times, at least about 6% of the unsaturated fatty acid moieties at the sn-1 and sn-3 positions are linoleic acid moieties.

[0086] In the various aspects and embodiments of the invention, the vegetable-derived fat source is characterized by having the following parameters: (i) at least 30%, at times, at least 33%, at times, at least 38%, and even at times, at least 40% of the total palmitic acid residues are at the sn-2 position of the glycerol backbone; (ii) at least 50%, at times, at least 70% of the fatty acid moieties at the sn-1 and sn-3 positions of the glycerol backbone are unsaturated; (iii) at least 35%, at times, at least 40%, of the unsaturated fatty acid moieties at the sn-1 and sn-3 positions are oleic acid moieties; and (iv) at least 4%, at times, at least 6%, of the unsaturated fatty acid moieties at the sn-1 and sn-3 positions are linoleic acid moieties.

[0087] In the various aspects and embodiments of the invention, the vegetable-derived fat source comprises triglyc-

erides with about 15% to about 40% palmitic acid moieties out of the total fatty acids. In some embodiments, the vegetable-derived fat source comprises triglycerides with about 15% to about 33% palmitic acid moieties out of the total fatty acids.

[0088] Thus, the palmitic acid content of the fat source may be 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54% or 55% of the total fatty acids.

[0089] In the various aspects and embodiments of the invention, in the vegetable-derived fat source according to the invention at least about 13% w/w, at times, at least about 15%, at times, at least about 18%, and even at times, at least about 22% of the total fatty acid residues at the sn-2 position of the glycerol backbone are palmitic acid residues.

[0090] A non-limiting example of a lipid composition or the vegetable-derived fat source according to all aspects of the invention comprises:

0%-10% - C8:0 fatty acid residue out of the total fatty acid residue content;
 0%-10% - C10:0 fatty acid residue out of the total fatty acid residue content;
 0%-22% - C12:0 fatty acid residue out of the total fatty acid residue content;
 0%-15% - C14:0 fatty acid residue out of the total fatty acid residue content;
 15%-55% - C16:0 fatty acid residue out of the total fatty acid residue content;
 1%-7% - C18:0 fatty acid residue out of the total fatty acid residue content;
 20%-75% - C18:1 fatty acid residue out of the total fatty acid residue content;
 2%-40% - C18:2 fatty acid residue out of the total fatty acid residue content; and
 0%-8% - C18:3 fatty acid residue out of the total fatty acid residue content, and
 wherein at least 30%, at times, at least 33%, and even at times, at least 40% of the C16:0 fatty acid residue out of the total fatty acid residue content is at sn-2 position the glycerol backbone.

[0091] In accordance with a more particular embodiment, the lipid composition or the vegetable-derived fat source according to all aspects of the invention comprises: 0%-2% - C8:0 fatty acid residue out of the total fatty acid residue content; 0%-2% - C10:0 fatty acid residue out of the total fatty acid residue content; 5%-15% - C12:0 fatty acid residue out of the total fatty acid residue content; 2%-10% - C14:0 fatty acid residue out of the total fatty acid residue content; 17%-25% - C16:0 fatty acid residue out of the total fatty acid residue content; 2%-5% - C18:0 fatty acid residue out of the total fatty acid residue content; 28%-48% - C 18:1 fatty acid residue out of the total fatty acid residue content; 5%-20% - C 18:2 fatty acid residue out of the total fatty acid residue content; 1%-3% - C18:3 fatty acid residue out of the total fatty acid residue content; and wherein at least 30%, at times, at least 33%, and even at times, at least 40% of the C16:0 fatty acid residue out of the total fatty acid residue content is at sn-2 position the glycerol backbone.

[0092] More specifically, the vegetable-derived fat source according to the invention comprises 0%-10% C8:0 fatty acids of the total fatty acids, preferably 0%-2%; 0%-10% C10:0 fatty acids of the total fatty acids, preferably 0%-2%; 0-22% C12:0 fatty acids of the total fatty acids, preferably 5-15%; 0-15% C14:0 fatty acids of the total fatty acids, preferably 2-10%; 15-55% C16:0 fatty acids of the total fatty acids, of which over 30% are esterified at the sn-2 position of the glycerol backbone; 1-7% C18:0 fatty acids of the total fatty acids, preferably 2-5%; 20-75% C18:1 fatty acids of the total fatty acids, preferably 28-48%; 2-40% C18:2 fatty acids of the total fatty acids, preferably 5-20%; 0-8% C18:3 fatty acids of the total fatty acids, preferably 1-3%; other fatty acids are each present in levels of less than 8% of the total fatty acids, preferably less than 5%.

[0093] Thus, the vegetable-derived fat source according to the invention may comprise: 0%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% or 10% of C8:0 fatty acids of the total fatty acids; 0%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% or 10% of C12:0 fatty acids of the total fatty acids; 0%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21% or 22% of C12:0 fatty acids of the total fatty acids; 0%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14% or 15% C14:0 fatty acids of the total fatty acids; 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54% or 55% C16:0 fatty acids of the total fatty acids; 1%, 1.2%, 1.4%, 1.6%, 1.8%, 2%, 2.2%, 2.4%, 2.6%, 2.8%, 3%, 3.2%, 3.4%, 3.6%, 3.8%, 4%, 4.2%, 4.4%, 4.6%, 4.8%, 5%, 5.2%, 5.4%, 5.6%, 5.8%, 6%, 6.2%, 6.4%, 6.6%, 6.8%, or 7% C18:0 fatty acids of the total fatty acids; 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74% or 75% C18:1 fatty acids of the total fatty acids; 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 22%, 23%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40% C18:2 fatty acids of the total fatty acids; 0%, 0.5%, 1%, 1.2%, 1.4%, 1.6%, 1.8%, 2%, 2.2%, 2.4%, 2.6%, 2.8%, 3%, 3.2%, 3.4%, 3.6%, 3.8%, 4%, 4.2%, 4.4%, 4.6%, 4.8%, 5%, 5.2%, 5.4%, 5.6%, 5.8%, 6%, 6.2%, 6.4%, 6.6%, 6.8%, 7%, 7.2%, 7.5%, 7.8% or 8% C18:3 fatty acids of the total fatty acids.

[0094] Specific vegetable-derived fat sources are described in WO05/036987 which is fully incorporated herein by

reference. These include fat concentrates (fat bases), fat blends, infant formulas comprising the concentrates/blends and other foods and food articles.

5 [0095] Of particular interest are vegetable-derived fat sources which are based on edible synthetic oils (which can be enzymatically produced), which mimic, as are, or when blended with edible vegetable oils (which may be randomized before blending) the triglyceride composition of human breast milk fat. Such fat sources have a high level of palmitic acid at the *sn*-2 position of the triglycerides, and a high level of unsaturated fatty acids at *sn*-1 and *sn*-3 positions. Oils of this type are applicant's various products marked *InFat*[™] (Enzymotec Ltd., Migdal HaEmeq, Israel). *InFat*[™] is used by applicant for a wide selection of fat bases (fat concentrates) for use, when diluted/blended with vegetable oils, in the preparation of infant formulas, dietary supplements and food articles, and also for resulting blends. Examples of *InFat*[™] compositions are shown in Table 1 below.

10 [0096] Thus, the vegetable-derived fat source used by the present invention may be a concentrate, particularly an enzymatically prepared fat base composition comprising a mixture of vegetable-derived triglycerides, with a total palmitic acid residues content of at most 38%, at times, at most 50%, of the total fatty acid residues; and with at least 50%, at times, at least 52.9%, even at times, at least 60% of the fatty acid moieties at the *sn*-2 position of the glycerol backbone being palmitic acid residues.

15 [0097] *InFat*[™] is an advanced fat-base ingredient for the production of fat preparations used in infant nutrition and in infant formulas. It is an exclusive fat base, designed and manufactured with a specific triglyceride compositions and structures, which has now been found to be efficient in promoting development of gut flora, particularly in infant populations prone to problems related to gut flora, as well as other such populations.

20 [0098] Thus, in the various aspects and embodiments of the invention, the vegetable-derived fat source is enzymatically made.

25 [0099] Further, in the various aspects and embodiments of the invention, the vegetable-derived fat source and/or at least one triglyceride of the fat source may be selected from the group consisting of naturally occurring triglycerides, synthetic triglycerides, semisynthetic triglycerides, and artificially produced triglycerides. In some further embodiments the triglyceride may be obtained from a vegetable source.

30 [0100] The vegetable-derived fat source according to the invention can also be a substitute human milk fat composition or human milk fat mimetic composition comprising a blend of at least 25% of the fat base concentrate with up to 75% of at least one vegetable oil. In some specific embodiments the fat source may comprise 25%, 30%, 36%, 50%, 52%, 60%, 63%, 73% and 83% of the fat base concentrate and 75%, 70%, 64%, 50%, 48%, 40%, 37%, 27% and 17%, respectively, of the at least one vegetable oil.

[0101] The following Examples present twelve blends, 1 to 12, wherein different amounts of the fat base concentrate (*InFat*[™]) were used, from 25% up to 83% of the content of the blend.

[0102] The vegetable oil used in the preparation of blends may be at least one of soy, palm tree, canola, coconut, palm kernel, sunflower, corn, safflower and rapeseed oil, as well as other vegetable oils and fats and mixtures thereof.

35 [0103] Thus, in the various aspects and embodiments of the invention, the vegetable-derived fat source comprises a fat base blended with a mixture of vegetable oils, wherein the mixture comprises oils selected from the group consisting of but not limited to soy, palm tree, canola, coconut, palm kernel, sunflower, corn, safflower and rapeseed oil. The vegetable oils (blending oils) may be chemically or enzymatically randomized before blending with the fat base (fat concentrate).

40 [0104] Most importantly, the vegetable-derived fat source of the present invention may be used in the preparation of infant formula. The infant formula used by the invention comprises in addition to the fat source at least one protein component and optionally at least one of carbohydrate source, vitamins, minerals, nucleotides and amino acids.

45 [0105] Thus, in the various aspects and embodiments of the invention, the infant formula comprises the vegetable-derived fat source, together with a protein source, a carbohydrate source, minerals, vitamins and optionally at least one of carrier, diluent; additive or excipient.

[0106] The terms "lipid" and "fat" are used herein synonymously.

50 [0107] The disclosed methods, not pertaining to the invention, are best practiced through administering to a subject, an infant formula or a food article prepared with and comprising the vegetable-derived fat source as described in the invention, either in the form of a concentrate base or in the form of a blend. Non-limiting examples of a fat concentrate/base are Fat Bases 1 to 11, and non-limiting examples of blends are Fat Blends 1 to 12.

[0108] Administration is usually via oral or enteral route, which may include the use of gavage feeding, with a gastric feeding tube, sonda, etc, particularly where adapted for infant feeding.

55 [0109] In another of its aspects, the present invention provides an edible vegetable-derived fat source as claimed in any of claims 1-12 for promoting development of beneficial gut flora in a subject, wherein the fat source is a triglyceride fat source comprising triglycerides with about 15 to about 55% palmitic acid moieties out of the total fatty acids, and wherein the level of palmitic acid moieties at the *sn*-2 position of the glycerol backbone is at least about 30% of total palmitic acid.

[0110] In some embodiments the vegetable-derived fat source has an effect on colonization of at least one pathogenic

bacteria in the gut of the subject, wherein the effect is selected from the group consisting of inhibiting, preventing and reducing colonization of the at least one pathogenic bacteria.

[0111] In some embodiments the vegetable-derived fat source may have an effect to the immune system of the subject.

[0112] In some embodiments the fat source has an effect on the immune system of the subject, wherein the subject suffers from at least one disorder of the immune system resulting from gut flora imbalance.

[0113] In some embodiments the at least one disorder in the immune system may be selected from inflammation, atopy, allergy, feeding intolerance and infection and the lipid composition is effective to treat the disorder.

[0114] In some embodiments the atopy may be selected from the group consisting of allergy, asthma, eczema, rhinitis and atopic dermatitis.

[0115] In some embodiments the vegetable-derived fat source promotes development of gut flora comprising predominantly bifidobacteria and lactobacilli.

[0116] In some further embodiments the vegetable-derived fat source may enhance colonization (increase the abundance) of the at least one beneficial bacterium in the gut of the subject. The bacteria may be selected from the group consisting of bifidobacteria and lactobacillus.

[0117] In some embodiments the vegetable-derived fat source inhibits colonization of the at least one pathogenic bacteria in the gut of the subject.

[0118] In some embodiments the pathogenic bacteria is selected from the group consisting of coliform organisms, enterobacteria, clostridia, veillonella, proteus, P. aeruginosa, clostridium, staphylococcus and streptococci. In some embodiments the pathogenic bacteria are clostridium and/or staphylococcus.

[0119] The lipid/fat source composition according to the invention may be formulated as or into an edible product. To this end, the lipid/fat source composition may be combined with at least one probiotic and prebiotic substance.

[0120] The edible product may be a nutritional composition, a pharmaceutical composition, a nutraceutical composition and/or a functional food. The edible product may be provided in fluid form (e.g. as a drink or beverage), as well as in a solid or semi solid form (e.g. as a porridge, or solid edible product).

[0121] The fat source according to the invention may be comprised in any one of food article and infant formula. The food article may be selected from bakery products, including bread, particularly biscuits and pastries, human milk fat substitute, dairy products, including milk and dairy drinks, ice cream, cereal products, sauces, soup, spreads, including margarine, fillings, oils and fats, soy products, meat products, fried food products, confectionery products, bars, candy bars, candies and chocolates, snacks, drinks and shakes, instant products, instant drink products, frozen food, prepared foods for infants, toddlers and young children, including prepared cooked mashed vegetables and/or fruits, condiment products, and cooking oils and fats.

[0122] Thus, in another of the aspects, not pertaining to the invention, it is disclosed a food article comprising the vegetable-derived fat source according to the invention for promoting development of beneficial gut flora in a subject, wherein said food article is selected from bakery products, including bread, particularly biscuits and pastries, human milk fat substitute, infant formula, dairy products, including milk and dairy drinks, ice cream, cereal products, sauces, soup, spreads, including margarine, fillings, oils and fats, soy products, meat products, fried food products, confectionery products, bars, candy bars, candies and chocolates, snacks, drinks and shakes, instant products, instant drink products, frozen food, prepared foods for infants, toddlers and young children and for adults, including prepared cooked mashed vegetables and/or fruits, condiment products, and cooking oils and fats.

[0123] In a further aspect, not pertaining to the invention, it is disclosed a commercial package for preparing an edible fat source or food article which is recommended for promoting development of beneficial gut flora in a subject and/or for reducing the frequency and duration of crying periods in a subject, in accordance with the invention. In addition to the active and non-active constituents, the commercial package contains instructions for use. These include terms of storage, instructions for preparation of the fat source or food article for administration, required dilutions, dosages, frequency of administration and the like. A commercial package in accordance with the invention may also contain the vegetable-derived fat source in a ready-to-use form, together with instructions for use. Dosages are usually determined according to age, weight, sex and condition of the subject, in accordance to good medical practice known to the attending physician and other medical personnel.

[0124] Thus, it is disclosed, not pertaining to the invention, a commercial package comprising

- a) a vegetable-derived fat source which upon enteral administration to a subject it promotes development of beneficial gut flora in the subject;
- b) optionally at least one of edible physiologically acceptable protein, carbohydrate, vitamin, mineral and active or non-active additive;
- c) optionally at least one edible physiologically acceptable carrier or diluent for carrying the constituent/s defined in a) and b);
- d) means and receptacles for admixing the constituents defined in a), b) and/or c); and
- e) instructions for use

[0125] In yet a further aspect, not pertaining to the invention, it is disclosed a commercial package comprising:

- a) a vegetable-derived fat source which upon enteral administration to a subject reduces the frequency and duration of crying periods in a subject, specifically an infant;
 - b) optionally at least one of edible physiologically acceptable protein, carbohydrate, vitamin, mineral and active or non-active additives;
 - c) optionally at least one edible physiologically acceptable carrier or diluent for carrying the constituent/s defined in a) and b);
 - d) means and receptacles for admixing the constituents defined in a), b) and/or c);
- and
- e) instructions for use.

[0126] In a specific embodiment of the disclosed commercial packages, the vegetable-derived fat source is a triglyceride fat source comprising triglycerides with about 15% to about 55% palmitic acid moieties out of the total fatty acids, and wherein the level of palmitic acid moieties at the *sn*-2 position of the glycerol backbone is at least 30% of total palmitic acid.

[0127] In some embodiments the lipid composition may be artificially enriched with at least one triglyceride. As used herein, the term "*artificially enriched*" is used to denote that the lipid composition, while typically originated from a natural lipid source, is subjected to at least one modification, typically an enzymatic processing step, albeit not limited thereto, that promotes enrichment of the lipids with at least one triglyceride as defined.

[0128] The natural lipid source may be any edible lipid source, preferably, a vegetable oil, including, without being limited thereto, soy oil, palm tree oil, canola oil, coconut oil, palm kernel oil, sunflower oil, corn oil, safflower and rapeseed oil.

[0129] The lipid composition is preferably provided to the subject orally, e.g. as an edible product, as discussed herein.

[0130] The disclosed methods, not pertaining to the invention, may be short-term methods as well as long-term methods. In other words, the subject, in particular, the infant, toddler or child subject, may receive a single dose of the lipid composition or an edible product comprising the lipid composition, as well as a series of doses of the lipid composition, per day, a series of doses along a period of several days, weeks, months and 1, 2, 3 or more years. It is appreciated that when the disclosed methods are conducted for a long period of time, the composition of the fat source and/or the product may vary depending on the age of the subject, as well as other considerations such as nutritional needs. Administration may commence at any time from day one after birth. Administration may also be to a breastfed subject, as supplementary feedings, or during or after weaning, or when the breastfeeding person (usually mother) is absent or unable to breastfeed.

[0131] Thus, the disclosed method, not pertaining to the invention, of promoting development of beneficial gut flora in a subject provides for maintaining an advantageous gut flora profile, for example but not limited to the profile of the gut flora of a breastfed infant, baby or toddler.

[0132] In some embodiments the triglyceride according to the invention is selected from the group consisting of naturally occurring triglycerides, synthetic triglycerides semisynthetic triglycerides, and artificially produced triglycerides, all derived from a vegetable source.

[0133] As used herein, the forms "*a*", "*an*" and "*the*" include singular as well as plural references unless the context clearly dictates otherwise. For example, the term "*a triglyceride*" includes one or more triglycerides which may form together a lipid base or a lipid blend. The term "*consisting essentially of*" is used to define the lipid composition which include the recited elements but exclude other elements, i.e. the term lipid composition is used to define a composition consisting essentially only lipids. "*Consisting of*" shall thus mean excluding more than trace elements of other elements. Embodiments defined by each of these transition terms are within the scope of this invention.

[0134] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "*comprise*", and variations such as "*comprises*" and "*comprising*", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps. Further, all numerical values, e.g. when referring the amounts or ranges of the elements constituting the various lipid compositions herein are approximations which are varied (+) or (-) by up to 20%, at times by up to 10% of the stated values. It is to be understood, even if not always explicitly stated that all numerical designations are preceded by the term "*about*".

[0135] It should be noted that where various embodiments are described by using a given range, the range is given as such merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range.

[0136] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable

sub-combination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

[0137] It is noted that features of certain embodiments of the invention which are described in detail in the context of one aspect of the invention, may be applicable in other aspects of the invention.

DESCRIPTION OF NON-LIMITING EXAMPLES

[0138] In the present description as well as in the non-limiting examples provided below reference is made to fat bases and fat blends. It is to be understood that the term "**fat base**" or "**fat concentrate**" or "**fat base concentrate**" is used to denote the enzymatically prepared lipid composition comprising a mixture of vegetable-derived triglycerides with high sn-2 palmitic acid; while the term "**fat blend**" is used to denote a lipid composition comprising a fat base and a mixture of vegetable-derived triglycerides. The fat blend is at time referred to by the term "**InFat**". As shown below, the fat blend is a fat base comprising mainly triglycerides with oleic-palmitic-oleic (OPO) fatty acids, with high total palmitic and high sn-2 palmitic acid mixed with other vegetable oils. Generally, this fat blend is used as a fat fraction in infant formulas and can be used in other baby foods such as biscuits, bar, etc.

Example 1 - Preparing fat bases and fat blends

[0139] **Table 1** details the contents of several fat bases enriched with a high content of palmitic acid at the sn-2 position. The fat bases comprise a high percentage of palmitic acid, C16:0, at the sn-2 position of triacylglycerol (TAG), and high percentage of unsaturated fatty acids at the sn-1 and sn-3 positions.

[0140] The fat bases are prepared as described in WO05/036987 which publication is fully incorporated herein by reference. Generally, a mixture of triglycerides, rich in palmitic acid (preferably above 78%) are reacted with a mixture of free fatty acids rich in oleic acid (preferably above 75%), with a low content of palmitic and stearic acids (preferably below 6%).

[0141] Briefly, the triglyceride mixture may be produced from double-fractionated palm stearin and the free fatty acids (FFA) mixture is obtained from palm kernel oil after fractionation, or from high oleic sunflower oil. The two mixtures are blended in stirred (optionally large scale) reactors with no additional solvent. To this mixture is added a suitable lipase and the mixture of triglycerides, FFA and catalyst is stirred at 50°C-60°C for about 3-9 hours, to yield the final and desired triglycerides mixture. Any excess FFAs are removed.

[0142] The triglyceride product may be further treated in order to improve color, odor and taste with bleaching and deodorization stages. Optionally, the product is fortified with natural antioxidants to increase the shelf life of the product. The catalyst can be further recycled, to be re-used in further batches.

Table 1 - Fat bases composition

Fat Base No. /fatty acid*	1	2	3	4	5	6	7	8	9	10	11
C16:0	32	29.4	29.6	32.6	32.2	30.6	29	29	30	33	30
C16:0 at sn-2 of total fatty acids at sn-2	67.2	59.7	61.3	66.1	66	62.9	53.9	55.6	59	52.9	55.8
Ratio (%) of C16:0 at sn-2 out of total C16:0	70.0	67.7	69.0	67.6	68.3	68.5	62	64	64	53.5	62
C18:0	4	4.4	4.4	4	4.1	3.8	2.6	2.6	3	3	3
C18:1	53.1	55.9	55.5	53.1	53.4	55	55.5	56	56.1	52	56.1
C18:2	8	7.8	8.2	8	7.9	8.3	9	9	8.5	10	8.5
*All numbers represent % (w/w), except the ratio which is defined as %. " C16:0 " represents the total palmitic, acid content out of total fatty acids. " C16:0 at sn-2 " represents the % palmitic acid at sn-2 out of total sn-2 positioned fatty acids. " Ratio " represents % of C16:0 at sn-2 palmitic acid out of total C16:0 [(% of C16:0 at sn-2 out of total sn-2 positioned fatty acids)/3]/(%total C16:0)]x100											

[0143] The fat bases are then used to form the fat blends which comprise also other oils. The fat base may represent from about 30% up to about 83% of the fat blends suitable for use in a formula for use in the invention. The blends comprising the fat bases of Table 1 in combination with other fats are provided in Table 2.

[0144] Specifically, **Table 2** details the contents of blends comprising one of fat bases 1, 7, 8, 9, 10 or 11. The fat

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blends are prepared by blending the selected fat base with other oils. As such, the fatty acids composition of the blends results from the fatty acids composition of both the fat base and of the other oils mixed with the fat base.

Table 2 - Fat blends composition

Fat Blend No. /fat*	Fat blend 1	Fat blend 2	Fat blend 3	Fat blend 4	Fat blend 5	Fat blend 6	Fat blend 7	Fat blend 8	Fat blend 9	Fat blend 10
C12:0	11.1	7.2	7.8	6.5	4.4	8.14	8.7	13.4	10.4	10
C14:0	4.5	3.1	3.3	2.8	2.1	2.94	3.54	5.3	4.3	4.2
C16:0	22.8	25.4	26.9	25.1	27.7	21.60	20.99	15	22.3	17
C16:0 at <i>sn</i> -2 of total fatty acids at <i>sn</i> -2	33.4	42.9	48.9	50.8	56.9	31.3	31.8	25	28.8	16
Ratio (%) <i>sn</i>-2 C16:0 of total C16:0	48.7	56.3	60.7	67.4	68.5	48.31	50.46	55	43	31.5
C18:0	2.3	3.0	3.1	3.5	4.0	2.65	2.65	2.9	4.4	3.2
C18:1	38.4	40.8	41.6	47.9	46.6	42.71	44.37	39.7	38.5	41.7
C18:2	13.5	15.6	12.8	8.6	11.7	17.96	16.43	15.3	14.0	18.2
C18:3	1.7	0.6		1.4		1.69	1.52	2	1.5	2.1
% Fat base 1	30	50	63	73	83					
% Fat base 7						60				
% Fat base 8							60			
% Fat base 9								36		
% Fat base 10									52	
% Fat base 11										25
Vegetable Oil										
Palm kernel oil						18				
Coconut oil	23	15	16	13.5	9.3		17	28	21	21
Palm oil	21	15	9							14
Sunflower		5			7.7			11		14
Corn oil	10	10	12						11	
Safflower								3		5
Rapeseed	16	5		13.5		4	6	20	16	21
Soybean						18	17			
Total	100	100	100	100	100	100	100	100	100	100
*All numbers represent % (w/w), except the ratio which is defined as %. " C16:0 " represents the total palmitic acid content of total fatty acids. " <i>C16:0 at sn-2</i> " represents the % palmitic acid at <i>sn-2</i> out of total <i>sn-2</i> positioned fatty acids. " <i>Ratio</i> " represents % of C16:0 at <i>sn-2</i> palmitic acid out of total C16:0 [(% of C16:0 at <i>sn-2</i> out of total <i>sn-2</i> positioned fatty acids)/3]/(%total C16:0)]x100										

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Table 3 - Fat blend 11 composition (with 30% fat base)

Fatty acid	% of fatty acids
C10:0	1.3
C12:0	10.3
C14:0	4.3
C16:0	23.5
C16:0 at <i>sn</i> -2 of total fatty acids at <i>sn</i> -2	30.3
Ratio (%) of C16:0 at <i>sn</i> -2 of total C16:0	43
C18:0	3.2
C18:1	39.2
C18:2	13.6
C18:3	1.7
C20:0	0.3
C20:1	0.3
C22:0	0.2
% fat base in fat blend 11	30
*All numbers represent % (w/w), except the ratio which is defined as %. " C-16:0 " represents the total palmitic acid content of total fatty acids. " C16:0 at <i>sn</i>-2 " represents the % palmitic acid at <i>sn</i> -2 out of total <i>sn</i> -2 positioned fatty acids. " Ratio " represents % of C16:0 at <i>sn</i> -2 palmitic acid out of total C16:0 [(% of C16:0 at <i>sn</i> -2 out of total <i>sn</i> -2 positioned fatty acids)/3]/(%total C16:0)]x100	

Table 4 - Fat Blend 12 composition (with 43% fat base)

Fatty acid	% from total Fatty acids
C8:0	1.6
C10:0	1.5
C12:0	10.6
C14:0	3.9
C16:0	17.2
C16:0 at <i>sn</i> -2 of total fatty acids at <i>sn</i> -2	26.3
Ratio (%) of <i>sn</i> -2 palmitic acid of total palmitic acid	51
C18:0	2.4
C18:1	41.1
C18:2	18.2
C18:3	2.2
% fat base (concentrate) in fat blend	43
Vegetable Oil	
Randomized Coconut oil	22
Randomized Sunflower	15

(continued)

Fatty acid	% from total Fatty acids
Randomized Rapeseed	20
*All numbers represent % (w/w), except the ratio which is defined as %. "C16:0" represents the total palmitic acid content of total fatty acids. "C16:0 at sn-2" represents the % palmitic acid at sn-2 out of total sn-2 positioned fatty acids. "Ratio" represents % of C16:0 at sn-2 palmitic acid out of total C16:0 [(% of C16:0 at sn-2 out of total sn-2 positioned fatty acids)/3]/(%total C16:0)]x100]	

Example 2 - Infant formula preparation

[0145] The infant formulas comprising a lipid composition (fat blend) were prepared as follows:

[0146] The fat fraction (fat blend) produced by the blending of fat base with other oils and fats as described above was further blended with other nutrients such as proteins, minerals, vitamins and carbohydrates to yield a food product supplying an infant with the major nutrients also found in human milk. The nutrients and fats were homogenized using pressure homogenization and spray dried to yield a homogenous powder. The powder was further re-dispersed in water (approx. 9 g powder per 60 ml water) to yield a ready-to-feed formula. The fat content of the ready feed was approx. 3.5 g per 100 ml which corresponds to the fat content of human breast milk, which is in the range of 30-40g/L.

[0147] Fat blend 9 was then mixed with other components as detailed in **Table 5** below to form infant formulas based on Fat Blend 9.

Table 5 - Composition of the infant formula based on Fat Blend 9

Formula	Per 100 g powder	Per 100 ml ready to feed (after mixing 15gr in 100 ml water)
Energy/Calories (kcal)	510	76.5
Sodium (mg)	160	24.0
Protein (g) (Lactalbumin/Casein 60/40)	12	1.8
Total Fat Blend in Infant Formula (gr)	26	3.9
Total Saturated fat (gr)	11	1.7
Linoleic acid (mg)	3540	531.0
Alpha-linolenic acid (mg)	355	53.3
Arachidonic acid (mg)	99	14.9
Docosahexaenoic acid (mg)	99	14.9
Cholesterol (mg)	27	4.1
Lactose (gr)	57	8.6
Calcium (mg)	455	68.3
Phosphorus (mg)	235	35.3
Potassium (mg)	410	61.5
Chloride (mg)	285	42.8
Iron (mg)	5.1	0.8
Magnesium (mg)	53	8.0
Zinc (mg)	3.5	0.5
Copper (mcg)	260	39.0

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(continued)

Formula	Per 100 g powder	Per 100 ml ready to feed (after mixing 15gr in 100 ml water)
Manganese (mcg)	25	3.8
Iodine (mcg)	77	11.6
Taurine (mg)	37	5.6
vitamin A I.U.	1570	235.5
Vitamin D I.U.	365	54.8
Vitamin E (mg)	7.5	1.1
Vitamin K (mcg)	59	8.9
Vitamin C (mg)	99	14.9
Vitamin B ₁ (mcg)	550	82.5
Vitamin B ₂ (mcg)	1660	249.0
Vitamin B ₆ (mcg)	420	63.0
Vitamin B ₁₂ (mcg)	3.3	0.5
Niacin (mg)	6.8	1.0
Panthenic acid (mg)	5.6	0.8
Folic acid (mcg)	92	13.8
Biotin (mcg)	17	2.6
Choline (mg)	115	17.3
Inositol (mg)	46	6.9
Moisture %	3	

[0148] Similarly, another infant formula was prepared using fat blend 11, as detailed in **Table 6** below.

Table 6 - Composition of the infant formula based on Fat Blend 11

Formula	Per 100 g powder	Per 100 ml ready to feed (after mixing 15gr in 100 ml water)
Energy/Calories (kcal)	508	68
Sodium (mg)	140	18.8
Protein (g) (Lactalbumin/Casein 60/40)	11.4	1.5
Total Fat Blend in Infant Formula (gr)	26.5	3.5
Total Saturated fat (gr)	11.3	1.49
Linoleic acid (mg)	5000	670
Alpha-linolenic acid (mg)	530	71
Arachidonic acid (mg)	115	15.3

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Formula	Per 100 g powder	Per 100 ml ready to feed (after mixing 15gr in 100 ml water)
Docosahexaenoic acid (mg)	108	14.4
Cholesterol (mg)	2	0.3
Lactose (gr)	56	7.5
Calcium (mg)	430	57.3
Phosphorus (mg)	250	33.5
Potassium (mg)	420	56.3
Chloride (mg)	300	40.2
Iron (mg)	5.25	0.7
Magnesium (mg)	50	6.7
Zinc (mg)	3.5	0.47
Copper (mcg)	300	40.2
Manganese (mcg)	45	6
Iodine (mcg)	45	6
Taurine (mg)	45	6
Vitamin A I.U.	1500	200
Vitamin D I.U.	300	40.2
Vitamin E (mg)	10	1.3
Vitamin K (mcg)	45	6
Vitamin C (mg)	60	8
Vitamin B ₁ (mcg)	400	53
Vitamin B ₂ (mcg)	800	127
Vitamin B ₆ (mcg)	375	50
Vitamin B ₁₂ (mcg)	1.15	0.2
Niacin (mg)	6	0.8
Panthenic acid (mg)	3	0.4
Folic acid (mcg)	67	9
Biotin (mcg)	14.3	1.9
Choline (mg)	37.5	5
Inositol (mg)	22.5	3
Moisture %	3	

[0149] The level of fat in an infant formula and the exact composition of the fat blend can be controlled in order to yield a final formulation which optimally mimics the human milk fat at different lactation periods. Generally, as appreciated, composition of mammalian milk changes in terms of fat content during lactation stages (set according to the age of the infant) and the infant formula, and in particular, the fat blend content, may thus be adapted according to the desired stage that needs to be mimicked.

Example 3 - The effect of infant formula with different fat components on intestinal flora in formula-fed infants**Study design**

5 **[0150]** The effect of the fat component in the infant formula on intestinal flora was examined in a double blind randomized clinical trial in human term formula fed infants with a reference arm of human breastfed infants.

[0151] Following screening, 36 healthy, growing, term infants were randomized to one of two treatment groups, 8 infants in the control group and 14 in the InFat group, with additional 14 breastfed infants as reference.

10 **Diets**

[0152] The efficacy of infant formula with fat blend 9 was investigated in a double-blind, randomized, controlled 6 weeks duration trial in healthy term infants. The study demonstrated the effect of the infant formula with InFat blend 9 compared to infant formula with standard vegetable oil (having high total palmitic mostly at sn-1 and sn-3 positions) and to breastfed milk, on intestinal microflora in term infants.

[0153] The three study groups were:

Group I - the *InFat group*, infants fed with the infant formula comprising InFat blend No. 9, enriched with palmitic acid at the sn-2 position;

20 Group II - the *control group*, infants fed with the vegetable oil mixture which comprises the same total amount of palmitic acid but mostly esterified to sn-1 and sn-3 positions;

Group III - the *reference breastfeeding group*, infants being breastfed.

25 **[0154]** The InFat infant formula (of Group I) and the control infant formula (of Group II) were essentially similar with respect to nutrient content and differ only in the position of palmitic acid in the triglyceride. Both the formula of Group I and the formula of Group II do not include any probiotics or prebiotics.

Table 7: Fatty acids composition comparison between tested Groups (% of weight of total fatty acids)

Fatty acid	Group I InFat based Infant Formula	Group II Vegetable oil mix based infant formula	Group III Human milk (Jensen 1999*)
C8:0	0.9	3.0	
C10:0	0.8	2.2	0.05-2.21
C12:0	10.4	9.4	2.01-11.77
C14:0	4.3	4.2	2.26-11.68
C16:0	22.3	18.7	12.9-27.50
Ratio of C16:0 at sn-2 position of total C16	43	13.6	~70
C18:0	4.4	6.4	3.49-10.65
C18:1	38.5	34.4	23.55-55.25
C18:2	14.0	15.1	5.79-27.55
C18:3	1.5	1.5	0.25-1.9
C20:4	0.5	0.4	0.05-0.87
C22:6	0.4	0.4	0-1.03
*Jensen, R. G. Lipids 34(12): 1243-71, 1999			

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Gut flora evaluation

[0155] **Intestinal flora:** Stool samples of at least 1gr were collected from each infant in the study at baseline (inclusion to the study) and after 6 weeks. Samples were stored at 4°C for up to 24 hrs before examination.

[0156] The tests performed on each sample included specific bacterial counts on specific plates for a general estimation of different bacteria types such as:

1. Lactobacillus on MRS+cys plate
2. Bifidobacteria on MRS+++ plate
3. Staphylococcus on BP plate
4. Clostridium on SPS plate
5. Pseudomonas on pseudo-cent plate

[0157] Further, parents were asked to fill a 3 days diary of their infant feedings and significant crying periods. Additionally, each infant was monitored for anthropometric parameters, general health and well being for safety assessment.

Results

[0158] 30 healthy, growing, term infants completed the 6 weeks study without protocol violation, 7 infants in the control group and 11 in the InFat group, with additional 12 breastfed infants as reference.

[0159] Both formulas were well tolerated, safe and did not produce any significant negative effect in the tested parameters.

Feeding

[0160] Infants that were fed with infant formula with InFat[™] had less feedings per day at age of 6 weeks compared to infants that were fed with formula with standard vegetable oil (8 vs. 7.5 periods per day). Though, the amount of formula that was consumed was lower for the infants that were fed with infant formula with InFat[™], the amount per feeding was higher compared to infants that were fed with formula with standard vegetable oil.

Table 8 - Formula consumption

	Control (n=7)		InFat (n=11)		significance
	Mean	SEM*	Mean	SEM*	2 formula groups
formula consumption per day (ml/feeding)	740.0	66.1	588.3	117.3	.350
formula consumption per kg per day	150.0	10.0	133.0	26.0	.599
number of feedings per day	8.0	.5	7.5	.8	.614
ml formula per feeding	94.0	9.2	119.0	6.7	.039
*SEM= standard error of the mean					

Intestinal flora

[0161] Infants that were fed with infant formula with InFat[™] had intestinal flora with higher abundance of lactobacillus and bifidobacteria after 6 weeks of feeding compared to infants that were fed with formula with standard vegetable oil and comparable to that of infants that were breastfed.

[0162] Infants that were fed with infant formula with InFat[™] had intestinal flora with decreased abundance of pathogenic bacteria such as clostridia after 6 weeks of feeding compared to infants that were fed with formula with standard vegetable oil and comparable to that of infants that were breastfed.

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Table 9 - Results of beneficial bacteria count (the results are graphically presented in Figure 1)

Beneficial Bacteria									
	Control (n=7)		InFat (n=11)		Breastfed (n=12)		significance		
	Mean	SEM*	Mean	SEM*	Mean	SEM*	3 groups	2 formulated groups	
Lactobacillus counts at baseline	1.1E+09	6.5E+08	6.1E+09	3.2E+09	3.4E+09	1.8E+09	.065	.020	
Lactobacillus counts after 6 weeks feeding	1.2E+10	3.2E+09	1.2E+11	7.8E+10	5.6E+10	2.0E+10	.000	.001	
Fold change of Lactobacillus from baseline	11.2		18.8		16.6				
Bifidobacteria counts at baseline	1.5E+11	9.8E+10	5.2E+10	4.5E+10	1.6E+09	7.1E+08	.000	.291	
Bifidobacteria counts after 6 weeks feeding	5.1E+09	12E+09	1.2E+11	7.8E+10	3.9E+10	2.3E+10	.000	.000	
Fold change of Bifidobacteria from baseline	0.0		2.4		25.1				
*SEM= standard error of the mean									

Table 10 - Results of pathogenic bacteria count

Pathogenic Bacteria								
	Control (n=7)		InFat (0=11)		Breastfed (n=12)		significance	
	Mean	SEM*	Mean	SEM*	Mean	SEM*	3 groups	2 formulasfed groups
Clostridium counts at baseline	3.3E+07	3.3E+07	9.1E+10	6.1E+10	4.3E+10	4.2E+10		.000
Change of Clostridium counts from baseline	2.1E+07	-2.1E+07	- 9.1E+10	6.1E+10	- 3.4E+10	4.3E+10	.463	.258
*SEM= standard error of the mean								

20 Comfort and stool characteristics

[0163] Parents of infants that were fed with infant formula with InFat™ did not report any significant difference of their infant stool characteristics or comfort compared to those of infants that were fed with formula with standard vegetable oil.

25 Crying

[0164] Infants that were fed with infant formula with InFat™ had less significant crying periods at age of 6 weeks compared to infants that were fed with formula with standard vegetable oil (0.49 vs. 0.8 periods per day or 1.5 vs. 2.4 crying periods per 3 days).

30 Conclusion

[0165] Infants that were fed with infant formula with InFat™ demonstrated:

- Intestinal flora that was similar to that of infants that were breastfed and more favorable compared to that of infants fed infant formula with standard vegetable oil.
- Reduced number of significant crying periods that was not related to reduction in hard stools or to comfort as no significant difference in the infant's stool characteristics or comfort was observed in comparison with the control group.

40 Example 4 - The effect of infant formula with different fat components on intestinal flora in infants born by Caesarean Section.

Study design

[0166] The effect of the fat component in the infant formula on intestinal flora was examined in a clinical trial in human term formula fed infants born by Caesarean Section, known to be prone to intestinal flora which is not optimal, with a reference arm of human breastfed infants.

[0167] Following screening, 4 healthy, growing, term infants born by cesarean delivery were fed with infant formula with InFat™, with additional 4 breastfed infants as reference.

Diets

[0168] The efficacy of infant formula with fat blend 9 was investigated in a double-blind, randomized, controlled 6 weeks duration trial in healthy term infants. The study demonstrated the effect of the infant formula with InFat™ blend 9 (the formula as in example 3) compared to breastfed milk, on intestinal microflora in term infants.

[0169] The two study groups were:

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Group I - the *InFat*TM group, infants fed with the infant formula comprising InFat blend No. 9, enriched with palmitic acid at the sn-2 position;

Group II - the *reference breastfeeding* group, infants being breastfed.

5 [0170] The formula of Group I did not include any probiotics or prebiotics.

Gut flora evaluation

10 [0171] Intestinal flora was evaluated as detailed in example 3.

Results

Intestinal flora

15 [0172] Infants born by cesarean section that were fed with infant formula with InFatTM had intestinal flora with higher abundance of lactobacillus and bifidobacteria after 6 weeks of feeding compared to baseline and comparable to that of infants born by cesarean section that were breastfed.

20 **Table 11: Results of beneficial bacteria count**

Beneficial Bacteria					
	InFat (n=4)		Breastfed (n=4)		significance
	Mean	SEM*	Mean	SEM*	2 groups
Lactobacillus counts at baseline	7.5E+08	5.8E+08	2.3E+09	1.6E+09	0.22
Change of Lactobacillus counts from baseline	1.1E+09	7.8E+08	4.1E+10	3.1E+10	0.236
*SEM= standard error of the mean					

30 [0173] Infants that were fed with infant formula with InFatTM had intestinal flora with decreased abundance of pathogenic bacteria as clostridia and staphylococcus after 6 weeks of feeding compared to baseline and comparable to that of infants that were breastfed.

35 **Table 12 - Results of pathogenic bacteria count**

Pathogenic Bacteria					
	InFat (n=4)		Breastfed (n=4)		significance
	Mean	SEM*	Mean	SEM*	2 groups
Clostridium counts at baseline	1.3E+11	1.2E+11	1.3E+11	1.2E+11	0.991
Change of Clostridium counts from baseline	-1.2E+11	1.3E+11	-1.3E+11	1.2E+11	0.998
Staphylococcus counts at baseline	1.3E+11	1.2E+11	7.9E+09	5.3E+09	0.008
Change of Staphylococcus counts from baseline	-1.3E+11	1.2E+11	-7.8E+09	5.3E+09	0.384
Pseudomonas counts at baseline	1.3E+11	1.2E+11	6.9E+09	6.7E+09	0.016
Change of Pseudomonas counts from baseline	-1.3E+11	1.2E+11	-6.3E+09	6.8E+09	0.375
*SEM= standard error of the mean					

Crying

55 [0174] Infants that were fed with infant formula with InFatTM had crying periods and duration at age of 6 weeks comparable to those of breastfed infants (0.67 and 0.8 crying periods per day and 10.4 and 19.4 minutes per day, respectively).

Conclusion

[0175] Infants born by Cesarean Section that were fed with infant formula with InFat™ demonstrated similar change in the intestinal flora to that of infants born by Cesarean Section that were breastfed; the pathogenic bacteria were reduced and the beneficial bacteria were increased.

Example 5 - The effect of infant formula with different fat components on crying in formula-fed infants.

Study design

[0176] The effect of the fat component in the infant formula on crying duration and frequency was examined in a double blind randomized clinical trial in human term formula fed infants with a reference arm of human breastfed infants.

[0177] Following screening, 83 healthy, growing, term infants were randomized to one of two treatment groups, 28 infants in the control group and 30 in the InFat group, with additional 25 breastfed infants as reference.

Diets

[0178] The efficacy of infant formula with fat blend 9 was investigated in a double-blind, randomized, controlled 12 weeks duration trial in healthy term infants. The study demonstrated the effect of the infant formula with InFat blend 9 compared to infant formula with standard vegetable oil (having high total palmitic mostly at sn-1 and sn-3 positions) and to breastfed milk, on crying time in term infants.

[0179] The three study groups were:

Group I - the *InFat* group, infants fed with the infant formula comprising InFat blend No. 9, enriched with palmitic acid at the sn-2 position;

Group II - the *control* group, infants fed with the vegetable oil mixture which comprises the same total amount of palmitic acid but mostly esterified to sn-1 and sn-3 positions;

Group III - the *reference breastfeeding* group, infants being breastfed.

[0180] The InFat infant formula (of Group I) and the control infant formula (of Group II) were essentially similar with respect to nutrient content and differ only in the position of palmitic acid in the triglyceride. Both the formula of Group I and the formula of Group II do not include any probiotics or prebiotics.

Crying evaluation

[0181] During the 12 weeks study parents were asked to fill 3 days diary of their infant feedings and significant crying periods at age of 6 weeks and at age of 12 weeks.

Results

[0182] 66 healthy, growing, term infants completed the 12 weeks study, 21 infants in the control group and 23 in the InFat group, with additional 22 breastfed infants as reference.

[0183] Both formulas were well tolerated, safe and did not produce any significant negative effect in the tested parameters.

Comfort and stool characteristics

[0184] Infants that were fed with infant formula with InFat™ had stool characteristics that did not differ significantly from those of infants that were fed with formula with standard vegetable oil at age of 6 weeks (9.6% and 10.4% hard stools, respectively, $p=0.6$) and at age of 12 weeks (8.5% and 12.5% hard stools, respectively, $p=0.3$).

[0185] Parents of infants that were fed with infant formula with InFat™ did not report any significant difference of their infant stool characteristics or comfort compared to those of infants that were fed with formula with standard vegetable oil.

Crying

[0186] Infants that were fed with infant formula with InFat™ had statistically significant reduced crying time and frequency.

[0187] At age of 6 weeks infants that were fed with infant formula with InFat™ had statistically significant reduced

number of crying periods compared to infants that were fed with infant formula with standard vegetable oil.

[0188] At age of 12 weeks infants that were fed with infant formula with InFat™ had statistically significant reduced crying duration and number of crying periods compared to infants that were fed with infant formula with standard vegetable oil.

[0189] The results also show that infants that were fed with infant formula with InFat™ had larger reduction in crying time per day from age of 6 weeks to age of 12 weeks.

Table 13 - crying pattern at 6 and 12 weeks

	Control		InFat™		Statistical significance
	Mean	SEM*	Mean	SEM*	2 formula groups
6 weeks	n=24		n=26		
number of significant crying periods per day	1.3	0.27	0.7	0.14	0.083
12 weeks	n=21		n=23		
number of significant crying periods per day	0.8	0.17	0.3	0.06	0.033
total crying duration per day (minutes)	23.6	5.15	3.8	0.79	0.043
Change in crying duration from 6 to 12 weeks	-4.8	-1.05	-32.1	-6.69	0.12
*SEM= standard error of the mean					

[0190] This reduction in crying was not related to hard stools and comfort since no statistical significance was shown for the hard stool reduction.

Conclusion

[0191] Infants that were fed with infant formula with InFat™ demonstrated reduced number of significant crying periods per day and reduced daily crying time duration.

Example 6 - The effect of infant formula with different fat components on immune effects in healthy formula-fed infants.

Sturdy design

[0192] The effect of the fat component in the infant formula on immune effect is examined in a double blind randomized clinical trial in human term formula fed infants with a reference arm of human breastfed infants.

[0193] Following screening, growing, term infants are randomized to one of two treatment groups, with additional breastfed infants as reference.

Diets

[0194] The efficacy of infant formula with InFat is investigated in a double-blind, randomized, controlled trial in healthy term infants. The study demonstrates the effect of the infant formula with InFat compared to infant formula with standard vegetable oil (having high total "palmitic mostly at sn-1 and sn-3 positions) and to breastfed milk, on immune health in term infants.

[0195] The three study groups are:

Group I the *InFat* group, infants fed with the infant formula comprising InFat, enriched with palmitic acid at the sn-2 position;

[0196] Group II - the *control* group, infants fed with the vegetable oil mixture which comprises the same total amount of palmitic acid but mostly esterified to sn-1 and sn-3 positions; Group III - the *reference breastfeeding* group, infants being breastfed.

[0197] The InFat infant formula (of Group I) and the control infant formula (of Group II) are essentially similar with respect to nutrient content and differ only in the position of palmitic acid in the triglyceride.

Immune health evaluation

[0198] Immune health consists of few elements:

- 5
1. incidence of allergy or atopy
 2. incidence of infectious episodes
 3. immune response to regular vaccination

[0199] Data is collected through follow-up visits, diaries written by parents, and telephone calls by trained personal.

10 **[0200]** In general, any sign or symptom related to allergy (Atopic dermatitis, wheezing episodes, and allergic urticaria) and infection (fever, cough, runny nose, and watery stools) is recorded, as well as any medical document.

[0201] During the study, parents are instructed to record allergic and infectious symptoms, every episode of fever, clinic visits, tests, physician's diagnosis, prescription of medications, particularly antibiotics, etc.

15 **[0202]** Additionally, each infant is monitored for anthropometric parameters, general health and well being.

Example 7 - The effect of infant formula with different fat components on protection from allergy in formula-fed infants prone to allergy.

Study design

20 **[0203]** The protective effect of the fat component in the infant formula against allergy and infections is examined in a prospective double blind randomized clinical trial in human term formula fed infants with a parental history of atopy and with a reference arm of human breastfed infants with a parental history of atopy.

25 **Diets**

[0204] The efficacy of infant formula with InFat is investigated in a double-blind, randomized, controlled trial in healthy term infants. The study demonstrates the protective effect of the infant formula with InFat compared to infant formula with standard vegetable oil (having high total palmitic mostly at sn-1 and sn-3 positions) and to breastfed milk, against allergy in term infants.

30 **[0205]** The three study groups are:

Group I - the *InFat* group, infants fed with the infant formula comprising InFat, enriched with palmitic acid at the sn-2 position;

35 Group II - the *control* group, infants fed with the vegetable oil mixture which comprises the same total amount of palmitic acid but mostly esterified to sn-1 and sn-3 positions;

Group III - the *reference breastfeeding* group, infants being breastfed.

40 **[0206]** The InFat infant formula (of Group I) and the control infant formula (of Group II) are essentially similar with respect to nutrient content and differ only in the position of palmitic acid in the triglyceride.

Immune health evaluation

45 **[0207]** Immune health consists of few elements:

1. incidence of allergy or atopy
2. incidence of infectious episodes

[0208] Data is collected through follow-up visits, diaries written by parents, and telephone calls by trained personal.

50 **[0209]** In general, any sign or symptom related to allergy (Atopic dermatitis, wheezing episodes, and allergic urticaria) and infection (fever, cough, runny nose, and watery stools) is recorded, as well as any medical document.

[0210] During the study, parents are instructed to record allergic and infectious symptoms, every episode of fever, clinic visits, tests, physician's diagnosis, prescription of medications, particularly antibiotics, etc.

55 **[0211]** Additionally, each infant is monitored for anthropometric parameters, general health and well being.

Example 8 - The effect of infant formula with different fat components on intestinal flora and crying in formula-fed Chinese infants

Study design

[0212] The effect of the fat component in the infant formula on intestinal flora and crying is examined in a double blind randomized clinical trial in Chinese term formula fed infants with a reference arm of human breastfed infants.

[0213] Following screening, 114 healthy, growing, term infants are randomized to one of two treatment groups, 57 infants in the control group and 57 in the InFat group, with additional 57 breastfed infants as reference group.

Diets

[0214] The efficacy of infant formula with InFat™ is investigated in a double-blind, randomized, controlled 24 weeks duration trial in healthy term infants. The study is to demonstrate the effect of the infant formula with InFat™ compared to infant formula with standard vegetable oil (having high total palmitic mostly at sn-1 and sn-3 positions) and to breastfed milk, on intestinal microflora and crying in Chinese term infants.

[0215] The three study groups are:

Group I - the *InFat* group, infants fed with the infant formula comprising InFat™, enriched with palmitic acid at the sn-2 position (20% total palmitic of which 44% is esterified to sn-2 position);

Group II - the *control* group, infants fed with the vegetable oil mixture which comprises the same total amount of palmitic acid but mostly esterified to sn-1 and sn-3 positions (20% total palmitic of which 13% is esterified to sn-2 position);

Group III - the *reference breastfeeding* group, infants being breastfed.

[0216] The InFat infant formula (of Group I) and the control infant formula (of Group II) are essentially similar with respect to nutrient content and differ only in the position of palmitic acid in the triglyceride. Both the formula of Group I and the formula of Group II include prebiotics. Therefore, the aim of the study is to show the effect of InFat™ on top of the effect of prebiotics.

Gut flora evaluation

[0217] Intestinal flora: Stool samples of at least 1gr are collected from each infant in the study at baseline (inclusion to the study) and after 6 weeks. Samples are stored at 4°C for up to 24 hrs before examination.

[0218] The tests performed on each sample include:

1. pH measurement to be determined based on pH level of a stool sample from the tested infant;
2. Specific bacterial counts on specific plates for a general estimation of different bacteria types such as:

- a. Lactobacilli on MRS+cys plate
- b. Bifidobacteria on MRS+++ plate
- c. Coliforms on Mackonkey plate
- d. E. coli on TBX plate
- e. Enterobacteria on SB plate
- f. Staphylococcus on BP plate
- g. Clostridium on SPS plate
- h. Pseudomonas on pseudo-cent plate

[0219] Further, parents are asked to fill 3 days diary of their infant feedings and significant crying periods.

[0220] Additionally, each infant is monitored for anthropometric parameters, general health and well being for safety assessment.

Claims

1. An edible enzymatically prepared vegetable-derived fat source for promoting development of beneficial gut flora in a subject; wherein said fat source is a triglyceride fat source comprising triglycerides with 15-55% palmitic acid moieties out of the total fatty acids, and wherein the level of palmitic acid moieties at the sn-2 position of the glycerol

backbone is at least 30% of total palmitic acid; and
wherein said fat source has an effect on colonization of at least one pathogenic bacteria in the gut of the subject,
said effect being selected from the group consisting of inhibiting, preventing and reducing colonization of the at least
one pathogenic bacteria.

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2. The fat source of Claim 1, wherein said fat source has an effect on the immune system of the subject.
3. The fat source of Claim 1 or 2, wherein said subject suffers from at least one disorder of the immune system resulting
from gut flora imbalance.
4. The fat source of Claim 3, wherein the at least one disorder in the immune system is selected from inflammation,
atopy, allergy, feeding intolerance and infection and the lipid composition is effective to treat the disorder.
5. The fat source of any one of Claims 1 to 4, wherein the fat source enhances colonization of the at least one bacteria
in the gut of the subject, wherein bacteria is selected from the group consisting of bifidobacteria and lactobacilli.
6. The fat source of any one of Claims 1 to 5, wherein said pathogenic bacteria is selected from the group consisting
of coliform organisms, enterobacteria, clostridia, veillonella, proteus, P. aeruginosa, staphylococci and streptococci.
7. The fat source of any one of Claims 1 to 6, wherein said subject is a child.
8. The fat source of Claim 7, wherein said child is an infant or a toddler.
9. The fat source of Claim 7 or 8, wherein said infant was delivered by a Caesarean Section.
10. The fat source of Claim 8 or 9, wherein said infant is a newborn selected from pre-term infant and term infant.
11. The fat source of any one of Claim 1 to 10, wherein said subject is at risk of developing an imbalance in the profile
of the gut flora population.
12. The fat source of any one of Claims 1 to 11, wherein said fat source being blended with a mixture of vegetable oils,
wherein said mixture comprises oils selected from the group consisting of soy, palm tree, canola, coconut, palm
kernel, sunflower, corn, safflower and rapeseed oil; and wherein said vegetable oils may optionally be randomized
before blending with the fat base.

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Patentansprüche

1. Essbare, enzymatisch hergestellte, aus Pflanzen gewonnene Fettquelle zum Fördern der Entwicklung einer günstigen
Darmflora in einer Person; wobei die besagte Fettquelle eine Triglycerid-Fettquelle ist, die Triglyceride mit 15
- 55 % Palmitinsäure-Einheiten von den gesamten Fettsäuren umfasst, und wobei die Konzentration von Palmitin-
säure-Einheiten an der sn2-Position der Glycerinhauptkette mindestens 30 % der gesamten Palmitinsäure beträgt;
und
wobei die besagte Fettquelle eine Wirkung auf die Kolonisation mindestens einer pathogenen Bakterie in dem Darm
der Person hat, wobei die Wirkung aus der Gruppe bestehend aus Hemmen, Verhindern und Verringern der Kolo-
nisation der mindestens einen pathogenen Bakterie ausgewählt ist.
2. Fettquelle nach Anspruch 1, wobei die besagte Fettquelle eine Wirkung auf das Immunsystem der Person hat.
3. Fettquelle nach Anspruch 1 oder 2, wobei die besagte Person an mindestens einer Erkrankung des Immunsystems
leidet, die aus einem Ungleichgewicht der Darmflora resultiert.
4. Fettquelle nach Anspruch 3, wobei die mindestens eine Erkrankung in dem Immunsystem aus Entzündung, Atopie,
Allergie, Fütterungsintoleranz und Infektion ausgewählt ist und die Lipidzusammensetzung zur Behandlung der
Erkrankung wirksam ist.
5. Fettquelle nach einem der Ansprüche 1 bis 4, wobei die Fettquelle die Kolonisation der mindestens einen Bakterie
in dem Darm der Person fördert, wobei die Bakterie aus der Gruppe bestehend aus Bifidobakterien und Lactobazillen

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ausgewählt ist.

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6. Fettquelle nach einem der Ansprüche 1 bis 5, wobei die besagte pathogene Bakterie aus der Gruppe bestehend aus Koliform-Organismen, Enterobakterien, Clostridien, Veillonellen, Proteus, *P. aeruginosa*, Staphylokokken und Streptokokken ausgewählt ist.
7. Fettquelle nach einem der Ansprüche 1 bis 6, wobei die besagte Person ein Kind ist.
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8. Fettquelle nach Anspruch 7, wobei das besagte Kind ein Säugling oder ein Kleinkind ist.
9. Fettquelle nach Anspruch 7 oder 8, wobei der besagte Säugling durch Kaiserschnitt entbunden wurde.
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10. Fettquelle nach Anspruch 8 oder 9, wobei der besagte Säugling ein Neugeborenes ist, das aus einem Frühgeborenen und einem Termingeborenen ausgewählt ist.
11. Fettquelle nach einem der Ansprüche 1 bis 10, wobei die besagte Person ein Risiko aufweist, ein Ungleichgewicht des Profils der Darmflorapopulation zu entwickeln.
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12. Fettquelle nach einem der Ansprüche 1 bis 11, wobei die besagte Fettquelle mit einer Mischung von Pflanzenölen vermischt wird, wobei die besagte Mischung Öle umfasst, die aus der Gruppe bestehend aus Soja-, Palm-, Raps-, Kokos-, Palmkern-, Sonnenblumen-, Mais-, Saflor- und Rapskernöl ausgewählt sind; und wobei die besagten Pflanzenöle gegebenenfalls vor dem Vermischen mit der Fettbasis willkürlich ausgewählt werden können.

25 **Revendications**

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1. Source de matière grasse comestible d'origine végétale préparée par voie enzymatique pour favoriser le développement de la flore intestinale bénéfique chez un sujet ; où ladite source de matière grasse est une source de matière grasse à base de triglycérides comprenant des triglycérides avec des fragments d'acide palmitique allant de 15 à 55% par rapport aux acides gras totaux et où le niveau de fragments d'acide palmitique à la position sn-2 du squelette de glycérol est d'au moins 30% d'acide palmitique total; et dans laquelle ladite source de matière grasse a un effet sur la colonisation d'au moins une bactérie pathogène dans l'intestin du sujet, ledit effet étant sélectionné dans le groupe constitué de l'inhibition, la prévention et la réduction de colonisation de l'au moins une bactérie pathogène.
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2. Source de matière grasse de la revendication 1, dans laquelle ladite source de matière grasse a un effet sur le système immunitaire du sujet.
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3. Source de matière grasse de la revendication 1 ou 2, dans laquelle ledit sujet souffre d'au moins un trouble du système immunitaire résultant d'un déséquilibre de la flore intestinale.
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4. Source de matière grasse de la revendication 3, dans laquelle l'au moins un trouble dans le système immunitaire est choisi parmi une inflammation, une atopie, une allergie, une intolérance alimentaire et une infection et la composition lipidique est efficace pour traiter le trouble.
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5. Source de matière grasse de l'une quelconque des revendications 1 à 4, dans laquelle la source de matière grasse améliore la colonisation de l'au moins une bactérie dans l'intestin du sujet, où la bactérie est choisie dans le groupe constitué de bifidobactéries et de lactobacilles.
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6. Source de matière grasse de l'une quelconque des revendications 1 à 5, dans laquelle ladite bactérie pathogène est choisie dans le groupe constitué d'organismes coliformes, d'entérobactéries, de clostridia, de veillonella, de proteus, de *P. aeruginosa*, de staphylocoques et de streptocoques.
7. Source de matière grasse de l'une quelconque des revendications 1 à 6, dans laquelle ledit sujet est un enfant.
8. Source de matière grasse de la revendication 7, dans laquelle ledit enfant est un nourrisson ou un enfant en bas âge.
9. Source de matière grasse de la revendication 7 ou 8, dans laquelle ledit nourrisson est né par césarienne.

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10. Source de matière grasse de la revendication 8 ou 9, dans laquelle ledit nourrisson est un nouveau-né choisi parmi un nourrisson prématuré et un nourrisson à terme.
- 5 11. Source de matière grasse de l'une quelconque des revendications 1 à 10, dans laquelle ledit sujet risque de développer un déséquilibre dans le profil de la population de la flore intestinale.
- 10 12. Source de matière grasse de l'une quelconque des revendications 1 à 11, dans laquelle ladite source de matière grasse est mélangée avec un mélange d'huiles végétales, où ledit mélange comprend des huiles choisies dans le groupe constitué d'huile de soja, de palmier, de canola, de noix de coco, de palmiste, de tournesol, de maïs, de carthame et de colza ; et où lesdites huiles végétales peuvent facultativement être réparties de manière aléatoire avant le mélange avec la base de matière grasse.

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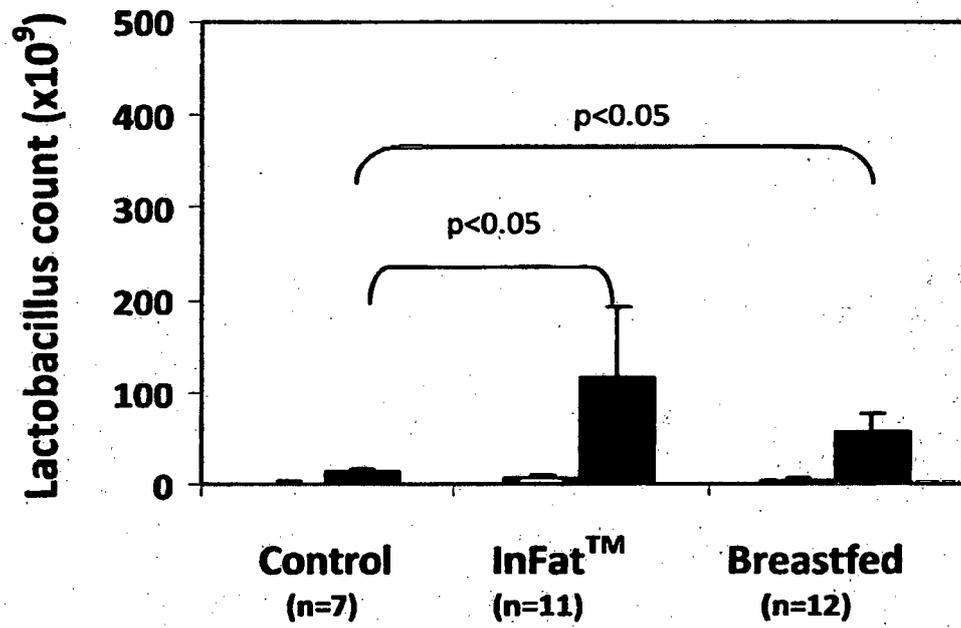


Figure 1

REFERENCES CITED IN THE DESCRIPTION

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