DESCRIPTION

PEDIATRIC NUTRITIONAL COMPOSITION WITH HUMAN MILK OLIGOSACCHARIDES, PREBIOTICS AND PROBIOTICS

TECHNICAL FIELD

[0001] The present disclosure generally provides nutritional compositions that are useful for promoting beneficial bacteria in the gastrointestinal tract of subjects, particularly pediatric subjects, wherein the nutritional composition includes a prebiotic composition comprising galacto-oligosaccharides (GOS) and/or polydextrose (PDX), human milk oligosaccharides (HMO) and a probiotic such as *Lactobacillus rhamnosus* GG (LGG). The present disclosure also provides methods for promoting the growth of beneficial microbiota in the gastrointestinal tract of pediatric subjects comprising administering to a subject the disclosed nutritional composition.

BACKGROUND

[0002] Human infant gut microbiota is rapidly established in the first few weeks following birth. Gut microbiota development in infants is understood to be initiated by exposure to maternal and environmental bacteria during birth. Further development of gut microbiota is affected by a newborn infant's diet. Whether the infant is breast fed or formula fed has a strong influence on the intestinal bacterial population and composition. Human milk contains numerous macro and micronutrient components, the identity and function of which are still being discovered and studied. Among these components, human milk oligosaccharides are believed to play an important role in the growth of beneficial bacteria in infants. In the breast fed infant, for example, *Bifidobacterium* species dominate among intestinal bacteria, while *Streptococcus* species and *Lactobacillus* species are less common. In contrast, the microflora of formula fed infants is more diverse, containing *Bifidobacterium* species and *Bacteroides* species as well as more pathogenic organisms such as *Staphylococcus, Escherichia coli* and *Clostridium* species. The species of *Bifidobacterium* in the stools of breast fed and formula fed infants vary as well. *Bifidobacterium* species are generally considered beneficial bacteria and are known to protect against colonization by pathogenic bacteria.

[0003] Gut microbiota is also important for healthy brain function, and it is believed that gut microbiota communicate with the brain via the gut-brain axis, and thus have an impact on brain development and function. More specifically, gut microbiota interact with enteric and central nervous systems via neural, neuroendocrine, neuroimmune and hormonal links. Brain development and growth exceeds that of any other organ or body tissue,

reaching its peak at 26 weeks of gestation and continuing at a rapid rate throughout the first three years of life. Sub-optimal nutrition during this phase may have irreversible consequences for cognitive function.

[0004] Accordingly, there is a need to provide nutritional compositions, such as infant formulas, that promote the growth of healthy gut microbiota, and promote a healthy gut-brain axis. Such compositions may provide improved cognitive development in infants and children, and thus provide lifelong brain benefits. The present disclosure addresses this need by providing nutritional compositions comprising a prebiotic, HMO and a probiotic.

DISCLOSURE OF THE INVENTION

[0005] The present disclosure is directed, in some embodiments, to a nutritional composition comprising a GOS and/or PDX, HMO or one or more precursors thereof, and a probiotic, such as LGG. While not being bound by any particular theory, it is believed that PDX, GOS, HMO and a probiotic may act synergistically when included in nutritional compositions, such as infant formulas, to promote the growth and/or function of beneficial gut microbiota, thereby stimulating the gut-brain axis. Such compositions may therefore promote healthy cognitive development in infants and children. More specifically, the nutritional compositions provided herein comprise in some embodiments: (i) a protein source, (ii) a lipid source, (iii) a carbohydrate source, (iv) HMO or a precursor thereof, (v) a prebiotic comprising galacto-oligosaccharide and/or polydextrose, and (vi) a probiotic.

[0005] The HMO useful in the present compositions include, but are not limited to, 2'-fucosyllactose, 3'-fucosyllactose, 3'sialyllactose, 6'sialyllactose, lacto-N-biose, lacto-N-neotetraose, lacto-N-tetraose, or any combination thereof. Precursors of HMO, such as sialic acid, fucose, or a combination thereof, also may be included in the present compositions.

[0006] Compositions of the present disclosure may also include, in some embodiments, a source of long chain polyunsaturated fatty acids, such as docosahexaenoic acid (DHA) and/or arachidonic acid (ARA), a source of β-glucan, lactoferrin, or any combination thereof.

[0007] The present disclosure further provides, in certain embodiments, a method for promoting the growth and/or function of beneficial microbiota in the gastrointestinal tract of a pediatric subject in need thereof comprising administering to the subject an effective amount of a nutritional composition comprising: (i) a protein source, (ii) a lipid source, (iii) a carbohydrate source, (iv) a human milk oligosaccharide or a precursor thereof, (v) a prebiotic, and (vi) a probiotic. In certain embodiments, the gut microbiota comprise *Lactobacillus* species, *Bifidobacterium* species, *Allobaculum* species or combinations thereof.

[0008] In certain embodiments, the method of promoting the growth and/or function of beneficial gut microbiota also promotes cognitive development in the subject. The

method may further reduce the growth of harmful gut microbiota in the subject, such as *Clostridium* species.

[0009] It is to be understood that both the foregoing general description and the following detailed description present embodiments of the disclosure and are intended to provide an overview or framework for understanding the nature and character of the disclosure as it is claimed. The description serves to explain the principles and operations of the claimed subject matter. Other and further features and advantages of the present disclosure will be readily apparent to those skilled in the art upon a reading of the following disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] **Figure 1** depicts a graph showing the effect of a diet containing 3'-sialyllactose (3SL) and 6'-siallylactose (6SL) on mice subjected to stress (SDR) compared to non-stressed mice (HCC) using a light:dark preference test.

[0011] **Figure 2** depicts a graph showing the effect of a diet containing 3'-sialyllactose (3SL) and 6'-siallylactose (6SL) on mice subjected to stress (SDR) compared to non-stressed mice (HCC) using an open field task test.

[0012] **Figure 3** depicts the representative movement tracks of mice on a diet containing 3'-sialyllactose (3SL) and 6'-siallylactose (6SL) and then subjected to stress (SDR) compared to non-stressed mice (HCC) using an open field task test.

[0013] **Figure 4** is a graph indicating the amount of sialic acid per gram of corpus callosum tissue of pigs fed six different diets (control, 2g/L 3'-SL, 4g/L 3'-SL, 2g/L 6'-SL, or 4g/L 6'-SL; 2 g/L PDX + 2 g/L GOS; n=9).

[0014] **Figure 5** is a graph indicating the amount of sialic acid per gram of cerebellum tissue of pigs fed six different diets (control, 2g/L 3'-SL, 4g/L 3'-SL, 2g/L 6'-SL, or 4g/L 6'-SL; 2 g/L PDX + 2 g/L GOS; n=9).

DETAILED DESCRIPTION OF THE DISCLOSURE

[0015] Reference now will be made in detail to the embodiments of the present disclosure, one or more examples of which are set forth herein below. Each example is provided by way of explanation of the nutritional composition of the present disclosure and is not a limitation. In fact, it will be apparent to those skilled in the art that various modifications and variations can be made to the teachings of the present disclosure without departing from the scope or spirit of the disclosure. For instance, features illustrated or described as part of one embodiment, can be used with another embodiment to yield a still further embodiment.

[0016] Thus, it is intended that the present disclosure covers such modifications and variations as come within the scope of the appended claims and their equivalents. Other objects, features and aspects of the present disclosure are disclosed in or are obvious from the following detailed description. It is to be understood by one of ordinary skill in the art that the present discussion is a description of exemplary embodiments only and is not intended as limiting the broader aspects of the present disclosure.

[0017] "Nutritional composition" means a substance or formulation that satisfies at least a portion of a subject's nutrient requirements. The terms "nutritional(s)," "nutritional formula(s)," "enteral nutritional(s)," "nutritional composition(s)," and "nutritional supplement(s)" are used interchangeably throughout the present disclosure to refer to liquids, powders, gels, pastes, solids, concentrates, suspensions, or ready-to-use forms of enteral formulas, oral formulas, formulas for infants, formulas for pediatric subjects, formulas for children, growing-up milks and/or formulas for adults, such as women who are lactating or pregnant. In particular embodiments, the nutritional compositions are for pediatric subjects, including infants and children.

[0018] The term "enteral" means through or within the gastrointestinal, or digestive, tract. "Enteral administration" includes oral feeding, intragastric feeding, transpyloric administration, or any other administration into the digestive tract.

[0019] "Pediatric subject" includes both infants and children, and refers herein to a human that is less than thirteen years of age. In some embodiments, a pediatric subject refers to a human subject that is less than eight years old. In other embodiments, a pediatric subject refers to a human subject between about one and about six years of age or about one and about three years of age. In still further embodiments, a pediatric subject refers to a human subject between about 6 and about 12 years of age.

[0020] "Infant" means a subject having an age of not more than about one year and includes infants from about zero to about twelve months. The term infant includes low birth weight infants, very low birth weight infants, and preterm infants. "Preterm" means an infant born before the end of the 37th week of gestation, while "full term" means an infant born after the end of the 37th week of gestation.

[0021] "Child" means a subject ranging in age from about twelve months to about thirteen years. In some embodiments, a child is a subject between the ages of one and twelve years old. In other embodiments, the terms "children" or "child" refer to subjects that are between about one and about six years old, between about one and about three years old, or between about seven and about twelve years old. In other embodiments, the

terms "children" or "child" refer to any range of ages between about 12 months and about 13 years.

[0022] "Children's nutritional product" refers to a composition that satisfies at least a portion of the nutrient requirements of a child. A growing-up milk is an example of a children's nutritional product.

[0023] "Infant formula" means a composition that satisfies at least a portion of the nutrient requirements of an infant. In the United States, the content of an infant formula is dictated by the federal regulations set forth at 21 C.F.R. Sections 100, 106, and 107. These regulations define macronutrient, vitamin, mineral, and other ingredient levels in an effort to simulate the nutritional and other properties of human breast milk.

[0024] The term "growing-up milk" refers to a broad category of nutritional compositions intended to be used as a part of a diverse diet in order to support the normal growth and development of a child between the ages of about 1 and about 6 years of age.

[0025] "Milk-based" means comprising at least one component that has been drawn or extracted from the mammary gland of a mammal. In some embodiments, a milk-based nutritional composition comprises components of milk that are derived from domesticated ungulates, ruminants or other mammals or any combination thereof. Moreover, in some embodiments, milk-based means comprising bovine casein, whey, lactose, or any combination thereof. Further, "milk-based nutritional composition" may refer to any composition comprising any milk-derived or milk-based product known in the art.

[0026] "Nutritionally complete" means a composition that may be used as the sole source of nutrition, which would supply essentially all of the required daily amounts of vitamins, minerals, and/or trace elements in combination with proteins, carbohydrates, and lipids. Indeed, "nutritionally complete" describes a nutritional composition that provides adequate amounts of carbohydrates, lipids, essential fatty acids, proteins, essential amino acids, conditionally essential amino acids, vitamins, minerals and energy required to support normal growth and development of a subject.

[0027] Therefore, a nutritional composition that is "nutritionally complete" for a preterm infant will, by definition, provide qualitatively and quantitatively adequate amounts of carbohydrates, lipids, essential fatty acids, proteins, essential amino acids, conditionally essential amino acids, vitamins, minerals, and energy required for growth of the preterm infant.

[0028] A nutritional composition that is "nutritionally complete" for a term infant will, by definition, provide qualitatively and quantitatively adequate amounts of all carbohydrates,

lipids, essential fatty acids, proteins, essential amino acids, conditionally essential amino acids, vitamins, minerals, and energy required for growth of the term infant.

[0029] A nutritional composition that is "nutritionally complete" for a child will, by definition, provide qualitatively and quantitatively adequate amounts of all carbohydrates, lipids, essential fatty acids, proteins, essential amino acids, conditionally essential amino acids, vitamins, minerals, and energy required for growth of a child.

[0030] As applied to nutrients, the term "essential" refers to any nutrient that cannot be synthesized by the body in amounts sufficient for normal growth and to maintain health and that, therefore, must be supplied by the diet. The term "conditionally essential" as applied to nutrients means that the nutrient must be supplied by the diet under conditions when adequate amounts of the precursor compound is unavailable to the body for endogenous synthesis to occur.

[0031] "Nutritional supplement" or "supplement" refers to a formulation that contains a nutritionally relevant amount of at least one nutrient. For example, supplements described herein may provide at least one nutrient for a human subject, such as a lactating or pregnant female.

[0032] "Probiotic" means a microorganism with low or no pathogenicity that exerts at least one beneficial effect on the health of the host. An example of a probiotic is LGG.

[0033] In an embodiment, the probiotic(s) may be viable or non-viable. As used herein, the term "viable", refers to live microorganisms. The term "non-viable" or "non-viable probiotic" means non-living probiotic microorganisms, their cellular components and/or metabolites thereof. Such non-viable probiotics may have been heat-killed or otherwise inactivated, but they retain the ability to favorably influence the health of the host. The probiotics useful in the present disclosure may be naturally-occurring, synthetic or developed through the genetic manipulation of organisms, whether such source is now known or later developed.

[0034] The term "non-viable probiotic" means a probiotic wherein the metabolic activity or reproductive ability of the referenced probiotic has been reduced or destroyed. More specifically, "non-viable" or "non-viable probiotic" means non-living probiotic microorganisms, their cellular components and/or metabolites thereof. Such non-viable probiotics may have been heat-killed or otherwise inactivated. The "non-viable probiotic" does, however, still retain, at the cellular level, its cell structure or other structure associated with the cell, for example exopolysaccharide and at least a portion its biological glycol-protein and DNA/RNA structure and thus retains the ability to favorably influence the health

of the host. Contrariwise, the term "viable" refers to live microorganisms. As used herein, the term "non-viable" is synonymous with "inactivated".

[0035] The term "cell equivalent" refers to the level of non-viable, non-replicating probiotics equivalent to an equal number of viable cells. The term "non-replicating" is to be understood as the amount of non-replicating microorganisms obtained from the same amount of replicating bacteria (cfu/g), including inactivated probiotics, fragments of DNA, cell wall or cytoplasmic compounds. In other words, the quantity of non-living, non-replicating organisms is expressed in terms of cfu as if all the microorganisms were alive, regardless whether they are dead, non-replicating, inactivated, fragmented etc.

[0036] "Prebiotic" means a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of beneficial gut bacteria in the digestive tract, selective reduction in gut pathogens, or favorable influence on gut short chain fatty acid profile that can improve the health of the host.

[0037] "β-glucan" means all β-glucan, including both β-1,3-glucan and β-1,3;1,6-glucan, as each is a specific type of β-glucan. Moreover, β-1,3;1,6-glucan is a type of β-1,3-glucan. Therefore, the term "β-1,3-glucan" includes β-1,3;1,6-glucan.

[0038] All percentages, parts and ratios as used herein are by weight of the total formulation, unless otherwise specified.

[0039] The nutritional composition of the present disclosure may be free of substantially free of any optional or selected ingredients described herein. In this context, and unless otherwise specified, the term "substantially free" means that the selected composition may contain less than a functional amount of the optional ingredient, typically less than 0.1% by weight, and also, including zero percent by weight of such optional or selected ingredient.

[0040] All references to singular characteristics or limitations of the present disclosure shall include the corresponding plural characteristic or limitation, and vice versa, unless otherwise specified or clearly implied to the contrary by the context in which the reference is made.

[0041] All combinations of method or process steps as used herein can be performed in any order, unless otherwise specified or clearly implied to the contrary by the context in which the referenced combination is made.

[0042] The compositions and methods of the present disclosure, including components thereof, can comprise, consist of, or consist essentially of the essential elements and limitations of the embodiments described herein, as well as any additional or optional

ingredients, components or limitations described herein or otherwise useful in nutritional compositions.

[0043] As used herein, the term "about" should be construed to refer to both of the numbers specified in any range. Any reference to a range should be considered as providing support for any subset within that range.

[0044] The present disclosure generally relates to pediatric nutritional compositions comprising a prebiotic such as GOS, PDX or, in a preferred embodiment, a combination of GOS and PDX; HMO; and a probiotic, such as LGG, that are capable of modulating the gut-brain axis in pediatric subjects, including preterm and term infants, toddlers and children. The GOS/PDX, HMO and probiotics are believed to work together in a complementary and/or synergistic manner by stimulating the growth and activity of beneficial gut microbiota. Gut microbiota are important in normal healthy brain function and development in human infants. Accordingly, the present compositions are believed to promote healthy brain development and function. More particularly, the present compositions, in some embodiments, improve gut microbiota composition and/or activity by increasing proliferation of *Bifidobacterium, Lactobacillus* and or *Allobaculum* species, while reducing proliferation of harmful microbiota such as *Clostridium* species.

[0045] More specifically, the present compositions may modulate a subject's neural development and function, both centrally and peripherally via the enteric nervous system. While not being bound by theory, it is believed that the interactions across the developing gut-brain axis promote neurological development and function in pediatric populations. Additional neurologic benefits may include promoting visual function, sensorimotor development, exploration and manipulation, object relatedness, object recognition, social and emotional development, healthy sleep patterns and stress reduction.

[0046] Accordingly, the present disclosure provides in some embodiments a nutritional composition comprising: (i) a protein source, (ii) a lipid source, (iii) a carbohydrate source, (iv) a human milk oligosaccharide or a precursor thereof, (v) a prebiotic comprising GOS and/or PDX, and (vi) a probiotic.

[0047] The term "HMO" or "human milk oligosaccharides" refers generally to a number of complex carbohydrates found in human breast milk that can be in either acidic or neutral form. In certain embodiments, the HMO is 2'-fucosyllactose, 3'-fucosyllactose, 3'sialyllactose, 6'sialyllactose, lacto-N-biose, lacto-N-neotetraose, lacto-N-tetraose, or any combination thereof. 3'sialyllactose, 6'sialyllactose contribute sialic acid, which is an important nutrient for brain development and cognitive function. HMO may be isolated or enriched from milk or produced via microbial fermentation, enzymatic processes, chemical

synthesis, or a combination thereof. Exemplary HMO precursors include sialic acid, fucose, or a combination thereof.

[0048] HMO are believed to correlate with the presence of beneficial infant specific *Bifidobacterium* species, such as *B. longum*, *B. infantis*, *B. breve*, and *B. bifidium* in breast fed infants. Accordingly, the HMO used in the present compositions may provide infant formulas that are functionally closer to human milk. Furthermore, the HMO may work synergistically with GOS/PDX and LGG to further promote the gut-brain axis, thereby providing immediate and lifelong gastrointestinal and neurological benefits to pediatric subjects. The HMO, in certain embodiments, is present in the compositions in an amount ranging from about 0.005 g/100 kcal to about 1 g/100 kcal. In other embodiments, the HMO may be present in an amount ranging from about 0.01 g/100 kcal to about 0.1 g/100 kcal, about 0.015 g/100 kcal to about 0.05 g/100 kcal.

[0049] The disclosed nutritional composition also comprises a source of prebiotics, specifically GOS and/or PDX. In one embodiment, at least 20% of the prebiotics comprises GOS. In other embodiments, the prebiotic component comprises both GOS and PDX. The GOS and PDX may be present in a ratio of about 1:9 to about 9:1 by weight. In other embodiments, the GOS and PDX are present in a ratio of about 1:4 to 4:1, or about 1:1.

[0050] In some embodiments, the amount of GOS in the nutritional composition may be from about 0.1 g/100 kcal to about 1.0 g/100 kcal. In another embodiment, the amount of GOS in the nutritional composition may be from about 0.1 g/100 kcal to about 0.5 g/100 kcal. The amount of PDX in the nutritional composition may, in some embodiments, be within the range of from about 0.1 g/100 kcal to about 0.5 g/100 kcal. In other embodiments, the amount of PDX may be about 0.3 g/100 kcal.

[0051] In a particular embodiment, GOS and PDX are supplemented into the nutritional composition in a total amount of about at least about 0.2 g/100 kcal and can be about 0.2 g/100 kcal to about 1.5 g/100 kcal. In some embodiments, the nutritional composition may comprise GOS and PDX in a total amount of from about 0.6 to about 0.8 g/100 kcal.

[0052] In some embodiments the nutritional composition comprises *Lactobacillus rhamnosus* GG (ATCC number 53103). Other probiotics useful in the present nutritional compositions include, but are not limited to, *Bifidobacterium* species such as *Bifidobacterium longum* BB536 (BL999, ATCC: BAA-999), and *Bifidobacterium animalis* subsp. lactis BB-12 (DSM No. 10140) or any combination thereof.

[0053] LGG and prebiotics, such as GOS and PDX, are believed to significantly and surprisingly improve brain development, cognitive function, and even social and emotional

skills. Additionally, the administration of a combination of GOS, PDX and LGG may alter the production of neurotransmitters, such as serotonin, 5-hydroxytryptophan, noradrenaline and/or 5-hydroxyindoleacetic acid. The ability of the compositions to modulate neurotransmitters may explain the beneficial effects of the present compositions on social skills, anxiety and memory function.

[0054] In some embodiments, the nutritional composition includes a probiotic, and more particularly, LGG, in an amount of from about 1 x 10^4 cfu/100 kcal to about 1.5 x 10^{10} cfu/100 kcal. In other embodiments, the nutritional composition comprises LGG in an amount of from about 1 x 10^6 cfu/100 kcal to about 1 x 10^9 cfu/100 kcal. Still, in certain embodiments, the nutritional composition may include LGG in an amount of from about 1 x 10^7 cfu/100 kcal to about 1 x 10^8 cfu/100 kcal. In some embodiments, where LGG is not included at the upper limit of the concentration range, additional probiotics may be included up to the upper limit concentration specified. The probiotic may be either non-viable or viable.

[0055] In some embodiments, the probiotic functionality in the nutritional composition of the present disclosure is provided by including a culture supernatant from a late-exponential growth phase of a probiotic batch-cultivation process, as disclosed in international published application no. WO 2013/142403, which is hereby incorporated by reference in its entirety. Without wishing to be bound by theory, it is believed that the activity of the culture supernatant can be attributed to the mixture of components (including proteinaceous materials, and possibly including (exo)polysaccharide materials) as found released into the culture medium at a late stage of the exponential (or "log") phase of batch cultivation of the probiotic. The term "culture supernatant" as used herein, includes the mixture of components found in the culture medium. The stages recognized in batch cultivation of bacteria are known to the skilled person. These are the "lag," the "log" ("logarithmic" or "exponential"), the "stationary" and the "death" (or "logarithmic decline") phases. In all phases during which live bacteria are present, the bacteria metabolize nutrients from the media, and secrete (exert, release) materials into the culture medium. The composition of the secreted material at a given point in time of the growth stages is not generally predictable.

[0056] In an embodiment, a culture supernatant is obtainable by a process comprising the steps of (a) subjecting a probiotic such as LGG to cultivation in a suitable culture medium using a batch process; (b) harvesting the culture supernatant at a late exponential growth phase of the cultivation step, which phase is defined with reference to the second half of the time between the lag phase and the stationary phase of the batch-cultivation process; (c) optionally removing low molecular weight constituents from the supernatant so as to retain

molecular weight constituents above 5-6 kiloDaltons (kDa); (d) removing liquid contents from the culture supernatant so as to obtain the composition.

[0057] The culture supernatant may comprise secreted materials that are harvested from a late exponential phase. The late exponential phase occurs in time after the mid exponential phase (which is halftime of the duration of the exponential phase, hence the reference to the late exponential phase as being the second half of the time between the lag phase and the stationary phase). In particular, the term "late exponential phase" is used herein with reference to the latter quarter portion of the time between the lag phase and the stationary phase of the LGG batch-cultivation process. In some embodiments, the culture supernatant is harvested at a point in time of 75% to 85% of the duration of the exponential phase, and may be harvested at about 5/6 of the time elapsed in the exponential phase.

[0058] The nutritional composition of the disclosure may contain a source of long chain polyunsaturated fatty acid (LCPUFA) that comprises docosahexaenoic acid. Other suitable LCPUFAs include, but are not limited to, α-linoleic acid, γ-linoleic acid, linoleic acid, linolenic acid, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (ARA).

[0059] In an embodiment, especially if the nutritional composition is an infant formula, the nutritional composition is supplemented with both DHA and ARA. In this embodiment, the weight ratio of ARA:DHA may be between about 1:3 and about 9:1. In a particular embodiment, the ratio of ARA:DHA is from about 1:2 to about 4:1.

[0060] If included, the source of DHA and/or ARA may be any source known in the art such as marine oil, fish oil, single cell oil, egg yolk lipid, and brain lipid. In some embodiments, the DHA and ARA are sourced from single cell Martek oils, DHASCO® and ARASCO®, or variations thereof. The DHA and ARA can be in natural form, provided that the remainder of the LCPUFA source does not result in any substantial deleterious effect on the subject. Alternatively, the DHA and ARA can be used in refined form.

[0061] In an embodiment, sources of DHA and ARA are single cell oils as taught in U.S. Pat. Nos. 5,374,657; 5,550,156; and 5,397,591, the disclosures of which are incorporated herein in their entirety by reference. Nevertheless, the present disclosure is not limited to only such oils.

[0062] The nutritional composition may also comprise a source of ß-glucan. Glucans are polysaccharides, specifically polymers of glucose, which are naturally occurring and may be found in cell walls of bacteria, yeast, fungi, and plants. Beta glucans (β-glucans) are themselves a diverse subset of glucose polymers, which are made up of chains of glucose monomers linked together via beta-type glycosidic bonds to form complex carbohydrates.

[0063] β-1,3-glucans are carbohydrate polymers purified from, for example, yeast, mushroom, bacteria, algae, or cereals. (Stone BA, Clarke AE. Chemistry and Biology of (1-3)-Beta-Glucans. London:Portland Press Ltd; 1993.) The chemical structure of β-1,3-glucan depends on the source of the β-1,3-glucan. Moreover, various physiochemical parameters, such as solubility, primary structure, molecular weight, and branching, play a role in biological activities of β-1,3-glucans. (Yadomae T., Structure and biological activities of fungal beta-1,3-glucans. Yakugaku Zasshi. 2000;120:413-431.)

[0064] β-1,3-glucans are naturally occurring polysaccharides, with or without β-1,6-glucose side chains that are found in the cell walls of a variety of plants, yeasts, fungi and bacteria. β-1,3;1,6-glucans are those containing glucose units with (1,3) links having side chains attached at the (1,6) position(s). β-1,3;1,6 glucans are a heterogeneous group of glucose polymers that share structural commonalities, including a backbone of straight chain glucose units linked by a β-1,3 bond with β-1,6-linked glucose branches extending from this backbone. While this is the basic structure for the presently described class of β-glucans, some variations may exist. For example, certain yeast β-glucans have additional regions of β(1,3) branching extending from the β(1,6) branches, which add further complexity to their respective structures.

[0065] β-glucans derived from baker's yeast, Saccharomyces cerevisiae, are made up of chains of D-glucose molecules connected at the 1 and 3 positions, having side chains of glucose attached at the 1 and 6 positions. Yeast-derived β-glucan is an insoluble, fiber-like, complex sugar having the general structure of a linear chain of glucose units with a β-1,3 backbone interspersed with β-1,6 side chains that are generally 6-8 glucose units in length. More specifically, β-glucan derived from baker's yeast is poly-(1,6)-β-D-glucopyranosyl-(1,3)-β-D-glucopyranose.

[0066] Furthermore, β-glucans are well tolerated and do not produce or cause excess gas, abdominal distension, bloating or diarrhea in pediatric subjects. Addition of β-glucan to a nutritional composition for a pediatric subject, such as an infant formula, a growing-up milk or another children's nutritional product, will improve the subject's immune response by increasing resistance against invading pathogens and therefore maintaining or improving overall health.

[0067] In a particular embodiment, a nutritional composition comprises per 100 kcal: (i) between about 1 g and about 7 g of a protein source, (ii) between about 1 g and about 10 g of a lipid source, (iii) between about 6 g and about 22 g of a carbohydrate source, (iv) between about 0.005 g and about 1 g of a human milk oligosaccharide, (v) between about 0.1 mg and 1.0 mg of a galacto-oligosaccharide, (vi) between about 0.1 mg and about 0.5 mg

of polydextrose, and (vii) between about $1x10^5$ cfu/100 kcals to about 1.5 x 10^9 cfu/100 kcals of *Lactobacillus rhamnosus* GG or about $1x10^5$ equivalent cfu/100 kcals to about 1.5 x 10^9 equivalent cfu/100 kcals of dry composition of *Lactobacillus rhamnosus* GG. In some embodiments, the nutritional composition comprises the culture supernatant from about 0.015 g per 100 kcal to about 1.5 g per 100 kcal.

[0068] The present disclosure also provides a method for promoting the growth of beneficial microbiota in the gastrointestinal tract of pediatric subject in need thereof comprising administering to the subject an effective amount of any of the nutritional compositions described herein, for example a nutritional composition comprising PDX, GOS, HMO and a probiotic such as LGG. More particularly, the present disclosure provides a method for promoting the growth of beneficial microbiota in the gastrointestinal tract of pediatric subject in need thereof comprising administering to the subject an effective amount of a nutritional composition comprising: (i) a protein source, (ii) a lipid source, (iii) a carbohydrate source, (iv) a human milk oligosaccharide or a precursor thereof, (v) a prebiotic comprising polydextrose and galacto-oligosaccharide, and (vi) a probiotic.

[0069] In certain embodiments, the administration of the composition to the subject stimulates the growth of gut bacteria in the subject, wherein the gut bacteria comprise *Lactobacillus* species, *Bifidobacterium* species, *Allobaculum* species or combinations thereof. In another embodiment, the method reduces the growth of *Clostridium* species in the gut of the subject. In still other embodiments, the method promotes cognitive development in the subject.

[0070] More specifically, the present compositions and methods, in some embodiments, improve the normal mental performance, learning, memory, cognition and visual function in a subject. In other embodiments, the present compositions and methods support healthy, normal or improved behavioral, psychomotor and emotional development in a subject. In yet further embodiments, the present compositions and methods promote sensorimotor development, exploration and manipulation, object relatedness, visual acuity, objection recognition, visual attention and/or other aspects of cognitive processing.

[0071] While not being bound by any particular theory, several mechanisms of action may contribute to the beneficial gastrointestinal and neurological benefits of the nutritional compositions and methods of the present disclosure. For example, the compositions beneficial by products of gut microbiota may affect brain and influence behavior. Additionally, the compositions may promote activation of the hypothalamic-pituitary-adrenal (HPA) axis and hippocammal neurogenesis. The HPA axis is a major part of the neuroendocrine system that controls reactions to stress and regulates many body processes,

including digestion, the immune system, mood and emotions, sexuality and energy storage and expenditure. It is the common mechanism for interactions among glands, hormones, and parts of the midbrain that mediate the general adaptation syndrome.

[0072] Another mechanism is modulation of brain derived neurothrophic factor (BDNF) in the hippocampus. BDNF acts on certain neurons of the central nervous system and the peripheral nervous system, helping to support the survival of existing neurons, and encourage the growth and differentiation of new neurons and synapses. In the brain, BDNF is active in the hippocampus, cortex, and basal forebrain, which are areas vital to learning, memory, and higher thinking. BDNF itself is important for long-term memory. Although the vast majority of neurons in the mammalian brain are formed prenatally, parts of the adult brain retain the ability to grow new neurons from neural stem cells in a process known as neurogenesis. Neurotrophins are chemicals that help to stimulate and control neurogenesis, BDNF being one of the most active. Mice born without the ability to make BDNF suffer developmental defects in the brain and sensory nervous system, and usually die soon after birth, suggesting that BDNF plays an important role in normal neural development.

[0073] The present compositions also may limit the amount of circulating interleukin 6 (IL-6) and chemokine (C-C motif) ligand 2 (CCL2). IL-6 is a proinflammatory cytokine that is known to stimulate the inflammatory and auto-immune processes in many diseases such as diabetes, atherosclerosis, depression, Alzheimer's Disease, systemic lupus erythematosus, multiple myeloma, prostate cancer, Behçet's disease, and rheumatoid arthritis. Additionally, advanced/metastatic cancer patients have higher levels of IL-6 in their blood. IL-6 has also been shown to lead to several neurological diseases through its impact on epigenetic modification within the brain. IL-6 activates the Phosphoinositide 3-kinase (PI3K) pathway, and a downstream target of this pathway is the protein kinase B (PKB). IL-6 is also implicated in pathways associated with mental disorders, such as schizophrenia and depression. CCL2 is a small cytokine that belongs to the CC chemokine family. CCL2 recruits monocytes, memory T cells, and dendritic cells to the sites of inflammation produced by either tissue injury or infection.

[0074] The present compositions may additionally promote the release of gut hormones such as 5-hydroxytryptophan (5-HT) from endocrine cells. 5-HT is a naturally occurring amino acid and a metabolic intermediate in the biosynthesis of the neurotransmitters serotonin and melatonin.

[0075] Yet another possible mechanism that may contribute to the beneficial gastrointestinal and neurological benefits of the present nutritional compositions is to stimulate the afferent (sensory) neural pathway, including the vagus nerve or sympathetic

neurotransmitters, thereby promoting healthy brain development. The present compositions also may modulate the pathways of genes involved in cognition.

[0076] The disclosed nutritional composition(s) may be provided in any form known in the art, such as a powder, a gel, a suspension, a paste, a solid, a liquid, a liquid concentrate, a reconstitutable powdered milk substitute or a ready-to-use product. The nutritional composition may, in certain embodiments, comprise a nutritional supplement, children's nutritional product, infant formula, human milk fortifier, growing-up milk or any other nutritional composition designed for a pediatric subject. Nutritional compositions of the present disclosure include, for example, orally-ingestible, health-promoting substances including, for example, foods, beverages, tablets, capsules and powders. Moreover, the nutritional composition of the present disclosure may be standardized to a specific caloric content, it may be provided as a ready-to-use product, or it may be provided in a concentrated form. In some embodiments, the nutritional composition is in powder form with a particle size in the range of 5 μm to 1500 μm, more preferably in the range of 10 μm to 1000μm, and even more preferably in the range of 50 μm to 300μm.

[0077] In some embodiments, the nutritional composition is an infant formula suitable for infants ranging in age from 0 to 12 months, from 0 to 3 months, 0 to 6 months or 6 to 12 months. In other embodiments, the disclosure provides a fortified milk-based growing-up milk designed for children ages 1-3 years and/or 4-6 years, wherein the growing-up milk supports growth and development and life-long health.

[0078] In one embodiment, where the nutritional composition is an infant formula, the combination of HMOs, probiotic, GOS, and PDX may be added to a commercially available infant formula. For example, Enfalac, Enfamil®, Enfamil® Premature Formula, Enfamil® with Iron, Enfamil® LIPIL®, Lactofree®, Nutramigen®, Pregestimil®, and ProSobee® (available from Mead Johnson & Company, Evansville, IN, U.S.A.) may be supplemented with PDX, GOS, HMO and LGG, and used in practice of the current disclosure.

[0079] As noted, the nutritional composition(s) of the disclosure may comprise a protein source. The protein source can be any used in the art, e.g., nonfat milk, whey protein, casein, soy protein, hydrolyzed protein, amino acids, and the like. Bovine milk protein sources useful in practicing the present disclosure include, but are not limited to, milk protein powders, milk protein concentrates, milk protein isolates, nonfat milk solids, nonfat milk, nonfat dry milk, whey protein, whey protein isolates, whey protein concentrates, sweet whey, acid whey, casein, acid casein, caseinate (e.g. sodium caseinate, sodium calcium caseinate, calcium caseinate) and any combinations thereof.

[0080] In one embodiment, the proteins of the nutritional composition are provided as intact proteins. In other embodiments, the proteins are provided as a combination of both intact proteins and partially hydrolyzed proteins, with a degree of hydrolysis of between about 4% and 10%. In certain other embodiments, the proteins are more completely hydrolyzed. In still other embodiments, the protein source comprises amino acids as a protein equivalent. In yet another embodiment, the protein source may be supplemented with glutamine-containing peptides.

[0081] In a particular embodiment of the nutritional composition, the whey:casein ratio of the protein source is similar to that found in human breast milk. In an embodiment, the protein source comprises from about 40% to about 90% whey protein and from about 10% to about 60% casein.

[0082] In some embodiments, the nutritional composition comprises between about 1 g and about 7 g of a protein source per 100 kcal. In other embodiments, the nutritional composition comprises between about 3.5 g and about 4.5 g of protein per 100 kcal.

[0083] Suitable fat or lipid sources for the nutritional composition of the present disclosure may be any known or used in the art, including but not limited to, animal sources, e.g., milk fat, butter, butter fat, egg yolk lipid; marine sources, such as fish oils, marine oils, single cell oils; vegetable and plant oils, such as corn oil, canola oil, sunflower oil, soybean oil, palm olein oil, coconut oil, high oleic sunflower oil, evening primrose oil, rapeseed oil, olive oil, flaxseed (linseed) oil, cottonseed oil, high oleic safflower oil, palm stearin, palm kernel oil, wheat germ oil; medium chain triglyceride oils and emulsions and esters of fatty acids; and any combinations thereof.

[0084] Carbohydrate sources can be any used in the art, e.g., lactose, glucose, fructose, corn syrup solids, maltodextrins, sucrose, starch, rice syrup solids, and the like. The amount of carbohydrate in the nutritional composition typically can vary from between about 5 g and about 25 g/100 kcal.

[0085] In some embodiments, the nutritional composition may include prebiotics in addition to GOS and PDX. In some embodiments, additional prebiotics useful in the present disclosure may include: lactulose, lactosucrose, raffinose, gluco-oligosaccharide, inulin, fructo-oligosaccharide, isomalto-oligosaccharide, soybean oligosaccharides, lactosucrose, xylo-oligosaccharide, chito-oligosaccharide, manno-oligosaccharide, aribino-oligosaccharide, siallyl-oligosaccharide, fuco-oligosaccharide, and gentio-oligosaccharides. In embodiments where GOS and PDX are not included at the upper limit of their respective concentration range, additional prebiotics may be included up to the upper limit concentration specified.

[0086] The nutritional composition of the present disclosure may comprise lactoferrin in some embodiments. Lactoferrins are single chain polypeptides of about 80 kD containing 1 – 4 glycans, depending on the species. The 3-D structures of lactoferrin of different species are very similar, but not identical. Each lactoferrin comprises two homologous lobes, called the N- and C-lobes, referring to the N-terminal and C-terminal part of the molecule, respectively. Each lobe further consists of two sub-lobes or domains, which form a cleft where the ferric ion (Fe3+) is tightly bound in synergistic cooperation with a (bi)carbonate anion. These domains are called N1, N2, C1 and C2, respectively. The N-terminus of lactoferrin has strong cationic peptide regions that are responsible for a number of important binding characteristics. Lactoferrin has a very high isoelectric point (~pI 9) and its cationic nature plays a major role in its ability to defend against bacterial, viral, and fungal pathogens. There are several clusters of cationic amino acids residues within the N-terminal region of lactoferrin mediating the biological activities of lactoferrin against a wide range of microorganisms.

[0087] Lactoferrin for use in the present disclosure may be, for example, isolated from the milk of a non-human animal or produced by a genetically modified organism. The oral electrolyte solutions described herein can, in some embodiments comprise non-human lactoferrin, non-human lactoferrin produced by a genetically modified organism and/or human lactoferrin produced by a genetically modified organism.

[0088] Suitable non-human lactoferrins for use in the present disclosure include, but are not limited to, those having at least 48% homology with the amino acid sequence of human lactoferrin. For instance, bovine lactoferrin ("bLF") has an amino acid composition which has about 70% sequence homology to that of human lactoferrin. In some embodiments, the non-human lactoferrin has at least 65% homology with human lactoferrin and in some embodiments, at least 75% homology. Non-human lactoferrins acceptable for use in the present disclosure include, without limitation, bLF, porcine lactoferrin, equine lactoferrin, buffalo lactoferrin, goat lactoferrin, murine lactoferrin and camel lactoferrin.

[0089] In some embodiments, the nutritional composition of the present disclosure comprises non-human lactoferrin, for example bLF. bLF is a glycoprotein that belongs to the iron transporter or transferring family. It is isolated from bovine milk, wherein it is found as a component of whey. There are known differences between the amino acid sequence, glycosylation patters and iron-binding capacity in human lactoferrin and bLF. Additionally, there are multiple and sequential processing steps involved in the isolation of bLF from cow's milk that affect the physiochemical properties of the resulting bLF preparation. Human

lactoferrin and bLF are also reported to have differences in their abilities to bind the lactoferrin receptor found in the human intestine.

[0090] Though not wishing to be bound by this or any other theory, it is believe that bLF that has been isolated from whole milk has less lipopolysaccharide (LPS) initially bound than does bLF that has been isolated from milk powder. Additionally, it is believed that bLF with a low somatic cell count has less initially-bound LPS. A bLF with less initially-bound LPS has more binding sites available on its surface. This is thought to aid bLF in binding to the appropriate location and disrupting the infection process.

[0091] bLF suitable for the present disclosure may be produced by any method known in the art. For example, in U.S. Patent No. 4,791,193, incorporated by reference herein in its entirety, Okonogi et al. discloses a process for producing bovine lactoferrin in high purity. Generally, the process as disclosed includes three steps. Raw milk material is first contacted with a weakly acidic cationic exchanger to absorb lactoferrin followed by the second step where washing takes place to remove nonabsorbed substances. A desorbing step follows where lactoferrin is removed to produce purified bovine lactoferrin. Other methods may include steps as described in U.S. Patent Nos. 7,368,141, 5,849,885, 5,919,913 and 5,861,491, the disclosures of which are all incorporated by reference in their entirety.

[0092] In certain embodiments, lactoferrin utilized in the present disclosure may be provided by an expanded bed absorption ("EBA") process for isolating proteins from milk sources. EBA, also sometimes called stabilized fluid bed adsorption, is a process for isolating a milk protein, such as lactoferrin, from a milk source comprises establishing an expanded bed adsorption column comprising a particulate matrix, applying a milk source to the matrix, and eluting the lactoferrin from the matrix with an elution buffer comprising about 0.3 to about 2.0 M sodium chloride. Any mammalian milk source may be used in the present processes, although in particular embodiments, the milk source is a bovine milk source. The milk source comprises, in some embodiments, whole milk, reduced fat milk, skim milk, whey, casein, or mixtures thereof.

[0093] In particular embodiments, the target protein is lactoferrin, though other milk proteins, such as lactoperoxidases or lactalbumins, also may be isolated. In some embodiments, the process comprises the steps of establishing an expanded bed adsorption column comprising a particulate matrix, applying a milk source to the matrix, and eluting the lactoferrin from the matrix with about 0.3 to about 2.0M sodium chloride. In other embodiments, the lactoferrin is eluted with about 0.5 to about 1.0 M sodium chloride, while in further embodiments, the lactoferrin is eluted with about 0.7 to about 0.9 M sodium chloride.

[0094] The expanded bed adsorption column can be any known in the art, such as those described in U.S. Patent Nos. 7,812,138, 6,620,326, and 6,977,046, the disclosures of which are hereby incorporated by reference herein. In some embodiments, a milk source is applied to the column in an expanded mode, and the elution is performed in either expanded or packed mode. In particular embodiments, the elution is performed in an expanded mode. For example, the expansion ratio in the expanded mode may be about 1 to about 3, or about 1.3 to about 1.7. EBA technology is further described in international published application nos. WO 92/00799, WO 02/18237, WO 97/17132, which are hereby incorporated by reference in their entireties.

[0095] The isoelectric point of lactoferrin is approximately 8.9. Prior EBA methods of isolating lactoferrin use 200 mM sodium hydroxide as an elution buffer. Thus, the pH of the system rises to over 12, and the structure and bioactivity of lactoferrin may be comprised, by irreversible structural changes. It has now been discovered that a sodium chloride solution can be used as an elution buffer in the isolation of lactoferrin from the EBA matrix. In certain embodiments, the sodium chloride has a concentration of about 0.3 M to about 2.0 M. In other embodiments, the lactoferrin elution buffer has a sodium chloride concentration of about 0.3 M to about 1.5 M, or about 0.5 m to about 1.0 M.

[0096] In other embodiments, lactoferrin for use in the composition of the present disclosure can be isolated through the use of radial chromatography or charged membranes, as would be familiar to the skilled artisan.

[0097] The lactoferrin that is used in certain embodiments may be any lactoferrin isolated from whole milk and/or having a low somatic cell count, wherein "low somatic cell count" refers to a somatic cell count less than 200,000 cells/mL. By way of example, suitable lactoferrin is available from Tatua Co-operative Dairy Co. Ltd., in Morrinsville, New Zealand, from FrieslandCampina Domo in Amersfoort, Netherlands or from Fonterra Co-Operative Group Limited in Auckland, New Zealand.

[0098] Surprisingly, lactoferrin included herein maintains certain bactericidal activity even if exposed to a low pH (i.e., below about 7, and even as low as about 4.6 or lower) and/or high temperatures (i.e., above about 65 °C, and as high as about 120 °C), conditions which would be expected to destroy or severely limit the stability or activity of human lactoferrin. These low pH and/or high temperature conditions can be expected during certain processing regimen for nutritional compositions of the types described herein, such as pasteurization. Therefore, even after processing regimens, lactoferrin has bactericidal activity against undesirable bacterial pathogens found in the human gut. The nutritional composition may, in some embodiments, comprise lactoferrin in an amount from about 25

mg/100 mL to about 150 mg/100 mL. In other embodiments lactoferrin is present in an amount from about 60 mg/100 mL to about 120 mg/100 mL. In still other embodiments lactoferrin is present in an amount from about 85 mg/100 mL to about 110 mg/100 mL.

[0099] In an embodiment, the nutritional composition(s) of the present disclosure comprises choline. Choline is a nutrient that is essential for normal function of cells. It is a precursor for membrane phospholipids, and it accelerates the synthesis and release of acetylcholine, a neurotransmitter involved in memory storage. Moreover, though not wishing to be bound by this or any other theory, it is believed that dietary choline and docosahexaenoic acid (DHA) act synergistically to promote the biosynthesis of phosphatidylcholine and thus help promote synaptogenesis in human subjects. Additionally, choline and DHA may exhibit the synergistic effect of promoting dendritic spine formation, which is important in the maintenance of established synaptic connections. In some embodiments, the nutritional composition(s) of the present disclosure includes about 40 mg choline per serving to about 100 mg per 8 oz. serving.

[0100] In an embodiment, the nutritional composition comprises a source of iron. In an embodiment, the source of iron is ferric pyrophosphate, ferric orthophosphate, ferrous fumarate or a mixture thereof and the source of iron may be encapsulated in some embodiments.

[0101] One or more vitamins and/or minerals may also be added in to the nutritional composition in amounts sufficient to supply the daily nutritional requirements of a subject. It is to be understood by one of ordinary skill in the art that vitamin and mineral requirements will vary, for example, based on the age of the subject. For instance, an infant may have different vitamin and mineral requirements than a child between the ages of one and thirteen years. Thus, the embodiments are not intended to limit the nutritional composition to a particular age group but, rather, to provide a range of acceptable vitamin and mineral components.

[0102] In certain embodiments, the composition may optionally include, but is not limited to, one or more of the following vitamins or derivations thereof: vitamin B1 (thiamin, thiamin pyrophosphate, TPP, thiamin triphosphate, TTP, thiamin hydrochloride, thiamin mononitrate), vitamin B2 (riboflavin, flavin mononucleotide, FMN, flavin adenine dinucleotide, FAD, lactoflavin, ovoflavin), vitamin B3 (niacin, nicotinic acid, nicotinamide, niacinamide, nicotinamide adenine dinucleotide, NAD, nicotinic acid mononucleotide, NicMN, pyridine-3-carboxylic acid), vitamin B3-precursor tryptophan, vitamin B6 (pyridoxine, pyridoxal, pyridoxamine, pyridoxine hydrochloride), pantothenic acid (pantothenate, panthenol), folate (folic acid, folacin, pteroylglutamic acid), vitamin B12 (cobalamin, methylcobalamin,

deoxyadenosylcobalamin, cyanocobalamin, hydroxycobalamin, adenosylcobalamin), biotin, vitamin C (ascorbic acid), vitamin A (retinol, retinyl acetate, retinyl palmitate, retinyl esters with other long-chain fatty acids, retinal, retinoic acid, retinol esters), vitamin D (calciferol, cholecalciferol, vitamin D3, 1,25,-dihydroxyvitamin D), vitamin E (α-tocopherol, α-tocopherol acetate, α-tocopherol succinate, α-tocopherol nicotinate, α-tocopherol), vitamin K (vitamin K1, phylloquinone, naphthoquinone, vitamin K2, menaquinone-7, vitamin K3, menaquinone-4, menadione, menaquinone-8, menaquinone-8H, menaquinone-9, menaquinone-9H, menaquinone-10, menaquinone-11, menaquinone-12, menaquinone-13), choline, inositol, β-carotene and any combinations thereof.

[0103] In other embodiments, the composition may optionally include, but is not limited to, one or more of the following minerals or derivations thereof: boron, calcium, calcium acetate, calcium gluconate, calcium chloride, calcium lactate, calcium phosphate, calcium sulfate, chloride, chromium, chromium chloride, chromium picolonate, copper, copper sulfate, copper gluconate, cupric sulfate, fluoride, iron, carbonyl iron, ferric iron, ferrous fumarate, ferric orthophosphate, iron trituration, polysaccharide iron, iodide, iodine, magnesium, magnesium carbonate, magnesium hydroxide, magnesium oxide, magnesium stearate, magnesium sulfate, manganese, molybdenum, phosphorus, potassium, potassium phosphate, potassium iodide, potassium chloride, potassium acetate, selenium, sulfur, sodium, docusate sodium, sodium chloride, sodium selenate, sodium molybdate, zinc, zinc oxide, zinc sulfate and mixtures thereof. Non-limiting exemplary derivatives of mineral compounds include salts, alkaline salts, esters and chelates of any mineral compound.

[0104] The minerals can be added to growing-up milks or to other children's nutritional compositions in the form of salts such as calcium phosphate, calcium glycerol phosphate, sodium citrate, potassium chloride, potassium phosphate, magnesium phosphate, ferrous sulfate, zinc sulfate, cupric sulfate, manganese sulfate, and sodium selenite. Additional vitamins and minerals can be added as known within the art.

[0105] In an embodiment, the children's nutritional composition may contain between about 10 and about 50% of the maximum dietary recommendation for any given country, or between about 10 and about 50% of the average dietary recommendation for a group of countries, per serving of vitamins A, C, and E, zinc, iron, iodine, selenium, and choline. In another embodiment, the children's nutritional composition may supply about 10 – 30% of the maximum dietary recommendation for any given country, or about 10 – 30% of the average dietary recommendation for a group of countries, per serving of B-vitamins. In yet another embodiment, the levels of vitamin D, calcium, magnesium, phosphorus, and potassium in the children's nutritional product may correspond with the average levels found

in milk. In other embodiments, other nutrients in the children's nutritional composition may be present at about 20% of the maximum dietary recommendation for any given country, or about 20% of the average dietary recommendation for a group of countries, per serving.

[0106] The children's nutritional composition of the present disclosure may optionally include one or more of the following flavoring agents, including, but not limited to, flavored extracts, volatile oils, cocoa or chocolate flavorings, peanut butter flavoring, cookie crumbs, vanilla or any commercially available flavoring. Examples of useful flavorings include, but are not limited to, pure anise extract, imitation banana extract, imitation cherry extract, chocolate extract, pure lemon extract, pure orange extract, pure peppermint extract, honey, imitation pineapple extract, imitation rum extract, imitation strawberry extract, or vanilla extract; or volatile oils, such as balm oil, bay oil, bergamot oil, cedarwood oil, cherry oil, cinnamon oil, clove oil, or peppermint oil; peanut butter, chocolate flavoring, vanilla cookie crumb, butterscotch, toffee, and mixtures thereof. The amounts of flavoring agent can vary greatly depending upon the flavoring agent used. The type and amount of flavoring agent can be selected as is known in the art.

[0107] The nutritional compositions of the present disclosure may optionally include one or more emulsifiers that may be added for stability of the final product. Examples of suitable emulsifiers include, but are not limited to, lecithin (e.g., from egg or soy), alpha lactalbumin and/or mono- and di-glycerides, and mixtures thereof. Other emulsifiers are readily apparent to the skilled artisan and selection of suitable emulsifier(s) will depend, in part, upon the formulation and final product.

[0108] The nutritional compositions of the present disclosure may optionally include one or more preservatives that may also be added to extend product shelf life. Suitable preservatives include, but are not limited to, potassium sorbate, sodium sorbate, potassium benzoate, sodium benzoate, calcium disodium EDTA, and mixtures thereof.

[0109] The nutritional compositions of the present disclosure may optionally include one or more stabilizers. Suitable stabilizers for use in practicing the nutritional composition of the present disclosure include, but are not limited to, gum arabic, gum ghatti, gum karaya, gum tragacanth, agar, furcellaran, guar gum, gellan gum, locust bean gum, pectin, low methoxyl pectin, gelatin, microcrystalline cellulose, CMC (sodium carboxymethylcellulose), methylcellulose hydroxypropyl methyl cellulose, hydroxypropyl cellulose, DATEM (diacetyl tartaric acid esters of mono- and diglycerides), dextran, carrageenans, and mixtures thereof.

[0110] The nutritional compositions of the disclosure may provide minimal, partial or total nutritional support. The compositions may be nutritional supplements or meal replacements. The compositions may, but need not, be nutritionally complete. In an

embodiment, the nutritional composition of the disclosure is nutritionally complete and contains suitable types and amounts of lipid, carbohydrate, protein, vitamins and minerals. The amount of lipid or fat typically can vary from about 2 to about 7 g/100 kcal. The amount of protein typically can vary from about 1 to about 5 g/100 kcal. The amount of carbohydrate typically can vary from about 8 to about 14 g/100 kcal.

[0111] In some embodiments, the nutritional composition of the present disclosure is a growing-up milk. Growing-up milks are fortified milk-based beverages intended for children over 1 year of age (typically from 1-6 years of age). They are not medical foods and are not intended as a meal replacement or a supplement to address a particular nutritional deficiency. Instead, growing-up milks are designed with the intent to serve as a complement to a diverse diet to provide additional insurance that a child achieves continual, daily intake of all essential vitamins and minerals, macronutrients plus additional functional dietary components, such as non-essential nutrients that have purported health-promoting properties.

[0112] The exact composition of an infant formula or a growing-up milk or other nutritional composition according to the present disclosure can vary from market-to-market, depending on local regulations and dietary intake information of the population of interest. In some embodiments, nutritional compositions according to the disclosure consist of a milk protein source, such as whole or skim milk, plus added sugar and sweeteners to achieve desired sensory properties, and added vitamins and minerals. The fat composition is typically derived from the milk raw materials. Total protein can be targeted to match that of human milk, cow milk or a lower value. Total carbohydrate is usually targeted to provide as little added sugar, such as sucrose or fructose, as possible to achieve an acceptable taste. Typically, Vitamin A, calcium and Vitamin D are added at levels to match the nutrient contribution of regional cow milk. Otherwise, in some embodiments, vitamins and minerals can be added at levels that provide approximately 20% of the dietary reference intake (DRI) or 20% of the Daily Value (DV) per serving. Moreover, nutrient values can vary between markets depending on the identified nutritional needs of the intended population, raw material contributions and regional regulations.

[0113] The pediatric subject may be a child or an infant. For example, the subject may an infant ranging in age from 0 to 3 months, about 0 to 6 months, 0 to 12 months, 3 to 6 months, or 6 to 12 months. The subject may alternatively be a child ranging in age from 1 to 13 years, 1 to 6 years or 1 to 3 years. In an embodiment, the composition may be administered to the pediatric subject prenatally, during infancy, and during childhood.

[0114] Examples are provided to illustrate some embodiments of the nutritional composition of the present disclosure but should not be interpreted as any limitation thereon. Other embodiments within the scope of the claims herein will be apparent to one skilled in the art from the consideration of the specification or practice of the nutritional composition or methods disclosed herein. It is intended that the specification, together with the example, be considered to be exemplary only, with the scope and spirit of the disclosure being indicated by the claims which follow the example.

EXAMPLES

EXAMPLE 1:

[0115] This study sought to determine whether prebiotic oligosaccharides that support the growth of beneficial commensal microbes would attenuate stressor-induced anxiety-like behavior. Briefly, mice (6-8 weeks old) were fed standard laboratory chow, or laboratory chow containing milk oligosaccharides 3'Sialyllactose (3SL) or 6'Sialyllactose (6SL) for two weeks prior to being exposed to either a social disruption stressor or a non-stressed control condition. In social disruption stressor model, mice are described as having anxiety-like behavior in the open field if they spend less time in the center of the open field and more time in the periphery. In our study, exposure to the stressor resulted in anxiety-like behavior in mice fed a control diet. In comparison to non-stressed control mice, stressor-exposed mice spent significantly more time in the dark in the light:dark preference test (Figure 1) and spent more time in the periphery of the open field (Figure 2). However, the behavior of stressor-exposed and non-stressed mice fed 3SL or 6SL was similar in both the light:dark preference task and the open field, indicating that these prebiotics can attenuate the effects of the stressor on anxiety-like behavior. The representative movement tracks of the animals treated with experimental diets is presented in Figure 3. The 3SL diet caused most movement in the light area which is indicative of the reduction of the effects of the stressor on anxiety-like behavior.

EXAMPLE 2:

[0116] Sialic acid (SA) is a key component of human milk oligosaccharides and neural tissues. SA accumulates in the brain rapidly during neonatal development and is thought to play an important role in brain development. This study aimed to determine if different isomers of sialyllactose (3'sialyllactose and 6'sialyllactose) enrich brain SA acid of developing neonatal piglets. Day-old pigs were randomized among 6 diets (control, 2g/L 3'-SL, 4g/L 3'-SL, 2g/L 6'-SL, or 4g/L 6'-SL; 2 g/L PDX + 2 g/L GOS; n=9) and fed three times per day for 21 d. A basal diet was patterned after term human infant formula, adjusted to meet the nutrient requirements

of neonatal pigs. Piglets readily consumed the formula, grew at normal rates and remained clinically healthy throughout the experiment. Dietary SL did not affect feed intake, growth or fecal consistency. On d21 pigs were euthanized and the left hemisphere of the brain was dissected into cerebrum, cerebellum, corpus callosum, and hippocampus regions. Total and lipid-bound (ganglioside) SA were assayed following extraction with chloroform:methanol (2:1), and free SA was calculated by difference. Protein-bound SA was measured in the insoluble residue following suspension in PBS containing 1% Triton X-100. SA was determined using a modified periodic acid-resorcinol reaction. Ganglioside-bound SA in the corpus callosum of pigs fed 2g/L of 3'-SL (359±16 ug SA/g wet tissue) or 6'-SL (361±16 ug SA/g) was increased by 15% over control pigs (314±16 ug SA/g; $P<0.05$; Figure 4). Similarly, ganglioside-bound SA in the cerebellum of pigs fed 4g/L of 3'-SL (416±14 ug SA/g) was increased by 10% over control pigs (377±14 ug SA/g; $P < 0.05$; Figure 5). In conclusion, supplementation of formula with 3'- or 6'-SL can enrich ganglioside SA in the brain of suckling piglets.

FORMULATION EXAMPLE 1:

	per 100 kcal	
Nutrient/Lipid		
	Minimum	**Maximum**
Protein (g)	1	7
Fat (g)	1	10
Carbohydrates (g)	5	25
DHA (mg)	5	100
GOS (g)	0.1	1.0
PDX (g)	0.1	0.5
LGG (CFU)	$1x10^4$	$1.5x10^{10}$
Milk oligosaccharides (e.g. sialyllactose) (g)	0.005	1
Vitamin A (IU)	134	921
Vitamin D (IU)	22	126
Vitamin E (IU)	0.8	5.4
Vitamin K (mcg)	2.9	18
Thiamin (mcg)	63	328
Riboflavin (mcg)	68	420
Vitamin B6 (mcg)	52	397

Vitamin B12 (mcg)	0.2	0.9
Niacin (mcg)	690	5881
Folic acid (mcg)	8	66
Panthothenic acid (mcg)	232	1211
Biotin (mcg)	1.4	5.5
Vitamin C (mg)	4.9	24
Choline (mg)	4.9	43
Calcium (mg)	68	297
Phosphorus (mg)	54	210
Magnesium (mg)	4.9	34
Sodium (mg)	24	88
Potassium (mg)	82	346
Chloride (mg)	53	237
Iodine (mcg)	8.9	79
Iron (mg)	0.7	2.8
Zinc (mg)	0.7	2.4
Manganese (mcg)	7.2	41
Copper (mcg)	16	331

CLAIMS

What is claimed is:

1. A nutritional composition comprising:

(i) a protein source,

(ii) a lipid source,

(iii) a carbohydrate source,

(iv) a human milk oligosaccharide or a precursor thereof,

(v) a prebiotic comprising polydextrose, galacto-oligosaccharide, or combinations thereof, and

(vi) a probiotic.

2. The composition of claim 1, wherein the at least one human milk oligosaccharide comprises 2'-fucosyllactose, 3'-fucosyllactose, 3'sialyllactose, 6'sialyllactose, lacto-N-biose, lacto-N-neotetraose, lacto-N-tetraose, or any combination thereof.

3. The composition of claim 1, wherein the human milk oligosaccharide precursor comprises sialic acid, fucose, or a combination thereof.

4. The composition of claim 1, wherein the human milk oligosaccharide is present at a concentration ranging from about 0.005 g/100 kcal to about 1 g/100 kcal.

5. The composition of claim 1, wherein the prebiotic comprises polydextrose and galactooligosaccharides in a ratio ranging from about 1:4 to about 4:1 by weight.

6. The composition of claim 1, wherein the polydextrose is present in an amount ranging from about 0.1 g/100kcal to about 0.5 g/00 kcal.

7. The composition of claim 1, wherein the galacto-oligosaccharide is present in the composition in an amount ranging from about 0.1 g/100 kcal to about 1.0 g/100kcal.

8. The composition of claim 1, wherein the probiotic comprises a *Lactobacillus* species.

9. The composition of claim 1, wherein the probiotic comprises *Lactobacillus rhamnosus* GG.

10. The composition of claim 1, wherein the probiotic is non-viable.

11. The composition of claim 1, wherein the probiotic is viable.

12. The composition of claim 1, wherein the probiotic is present in an amount ranging from about $1x10^5$ cfu/100 kcals to about 1.5×10^9 cfu/100 kcals of *Lactobacillus rhamnosus* GG.

13. The composition of claim 1, further comprising a source of long chain polyunsaturated fatty acids.

14. The composition of claim 13, wherein the source long chain polyunsaturated fatty acids comprises docosahexaenoic acid, arachidonic acid, or a combination thereof.

15. The composition of claim 1, further comprising a source of β-glucan.

16. The composition of claim 15, wherein the source of b-glucan comprises a 1,3-□-glucan.

17. The composition of claim 1, wherein the composition comprises per 100 kcal:

(i) between about 1 g and about 7 g of a protein source,

(ii) between about 1 g and about 10 g of a lipid source,

(iii) between about 6 g and about 22 g of a carbohydrate source,

(iv) between about 0.005 g and about 1 g of a human milk oligosaccharide,

(v) between about 0.1 g and 1.0 g of a galacto-oligosaccharide,

(vi) between about 0.1 g and about 0.5 g of a polydextrose, and

(vii) between about $1x10^5$ cfu/100 kcals to about 1.5×10^9 cfu/100 kcals of *Lactobacillus rhamnosus* GG.

18. A method for promoting the growth of beneficial microbiota in the gastrointestinal tract of pediatric subject in need thereof comprising administering to the subject an effective amount of a nutritional composition comprising:

(i) a protein source,

(ii) a lipid source,

(iii) a carbohydrate source,

(iv) a human milk oligosaccharide or a precursor thereof,

(v) a prebiotic comprising polydextrose, galacto-oligosaccharide, or combinations thereof, and

(vi) a probiotic.

19. The method of claim 18, wherein administration of the composition stimulates the growth of gut bacteria in the subject, wherein the gut bacteria comprise *Lactobacillus* species, *Bifidobacterium* species, *Allobaculum* species or combinations thereof.

20. The method of claim 18, wherein the method further promotes cognitive development in the subject.

21. The method of claim 18, wherein the method further reduces the growth of *Clostridium* species in the gut of the subject.