METHODS FOR DECREASING THE INCIDENCE OF NECROTIZING ENTEROCOLITIS IN INFANTS, TODDLERS, OR CHILDREN USING HUMAN MILK OLIGOSACCHARIDES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 61/428,863 filed on December 31, 2010; and U.S. Provisional Application No. 61/428,868 filed on December 31, 2010, which disclosures are incorporated by reference in their entirety.

FIELD OF THE DISCLOSURE

[0002] The present disclosure relates to human milk oligosaccharides (HMOs) for improving gastrointestinal function and tolerance in infants, toddlers, and children. More particularly, the present disclosure relates to human milk fortifiers, preterm and term infant formulas, and pediatric formulas comprising HMOs that can stimulate enteric nerve cells in the gastrointestinal tract, thereby treating and/or preventing numerous gastrointestinal-related conditions and diseases.

BACKGROUND OF THE DISCLOSURE

[0003] During postnatal development, a newborn's intestine experiences a process of maturation that ends with the production of gastrointestinal epithelium that functions as a selective barrier (i.e., gut barrier). The gastrointestinal epithelium permits the absorption of nutrients, electrolytes and water, while preventing exposure to dietary and microbial antigens, including food allergens. Specifically, this barrier limits the passage of antigens to the systemic circulation, thereby preventing infection, inflammatory reactions, and other gastrointestinal diseases and disorders that may occur during infancy and later in life. For very young infants, and particularly, preterm infants, who have an immature immune system and intestinal tract, development of suboptimal intestinal flora may result in infection, diarrhea, allergies, and food intolerance.

[0004] Barrier formation and maintenance has been found to be affected by the diet. Breast milk contains components that not only act as pathogen receptor analogues, but also activate immune factors by infant intestinal epithelial cells and/or associated immune cell populations to enhance development and maturation of the infant's gastrointestinal and immune systems.

[0005] Not all infants, however, are in a position to receive human breast milk. It would therefore be desirable to provide nutritional compositions, and synthetic infant formulas in particular, that can produce nutritional benefits including improved gastrointestinal growth, development, and maturation. It would additionally be beneficial if the nutritional compositions could enhance immunity against microbial infections and other gastrointestinal diseases, conditions, and disorders.

SUMMARY OF THE DISCLOSURE

[0006] The present disclosure is directed to nutritional compositions, including synthetic infant formulas, synthetic pediatric formulas, and synthetic child formulas including at least one HMO alone or in combination with other components such as prebiotic oligosaccharides and/or probiotics, for improving gut function and immunity in an infant, toddler, child, or adult, along with related methods of use. More particularly, the nutritional compositions can improve growth and maturation of the gut barrier, thereby treating and/or preventing formula intolerance or other gastrointestinal diseases and/or disorders resulting from a loss of dysfunction of the gut barrier.

[0007] One embodiment is directed to a method of reducing the incidence of necrotizing enterocolitis in an infant, toddler, or child in need thereof. The method comprises administering to the infant, toddler, or child a composition comprising human milk oligosaccharides in a total amount of from about 0.001 mg/mL to about 20 mg/mL.

[0008] Another embodiment is directed to a method of reducing the incidence of necrotizing enterocolitis in an infant, toddler, or child in need thereof. The method comprises administering to the infant, toddler, or child a nutritional composition comprising 6'-sialyllactose, 2'-fucosyllactose, and lacto-N-neotetraose.

[0009] Another embodiment is directed to a synthetic formula for improving the feeding intolerance of an infant, toddler, or child in need thereof. The synthetic formula comprises from about 0.2 mg/mL to about 20 mg/mL of an oligosaccharide blend, wherein the oligosaccharide blend comprises a first oligosaccharide selected from the group consisting of a fructooligosaccharide, a galactooligosaccharide, lacto-N-neotetraose, 2'-fucosyllactose, and combinations thereof; a second oligosaccharide selected from the group consisting of 3'-sialyllactose, 6'-sialyllactose, and combinations thereof; and a third oligosaccharide selected from the group consisting of inulin, a gum, polydextrose, and combinations thereof.

[0010] Another embodiment is directed to a synthetic formula for improving the feeding intolerance of an infant, toddler, or child in need thereof. The synthetic formula comprises from about 0.2 mg/mL to about 20 mg/mL of an oligosaccharide blend, wherein the oligosaccharide blend comprises a first oligosaccharide selected from the group consisting of a fructooligosaccharide, a galactooligosaccharide, lacto-N-neotetraose, 2'-fucosyllactose, and combinations thereof; and a second oligosaccharide selected from the group consisting of 3'-sialyllactose, 6'-sialyllactose, and combinations thereof.

[0011] Another embodiment is directed to a method of improving the feeding tolerance of an infant, toddler, or child in need thereof. The method comprises administering to the infant, toddler, or child a nutritional composition comprising from about 0.2 mg/mL to about 20 mg/mL of an oligosaccharide blend, wherein the oligosaccharide blend comprises a first oligosaccharide selected from the group consisting of a fructooligosaccharide, a galactooligosaccharide, lacto-N-neotetraose, 2'-fucosyllactose, and combinations thereof; a second oligosaccharide selected from the group consisting of 3'-sialyllactose, 6'-sialyllactose, and combinations thereof; and a third oligosaccharide selected from the group consisting of inulin, a gum, polydextrose, and combinations thereof.

[0012] Another embodiment is directed to a method of improving the feeding tolerance of an infant, toddler, or child in need thereof. The method comprises administering to the infant, toddler, or child a synthetic pediatric formula comprising from about 0.2 mg/mL to about 20 mg/mL of an oligosaccharide blend, wherein the oligosaccharide blend comprises a first oligosaccharide selected from the group consisting

of a fructooligosaccharide, a galactooligosaccharide, lacto-N-neotetraose, 2'-fucosyllactose, and combinations thereof; a second oligosaccharide selected from the group consisting of 3'-sialyllactose, 6'-sialyllactose, and combinations thereof; and a third oligosaccharide selected from the group consisting of inulin, a gum, polydextrose, and combinations thereof.

[0013] It has been discovered that HMOs that are delivered to the gut tissue stimulate the gut-brain-immune axis, and improve the immune system and enteric nervous system. Specifically, it has been found that 2'-fucosyllactose stimulates enteric nerve cells in the gastrointestinal tract such that gut function may be improved and gastrointestinal issues minimized.

[0014] Additionally, it has been found that the digestive tolerance of an infant, toddler, child, or adult can be significantly increased by administering to the infant, toddler, child or adult a select blend of carbohydrates including HMOs. Specifically, the carbohydrate blend includes a combination of fast, medium, and slowly digested carbohydrates including specific HMOs such as lacto-N-neotetraose, 2'-fucosyllactose, 3'-fucosyllactose, 3'-sialyllactose and/or 6'-sailyllactose.

[0015] Moreover, it has been found that intestinal barrier integrity of an infant, toddler, child, or adult can be significantly improved by administering to the infant, toddler, child, or adult a synbiotic composition including HMOs. Specifically, the synbiotic combination includes a probiotic, at least one of a galactooligosaccharide and a fructooligosaccharide (such as a short chain fructooligosaccharide) and at least one HMO. The synbiotic composition promotes the colonization of beneficial intestinal microbiota in order to discourage the growth of harmful bacteria.

[0016] Although the nutritional compositions and methods are primarily discussed herein in relation to preterm infants and infants in general, it should be understood that many of the benefits discussed herein may be provided to toddlers, children, and adults administered combinations of the HMOs alone, or with other components as described herein, such as prebiotic oligosaccharides and/or probiotics, for example. Particularly, in some embodiments, the incidence of gastrointestinal diseases and disorders that generally affect adults, such as Crohn's disease, irritable bowel syndrome and

the like, can be reduced with the use of the nutritional compositions of the present disclosure including HMOs.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] FIG. 1 is a graph depicting the effect of 2'FL and 3'FL on gut motility as measured in Example 35.

[0018] FIG. 2 is a table setting forth the microbiological medium used in the *in vitro* experiment of Example 36.

[0019] FIG. 3 is a graph depicting the change in pH over time as affected by the various oligosaccharide substrates as tested in Example 36.

[0020] FIG. 4 is a graph depicting change in short chain fatty acid production over time as affected by the various oligosaccharide substrates as tested in Example 36.

[0021] FIG. 5 depicts growth curves of various *Bifidobacterium spp.* as evaluated in Example 37.

[0022] FIG. 6 depicts growth curves of various *Bifidobacterium spp.* as evaluated in Example 37.

[0023] FIG. 7 depicts growth curves of various *Bifidobacterium spp.* as evaluated in Example 37.

[0024] FIGs. 8-10 are graphs showing Pre-confluent HT-29 epithelial cell differentiation in the presence of LNnT, 2'FL, and 6'SL.

[0025] FIGs. 11-13 are graphs showing Confluent HT-29 epithelial cell resistance in the presence of LNnT, 2'FL, and 6'SL.

[0026] FIGs. 14-16 are graphs showing Post-confluent CaCo-2Bbe epithelial cell differentiation in the presence of LNnT, 2'FL, and 6'SL.

[0027] FIGs. 17-19 are graphs showing Post-confluent CaCo-2Bbe epithelial cell sucrase activity in the presence of LNnT, 2'FL, and 6'SL.

[0028] FIGs. 20-22 are graphs showing Post-confluent CaCo-2Bbe epithelial cell resistance in the presence of LNnT, 2'FL, and 6'SL.

[0029] FIGS. 23A-23E are charts depicting the effect of human milk oligosaccharides and dose dependency thereof on the expression of several genes involved in the healing response of the gastrointestinal tract as measured in Example 39.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0030] The nutritional compositions and methods described herein utilize HMOs alone or in combination with at least one other prebiotic oligosaccharide and/or a probiotic for controlling and reducing a number of diseases, disorders and conditions related to the gut-brain-immune system. These and other features of the nutritional compositions and methods, as well as some of the many optional variations and additions, are described in detail hereafter.

[0031] The terms "retort packaging" and "retort sterilizing" are used interchangeably herein, and unless otherwise specified, refer to the common practice of filling a container, most typically a metal can or other similar package, with a nutritional liquid and then subjecting the liquid-filled package to the necessary heat sterilization step, to form a sterilized, retort packaged, nutritional liquid product.

[0032] The term "aseptic packaging" as used herein, unless otherwise specified, refers to the manufacture of a packaged product without reliance upon the above-described retort packaging step, wherein the nutritional liquid and package are sterilized separately prior to filling, and then are combined under sterilized or aseptic processing conditions to form a sterilized, aseptically packaged, nutritional liquid product.

[0033] The terms "fat" and "oil" as used herein, unless otherwise specified, are used interchangeably to refer to lipid materials derived or processed from plants or animals. These terms also include synthetic lipid materials so long as such synthetic materials are suitable for oral administration to humans.

[0034] The term "human milk oligosaccharide" or "HMO", unless otherwise specified, refers generally to a number of complex carbohydrates found in human breast milk that can be in acidic or neutral form, and to precursors thereof. Exemplary non-

limiting human milk oligosaccharides include 3'-sialyllactose, 6'-sialyllactose, 3'-fucosyllactose, 2'-fucosyllactose, and lacto-N-neo-tetraose. Exemplary human milk oligosaccharide precursors includes sialic acid and/or fucose.

[0035] The term "shelf stable" as used herein, unless otherwise specified, refers to a nutritional product that remains commercially stable after being packaged and then stored at 18-24°C for at least 3 months, including from about 6 months to about 24 months, and also including from about 12 months to about 18 months.

[0036] The terms "nutritional formulation" or "nutritional composition" as used herein, are used interchangeably and, unless otherwise specified, refer to synthetic formulas including nutritional liquids, nutritional powders, nutritional solids, nutritional semi-solids, nutritional semi-liquids, nutritional supplements, and any other nutritional food product as known in the art. The nutritional powders may be reconstituted to form a nutritional liquid, all of which comprise one or more of fat, protein and carbohydrate and are suitable for oral consumption by a human. The terms "nutritional formulation" or "nutritional composition" do not include human breast milk.

[0037] The term "nutritional liquid" as used herein, unless otherwise specified, refers to nutritional compositions in ready-to-drink liquid form, concentrated form, and nutritional liquids made by reconstituting the nutritional powders described herein prior to use.

[0038] The term "nutritional powder" as used herein, unless otherwise specified, refers to nutritional compositions in flowable or scoopable form that can be reconstituted with water or another aqueous liquid prior to consumption and includes both spraydried and drymixed/dryblended powders.

[0039] The term "nutritional semi-solid," as used herein, unless otherwise specified, refers to nutritional products that are intermediate in properties, such as rigidity, between solids and liquids. Some semi-solids examples include puddings, gelatins, and doughs.

[0040] The term "nutritional semi-liquid," as used herein, unless otherwise specified, refers to nutritional products that are intermediate in properties, such as flow properties, between liquids and solids. Some semi-liquids examples include thick shakes and liquid gels.

[0041] The term "infant" as used herein, unless otherwise specified, refers to a person 12 months or younger. The term "preterm infant" as used herein, refers to a person born prior to 36 weeks of gestation.

[0042] The term "toddler" as used herein, unless otherwise specified, refers to a person greater than one year of age up to three years of age.

[0043] The term "child" as used herein, unless otherwise specified, refers to a person greater than three years of age up to twelve years of age.

[0044] The term "newborn" as used herein, unless otherwise specified, refers to a person from birth up to four weeks of age.

[0045] The terms "infant formula" or "synthetic infant formula" as used herein, unless otherwise specified, are used interchangeably and refer to liquid, semi-liquid, solid, and semi-solid human milk replacements or substitutes that are suitable for consumption by an infant. The synthetic formulas include components that are of semi-purified or purified origin. As used herein, unless otherwise specified, the terms "semi-purified" or "purified" refer to a material that has been prepared by purification of a natural material or by synthesis. The terms "infant formula" or "synthetic infant formula" do not include human breast milk.

[0046] The term "synthetic pediatric formula" as used herein, unless otherwise specified, refers to liquid, semi-liquid, solid, and semi-solid human milk replacements or substitutes that are suitable for consumption by an infant or toddler up to the age of 36 months (3 years). The synthetic formulas include components that are of semi-purified or purified origin. As used herein, unless otherwise specified, the terms "semi-purified" or "purified" refer to a material that has been prepared by purification of a natural material or by synthesis. The term "synthetic pediatric nutritional formula" does not include human breast milk.

[0047] The term "synthetic child formula" as used herein, unless otherwise specified, refers to liquid, semi-liquid, solid, and semi-solid human milk replacements or substitutes that are suitable for consumption by a child up to the age of 12 years. The synthetic formulas include components that are of semi-purified or purified origin. As used herein, unless otherwise specified, the terms "semi-purified" or "purified" refer to a material that has been prepared by purification of a natural material or by synthesis. The term "synthetic child nutritional formula" does not include human breast milk.

[0048] The term "preterm infant formula" as used herein, unless otherwise specified, refers to liquid and solid nutritional products suitable for consumption by a preterm infant.

[0049] The term "human milk fortifier" as used herein, unless otherwise specified, refers to liquid and solid nutritional products suitable for mixing with breast milk or preterm infant formula or infant formula for consumption by a preterm or term infant.

[0050] The terms "susceptible" and "at risk" as used herein, unless otherwise specified, mean having little resistance to a certain condition or disease, including being genetically predisposed, having a family history of, and/or having symptoms of the condition or disease.

[0051] The term "cognition" as used herein, unless otherwise specified, refers to an individual's ability for learning, memory acquisition, and memory recall.

[0052] The terms "growth of a virus" or "growth of bacteria" as used herein, unless otherwise specified, refer to the production, proliferation, or replication of a virus or bacteria.

[0053] All percentages, parts and ratios as used herein, are by weight of the total composition, unless otherwise specified. All such weights, as they pertain to listed ingredients, are based on the active level and, therefore, do not include solvents or by-products that may be included in commercially available materials, unless otherwise specified.

[0054] Numerical ranges as used herein are intended to include every number and subset of numbers within that range, whether specifically disclosed or not. Further, these numerical ranges should be construed as providing support for a claim directed to any number or subset of numbers in that range. For example, a disclosure of from 1 to 10 should be construed as supporting a range of from 2 to 8, from 3 to 7, from 5 to 6, from 1 to 9, from 3.6 to 4.6, from 3.5 to 9.9, and so forth.

[0055] 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.

[0056] 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.

[0057] The nutritional compositions and methods may comprise, consist of, or consist essentially of the essential elements of the compositions and methods as described herein, as well as any additional or optional element described herein or otherwise useful in nutritional composition applications.

Product Form

[0058] The nutritional compositions of the present disclosure may be formulated and administered in any known or otherwise suitable oral product form. Any solid, semi-solid, liquid, semi-liquid, or powder product form, including combinations or variations thereof, are suitable for use herein, provided that such forms allow for safe and effective oral delivery to the individual of the essential ingredients and any optional ingredients, as also defined herein.

[0059] The nutritional compositions of the present disclosure are desirably formulated as dietary product forms, which are defined herein as those embodiments comprising the ingredients of the present disclosure in a product form that then contains at least one of fat, protein, and carbohydrate, and preferably also contains vitamins, minerals, or combinations thereof. The nutritional compositions will comprise at least one HMO,

and many times at least two or more HMOs, desirably in combination with at least one of protein, fat, vitamins, and minerals, to produce a nutritional combination.

[0060] The nutritional composition may be formulated with sufficient kinds and amounts of nutrients to provide a sole, primary, or supplemental source of nutrition, or to provide a specialized nutritional composition for use in individuals afflicted with specific diseases, disorders, or conditions or with a targeted nutritional benefit as described below.

[0061] Specific non-limiting examples of product forms suitable for use with the HMO-containing compositions as disclosed herein include, for example, liquid and powdered dietary supplements, liquid and powdered human milk fortifiers, liquid and powdered preterm infant formulas, liquid and powdered infant formulas, liquid and powdered elemental and semi-elemental formulas, liquid and powdered pediatric formulas, liquid and powdered toddler formulas, liquid and powdered follow-on formulas, liquid, powdered and solid adult nutritional formulas suitable for use with individuals suffering from food intolerance, allergies, immune disorders, and other gastrointestinal diseases, conditions, and/or disorders.

Nutritional Liquids

[0062] Nutritional liquids include both concentrated and ready-to-feed nutritional liquids. These nutritional liquids are most typically formulated as suspensions or emulsions, although other liquid forms are within the scope of the present disclosure.

[0063] Nutritional emulsions suitable for use may be aqueous emulsions comprising proteins, fats, and carbohydrates. These emulsions are generally flowable or drinkable liquids at from about 1°C to about 25°C and are typically in the form of oil-in-water, water-in-oil, or complex aqueous emulsions, although such emulsions are most typically in the form of oil-in-water emulsions having a continuous aqueous phase and a discontinuous oil phase.

[0064] The nutritional emulsions may be and typically are shelf stable. The nutritional emulsions typically contain up to about 95% by weight of water, including from about 50% to about 95%, also including from about 60% to about 90%, and also including from about 70% to about 85%, by weight of water. The nutritional emulsions may have a

variety of product densities, but most typically have a density greater than about 1.03 g/mL, including greater than about 1.04 g/mL, including greater than about 1.055 g/mL, including from about 1.06 g/mL to about 1.12 g/mL, and also including from about 1.085 g/mL to about 1.10 g/mL.

[0065] The nutritional emulsions may have a caloric density tailored to the nutritional needs of the ultimate user, although in most instances the emulsions comprise generally at least 19 kcal/fl oz (660 kcal/liter), more typically from about 20 kcal/fl oz (675-680 kcal/liter) to about 25 kcal/fl oz (820 kcal/liter), even more typically from about 20 kcal/fl oz (675-680 kcal/liter) to about 24 kcal/fl oz (800-810 kcal/liter). Generally, the 22-24 kcal/fl oz formulas are more commonly used in preterm or low birth weight infants, and the 20-21 kcal/fl oz (675-680 to 700 kcal/liter) formulas are more often used in term infants. In some embodiments, the emulsion may have a caloric density of from about 50-100 kcal/liter to about 660 kcal/liter, including from about 150 kcal/liter to about 500 kcal/liter. In some specific embodiments, the emulsion may have a caloric density of 25, or 50, or 75, or 100 kcal/liter.

[0066] The nutritional emulsion may have a pH ranging from about 3.5 to about 8, but are most advantageously in a range of from about 4.5 to about 7.5, including from about 5.5 to about 7.3, including from about 6.2 to about 7.2.

[0067] Although the serving size for the nutritional emulsion can vary depending upon a number of variables, a typical serving size is generally at least about 1 mL, or even at least about 2 mL, or even at least about 5 mL, or even at least about 10 mL, or even at least about 25 mL, including ranges from about 2 mL to about 300 mL, including from about 4 mL to about 250 mL, and including from about 10 mL to about 240 mL.

Nutritional Solids

[0068] The nutritional solids may be in any solid form, but are typically in the form of flowable or substantially flowable particulate compositions, or at least particulate compositions. Particularly suitable nutritional solid product forms include spray dried, agglomerated and/or dryblended powder compositions. The compositions can easily be scooped and measured with a spoon or similar other device, and can easily be reconstituted by the intended user with a suitable aqueous liquid, typically water, to form a nutritional

composition for immediate oral or enteral use. In this context, "immediate" use generally means within about 48 hours, most typically within about 24 hours, preferably right after reconstitution.

[0069] The nutritional powders may be reconstituted with water prior to use to a caloric density tailored to the nutritional needs of the ultimate user, although in most instances the powders are reconstituted with water to form compositions comprising at least 19 kcal/fl oz (660 kcal/liter), more typically from about 20 kcal/fl oz (675-680 kcal/liter) to about 25 kcal/fl oz (820 kcal/liter), even more typically from about 20 kcal/fl oz (675-680 kcal/liter) to about 24 kcal/fl oz (800-810 kcal/liter). Generally, the 22-24 kcal/fl oz formulas are more commonly used in preterm or low birth weight infants, and the 20-21 kcal/fl oz (675-680 to 700 kcal/liter) formulas are more often used in term infants. In some embodiments, the reconstituted powder may have a caloric density of from about 50-100 kcal/liter to about 660 kcal/liter, including from about 150 kcal/liter to about 500 kcal/liter. In some specific embodiments, the emulsion may have a caloric density of 25, or 50, or 75, or 100 kcal/liter.

Human Milk Oligosaccharides (HMOs)

[0070] The nutritional compositions of the present disclosure include at least one HMO, and in many embodiments, a combination of two or more HMOs. Oligosaccharides are one of the main components of human breast milk, which contains, on average, 10 grams per liter of neutral oligosaccharides and 1 gram per liter of acidic oligosaccharides. The compositional structure of HMOs is very complex and more than 200 different oligosaccharide-like structures are known.

[0071] The HMO or HMOs may be included in the nutritional compositions alone, or in some embodiments, in combination with other components (e.g., prebiotic oligosaccharides, probiotics, etc.) as described herein. In many embodiments, HMOs are included in the nutritional compositions with multiple additional components. The HMO or HMOs may be isolated or enriched from milk(s) secreted by mammals including, but not limited to: human, bovine, ovine, porcine, or caprine species. The HMOs may also be produced via microbial fermentation, enzymatic processes, chemical synthesis, or combinations thereof.

[0072] Suitable HMOs for use in the nutritional compositions may include neutral oligosaccharides, acidic oligosaccharides, n-acetylglucosylated oligosaccharides, and HMO precursors. Specific non-limiting examples of HMOs that may be included individually or in combination in the compositions of the present disclosure include: sialic acid (i.e., free sialic acid, lipid-bound sialic acid, protein-bound sialic acid); D-glucose (Glc); D-galactose (Gal); N-acetylglucosamine (GlcNAc); L-fucose (Fuc); fucosyl oligosaccharides (i.e., Lacto-N-fucopentaose I; Lacto-N-fucopentaose II; 2'-Fucosyllactose; 3'-Fucosyllactose; Lacto-N-fucopentaose III; Lacto-N-difucohexaose I; and Lactodifucotetraose); non-fucosylated, non-sialylated oligosaccharides (i.e., Lacto-N-tetraose and Lacto-N-neotetraose); sialyl oligosaccharides (i.e., 3'-Sialyl-3-fucosyllactose; Disialomonofucosyllacto-N-neohexaose; Monofucosylmonosialyllacto-N-octaose (sialyl Lea); Sialyllacto-N-fucohexaose II; Disialyllacto-N-fucopentaose II; Monofucosyldisialyllacto-N-tetraose); and sialyl fucosyl oligosaccharides (i.e., 2'-Sialyllactose; 2-Sialyllactosamine; 3'-Sialyllactose; 3'-Sialyllactosamine; 6'-Sialyllactose; 6'-Sialyllactosamine; Sialyllacto-N-neotetraose c; Monosialyllacto-N-hexaose; Disialyllacto-N-hexaose I; Monosialyllacto-N-neohexaose I; Monosialyllacto-N-neohexaose II; Disialyllacto-N-neohexaose; Disialyllacto-N-tetraose; Disialyllacto-N-hexaose II; Sialyllacto-N-tetraose a; Disialyllacto-N-hexaose I; and Sialyllacto-N-tetraose b). Also useful are variants in which the glucose (Glc) at the reducing end is replaced by N-acetylglucosamine (e.g., 2'-fucosyl-N-acetylglucosamine (2'-FLNac) is such a variant to 2'-fucosyllactose). These HMOs are described more fully in U.S. Patent Application No. 2009/0098240, which is herein incorporated by reference in its entirety. Other suitable examples of HMOs that may be included in the compositions of the present disclosure include lacto-N-fucopentaose V, lacto-N-hexaose, para-lacto-N-hexaose, lacto-N-neohexaose, para-lacto-N-neohexaose, monofucosyllacto-N-hexaose II, isomeric fucosylated lacto-N-hexaose (1), isomeric fucosylated lacto-N-hexaose (3), isomeric fucosylated lacto-N-hexaose (2), difucosyl-para-lacto-N-neohexaose, difucosyl-para-lacto-N-hexaose, difucosyllacto-N-hexaose, lacto-N-neoocataose, para-lacto-N-octanose, iso-lacto-N-octaose, lacto-N-octaose, monofucosyllacto-neoocataose, monofucosyllacto-N-ocataose, difucosyllacto-N-octaose I, difucosyllacto-N-octaose II, difucosyllacto-N-neoocataose II, difucosyllacto-N-neoocataose I, lacto-N-decaose, trifucosyllacto-N-neooctaose, trifucosyllacto-N-octaose, trifucosyl-iso-lacto-N-octaose, lacto-N-difuco-hexaose II, sialyl-lacto-N-tetraose a, sialyl-lacto-N-tetraose b, sialyl-lacto-N-tetraose c,

sialyl-fucosyl-lacto-N-tetraose I, sialyl-fucosyl-lacto-N-tetraose II, and disialyl-lacto-N-tetraose, and combinations thereof. Particularly suitable nutritional compositions include at least one of the following HMOs or HMO precursors: sialic acid (SA); 2'-Sialyllactose (2'SL); 3'-Sialyllactose (3'SL); 6'-Sialyllactose (6'SL); 2'-Fucosyllactose (2'FL); 3'-Fucosyllactose (3'FL); and Lacto-N-tetraose and Lacto-N-neotetraose (LNnT), and in particular, combinations of 2'FL with at least one of 6'SL and 3'SL; and combinations of LNnT with at least one of 6'SL and 3'FL.

[0073] Other exemplary combinations include: SA, 3'SL, 6'SL, 3'FL, 2'FL, and LNnT; 3'SL, 6'SL, 3'FL, 2'FL, and LNnT; SA, 6'SL, 3'FL, 2'FL, and LNnT; SA, 3'SL, 3'FL, 2'FL, and LNnT; SA, 3'SL, 6'SL, 2'FL, and LNnT; SA, 3'SL, 6'SL, 3'FL, and LNnT; SA, 3'SL, 6'SL, 3'FL, and 2'FL; SA and 3'SL; SA and 6'SL; SA and 2'FL; SA and LNnT; SA, 3'SL, and 6'SL; SA, 3'SL and 3'FL; SA, 3'SL and 2'FL; SA, 3'SL and LNnT; SA, 6'SL and 3'FL; SA, 6'SL, and 2'FL; SA, 6'SL, and LNnT; SA, 3'FL, and 2'FL; SA, 3'FL, and LNnT; SA, 2'FL, and LNnT; SA, 3'SL, 6'SL, and 3'FL; SA, 3'SL, 6'SL and 2'FL; SA, 3'SL, 6'SL, and LNnT; SA, 3'SL, 3'FL, and 2'FL; SA, 3'SL, 3'FL, and LNnT; SA, 3'SL, 2'FL, and LNnT; SA, 6'SL, 3'FL, and 2'FL; SA, 6'SL, 2'FL, and LNnT; SA, 6'SL, 3'FL, and LNnT; SA, 3'FL, 2'FL, and LNnT; SA, 6'SL, 2'FL, and LNnT; SA, 3'SL, 3'FL, 2'FL, and LNnT; SA, 6'SL, 3'FL, 2'FL, and LNnT; SA, 3'SL, 6'SL, 3'FL, and LNnT; SA, 3'SL, 3'FL, 2'FL, and LNnT; SA, 3'SL, 6'SL, 2'FL, and LNnT; 3'SL, 6'SL, 3'FL, and 2'FL; 3'SL, 6'SL, 2'FL, and LNnT; 3'SL, 3'FL, 2'FL, and LNnT; 3'SL, 6'SL, 3'FL, and LNnT; 3'SL, 6'SL, and 3'FL; 3'SL, 3'FL, and 2'FL; 3'SL, 2'FL, and LNnT; 3'SL, 6'SL, and 2'FL; 3'SL, 6'SL, and LNnT; 3'SL and 3'FL; 3'SL and 2'FL; 3'SL and LNnT; 6'SL and 3'FL; 6'SL and 2'FL; 6'SL and LNnT; 6'SL, 3'FL, and LNnT; 6'SL, 3'FL, 2'FL, and LNnT; 3'FL, 2'FL, and LNnT; 3'FL and LNnT; and 2'FL and LNnT.

[0074] The HMOs are present in the nutritional compositions in total amounts of HMO in the composition (mg of HMO per mL of composition) of at least about 0.001 mg/mL, including at least about 0.01 mg/mL, including from about 0.001 mg/mL to about 20 mg/mL, including from about 0.01 mg/mL to about 20 mg/mL, including from about 0.001 mg/mL to about 15 mg/mL, including from about 0.01 mg/mL to about 15 mg/mL, including from about 0.001 mg/mL to about 10 mg/mL, including from about 0.01 mg/mL to about 10 mg/mL, including from about 0.001 mg/mL to about 5 mg/mL, including from about 0.01 mg/mL to about 5 mg/mL, and including from about 0.001 mg/mL to about 1

mg/mL of total HMO in the nutritional composition, including from about 0.001 mg/mL to about 0.23 mg/mL, and including from about 0.01 mg/mL to about 0.23 mg/mL. Typically, the amount of HMO in the nutritional composition will depend on the specific HMO or HMOs present and the amounts of other components in the nutritional compositions.

[0075] In one specific embodiment when the nutritional composition is a nutritional powder, the total concentration of HMOs in the nutritional powder is from about 0.0005% to about 5%, including from about 0.01% to about 1% (by weight of the nutritional powder).

[0076] In another specific embodiment, when the nutritional composition is a ready-to-feed nutritional liquid, the total concentration of HMOs in the ready-to-feed nutritional liquid is from about 0.0001% to about 0.50%, including from about 0.001% to about 0.15%, including from about 0.01% to about 0.10%, and further including from about 0.01% to about 0.03% (by weight of the ready-to-feed nutritional liquid).

[0077] In another specific embodiment, when the nutritional composition is a concentrated nutritional liquid, the total concentration of HMOs in the concentrated liquid is from about 0.0002% to about 0.60%, including from about 0.002% to about 0.30%, including from about 0.02% to about 0.20%, and further including from about 0.02% to about 0.06% (by weight of the concentrated nutritional liquid).

[0078] In one specific embodiment, the nutritional composition includes a neutral human milk oligosaccharide in an amount of from about 0.001 mg/mL to about 20 mg/mL, including from 0.01 mg/mL to about 20 mg/mL, including from about 0.001 mg/mL to less than 2 mg/mL, and including from about 0.01 mg/mL to less than 2 mg/mL.

[0079] In one specific embodiment of the present disclosure, a nutritional composition includes 2'FL. The 2'FL may be the only HMO included in the nutritional composition, or other additional HMOs may also be included in the nutritional composition (e.g., the 2'FL may be combined with 3'SL and/or 6'SL in some specific embodiments). In one embodiment, the 2'FL is included in the nutritional composition in an amount of from about 0.001 mg/mL to about 20 mg/mL, including from about 0.01 mg/mL to about 20 mg/mL, including from about 0.001 mg/mL to less than 2 mg/mL, and including from

about 0.01 mg/mL to less than 2 mg/mL. In another embodiment, the 2'FL is included in the nutritional composition in an amount of from about 0.001 mg/mL to about 20 mg/mL, including from about 0.01 mg/mL to about 20 mg/mL, including from greater than 2.5 mg/mL to about 20 mg/mL, including from greater than 2.5 mg/mL to about 15 mg/mL, and including from greater than 2.5 mg/mL to about 10 mg/mL.

[0080] In one specific embodiment, the nutritional composition includes 6'SL, alone or in combination with other HMOs, in an amount of from about 0.001 mg/mL to about 20 mg/mL, including from about 0.01 mg/mL to about 20 mg/mL, including from about 0.001 mg/mL to less than 0.25 mg/mL, and including from about 0.01 mg/mL to less than 0.25 mg/mL. In another embodiment, the nutritional composition includes 6'SL, alone or in combination with other HMOs, in an amount of from about 0.001 mg/mL to about 20 mg/mL, including from about 0.01 mg/mL to about 20 mg/mL, including from greater than 0.4 mg/mL to about 20 mg/mL, including from greater than 0.4 mg/mL to about 15 mg/mL, and including from greater than 0.4 mg/mL to about 10 mg/mL.

[0081] In one embodiment, when the nutritional composition includes 6'SL, the total amount of HMOs in the nutritional composition includes at least about 88% (by total weight HMOs) 6'SL, including from about 88% (by total weight HMOs) to about 96% (by total weight HMOs), including from about 88% (by total weight HMOs) to about 100% (by total weight HMOs), and including about 100% (by total weight HMOs) 6'SL.

[0082] In another embodiment, the nutritional composition includes 3'SL, alone or in combination with other HMOs, in an amount of from about 0.001 mg/mL to about 20 mg/mL, including from about 0.01 mg/mL to about 20 mg/mL, including from about 0.001 mg/mL to less than 0.15 mg/mL, including from about 0.01 mg/mL to less than 0.15 mg/mL, including from greater than 0.25 mg/mL to about 20 mg/mL, including from greater than 0.25 mg/mL to about 15 mg/mL, and including from greater than 0.25 mg/mL to about 10 mg/mL.

[0083] In one embodiment, when the nutritional composition includes 3'SL, the total amount of HMOs in the nutritional composition includes at least about 85% (by total weight HMOs) 3'SL, including from about 85% (by total weight HMOs) to about 88% (by total weight HMOs), including from about 88% (by total weight HMOs) to about 100% (by total weight HMOs), and including about 100% (by total weight HMOs) 3'SL.

[0084] In one specific embodiment, the nutritional composition includes LNnT, alone or in combination with other HMOs, in an amount of from about 0.001 mg/mL to about 20 mg/mL, including from about 0.01 mg/mL to about 20 mg/mL, including from about 0.001 mg/mL to less than 0.2 mg/mL, including from about 0.01 mg/mL to less than 0.2 mg/mL, including from greater than 0.32 mg/mL to about 20 mg/mL, including from greater than 0.32 mg/mL to about 15 mg/mL, and including from greater than 0.32 mg/mL to about 10 mg/mL.

Additional Prebiotic Oligosaccharides

[0085] The nutritional compositions of the present disclosure may, in addition to the HMOs described above, comprise an additional source or sources of prebiotic oligosaccharides (the total amount of oligosaccharides being referred to herein as an "oligosaccharide blend" of the nutritional composition). Suitable additional sources of prebiotic oligosaccharides for use in the nutritional compositions include any prebiotic oligosaccharide that is suitable for use in an oral nutritional composition and is compatible with the essential elements and features of such compositions. In some embodiments, the nutritional composition includes a combination of one or more HMOs and one or more additional prebiotic oligosaccharides such that the composition provides a synergistic benefit to the end user, such as a synergistic benefit in improving feeding intolerance in infants.

[0086] In some embodiments, the combinations of HMO or HMOs with the additional prebiotic oligosaccharides to provide the synergistic effect include HMOs and additional prebiotic oligosaccharides that ferment at a rapid rate ("rapidly-fermenting oligosaccharides"), oligosaccharides that ferment at a moderate rate ("medium-fermenting oligosaccharides"), and/or oligosaccharides that ferment at a slow rate ("slowly-fermenting oligosaccharides"). Some preferred embodiments provide a nutritional composition that includes at least one HMO in combination with a rapidly-fermenting oligosaccharide, a medium-fermenting oligosaccharide, and/or a slowly-fermenting oligosaccharide.

[0087] Non-limiting examples of suitable additional prebiotic oligosaccharides for use in the nutritional compositions described herein include prebiotic oligosaccharides that have a degree of polymerization (DP) of at least 2 monose units, which are not or only partially digested in the intestine by the action of acids or digestive enzymes present in the

human upper digestive tract (small intestine and stomach), but which are fermentable by the human intestinal flora. The term "monose units" refers to units having a closed ring structure, preferably hexose, e.g., the pyranose or furanose forms. Particularly preferred oligosaccharides for use in combination with the HMO or HMOs in the nutritional compositions of the present disclosure include galactooligosaccharides (GOS), fructooligosaccharides (FOS), short chain fructooligosaccharides, inulin, polydextrose (PDX), pectin hydrolysate, and gum fiber. In one specific embodiment, the gum fiber is gum arabic.

[0088] The oligosaccharide blend is present in the nutritional compositions in a total amount of at least about 0.001 mg/mL, including at least about 0.01 mg/mL, including at least about 0.1 mg/mL, including at least about 1 mg/mL, including from about 0.001 mg/mL to about 20 mg/mL, including from about 1 mg/mL to about 20 mg/mL, including from about 1 mg/mL to about 15 mg/mL, including from about 1 mg/mL to about 10 mg/mL, including from about 1 mg/mL to about 5 mg/mL, and including from about 2 mg/mL to about 20 mg/mL. In one embodiment, the oligosaccharide blend is present in the nutritional composition in a total amount of from about 1 mg/mL to about 4 mg/mL.

[0089] Typically, when used as an oligosaccharide blend, the nutritional compositions, in addition to the HMO or HMOs, include at least one rapidly-fermented oligosaccharide, at least one medium-fermented oligosaccharide, and, optionally, at least one slowly-fermented oligosaccharide to provide a nutritional composition that is tolerated well by preterm and term infants (i.e., reduced gassiness and/or stool frequency). Rapidly-fermented oligosaccharides generally have a fermentation rate of greater than 4,000 μg/g of dry matter/hour; medium-fermented oligosaccharides generally have a fermentation rate of from 1,500 μg/g of dry matter/hour to 4,000 μg/g of dry matter/hour; and slowly-fermented oligosaccharides generally have a fermentation rate of less than 1,500 μg/g of dry matter/hour.

[0090] By way of specific example, rapidly-fermented oligosaccharides include FOS, GOS (about 9,304 μg/g of dry matter/hour), LNnT (about 4,488 μg/g of dry matter/hour), 2'FL (about 4,872 μg/g of dry matter/hour), and combinations thereof. Medium-fermented oligosaccharides include 6'SL (about 1,809 μg/g of dry matter/hour), 3'SL, 2'FL, 3'FL, LNnT and combinations thereof. Slowly-fermented oligosaccharides

include longer chain carbohydrates such as inulin (about 1,435 μg/g of dry matter/hour), gum fibers (e.g., gum arabic (about 785 μg/g of dry matter/hour)), and combinations thereof.

[0091] When used in an oligosaccharide blend, the rapidly-fermented oligosaccharides can be included in the nutritional compositions in amounts of from about 0.05 mg/mL to about 20 mg/mL, including from about 0.5 mg/mL to about 15 mg/mL, including from about 0.5 mg/mL to about 10 mg/mL, including from about 1 mg/mL to about 15 mg/mL, including from about 1 mg/mL to about 10 mg/mL, including from about 2 mg/mL to about 8 mg/mL, and also including from about 3 mg/mL to about 5 mg/mL. The medium-fermented oligosaccharides can be included in the nutritional compositions in amounts of from about 0.05 mg/mL to about 20 mg/mL, including from about 0.05 mg/mL to about 15 mg/mL, including from about 0.05 mg/mL to about 10 mg/mL, including from about 0.05 mg/mL to about 5 mg/mL, including from about 0.05 mg/mL to about 2.5 mg/mL, including from about 0.05 mg/mL to about 1 mg/mL, including from about 0.05 mg/mL to about 0.5 mg/mL, and including from about 0.05 mg/mL to about 0.25 mg/mL. The slowly-fermented oligosaccharides can be included in the nutritional compositions in amounts of from about 0.05 mg/mL to about 20 mg/mL, including from about 0.05 mg/mL to about 15 mg/mL, including from about 0.05 mg/mL to about 10 mg/mL, including from about 0.05 mg/mL to about 5 mg/mL, and also including from about 0.05 mg/mL to about 2.5 mg/mL.

[0092] In one specific embodiment, the nutritional composition includes an oligosaccharide blend including LNnT, 6'SL and inulin in a total amount of oligosaccharide blend of from about 0.05 mg/mL to about 20 mg/mL.

[0093] In another specific embodiment, the nutritional composition includes an oligosaccharide blend including 2'FL, 6'SL and inulin in a total amount of oligosaccharide blend of from about 0.001 mg/mL to about 20 mg/mL, including from about 0.01 mg/mL to about 20 mg/mL.

[0094] Other exemplary combinations include: FOS, GOS, 2'FL, LNnT, 3'SL, and 6'SL; FOS, GOS, 2'FL, 3'SL, and 6'SL; FOS, GOS, LNnT, 3'SL, and 6'SL; FOS, 2'FL, LNnT, 3'SL, and 6'SL; GOS, 2'FL, LNnT, 3'SL, and 6'SL; FOS, GOS, 3'SL, and 6'SL; FOS, 2'FL, 3'SL, and 6'SL; FOS, LNnT, 3'SL, and 6'SL; GOS, 2'FL, 3'SL, and

6'SL; GOS, LNnT, 3'SL, and 6'SL; 2'FL, LNnT, 3'SL, and 6'SL; FOS, 3'SL, and 6'SL; GOS, 3'SL, and 6'SL; 2'FL, 3'SL, and 6'SL; LNnT, 3'SL, and 6'SL; FOS, GOS, 2'FL, LNnT, and 3'SL; FOS, GOS, 2'FL, and 3'SL; FOS, GOS, LNnT, and 3'SL; FOS, 2'FL, LNnT, and 3'SL; GOS, 2'FL, LNnT, and 3'SL; FOS, GOS, and 3'SL; FOS, 2'FL, and 3'SL; FOS, LNnT, and 3'SL; GOS, 2'FL, and 3'SL; GOS, LNnT, and 3'SL; 2'FL, LNnT, and 3'SL; FOS and 3'SL; GOS and 3'SL; 2'FL and 3'SL; LNnT and 3'SL; FOS, GOS, 2'FL, LNnT, and 6'SL; FOS, GOS, 2'FL, and 6'SL; FOS, GOS, LNnT, and 6'SL; FOS, 2'FL, LNnT, and 6'SL; GOS, 2'FL, LNnT, and 6'SL; FOS, GOS, and 6'SL; FOS, 2'FL, and 6'SL; FOS, LNnT, and 6'SL; GOS, 2'FL, and 6'SL; GOS, LNnT, and 6'SL; 2'FL, LNnT, and 6'SL; FOS and 6'SL; GOS and 6'SL; 2'FL and 6'SL; and LNnT and 6'SL.

[0095] Further exemplary combinations include: FOS, GOS, 2'FL, LNnT, 3'SL, 6'SL, inulin, a gum, and polydextrose; FOS, GOS, 2'FL, 3'SL, 6'SL, inulin, a gum, and polydextrose; FOS, GOS, LNnT, 3'SL, 6'SL, inulin, a gum, and polydextrose; FOS, 2'FL, LNnT, 3'SL, 6'SL, inulin, a gum, and polydextrose; GOS, 2'FL, LNnT, 3'SL, 6'SL, inulin, a gum, and polydextrose; FOS, GOS, 3'SL, 6'SL, inulin, a gum, and polydextrose; FOS, 2'FL, 3'SL, 6'SL, inulin, a gum, and polydextrose; FOS, LNnT, 3'SL, 6'SL, inulin, a gum, and polydextrose; GOS, 2'FL, 3'SL, 6'SL, inulin, a gum, and polydextrose; GOS, LNnT, 3'SL, 6'SL, inulin, a gum, and polydextrose; 2'FL, LNnT, 3'SL, 6'SL, inulin, a gum, and polydextrose; FOS, 3'SL, 6'SL, inulin, a gum, and polydextrose; GOS, 3'SL, 6'SL, inulin, a gum, and polydextrose; 2'FL, 3'SL, 6'SL, inulin, a gum, and polydextrose; LNnT, 3'SL, 6'SL, inulin, a gum, and polydextrose; FOS, GOS, 2'FL, LNnT, 3'SL, inulin, a gum, and polydextrose; FOS, GOS, 2'FL, 3'SL, inulin, a gum, and polydextrose; FOS, GOS, LNnT, 3'SL, inulin, a gum, and polydextrose; FOS, 2'FL, LNnT, 3'SL, inulin, a gum, and polydextrose; GOS, 2'FL, LNnT, 3'SL, inulin, a gum, and polydextrose; FOS, GOS, 3'SL, inulin, a gum, and polydextrose; FOS, 2'FL, 3'SL, inulin, a gum, and polydextrose; FOS, LNnT, 3'SL, inulin, a gum, and polydextrose; GOS, 2'FL, 3'SL, inulin, a gum, and polydextrose; GOS, LNnT, 3'SL, inulin, a gum, and polydextrose; 2'FL, LNnT, 3'SL, inulin, a gum, and polydextrose; FOS, 3'SL, inulin, a gum, and polydextrose; GOS, 3'SL, inulin, a gum, and polydextrose; 2'FL, 3'SL, inulin, a gum, and polydextrose; LNnT, 3'SL, inulin, a gum, and polydextrose; FOS, GOS, 2'FL, LNnT, 6'SL, inulin, a gum, and polydextrose; FOS, GOS, 2'FL, 6'SL, inulin, a gum, and polydextrose; FOS, GOS, LNnT, 6'SL, inulin, a gum, and polydextrose; FOS, 2'FL, LNnT, 6'SL, inulin, a gum, and

polydextrose; GOS, 2'FL, LNnT, 6'SL, inulin, a gum, and polydextrose; FOS, GOS, 6'SL, inulin, a gum, and polydextrose; FOS, 2'FL, 6'SL, inulin, a gum, and polydextrose; FOS, LNnT, 6'SL, inulin, a gum, and polydextrose; GOS, 2'FL, 6'SL, inulin, a gum, and polydextrose; GOS, LNnT, 6'SL, inulin, a gum, and polydextrose; 2'FL, LNnT, 6'SL, inulin, a gum, and polydextrose; FOS, 6'SL, inulin, a gum, and polydextrose; GOS, 6'SL, inulin, a gum, and polydextrose; 2'FL, 6'SL, inulin, a gum, and polydextrose; LNnT, 6'SL, inulin, a gum, and polydextrose; FOS, GOS, 2'FL, LNnT, 3'SL, 6'SL, inulin, and a gum; FOS, GOS, 2'FL, 3'SL, 6'SL, inulin, and a gum; FOS, GOS, LNnT, 3'SL, 6'SL, inulin, and a gum; FOS, 2'FL, LNnT, 3'SL, 6'SL, inulin, and a gum; GOS, 2'FL, LNnT, 3'SL, 6'SL, inulin, and a gum; FOS, GOS, 3'SL, 6'SL, inulin, and a gum; FOS, 2'FL, 3'SL, 6'SL, inulin, and a gum; FOS, LNnT, 3'SL, 6'SL, inulin, and a gum; GOS, 2'FL, 3'SL, 6'SL, inulin, and a gum; GOS, LNnT, 3'SL, 6'SL, inulin, and a gum; 2'FL, LNnT, 3'SL, 6'SL, inulin, and a gum; FOS, 3'SL, 6'SL, inulin, and a gum; GOS, 3'SL, 6'SL, inulin, and a gum; 2'FL, 3'SL, 6'SL, inulin, and a gum; LNnT, 3'SL, 6'SL, inulin, and a gum; FOS, GOS, 2'FL, LNnT, 3'SL, inulin, and a gum; FOS, GOS, 2'FL, 3'SL, inulin, and a gum; FOS, GOS, LNnT, 3'SL, inulin, and a gum; FOS, 2'FL, LNnT, 3'SL, inulin, and a gum; GOS, 2'FL, LNnT, 3'SL, inulin, and a gum; FOS, GOS, 3'SL, inulin, and a gum; FOS, 2'FL, 3'SL, inulin, and a gum; FOS, LNnT, 3'SL, inulin, and a gum; GOS, 2'FL, 3'SL, inulin, and a gum; GOS, LNnT, 3'SL, inulin, and a gum; 2'FL, LNnT, 3'SL, inulin, and a gum; FOS, 3'SL, inulin, and a gum; GOS, 3'SL, inulin, and a gum; 2'FL, 3'SL, inulin, and a gum; LNnT, 3'SL, inulin, and a gum; FOS, GOS, 2'FL, LNnT, 6'SL, inulin, and a gum; FOS, GOS, 2'FL, 6'SL, inulin, and a gum; FOS, GOS, LNnT, 6'SL, inulin, and a gum; FOS, 2'FL, LNnT, 6'SL, inulin, and a gum; GOS, 2'FL, LNnT, 6'SL, inulin, and a gum; FOS, GOS, 6'SL, inulin, and a gum; FOS, 2'FL, 6'SL, inulin, and a gum; FOS, LNnT, 6'SL, inulin, and a gum; GOS, 2'FL, 6'SL, inulin, and a gum; GOS, LNnT, 6'SL, inulin, and a gum; 2'FL, LNnT, 6'SL, inulin, and a gum; FOS, 6'SL, inulin, and a gum; GOS, 6'SL, inulin, and a gum; 2'FL, 6'SL, inulin, and a gum; LNnT, 6'SL, inulin, and a gum; FOS, GOS, 2'FL, LNnT, 3'SL, 6'SL, inulin, and polydextrose; FOS, GOS, 2'FL, 3'SL, 6'SL, inulin, and polydextrose; FOS, GOS, LNnT, 3'SL, 6'SL, inulin, and polydextrose; FOS, 2'FL, LNnT, 3'SL, 6'SL, inulin, and polydextrose; GOS, 2'FL, LNnT, 3'SL, 6'SL, inulin, and polydextrose; FOS, GOS, 3'SL, 6'SL, inulin, and polydextrose; FOS, 2'FL, 3'SL, 6'SL, inulin, and polydextrose; FOS, LNnT, 3'SL, 6'SL, inulin, and polydextrose; GOS, 2'FL, 3'SL, 6'SL, inulin, and polydextrose; GOS, LNnT, 3'SL, 6'SL, inulin, and

polydextrose; 2'FL, LNnT, 3'SL, 6'SL, inulin, and polydextrose; FOS, 3'SL, 6'SL, inulin, and polydextrose; GOS, 3'SL, 6'SL, inulin, and polydextrose; 2'FL, 3'SL, 6'SL, inulin, and polydextrose; LNnT, 3'SL, 6'SL, inulin, and polydextrose; FOS, GOS, 2'FL, LNnT, 3'SL, inulin, and polydextrose; FOS, GOS, 2'FL, 3'SL, inulin, and polydextrose; FOS, GOS, LNnT, 3'SL, inulin, and polydextrose; FOS, 2'FL, LNnT, 3'SL, inulin, and polydextrose; GOS, 2'FL, LNnT, 3'SL, inulin, and polydextrose; FOS, GOS, 3'SL, inulin, and polydextrose; FOS, 2'FL, 3'SL, inulin, and polydextrose; FOS, LNnT, 3'SL, inulin, and polydextrose; GOS, 2'FL, 3'SL, inulin, and polydextrose; GOS, LNnT, 3'SL, inulin, and polydextrose; 2'FL, LNnT, 3'SL, inulin, and polydextrose; FOS, 3'SL, inulin, and polydextrose; GOS, 3'SL, inulin, and polydextrose; 2'FL, 3'SL, inulin, and polydextrose; LNnT, 3'SL, inulin, and polydextrose; FOS, GOS, 2'FL, LNnT, 6'SL, inulin, and polydextrose; FOS, GOS, 2'FL, 6'SL, inulin, and polydextrose; FOS, GOS, LNnT, 6'SL, inulin, and polydextrose; FOS, 2'FL, LNnT, 6'SL, inulin, and polydextrose; GOS, 2'FL, LNnT, 6'SL, inulin, and polydextrose; FOS, GOS, 6'SL, inulin, and polydextrose; FOS, 2'FL, 6'SL, inulin, and polydextrose; FOS, LNnT, 6'SL, inulin, and polydextrose; GOS, 2'FL, 6'SL, inulin, and polydextrose; GOS, LNnT, 6'SL, inulin, and polydextrose; 2'FL, LNnT, 6'SL, inulin, and polydextrose; FOS, 6'SL, inulin, and polydextrose; GOS, 6'SL, inulin, and polydextrose; 2'FL, 6'SL, inulin, and polydextrose; LNnT, 6'SL, inulin, and polydextrose; FOS, GOS, 2'FL, LNnT, 3'SL, 6'SL, a gum, and polydextrose; FOS, GOS, 2'FL, 3'SL, 6'SL, a gum, and polydextrose; FOS, GOS, LNnT, 3'SL, 6'SL, a gum, and polydextrose; FOS, 2'FL, LNnT, 3'SL, 6'SL, a gum, and polydextrose; GOS, 2'FL, LNnT, 3'SL, 6'SL, a gum, and polydextrose; FOS, GOS, 3'SL, 6'SL, a gum, and polydextrose; FOS, 2'FL, 3'SL, 6'SL, a gum, and polydextrose; FOS, LNnT, 3'SL, 6'SL, a gum, and polydextrose; GOS, 2'FL, 3'SL, 6'SL, a gum, and polydextrose; GOS, LNnT, 3'SL, 6'SL, a gum, and polydextrose; 2'FL, LNnT, 3'SL, 6'SL, a gum, and polydextrose; FOS, 3'SL, 6'SL, a gum, and polydextrose; GOS, 3'SL, 6'SL, a gum, and polydextrose; 2'FL, 3'SL, 6'SL, a gum, and polydextrose; LNnT, 3'SL, 6'SL, a gum, and polydextrose; FOS, GOS, 2'FL, LNnT, 3'SL, a gum, and polydextrose; FOS, GOS, 2'FL, 3'SL, a gum, and polydextrose; FOS, GOS, LNnT, 3'SL, a gum, and polydextrose; FOS, 2'FL, LNnT, 3'SL, a gum, and polydextrose; GOS, 2'FL, LNnT, 3'SL, a gum, and polydextrose; FOS, GOS, 3'SL, a gum, and polydextrose; FOS, 2'FL, 3'SL, a gum, and polydextrose; FOS, LNnT, 3'SL, a gum, and polydextrose; GOS, 2'FL, 3'SL, a gum, and polydextrose; GOS, LNnT, 3'SL, a gum, and polydextrose; 2'FL, LNnT, 3'SL, a gum, and polydextrose; FOS, 3'SL, a

gum, and polydextrose; GOS, 3'SL, a gum, and polydextrose; 2'FL, 3'SL, a gum, and polydextrose; LNnT, 3'SL, a gum, and polydextrose; FOS, GOS, 2'FL, LNnT, 6'SL, a gum, and polydextrose; FOS, GOS, 2'FL, 6'SL, a gum, and polydextrose; FOS, GOS, LNnT, 6'SL, a gum, and polydextrose; FOS, 2'FL, LNnT, 6'SL, a gum, and polydextrose; GOS, 2'FL, LNnT, 6'SL, a gum, and polydextrose; FOS, GOS, 6'SL, a gum, and polydextrose; FOS, 2'FL, 6'SL, a gum, and polydextrose; FOS, LNnT, 6'SL, a gum, and polydextrose; GOS, 2'FL, 6'SL, a gum, and polydextrose; GOS, LNnT, 6'SL, a gum, and polydextrose; 2'FL, LNnT, 6'SL, a gum, and polydextrose; FOS, 6'SL, a gum, and polydextrose; GOS, 6'SL, a gum, and polydextrose; 2'FL, 6'SL, a gum, and polydextrose; LNnT, 6'SL, a gum, and polydextrose; FOS, GOS, 2'FL, LNnT, 3'SL, 6'SL, and inulin; FOS, GOS, 2'FL, 3'SL, 6'SL, and inulin; FOS, GOS, LNnT, 3'SL, 6'SL, and inulin; FOS, 2'FL, LNnT, 3'SL, 6'SL, and inulin; GOS, 2'FL, LNnT, 3'SL, 6'SL, and inulin; FOS, GOS, 3'SL, 6'SL, and inulin; FOS, 2'FL, 3'SL, 6'SL, and inulin; FOS, LNnT, 3'SL, 6'SL, and inulin; GOS, 2'FL, 3'SL, 6'SL, and inulin; GOS, LNnT, 3'SL, 6'SL, and inulin; 2'FL, LNnT, 3'SL, 6'SL, and inulin; FOS, 3'SL, 6'SL, and inulin; GOS, 3'SL, 6'SL, and inulin; 2'FL, 3'SL, 6'SL, and inulin; LNnT, 3'SL, 6'SL, and inulin; FOS, GOS, 2'FL, LNnT, 3'SL, and inulin; FOS, GOS, 2'FL, 3'SL, and inulin; FOS, GOS, LNnT, 3'SL, and inulin; FOS, 2'FL, LNnT, 3'SL, and inulin; GOS, 2'FL, LNnT, 3'SL, and inulin; FOS, GOS, 3'SL, and inulin; FOS, 2'FL, 3'SL, and inulin; FOS, LNnT, 3'SL, and inulin; GOS, 2'FL, 3'SL, and inulin; GOS, LNnT, 3'SL, and inulin; 2'FL, LNnT, 3'SL, and inulin; FOS, 3'SL, and inulin; GOS, 3'SL, and inulin; 2'FL, 3'SL, and inulin; LNnT, 3'SL, and inulin; FOS, GOS, 2'FL, LNnT, 6'SL, and inulin; FOS, GOS, 2'FL, 6'SL, and inulin; FOS, GOS, LNnT, 6'SL, and inulin; FOS, 2'FL, LNnT, 6'SL, and inulin; GOS, 2'FL, LNnT, 6'SL, and inulin; FOS, GOS, 6'SL, and inulin; FOS, 2'FL, 6'SL, and inulin; FOS, LNnT, 6'SL, and inulin; GOS, 2'FL, 6'SL, and inulin; GOS, LNnT, 6'SL, and inulin; 2'FL, LNnT, 6'SL, and inulin; FOS, 6'SL, and inulin; GOS, 6'SL, and inulin; FOS, GOS, 2'FL, LNnT, 3'SL, 6'SL, and polydextrose; FOS, GOS, 2'FL, 3'SL, 6'SL, and polydextrose; FOS, GOS, LNnT, 3'SL, 6'SL, and polydextrose; FOS, 2'FL, LNnT, 3'SL, 6'SL, and polydextrose; GOS, 2'FL, LNnT, 3'SL, 6'SL, and polydextrose; FOS, GOS, 3'SL, 6'SL, and polydextrose; FOS, 2'FL, 3'SL, 6'SL, and polydextrose; FOS, LNnT, 3'SL, 6'SL, and polydextrose; GOS, 2'FL, 3'SL, 6'SL, and polydextrose; GOS, LNnT, 3'SL, 6'SL, and polydextrose; 2'FL, LNnT, 3'SL, 6'SL, and polydextrose; FOS, 3'SL, 6'SL, and polydextrose; GOS, 3'SL, 6'SL, and polydextrose; 2'FL, 3'SL, 6'SL, and polydextrose;

LNnT, 3'SL, 6'SL, and polydextrose; FOS, GOS, 2'FL, LNnT, 3'SL, and polydextrose; FOS, GOS, 2'FL, 3'SL, and polydextrose; FOS, GOS, LNnT, 3'SL, and polydextrose; FOS, 2'FL, LNnT, 3'SL, and polydextrose; GOS, 2'FL, LNnT, 3'SL, and polydextrose; FOS, GOS, 3'SL, and polydextrose; FOS, 2'FL, 3'SL, and polydextrose; FOS, LNnT, 3'SL, and polydextrose; GOS, 2'FL, 3'SL, and polydextrose; GOS, LNnT, 3'SL, and polydextrose; 2'FL, LNnT, 3'SL, and polydextrose; FOS, 3'SL, and polydextrose; GOS, 3'SL, and polydextrose; 2'FL, 3'SL, and polydextrose; LNnT, 3'SL, and polydextrose; FOS, GOS, 2'FL, LNnT, 6'SL, and polydextrose; FOS, GOS, 2'FL, 6'SL, and polydextrose; FOS, GOS, LNnT, 6'SL, and polydextrose; FOS, 2'FL, LNnT, 6'SL, and polydextrose; GOS, 2'FL, LNnT, 6'SL, and polydextrose; FOS, GOS, 6'SL, and polydextrose; FOS, 2'FL, 6'SL, and polydextrose; FOS, LNnT, 6'SL, and polydextrose; GOS, 2'FL, 6'SL, and polydextrose; GOS, LNnT, 6'SL, and polydextrose; 2'FL, LNnT, 6'SL, and polydextrose; FOS, 6'SL, and polydextrose; GOS, 6'SL, and polydextrose; 2'FL, 6'SL, and polydextrose; LNnT, 6'SL, and polydextrose; FOS, GOS, 2'FL, LNnT, 3'SL, 6'SL, and a gum; FOS, GOS, 2'FL, 3'SL, 6'SL, and a gum; FOS, GOS, LNnT, 3'SL, 6'SL, and a gum; FOS, 2'FL, LNnT, 3'SL, 6'SL, and a gum; GOS, 2'FL, LNnT, 3'SL, 6'SL, and a gum; FOS, GOS, 3'SL, 6'SL, and a gum; FOS, 2'FL, 3'SL, 6'SL, and a gum; FOS, LNnT, 3'SL, 6'SL, and a gum; GOS, 2'FL, 3'SL, 6'SL, and a gum; GOS, LNnT, 3'SL, 6'SL, and a gum; 2'FL, LNnT, 3'SL, 6'SL, and a gum; FOS, 3'SL, 6'SL, and a gum; GOS, 3'SL, 6'SL, and a gum; 2'FL, 3'SL, 6'SL, and a gum; LNnT, 3'SL, 6'SL, and a gum; FOS, GOS, 2'FL, LNnT, 3'SL, and a gum; FOS, GOS, 2'FL, 3'SL, and a gum; FOS, GOS, LNnT, 3'SL, and a gum; FOS, 2'FL, LNnT, 3'SL, and a gum; GOS, 2'FL, LNnT, 3'SL, and a gum; FOS, GOS, 3'SL, and a gum; FOS, 2'FL, 3'SL, and a gum; FOS, LNnT, 3'SL, and a gum; GOS, 2'FL, 3'SL, and a gum; GOS, LNnT, 3'SL, and a gum; 2'FL, LNnT, 3'SL, and a gum; FOS, 3'SL, and a gum; GOS, 3'SL, and a gum; 2'FL, 3'SL, and a gum; LNnT, 3'SL, and a gum; FOS, GOS, 2'FL, LNnT, 6'SL, and a gum; FOS, GOS, 2'FL, 6'SL, and a gum; FOS, GOS, LNnT, 6'SL, and a gum; FOS, 2'FL, LNnT, 6'SL, and a gum; GOS, 2'FL, LNnT, 6'SL, and a gum; FOS, GOS, 6'SL, and a gum; FOS, 2'FL, 6'SL, and a gum; FOS, LNnT, 6'SL, and a gum; GOS, 2'FL, 6'SL, and a gum; GOS, LNnT, 6'SL, and a gum; 2'FL, LNnT, 6'SL, and a gum; FOS, 6'SL, and a gum; GOS, 6'SL, and a gum; 2'FL, 6'SL, and a gum; and LNnT, 6'SL, and a gum.

Probiotics

[0096] The nutritional compositions of the present disclosure may, in addition to HMOs (and, optionally, other prebiotic oligosaccharides as described above), comprise one or more probiotics. In some embodiments, the nutritional composition includes a combination of HMOs and probiotics such that the composition provides a synergistic benefit to the end user in promoting the growth of microbiota in the gastrointestinal tract of infants.

[0097] Probiotics are live microorganisms thought to be healthy for the host organism. Lactic acid bacteria (LAB) and bifidobacteria are the most common types of microbes used as probiotics. Probiotics maintain the microbial ecology of the gut and show physiological, immuno-modulatory and antimicrobial effects, such that the use of probiotics has been found to prevent and treat gastrointestinal diseases and/or disorders, pathogen-induced diarrhea and toxin-producing bacteria, urogenital infections, and atopic diseases.

[0098] In order for microbes to exhibit beneficial probiotic effects *in vivo*, the organisms should survive for extended time periods in the gastrointestinal tract. Therefore, it is important that probiotic strains be selected that possess qualities that prevent their rapid removal by gut contraction. Effective probiotic strains are able to survive gastric conditions and colonize the intestine, at least temporarily, by adhering to the intestinal epithelium.

[0099] Non-limiting examples of probiotic strains for use in the nutritional compositions herein include the genus *Lactobacillus* including *L. acidophilus*, *L. amylovorus*, *L. brevis*, *L. bulgaricus*, *L. casei spp. casei*, *L. casei spp. rhamnosus*, *L. crispatus*, *L. delbrueckii ssp. lactis*, *L. fermentum*, *L. helveticus*, *L. johnsonii*, *L. paracasei*, *L. pentosus*, *L. plantarum*, *L. reuteri*, and *L. sake*; the genus *Bifidobacterium* including: *B. animalis*, *B. bifidum*, *B. breve*, *B. infantis*, and *B. longum*; the genus *Pediococcus* including: *P. acidilactici*; the genus *Propionibacterium* including: *P. acidipropionici*, *P. freudenreichii*, *P. jensenii*, and *P. theonii*; and the genus *Streptococcus* including: *S. cremoris*, *S. lactis*, and *S. thermophilus*. Particularly preferred probiotics include probiotics of human infant origin such as *B. infantis* M-63, *B. infantis* ATCC 15697, *B. infantis*

35624, *B. infantis* CHCC2228, *B. infantis* BB-02, *B. infantis* DSM20088, and *B. infantis* R-0033.

[00100] The probiotic is present in the nutritional compositions in a total amount of at least about 10^3 CFU/g, including from about 10^3 CFU/g to about 10^{12} CFU/g, and including from about 10^6 CFU/g to about 10^7 CFU/g.

[00101] In some embodiments, the nutritional composition includes a probiotic in combination with a first oligosaccharide including fructooligosaccharide and/or a galactooligosaccharide further in combination with a second oligosaccharide including at least one HMO such as 2'FL, 3'FL, 3'SL, 6'SL, and/or LNnT. In these embodiments, the first oligosaccharide and the second oligosaccharide are present in the compositions in a weight ratio of first oligosaccharide:second oligosaccharide of about 10:1, or even from about 11:1 to about 8:1.

Macronutrients

[00102] The nutritional compositions including the HMO or HMOs may be formulated to include at least one of protein, fat, and carbohydrate. In many embodiments, the nutritional compositions will include the HMO or HMOs with protein, carbohydrate and fat.

[00103] Although total concentrations or amounts of the fat, protein, and carbohydrates may vary depending upon the product type (i.e., human milk fortifier, preterm infant formula, infant formula, toddler formula, pediatric formula, follow-on formula, adult nutritional, etc.), product form (i.e., nutritional solid, powder, ready-to-feed liquid, or concentrated liquid), and targeted dietary needs of the intended user, such concentrations or amounts most typically fall within one of the following embodied ranges, inclusive of any other essential fat, protein, and/or carbohydrate ingredients as described herein.

[00104] For the liquid preterm and term infant formulas, carbohydrate concentrations (including both HMOs and any other carbohydrate/oligosaccharide sources) most typically range from about 5% to about 40%, including from about 7% to about 30%, including from about 10% to about 25%, by weight of the preterm or term infant formula;

fat concentrations most typically range from about 1% to about 30%, including from about 2% to about 15%, and also including from about 3% to about 10%, by weight of the preterm or term infant formula; and protein concentrations most typically range from about 0.5% to about 30%, including from about 1% to about 15%, and also including from about 2% to about 10%, by weight of the preterm or term infant formula.

[00105] For the liquid human milk fortifiers, carbohydrate concentrations (including both HMOs and any other carbohydrate/oligosaccharide sources) most typically range from about 10% to about 75%, including from about 10% to about 50%, including from about 20% to about 40%, by weight of the human milk fortifier; fat concentrations most typically range from about 10% to about 40%, including from about 15% to about 37%, and also including from about 18% to about 30%, by weight of the human milk fortifier; and protein concentrations most typically range from about 5% to about 40%, including from about 10% to about 30%, and also including from about 15% to about 25%, by weight of the human milk fortifier.

[00106] For the adult nutritional liquids, carbohydrate concentrations (including both HMOs and any other carbohydrate/oligosaccharide sources) most typically range from about 5% to about 40%, including from about 7% to about 30%, including from about 10% to about 25%, by weight of the adult nutritional; fat concentrations most typically range from about 2% to about 30%, including from about 3% to about 15%, and also including from about 5% to about 10%, by weight of the adult nutritional; and protein concentrations most typically range from about 0.5% to about 30%, including from about 1% to about 15%, and also including from about 2% to about 10%, by weight of the adult nutritional.

[00107] The amount of carbohydrates, fats, and/or proteins in any of the liquid nutritional compositions described herein may also be characterized in addition to, or in the alternative, as a percentage of total calories in the liquid nutritional composition as set forth in the following table. These macronutrients for liquid nutritional compositions of the present disclosure are most typically formulated within any of the caloric ranges (embodiments A-F) described in the following table (each numerical value is preceded by the term "about").

Nutrient % Total Cal.	Embodiment A	Embodiment B	Embodiment C
Carbohydrate	0-98	2-96	10-75
Protein	0-98	2-96	5-70
Fat	0-98	2-96	20-85
	Embodiment D	**Embodiment E**	**Embodiment F**
Carbohydrate	30-50	25-50	25-50
Protein	15-35	10-30	5-30
Fat	35-55	1-20	2-20

[00108] In one specific example, liquid infant formulas (both ready-to-feed and concentrated liquids) include those embodiments in which the protein component may comprise from about 7.5% to about 25% of the caloric content of the formula; the carbohydrate component (including both HMOs and any other carbohydrate/oligosaccharide sources) may comprise from about 35% to about 50% of the total caloric content of the infant formula; and the fat component may comprise from about 30% to about 60% of the total caloric content of the infant formula. These ranges are provided as examples only, and are not intended to be limiting. Additional suitable ranges are noted in the following table (each numerical value is preceded by the term "about").

Nutrient % Total Cal.	Embodiment G	Embodiment H	Embodiment I
Carbohydrates:	20-85	30-60	35-55
Fat:	5-70	20-60	25-50
Protein:	2-75	5-50	7-40

[00109] When the nutritional composition is a powdered preterm or term infant formula, the protein component is present in an amount of from about 5% to about 35%, including from about 8% to about 12%, and including from about 10% to about 12% by weight of the preterm or term infant formula; the fat component is present in an amount of from about 10% to about 35%, including from about 25% to about 30%, and including from about 26% to about 28% by weight of the preterm or term infant formula; and the carbohydrate component (including both HMOs and any other

carbohydrate/oligosaccharide sources) is present in an amount of from about 30% to about 85%, including from about 45% to about 60%, including from about 50% to about 55% by weight of the preterm or term infant formula.

[00110] For powdered human milk fortifiers, the protein component is present in an amount of from about 1% to about 55%, including from about 10% to about 50%, and including from about 10% to about 30% by weight of the human milk fortifier; the fat component is present in an amount of from about 1% to about 30%, including from about 1% to about 25%, and including from about 1% to about 20% by weight of the human milk fortifier; and the carbohydrate component (including both HMOs and any other carbohydrate/oligosaccharide sources) is present in an amount of from about 15% to about 75%, including from about 15% to about 60%, including from about 20% to about 50% by weight of the human milk fortifier.

[0111] For powdered adult nutritionals, the protein component is present in an amount of from about 10% to about 90%, including from about 30% to about 80%, and including from about 40% to about 75% by weight of the adult nutritional; the fat component is present in an amount of from about 0.5% to about 20%, including from about 1% to about 10%, and including from about 2% to about 5% by weight of the adult nutritional; and the carbohydrate component (including both HMOs and any other carbohydrate/oligosaccharide sources) is present in an amount of from about 5% to about 40%, including from about 7% to about 30%, including from about 10% to about 25% by weight of the adult nutritional.

[0112] The total amount or concentration of fat, carbohydrate, and protein, in the powdered nutritional compositions of the present disclosure can vary considerably depending upon the selected composition and dietary or medical needs of the intended user. Additional suitable examples of macronutrient concentrations are set forth below. In this context, the total amount or concentration refers to all fat, carbohydrate, and protein sources in the powdered composition. For powdered nutritional compositions, such total amounts or concentrations are most typically and preferably formulated within any of the embodied ranges described in the following table (each numerical value is preceded by the term "about').

Nutrient % Total Cal.	Embodiment J	Embodiment K	Embodiment L
Carbohydrate	1-85	30-60	35-55
Fat	5-70	20-60	25-50
Protein	2-75	5-50	7-40

Fat

[0113] The nutritional compositions of the present disclosure may optionally comprise any source or sources of fat. Suitable sources of fat for use herein include any fat or fat source that is suitable for use in an oral nutritional composition and is compatible with the essential elements and features of such composition. For example, in one specific embodiment, the fat is derived from long chain polyunsaturated fatty acids (LCPUFAs).

[0114] Exemplary LCPUFAs for use in the nutritional compositions include, for example, ω-3 LCPUFAs and ω-6 LCPUFAs. Specific LCPUFAs include docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), arachidonic acid (ARA), linoleic acid, linolenic acid (alpha linolenic acid) and gamma-linolenic acid derived from oil sources such as plant oils, marine plankton, fungal oils, and fish oils. In one particular embodiment, the LCPUFAs are derived from fish oils such as menhaden, salmon, anchovy, cod, halibut, tuna, or herring oil. Particularly preferred LCPUFAs for use in the nutritional compositions with the HMOs include DHA, ARA, EPA, DPA, and combinations thereof.

[0115] In order to reduce potential side effects of high dosages of LCPUFAs in the nutritional compositions, the content of LCPUFAs preferably does not exceed 3% by weight of the total fat content, including below 2% by weight of the total fat content, and including below 1% by weight of the total fat content in the nutritional composition.

[0116] The LCPUFA may be provided as free fatty acids, in triglyceride form, in diglyceride form, in monoglyceride form, in phospholipid form, in esterfied form or as a mixture of one or more of the above, preferably in triglyceride form. In another specific embodiment, the fat is derived from short chain fatty acids.

[0117] Additional non-limiting examples of suitable fats or sources thereof for use in the nutritional compositions described herein include coconut oil, fractionated coconut oil, soybean oil, corn oil, olive oil, safflower oil, high oleic safflower oil, oleic acids (EMERSOL 6313 OLEIC ACID, Cognis Oleochemicals, Malaysia), MCT oil (medium chain triglycerides), sunflower oil, high oleic sunflower oil, palm and palm kernel oils, palm olein, canola oil, marine oils, fish oils, fungal oils, algae oils, cottonseed oils, and combinations thereof.

Protein

[0118] The nutritional compositions of the present disclosure may optionally further comprise protein. Any protein source that is suitable for use in oral nutritional compositions and is compatible with the essential elements and features of such compositions is suitable for use in the nutritional compositions.

[0119] Non-limiting examples of suitable proteins or sources thereof for use in the nutritional compositions include hydrolyzed, partially hydrolyzed or non-hydrolyzed proteins or protein sources, which may be derived from any known or otherwise suitable source such as milk (e.g., casein, whey), animal (e.g., meat, fish), cereal (e.g., rice, corn), vegetable (e.g., soy) or combinations thereof. Non-limiting examples of such proteins include milk protein isolates, milk protein concentrates as described herein, casein protein isolates, extensively hydrolyzed casein, whey protein, sodium or calcium caseinates, whole cow milk, partially or completely defatted milk, soy protein isolates, soy protein concentrates, and so forth. In one specific embodiment, the nutritional compositions include a protein source derived from milk proteins of human and/or bovine origin.

[0120] In one embodiment, the protein source is a hydrolyzed protein hydrolysate. In this context, the terms "hydrolyzed protein" or "protein hydrolysates" are used interchangeably herein and include extensively hydrolyzed proteins, wherein the degree of hydrolysis is most often at least about 20%, including from about 20% to about 80%, and also including from about 30% to about 80%, even more preferably from about 40% to about 60%. The degree of hydrolysis is the extent to which peptide bonds are broken by a hydrolysis method. The degree of protein hydrolysis for purposes of characterizing the extensively hydrolyzed protein component of these embodiments is easily determined by one of ordinary skill in the formulation arts by quantifying the amino nitrogen to total

nitrogen ratio (AN/TN) of the protein component of the selected liquid formulation. The amino nitrogen component is quantified by USP titration methods for determining amino nitrogen content, while the total nitrogen component is determined by the Tecator Kjeldahl method, all of which are well known methods to one of ordinary skill in the analytical chemistry art.

[0121] Suitable hydrolyzed proteins may include soy protein hydrolysate, casein protein hydrolysate, whey protein hydrolysate, rice protein hydrolysate, potato protein hydrolysate, fish protein hydrolysate, egg albumen hydrolysate, gelatin protein hydrolysate, combinations of animal and vegetable protein hydrolysates, and combinations thereof. Particularly preferred protein hydrolysates include whey protein hydrolysate and hydrolyzed sodium caseinate.

[0122] When used in the nutritional compositions, the protein source may include at least about 20% (by weight total protein) protein hydrolysate, including from about 30% to 100% (by weight total protein) protein hydrolysate, and including from about 40% to about 80% (by weight total protein) protein hydrolysate, and including about 50% (by weight total protein) protein hydrolysate. In one particular embodiment, the nutritional composition includes 100% (by weight total protein) protein hydrolysate.

Carbohydrate

[0123] The nutritional compositions of the present disclosure may further optionally comprise any carbohydrates that are suitable for use in an oral nutritional composition and are compatible with the essential elements and features of such compositions.

[0124] Non-limiting examples of suitable carbohydrates or sources thereof for use in the nutritional compositions described herein may include maltodextrin, hydrolyzed or modified starch or cornstarch, glucose polymers, corn syrup, corn syrup solids, rice-derived carbohydrates, pea-derived carbohydrates, potato-derived carbohydrates, tapioca, sucrose, glucose, fructose, lactose, high fructose corn syrup, honey, sugar alcohols (e.g., maltitol, erythritol, sorbitol), artificial sweeteners (e.g., sucralose, acesulfame potassium, stevia) and combinations thereof. A particularly desirable carbohydrate is a low dextrose equivalent (DE) maltodextrin.

Other Optional Ingredients

[0125] The nutritional compositions of the present disclosure may further comprise other optional components that may modify the physical, chemical, aesthetic or processing characteristics of the compositions or serve as pharmaceutical or additional nutritional components when used in the targeted population. Many such optional ingredients are known or otherwise suitable for use in medical food or other nutritional products or pharmaceutical dosage forms and may also be used in the compositions herein, provided that such optional ingredients are safe for oral administration and are compatible with the essential and other ingredients in the selected product form.

[0126] Non-limiting examples of such optional ingredients include preservatives, emulsifying agents, buffers, pharmaceutical actives, anti-inflammatory agents, additional nutrients as described herein, colorants, flavors, thickening agents and stabilizers, emulsifying agents, lubricants, and so forth.

[0127] The nutritional compositions may further comprise a sweetening agent, preferably including at least one sugar alcohol such as maltitol, erythritol, sorbitol, xylitol, mannitol, isolmalt, and lactitol, and also preferably including at least one artificial or high potency sweetener such as acesulfame K, aspartame, sucralose, saccharin, stevia, and tagatose. These sweetening agents, especially as a combination of a sugar alcohol and an artificial sweetener, are especially useful in formulating liquid beverage embodiments of the present disclosure having a desirable favor profile. These sweetener combinations are especially effective in masking undesirable flavors sometimes associated with the addition of vegetable proteins to a liquid beverage. Optional sugar alcohol concentrations in the nutritional composition may range from at least 0.01%, including from 0.1% to about 10%, and also including from about 1% to about 6%, by weight of the nutritional composition. Optional artificial sweetener concentrations may range from about 0.01%, including from about 0.05% to about 5%, also including from about 0.1% to about 1.0%, by weight of the nutritional composition.

[0128] A flowing agent or anti-caking agent may be included in the nutritional compositions as described herein to retard clumping or caking of the powder over time and to make a powder embodiment flow easily from its container. Any known flowing or anti-caking agents that are known or otherwise suitable for use in a nutritional powder or

product form are suitable for use herein, non-limiting examples of which include tricalcium phosphate, silicates, and combinations thereof. The concentration of the flowing agent or anti-caking agent in the nutritional composition varies depending upon the product form, the other selected ingredients, the desired flow properties, and so forth, but most typically range from about 0.1% to about 4%, including from about 0.5% to about 2%, by weight of the nutritional composition.

[0129] A stabilizer may also be included in the nutritional compositions. Any stabilizer that is known or otherwise suitable for use in a nutritional composition is also suitable for use herein, some non-limiting examples of which include gums such as xanthan gum. The stabilizer may represent from about 0.1% to about 5.0%, including from about 0.5% to about 3%, including from about 0.7% to about 1.5%, by weight of the nutritional composition.

[0130] Additionally, the nutritional compositions may comprise one or more antioxidants to provide nutritional support, as well as to reduce oxidative stress. Any antioxidants suitable for oral administration may be included for use in the nutritional compositions of the present disclosure, including, for example, vitamin A, vitamin E, vitamin C, retinol, tocopherol, and carotenoids.

[0131] In one specific embodiment, the antioxidants for use in the nutritional compositions include carotenoids such as lutein, zeaxanthin, lycopene, beta-carotene, and combinations thereof, and particularly, combinations of the carotenoids lutein, lycopene, and beta-carotene. Nutritional compositions containing these combinations, as selected and defined herein, can be used to modulate inflammation and/or levels of C-reactive protein in preterm and term infants.

[0132] The nutritional compositions may further comprise any of a variety of other vitamins or related nutrients, non-limiting examples of which include vitamin D, vitamin K, thiamine, riboflavin, pyridoxine, vitamin B_{12}, niacin, folic acid, pantothenic acid, biotin, choline, inositol, salts and derivatives thereof, and combinations thereof.

[0133] The nutritional compositions may further comprise any of a variety of other additional minerals, non-limiting examples of which include calcium, phosphorus, magnesium, iron, zinc, manganese, copper, sodium, potassium, molybdenum, chromium, chloride, and combinations thereof.

[0134] The nutritional compositions of the present disclosure may additionally comprise nucleotides and/or nucleotide precursors selected from the group consisting of nucleoside, purine base, pyrimidine base, ribose and deoxyribose to further improve intestinal barrier integrity and/or maturation. The nucleotide may be in monophosphate, diphosphate, or triphosphate form. The nucleotide may be a ribonucleotide or a deoxyribonucleotide. The nucleotides may be monomeric, dimeric, or polymeric (including RNA and DNA). The nucleotide may be present in the nutritional composition as a free acid or in the form of a salt, preferably a monosodium salt.

[0135] Suitable nucleotides and/or nucleosides for use in the nutritional compositions include one or more of cytidine 5'-monophosphate, uridine 5'-monophosphate, adenosine 5'-monophosphate, guanosine 5'-1-monophosphate, and/or inosine 5'-monophosphate, more preferably cytidine 5'-monophosphate, uridine 5'-monophosphate, adenosine 5'-monophosphate, guanosine 5'-monophosphate, and inosine 5'-monophosphate.

Methods of Manufacture

[0136] The nutritional compositions of the present disclosure may be prepared by any known or otherwise effective manufacturing technique for preparing the selected product solid or liquid form. Many such techniques are known for any given product form such as nutritional liquids or powders and can easily be applied by one of ordinary skill in the art to the nutritional compositions described herein.

[0137] The nutritional compositions of the present disclosure can therefore be prepared by any of a variety of known or otherwise effective formulation or manufacturing methods. In one suitable manufacturing process, for example, at least three separate slurries are prepared, including a protein-in-fat (PIF) slurry, a carbohydrate-mineral (CHO-MIN) slurry, and a protein-in-water (PIW) slurry. The PIF slurry is formed by heating and mixing the oil (e.g., canola oil, corn oil, etc.) and then adding an emulsifier (e.g., lecithin),

fat soluble vitamins, and a portion of the total protein (e.g., milk protein concentrate, etc.) with continued heat and agitation. The CHO-MIN slurry is formed by adding with heated agitation to water: minerals (e.g., potassium citrate, dipotassium phosphate, sodium citrate, etc.), trace and ultra trace minerals (TM/UTM premix), thickening or suspending agents (e.g. avicel, gellan, carrageenan). The resulting CHO-MIN slurry is held for 10 minutes with continued heat and agitation before adding additional minerals (e.g., potassium chloride, magnesium carbonate, potassium iodide, etc.), and/or carbohydrates (e.g., HMOs, fructooligosaccharide, sucrose, corn syrup, etc.). The PIW slurry is then formed by mixing with heat and agitation the remaining protein, if any.

[0138] The resulting slurries are then blended together with heated agitation and the pH adjusted to 6.6-7.0, after which the composition is subjected to high-temperature short-time (HTST) processing during which the composition is heat treated, emulsified and homogenized, and then allowed to cool. Water soluble vitamins and ascorbic acid are added, the pH is adjusted to the desired range if necessary, flavors are added, and water is added to achieve the desired total solid level. The composition is then aseptically packaged to form an aseptically packaged nutritional emulsion. This emulsion can then be further diluted, heat-treated, and packaged to form a ready-to-feed or concentrated liquid, or it can be heat-treated and subsequently processed and packaged as a reconstitutable powder, e.g., spray dried, drymixed, agglomerated.

[0139] The nutritional solid, such as a spray dried nutritional powder or drymixed nutritional powder, may be prepared by any collection of known or otherwise effective techniques, suitable for making and formulating a nutritional powder.

[0140] For example, when the nutritional powder is a spray dried nutritional powder, the spray drying step may likewise include any spray drying technique that is known for or otherwise suitable for use in the production of nutritional powders. Many different spray drying methods and techniques are known for use in the nutrition field, all of which are suitable for use in the manufacture of the spray dried nutritional powders herein.

[0141] One method of preparing the spray dried nutritional powder comprises forming and homogenizing an aqueous slurry or liquid comprising predigested fat, and optionally protein, carbohydrate, and other sources of fat, and then spray drying the slurry

or liquid to produce a spray dried nutritional powder. The method may further comprise the step of spray drying, drymixing, or otherwise adding additional nutritional ingredients, including any one or more of the ingredients described herein, to the spray dried nutritional powder.

[0142] Other suitable methods for making nutritional compositions are described, for example, in U.S. Pat. No. 6,365,218 (Borschel, et al.), U.S. Patent No. 6,589,576 (Borschel, et al.), U.S. Pat. No. 6,306,908 (Carlson, et al.), U.S. Patent Application No. 20030118703 A1 (Nguyen, et al.), which descriptions are incorporated herein by reference to the extent that they are consistent herewith.

Methods of Use

[0143] The nutritional compositions as described herein can be used to address one or more of the diseases, disorders, or conditions discussed herein, or can be used to provide one or more of the benefits described herein, to preterm infants, infants, toddlers, children, and adults, including pregnant women. The preterm infant, infant, toddler, child, adult and pregnant women utilizing the nutritional compositions described herein may actually have or be afflicted with the disease or condition described, or may be susceptible to, or at risk of, getting the disease or condition (that is, may not actually yet have the disease or condition, but is at elevated risk as compared to the general population for getting it due to certain conditions, family history, etc.) Whether the preterm infant, infant, toddler, child, adult, and pregnant women actually have the disease or condition, or is at risk or susceptible to the disease or condition, the preterm infant, infant, toddler, child, adult, and pregnant women are classified herein as "in need of" assistance in dealing with and combating the disease or condition. For example, the preterm infant, infant, toddler, child, adult and pregnant women may actually have respiratory inflammation or may be at risk of getting respiratory inflammation (susceptible to getting respiratory inflammation) due to family history or other medical conditions, for example. Whether the preterm infant, infant, toddler, child, adult, and pregnant women actually has the disease or condition, or is only at risk or susceptible to getting the disease or condition, it is within the scope of the present disclosure to assist the preterm infant, infant, toddler, child, adult and pregnant women with the nutritional compositions described herein.

[0144] Based on the foregoing, because some of the method embodiments of the present disclosure are directed to specific subsets or subclasses of identified individuals (that is, the subset or subclass of individuals "in need" of assistance in addressing one or more specific diseases or specific conditions noted herein), not all preterm infants, infants, toddlers, children, adults and pregnant women will fall within the subset or subclass of preterm infants, infants, toddlers, children, adults, and pregnant women as described herein for certain diseases or conditions.

[0145] The nutritional compositions as described herein comprise HMOs, alone or in combination with one or more additional components, to provide a nutritional source for improving at least the intestinal/gut function. Specifically, the nutritional compositions can stimulate enteric nerve cells in the gastrointestinal tract of an individual to improve intestinal/gut barrier integrity; improve feeding tolerance (e.g., reduced diarrhea, loose stools, gas, and bloating); reduce colic in infants; protect against necrotizing enterocolitis and other disorders of prematurity; address gastrointestinal diseases and disorders associated with the enteric nervous system; address gastrointestinal diseases and disorders of gut contractility and inflammation; correct effects of gut dysbiosis; and affect long-term modulation of allergic tolerance.

[0146] More particularly, in some embodiments, the nutritional compositions may be administered to an individual having, susceptible to, or at risk of, gastrointestinal diseases and disorders associated with the enteric nervous system and/or associated with gut contractility and inflammation, which may include, for example, irritable bowel syndrome, colitis (e.g., necrotizing enterocolitis, Crohn's disease, ischemic colitis, cryptosporidium enterocolitis, pseudomembranous colitis, cytomegalovirus, ulcerative colitis), food intolerance, and food allergies.

[0147] Along with improved growth and maturation of an individual's immune system as described above, the use of the nutritional compositions of the present disclosure may also function to enhance the individual's ability to resist microbial infection and to promote the growth of beneficial microbiota in the gastrointestinal tract of an infant, toddler, child, or adult.

[0148] Further, when used in combination with LCPUFAs and/or antioxidants, and particularly, with carotenoids, the HMOs can reduce oxidative stress, which is a metabolic condition in which there is an increased production and accumulation of oxidized biomolecules such as lipid peroxides and their catabolites, protein carbonyls, and oxidatively damaged DNA. The outcomes of oxidative stress range from unwanted changes in metabolism to inflammation and cell and tissue death. Accordingly, by reducing the incidence of unregulated inflammation and oxidation in the infant, damage to the tissue lining and cell death is reduced, further reducing the incidence of inflammatory diseases, such as necrotizing enterocolitis (NEC).

[0149] Additionally, the nutritional compositions of the present disclosure may also be used to improve cognition in individuals, particularly in individuals susceptible to, or at risk of, neurodegenerative diseases, which may include, for example, Alzheimer's disease, Huntington's disease, Parkinson's disease, and schizophrenia, or in individuals suffering from conditions caused by impaired cognitive development or neurodevelopmental conditions, such as attention deficit hyperactivity disorder and autism.

EXAMPLES

[0150] The following examples illustrate specific embodiments and/or features of the nutritional compositions and methods of the present disclosure. The examples are given solely for the purpose of illustration and are not to be construed as limitations of the present disclosure, as many variations thereof are possible without departing from the spirit and scope of the disclosure. All exemplified amounts are weight percentages based upon the total weight of the composition, unless otherwise specified.

[0151] The exemplified compositions are shelf stable nutritional compositions prepared in accordance with the manufacturing methods described herein, such that each exemplified composition, unless otherwise specified, includes an aseptically processed embodiment and a retort packaged embodiment.

[0152] The nutritional liquid embodiments are aqueous oil-in-water emulsions that are packaged in 240 mL plastic containers and remain physically stable for 12-18 months after composition/packaging at storage temperatures ranging from 1-25°C.

EXAMPLES 1-5

[0153] Examples 1-5 illustrate ready-to-feed nutritional emulsions of the present disclosure, the ingredients of which are listed in the table below. All ingredient amounts are listed as kilogram per 1000 kilogram batch of product, unless otherwise specified.

Ingredient	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Ex. 5
Water	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.
Condensed Skim Milk	86.64	86.64	86.64	86.64	86.64
Lactose	54.80	54.80	54.80	54.80	54.80
High oleic safflower oil	14.10	14.10	14.10	14.10	14.10
Soybean oil	10.6	10.6	10.6	10.6	10.6
Coconut oil	10.1	10.1	10.1	10.1	10.1
2' fucosyllactose (2'FL)	0.1896	0.1801	0.1706	0.1991	0.2086
Galactooligosaccharides (GOS)	8.630	8.630	8.630	8.630	8.630
Whey protein concentrate	6.40	6.40	6.40	6.40	6.40
Potassium citrate	478.9 g	478.9 g	478.9 g	478.9 g	478.9 g
Calcium carbonate	448.28 g	448.28 g	448.28 g	448.28 g	448.28 g
Soy lecithin	355.74 g	355.74 g	355.74 g	355.74 g	355.74 g
Stabilizer	355.74 g	355.74 g	355.74 g	355.74 g	355.74 g
ARA oil	368.01 g	368.01 g	368.01 g	368.01 g	368.01 g
Nucleotide/chloride premix	293.26 g	293.26 g	293.26 g	293.26 g	293.26 g
Potassium chloride	226.45 g	226.45 g	226.45 g	226.45 g	226.45 g
Ascorbic acid	445.94 g	445.94 g	445.94 g	445.94 g	445.94 g
Vitamin mineral premix	142.88 g	142.88 g	142.88 g	142.88 g	142.88 g
DHA oil	137.8 g	137.8 g	137.8 g	137.8 g	137.8 g
Carrageenan	180.0 g	180.0 g	180.0 g	180.0 g	180.0 g
Magnesium chloride	55.0 g	55.0 g	55.0 g	55.0 g	55.0 g
Ferrous sulfate	58.0 g	58.0 g	58.0 g	58.0 g	58.0 g
Choline chloride	53.9 g	53.9 g	53.9 g	53.9 g	53.9 g
Vitamin A, D_3, E, K_1 premix	47.40 g	47.40 g	47.40 g	47.40 g	47.40 g
Citric acid	29.77 g	29.77 g	29.77 g	29.77 g	29.77 g
Probiotic	1.0	1.0	1.0	1.0	1.0
Mixed carotenoid premix	26.40 g	26.40 g	26.40 g	26.40 g	26.40 g
Sodium chloride	AN	AN	AN	AN	AN
L-carnitine	3.31 g	3.31 g	3.31 g	3.31 g	3.31 g
Tricalcium phosphate	15.65 g	15.65 g	15.65 g	15.65 g	15.65 g
Potassium phosphate monobasic	13.67 g	13.67 g	13.67 g	13.67 g	13.67 g
Riboflavin	2.42 g	2.42 g	2.42 g	2.42 g	2.42 g
Potassium hydroxide	AN	AN	AN	AN	AN

AN = as needed

EXAMPLES 6-10

[0154] Examples 6-10 illustrate ready-to-feed nutritional emulsions of the present disclosure, the ingredients of which are listed in the table below. All ingredient amounts are listed as kilogram per 1000 kilogram batch of product, unless otherwise specified.

Ingredient	Ex. 6	Ex. 7	Ex. 8	Ex. 9	Ex. 10
Water	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.
Condensed Skim Milk	86.64	86.64	86.64	86.64	86.64
Lactose	54.80	54.80	54.80	54.80	54.80
High oleic safflower oil	14.10	14.10	14.10	14.10	14.10
Soybean oil	10.6	10.6	10.6	10.6	10.6
Coconut oil	10.1	10.1	10.1	10.1	10.1
2' fucosyllactose (2'FL)	0.0948	0.09005	0.0853	0.0995	0.1043
Lacto-N-neotetraose (LNnT)	0.0948	0.09005	0.0853	0.0995	0.1043
Galactooligosaccharides (GOS)	8.630	8.630	8.630	8.630	8.630
Whey protein concentrate	6.40	6.40	6.40	6.40	6.40
Potassium citrate	478.9 g	478.9 g	478.9 g	478.9 g	478.9 g
Calcium carbonate	448.28 g	448.28 g	448.28 g	448.28 g	448.28 g
Soy lecithin	355.74 g	355.74 g	355.74 g	355.74 g	355.74 g
Stabilizer	355.74 g	355.74 g	355.74 g	355.74 g	355.74 g
ARA oil	368.01 g	368.01 g	368.01 g	368.01 g	368.01 g
Nucleotide/chloride premix	293.26 g	293.26 g	293.26 g	293.26 g	293.26 g
Potassium chloride	226.45 g	226.45 g	226.45 g	226.45 g	226.45 g
Ascorbic acid	445.94 g	445.94 g	445.94 g	445.94 g	445.94 g
Vitamin mineral premix	142.88 g	142.88 g	142.88 g	142.88 g	142.88 g
DHA oil	137.8 g	137.8 g	137.8 g	137.8 g	137.8 g
Carrageenan	180.0 g	180.0 g	180.0 g	180.0 g	180.0 g
Magnesium chloride	55.0 g	55.0 g	55.0 g	55.0 g	55.0 g
Ferrous sulfate	58.0 g	58.0 g	58.0 g	58.0 g	58.0 g
Choline chloride	53.9 g	53.9 g	53.9 g	53.9 g	53.9 g
Vitamin A, D_3, E, K_1 premix	47.40 g	47.40 g	47.40 g	47.40 g	47.40 g
Citric acid	29.77 g	29.77 g	29.77 g	29.77 g	29.77 g
Probiotic	1.0	0.95	0.90	1.05	1.10
Mixed carotenoid premix	26.40 g	26.40 g	26.40 g	26.40 g	26.40 g
Sodium chloride	AN	AN	AN	AN	AN
L-carnitine	3.31 g	3.31 g	3.31 g	3.31 g	3.31 g
Tricalcium phosphate	15.65 g	15.65 g	15.65 g	15.65 g	15.65 g
Potassium phosphate monobasic	13.67 g	13.67 g	13.67 g	13.67 g	13.67 g
Riboflavin	2.42 g	2.42 g	2.42 g	2.42 g	2.42 g
Potassium hydroxide	AN	AN	AN	AN	AN

AN = as needed

EXAMPLES 11-15

[0155] Examples 11-15 illustrate concentrated liquid emulsions of the present disclosure, the ingredients of which are listed in the table below. All ingredient amounts are listed as kilogram per 1000 kilogram batch of product, unless otherwise specified.

Ingredient	Ex. 11	Ex. 12	Ex. 13	Ex. 14	Ex. 15
Water	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.
Condensed Skim Milk	166.6	166.6	166.6	166.6	166.6
Lactose	106.1	106.1	106.1	106.1	106.1
High oleic safflower oil	27.16	27.16	27.16	27.16	27.16
Soybean oil	20.42	20.42	20.42	20.42	20.42
Coconut oil	19.48	19.48	19.48	19.48	19.48
2' fucosyllactose (2'FL)	0.1896	0.1188	0.0853	0.2414	0.2560
Galactooligosaccharides (GOS)	16.71	16.71	16.71	16.71	16.71

Whey protein concentrate	12.20	12.20	12.20	12.20	12.20
Potassium citrate	894.5 g	894.5 g	894.5 g	894.5 g	894.5 g
Calcium carbonate	1.072	1.072	1.072	1.072	1.072
Monoglycerides	690.0 g	690.0 g	690.0 g	690.0 g	690.0 g
Soy lecithin	690.0 g	690.0 g	690.0 g	690.0 g	690.0 g
ARA oil	684.2 g	684.2 g	684.2 g	684.2 g	684.2 g
Nucleotide/chloride premix	568.9 g	568.9 g	568.9 g	568.9 g	568.9 g
Potassium chloride	480.8 g	480.8 g	480.8 g	480.8 g	480.8 g
Ascorbic acid	958.6 g	958.6 g	958.6 g	958.6 g	958.6 g
Vitamin mineral premix	276.9 g	276.9 g	276.9 g	276.9 g	276.9 g
DHA oil	256.1 g	256.1 g	256.1 g	256.1 g	256.1 g
Carrageenan	200.0 g	200.0 g	200.0 g	200.0 g	200.0 g
Magnesium chloride	174.7 g	174.7 g	174.7 g	174.7 g	174.7 g
Ferrous sulfate	112.7 g	112.7 g	112.7 g	112.7 g	112.7 g
Choline chloride	104.8 g	104.8 g	104.8 g	104.8 g	104.8 g
Vitamin A, D_3, E, K_1 premix	86.90 g	86.90 g	86.90 g	86.90 g	86.90 g
Citric acid	64.55 g	64.55 g	64.55 g	64.55 g	64.55 g
Mixed carotenoid premix	45.63 g	45.63 g	45.63 g	45.63 g	45.63 g
Sodium chloride	AN	AN	AN	AN	AN
L-carnitine	6.371 g	6.371 g	6.371 g	6.371 g	6.371 g
Riboflavin	2.921 g	2.921 g	2.921 g	2.921 g	2.921 g
Vitamin A Palmitate	1.504 g	1.504 g	1.504 g	1.504 g	1.504 g
Potassium hydroxide	659.8 g	659.8 g	659.8 g	659.8 g	659.8 g
Tricalcium phosphate	AN	AN	AN	AN	AN
Potassium phosphate monobasic	AN	AN	AN	AN	AN

AN = as needed

EXAMPLES 16-20

[0156] Examples 16-20 illustrate spray dried nutritional powders of the present disclosure, the ingredients of which are listed in the table below. All ingredient amounts are listed as kilogram per 1000 kilogram batch of product, unless otherwise specified.

Ingredient	Ex. 16	Ex. 17	Ex. 18	Ex. 19	Ex. 20
Nonfat dry milk	456.9	456.9	456.9	456.9	456.9
Lactose	259.0	259.0	259.0	259.0	259.0
High oleic sunflower oil	93.9	93.9	93.9	93.9	93.9
Soy oil	70.4	70.4	70.4	70.4	70.4
Coconut oil	67.1	67.1	67.1	67.1	67.1
2' fucosyllactose (2'FL)	0.7584	0.7204	0.6824	0.7964	0.8344
Galactooligosaccharide (GOS)	53.5	53.5	53.5	53.5	53.5
Probiotic	1.0	0.95	0.90	1.05	1.10
Flavoring agent	6.2	6.2	6.2	6.2	6.2
Calcium carbonate	4.8	4.8	4.8	4.8	4.8
Potassium citrate	4.7	4.7	4.7	4.7	4.7
Oligofructose (FOS)	2.9	2.9	2.9	2.9	2.9
Ascorbic acid	2.0	2.0	2.0	2.0	2.0
Nucleotide/Choline Premix	1.8	1.8	1.8	1.8	1.8
ARA oil	1.8	1.8	1.8	1.8	1.8
Vitamin/Trace Mineral Premix	1.5	1.5	1.5	1.5	1.5
Sodium chloride	1.3	1.3	1.3	1.3	1.3
Lecithin	1.2	1.2	1.2	1.2	1.2

Sodium citrate	982.2 g	982.2 g	982.2 g	982.2 g	982.2 g
DHA oil	882.1 g	882.1 g	882.1 g	882.1 g	882.1 g
Magnesium chloride	477.4 g	477.4 g	477.4 g	477.4 g	477.4 g
Vitamin A, D3, E, K1 Premix	314.7 g	314.7 g	314.7 g	314.7 g	314.7 g
Ascorbyl Palmitate	278.8 g	278.8 g	278.8 g	278.8 g	278.8 g
Antioxidant	137.3 g	137.3 g	137.3 g	137.3 g	137.3 g
Tocopheryl acetate	32.0 g	32.0 g	32.0 g	32.0 g	32.0 g
Beta-carotene 30%	11.0 g	11.0 g	11.0 g	11.0 g	11.0 g
Potassium iodide	2.5 g	2.5 g	2.5 g	2.5 g	2.5 g
Riboflavin	2.0 g	2.0 g	2.0 g	2.0 g	2.0 g
Magnesium sulfate	499.5 mg	499.5 mg	499.5 mg	499.5 mg	499.5 mg
Potassium phosphate dibasic	AN	AN	AN	AN	AN
Potassium chloride	AN	AN	AN	AN	AN
Tricalcium phosphate	AN	AN	AN	AN	AN
Potassium hydroxide	AN	AN	AN	AN	AN
Calcium hydroxide	AN	AN	AN	AN	AN
Sodium hydroxide	AN	AN	AN	AN	AN
Water	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.

AN = as needed

EXAMPLES 21-25

[0157] Examples 21-25 illustrate spray dried nutritional powders of the present disclosure, the ingredients of which are listed in the table below. All ingredient amounts are listed as kilogram per 1000 kilogram batch of product, unless otherwise specified.

Ingredient	Ex. 21	Ex. 22	Ex. 23	Ex. 24	Ex. 25
Water	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.
Corn syrup	308.9	308.9	308.9	308.9	308.9
Maltodextrin	297.1	297.1	297.1	297.1	297.1
Sucrose	112.4	112.4	112.4	112.4	112.4
High Oleic sunflower oil	84.9	84.9	84.9	84.9	84.9
Sodium caseinate	73.0	73.0	73.0	73.0	73.0
Calcium caseinate	50.2	50.2	50.2	50.2	50.2
2' fucosyllactose (2'FL)	0.7584	0.7204	0.6824	0.7964	0.8344
Inulin, oligofructose	47.0	47.0	47.0	47.0	47.0
Soy oil	38.3	38.3	38.3	38.3	38.3
Isolated soy protein	35.9	35.9	35.9	35.9	35.9
Milk protein isolate	16.3	16.3	16.3	16.3	16.3
Canola oil	13.7	13.7	13.7	13.7	13.7
Sodium citrate	9.8	9.8	9.8	9.8	9.8
Potassium citrate	9.7	9.7	9.7	9.7	9.7
Tricalcium phosphate	9.0	9.0	9.0	9.0	9.0
Flavoring agent	7.3	7.3	7.3	7.3	7.3
Magnesium chloride	6.2	6.2	6.2	6.2	6.2
Potassium chloride	5.5	5.5	5.5	5.5	5.5
Choline chloride	1.7	1.7	1.7	1.7	1.7
Vitamin premix	950.0 g	950.0 g	950.0 g	950.0 g	950.0 g
Ascorbic acid	755.0 g	755.0 g	755.0 g	755.0 g	755.0 g
Vitamin/trace mineral premix	465.0 g	465.0 g	465.0 g	465.0 g	465.0 g
Potassium hydroxide	215.9 g	215.9 g	215.9 g	215.9 g	215.9 g
Potassium phosphate dibasic	185.8 g	185.8 g	185.8 g	185.8 g	185.8 g

Ascorbyl palmitate	164.7 g	164.7 g	164.7 g	164.7 g	164.7 g
Antioxidant	82.3 g	82.3 g	82.3 g	82.3 g	82.3 g
Vitamin A, D3, E, K1 premix	82.3 g	82.3 g	82.3 g	82.3 g	82.3 g
Vitamin A palmitate	16.5 g	16.5 g	16.5 g	16.5 g	16.5 g
Ferrous sulfate	12.0 g	12.0 g	12.0 g	12.0 g	12.0 g
Beta carotene 30%	5.5 g	5.5 g	5.5 g	5.5 g	5.5 g
Vitamin D3 oil	1.0 g	1.0 g	1.0 g	1.0 g	1.0 g
Potassium iodide	800.0 mg	800.0 mg	800.0 mg	800.0 mg	800.0 mg
Citric acid	AN	AN	AN	AN	AN
Potassium hydroxide 40%	AN	AN	AN	AN	AN
Maltodextrin	AN	AN	AN	AN	AN
Magnesium sulfate	AN	AN	AN	AN	AN
Sodium chloride	AN	AN	AN	AN	AN
Calcium carbonate	AN	AN	AN	AN	AN

AN = as needed

EXAMPLES 26-30

[0158] Examples 26-30 illustrate ready-to-feed nutritional emulsions of the present disclosure, the ingredients of which are listed in the table below. All ingredient amounts are listed as kilogram per 1000 kilogram batch of product, unless otherwise specified.

Ingredient	Ex. 26	Ex. 27	Ex. 28	Ex. 29	Ex. 30
Water	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.
Condensed Skim Milk	86.64	86.64	86.64	86.64	86.64
Lactose	54.80	54.80	54.80	54.80	54.80
High oleic safflower oil	14.10	14.10	14.10	14.10	14.10
Soybean oil	10.6	10.6	10.6	10.6	10.6
Coconut oil	10.1	10.1	10.1	10.1	10.1
2' fucosyllactose (2'FL)	0.0948	0.09005	0.0853	0.0995	0.1043
6'-sialyllactose (6'SL)	0.0948	0.09005	0.0853	0.0995	0.1043
Galactooligosaccharides (GOS)	8.630	8.630	8.630	8.630	8.630
Whey protein concentrate	6.40	6.40	6.40	6.40	6.40
Potassium citrate	478.9 g	478.9 g	478.9 g	478.9 g	478.9 g
Calcium carbonate	448.28 g	448.28 g	448.28 g	448.28 g	448.28 g
Soy lecithin	355.74 g	355.74 g	355.74 g	355.74 g	355.74 g
Stabilizer	355.74 g	355.74 g	355.74 g	355.74 g	355.74 g
ARA oil	368.01 g	368.01 g	368.01 g	368.01 g	368.01 g
Nucleotide/chloride premix	293.26 g	293.26 g	293.26 g	293.26 g	293.26 g
Potassium chloride	226.45 g	226.45 g	226.45 g	226.45 g	226.45 g
Ascorbic acid	445.94 g	445.94 g	445.94 g	445.94 g	445.94 g
Vitamin mineral premix	142.88 g	142.88 g	142.88 g	142.88 g	142.88 g
DHA oil	137.8 g	137.8 g	137.8 g	137.8 g	137.8 g
Carrageenan	180.0 g	180.0 g	180.0 g	180.0 g	180.0 g
Magnesium chloride	55.0 g	55.0 g	55.0 g	55.0 g	55.0 g
Ferrous sulfate	58.0 g	58.0 g	58.0 g	58.0 g	58.0 g
Choline chloride	53.9 g	53.9 g	53.9 g	53.9 g	53.9 g
Vitamin A, D_3, E, K_1 premix	47.40 g	47.40 g	47.40 g	47.40 g	47.40 g
Citric acid	29.77 g	29.77 g	29.77 g	29.77 g	29.77 g
Probiotic	1.0	0.95	0.90	1.05	1.10
Mixed carotenoid premix	26.40 g	26.40 g	26.40 g	26.40 g	26.40 g
Sodium chloride	AN	AN	AN	AN	AN

L-carnitine	3.31 g	3.31 g	3.31 g	3.31 g	3.31 g
Tricalcium phosphate	15.65 g	15.65 g	15.65 g	15.65 g	15.65 g
Potassium phosphate monobasic	13.67 g	13.67 g	13.67 g	13.67 g	13.67 g
Riboflavin	2.42 g	2.42 g	2.42 g	2.42 g	2.42 g
Potassium hydroxide	AN	AN	AN	AN	AN

AN = as needed

EXAMPLES 31-34

[0159] Examples 31-34 illustrate concentrated liquid human milk fortifiers of the present disclosure, the ingredients of which are listed in the table below. All ingredient amounts are listed as kilogram per 1000 kilogram batch of product, unless otherwise specified.

Ingredient (Per 1000 Kg)	**Ex. 31**	**Ex. 32**	**Ex. 33**	**Ex. 34**
Water	Q.S.	Q.S.	Q.S.	Q.S.
Casein Hydrolysate	108	108	125	150
Maltodextrin	104	104	104	104
MCT Oil	17.3	17.3	17.3	17.3
Tricalcium Phosphate	16.0	16.0	16.0	16.0
Soy Oil	10.4	10.4	10.4	10.4
6' sialyllactose (6'SL)	0.0948	0.09005	0.0853	0.0995
Lacto-N-neotetraose (LNnT)	0.0948	0.09005	0.0853	0.0995
Galactooligosaccharides (GOS)	6.7704	6.7704	6.7704	6.7704
Gum Arabic	12.0	10.0	15.0	2.031
Starch	12.0	10.0	35.0	6.0
Coconut Oil	6.3	6.3	6.3	6.3
Potassium Citrate	6.9	6.9	6.9	6.9
Ascorbic Acid	2.9	2.9	2.9	2.9
Magnesium Chloride	4.0	4.0	4.0	4.0
ARA oil	2.6	2.6	2.6	2.6
Leucine	1.8	1.8	1.8	1.8
DHA oil	2.1	2.1	2.1	2.1
Potassium Chloride	1.1	1.1	1.1	1.1
Tyrosine	1.4	1.4	1.4	1.4
Monoglycerides	800 g	800 g	800 g	800 g
Mixed Carotenoid Premix	551 g	551 g	551 g	551 g
M-Inositol	529 g	529 g	529 g	529 g
Sodium Chloride	861 g	861 g	861 g	861 g
L-Carnitine	221 g	221 g	221 g	221 g
Tryptophan	331 g	331 g	331 g	331 g
Zinc Sulfate	309 g	309 g	309 g	309 g
Niacinamide	320 g	320 g	320 g	320 g
Tocopheryl Acetate	364 g	364 g	364 g	364 g
Gellan Gum	200 g	300 g	400 g	600 g
Ferrous Sulfate	106 g	106 g	106 g	106 g
Choline Chloride	353 g	353 g	353 g	353 g
Calcium Pantothenate	132 g	132 g	132 g	132 g
Vitamin A Palmitate	77 g	77 g	77 g	77 g

Riboflavin	33 g	33 g	33 g	33 g
Vitamin D3	13 g	13 g	13 g	13 g
Copper Sulfate	18 g	18 g	18 g	18 g
Pyridoxine Hydrochloride	20 g	20 g	20 g	20 g
Thiamin Hydrochloride	24 g	24 g	24 g	24 g
Folic Acid	3.3 g	3.3 g	3.3 g	3.3 g
Biotin	2.5 g	2.5 g	2.5 g	2.5 g
Manganese Sulfate	1.8 g	1.8 g	1.8 g	1.8 g
Phylloquinone	880 mg	880 mg	880 mg	880 mg
Sodium Selenate	90 mg	90 mg	90 mg	90 mg
Cyanocobalamin	88 mg	88 mg	88 mg	88 mg
Potassium Hydroxide	Q.S.	Q.S.	Q.S.	Q.S.

EXAMPLE 35

[0160] In this Example, the effect of 2'-fucosyllactose (2'FL) or 3'-fucosyllactose (3'FL) on stimulating enteric nerve cells in the gastrointestinal tract of rodents is analyzed.

[0161] Specifically, a peristalsis model using luminally perfused mouse colon is used to test the stimulation effect of 2'FL or 3'FL on enteric nerve cells. Colon muscle is perfused with 2'FL or 3'FL, at concentrations of 1 mg/mL, 0.5 mg/mL, and 0.1 mg/mL, for 15 minutes. The frequency and amplitude of contractions of the muscle is analyzed. The results are shown in FIG. 1.

[0162] As shown in the results, there is a direct stimulation of nerve cells by 2'FL or 3'FL without involving gut microbiota and/or their metabolites. Specifically, the frequency and amplitude of contraction are reduced consistently and in a dose response fashion. The results indicate that 3'FL is more effective than 2'FL.

EXAMPLE 36

[0163] In this Example, the fermentation rates of various non-digestible carbohydrates are measured.

[0164] Inclusion/exclusion criteria for choosing eight infant participants include: the infant was full term at birth with a gestational age of 38 to 42 weeks; the infant was at or above the fifth percentile for weight at birth; the infant has no maternal medical history of diabetes, tuberculosis, or perinatal infection with proven adverse effects on the fetus; was a vaginal birth; was at least 2 months of age at study entry, but not older than 4 months of age; has no known cardiac, respiratory, gastrointestinal, or other systemic disease such

as urinary tract infection or otitis media; is free of history of blood group incompatibility serious enough to result in hematological problems; and is not receiving any medications (except for supplemental vitamins) and has never received antibiotics. The eight infants are allowed to consume their normal diet of breast milk or infant formula. Four infants are exclusively breast fed and four infants are exclusively formula fed one of four commercially available infant formulas.

[0165] On the day of the *in vitro* experiments, a fecal sample is collected in the diaper and prepped within 15 minutes of defecation. For prepping, the sample is placed in a container with tepid water and analyzed. Fecal samples are diluted 1:10 (wt/vol) in anaerobic dilution solution by blending for 15 seconds in a Waring blender under a stream of CO_2. Blended, diluted feces are filtered through four layers of cheesecloth and sealed in 125-mL serum bottles under CO_2. Inoculum is stored at 37°C until inoculation of *in vitro* tubes.

[0166] Oligosaccharide substrates suitable for growing the bacterium include galactooligosaccharides (GOS) 95 (GOS; Inalco Pharmaceuticals, San Luis Obispo, California), α-(2-6')-N-Acetylneuraminyl-lactose sodium salt (6'SL; Inalco Pharmaceuticals, San Luis Obispo, California); 2'- α-L-Fucopyranosyl-D-Lactose (2'FL; Inalco Pharmaceuticals, San Luis Obispo, California); LNnT; Orafti® HP inulin (HP inulin) (BENEO-Orafti, Belgium); and gum arabic (Fisher Scientific, Pittsburgh, Pennsylvania).

In vitro fermentation model

[0167] Approximately 80 mg of each substrate is weighed in triplicate for each pull time into 16-mL Balch tubes that are used in a model that simulates large bowel fermentation. An aliquot (7.2 mL) of medium (Table 1; FIG. 2) is aseptically transferred into the Balch tubes, capped with butyl rubber stoppers, and sealed with aluminum caps. Tubes containing HP inulin and gum arabic are stored at 4°C for approximately 12 h to enable hydration of the substrates before initiating fermentation. These tubes are placed in a 37°C water bath approximately 30 min before inoculation. Due to the cost of the substrates and difficulty in obtaining samples from infants, tubes containing GOS, 6'SL, 2'FL, and LNnT are hydrated upon obtaining a fecal sample and placed in a 37°C water bath until inoculation.

[0168] Sample and blank tubes are aseptically inoculated with 0.8 ml of diluted feces. Tubes are incubated at 37°C with periodic mixing every 2 h for up to 12 h. At 0, 3, 6, and 12 h after inoculation, tubes are removed from the 37°C incubator and processed immediately for analyses. The pH of tube contents is measured with a standard pH meter. A 3-ml subsample of fluid is collected and used for short-chain fatty acid and lactate analyses. A 2-mL subsample is taken and frozen at -80°C for bacterial analysis.

Short-chain fatty acid (SCFA) analyses

[0169] The 3-mL aliquot of fluid removed from the sample tubes for SCFA analysis is immediately added to 0.75 mL of 25% metaphosphoric acid. Concentrations of acetate, propionate, and butyrate are determined using a Hewlett-Packard 5890A series II gas chromatograph and a glass column (180 cm x 4 mm i.d.) packed with 10% SP-1200/1% H_3PO_4 on 80/100+ mesh Chromosorb WAW (Supelco Inc., Bellefonte, PA). Oven temperature, detector temperature, and injector temperature are 125, 175, and 180°C, respectively. SCFA concentration values are corrected for blank tube production of SCFA and 0 h concentrations for each substrate. Total SCFA are calculated as the total amount of acetate, propionate, and butyrate.

[0170] Data is analyzed as a split-split-plot in a completely randomized block design using the Mixed procedure of SAS (SAS Inst., Inc., Cary, NC). Block is defined as the diet of the baby (breast milk or formula). Fixed effects tested include diet, substrate, and time, and the interactions are investigated if significant. Infant and period are included as random effects in the model. Means are separated using a protected LSD with a Tukey adjustment to control for experiment-wise error. Least square means are reported along with the pooled SEM for all response criteria. A probability of $P<0.05$ is accepted as statistically significant.

Results and Discussion

[0171] The pH change from baseline decreases ($P<0.0001$) over time for all substrates except gum arabic (FIG. 3). At 3, 6, and 12 h after inoculation, pH change from baseline is smallest ($P<0.0001$) with the gum arabic substrate, and greatest in the LNnT, 2'FL, and GOS substrates. A decrease in pH is an indicator of fermentation, and these data are reflective of SCFA production.

[0172] Total SCFA production differs among substrates (FIG. 4) at 3, 6, and 12 h of fermentation ($P<0.0001$). Gum arabic produces the least amount of SCFA and does not change over time. After 3 and 6 h of fermentation, total SCFA production is lower ($P<0.05$) with HP inulin compared to all other substrates and is lower ($P<0.05$) with 6'SL compared to GOS. By 12 h of fermentation, total SCFA production remains lower ($P<0.05$) with HP inulin relative to 2'FL, 6'SL, GOS, and LNnT substrates. Also, after 12 h of fermentation, total SCFA production is greater ($P<0.05$) for the 6'SL and GOS substrates compared to 2'FL.

EXAMPLE 37

[0173] In this Example, probiotic fermentation parameters are determined for purified HMOs, HMO precursors, and other prebiotic oligosaccharides.

Bacterial Cultures

[0174] All bifidobacteria strains are initially inoculated from frozen stocks, grown in deMan Rogosa Sharpe (MRS) broth (Difco, Detroit, MI) supplemented with 0.5 g/L L-cysteine/HCl and incubated at 37°C for 24 h in an anaerobic chamber (90% N_2, 5% CO_2 and 5% H_2). Subsequently, the cultures are passed twice on a semi-synthetic MRS medium (sMRS) + 0.5 g/L L-cysteine/HCl which is supplemented with 1% (w/v) filter-sterilized glucose as the sole carbohydrate source. After the 2nd pass, cultures are prepared to use as inoculums for growth assays described below. All bacterial strains for use in this Example are listed in the table below.

Table: Microorganisms

	Culture Collection Number	Genus	Species	Strain
1	MJM29	*Bifidobacterium*	*adolescentis*	ATCC 15703
2	MJM30	*Bifidobacterium*	*infantis*	S12; ATCC 15697
3	MJM32	*Bifidobacterium*	*animalis* subsp. *lactis*	DSM 10140
4	MJM33	*Bifidobacterium*	*Animalis* subsp. *Animalis*	ATCC 25527
5	MJM34	*Bifidobacterium*	*Bifidum*	ATCC 29521
6	MJM35	*Bifidobacterium*	*Breve*	ATCC 15700
7	MJM37	*Bifidobacterium*	*Bifidum*	ATCC 11617
8	MJM88	*Bifidobacterium*	*Lactis*	Bf-6 (Cargill)
9	MJM92	*Bifidobacterium*	*Longum*	BB536 (Morinaga)
10	MJM93	*Bifidobacterium*	*Infantis*	M-63 (Morinaga)
11	MJM94	*Bifidobacterium*	*Breve*	M-16V (Morinaga)
12	MJM95	*Bifidobacterium*	*Lactis*	Bb12; (Chr. Hansen)

Bacterial Growth Assays

[0175] After the 2nd pass in sMRS + glucose + cysteine, the cultures are washed once with 10 mL of sterile sMRS + cysteine (no carbohydrate), resuspended in 10 ml of sterile sMRS + cysteine (no carbohydrate) and then used as a 1% inoculum. Carbohydrates for use in this Example are shown in the table below. The carbohydrates are sterilized with a 0.22 micron filter and used at a 1% final concentration. Cell growth is performed in 250 μL of sMRS + cysteine covered with 50 μL of mineral oil in a Bioscreen 100-well Honeycomb plate. Cell growth is monitored by measuring optical density at 600 nm (OD600) using a Bioscreen C Automated Microbiology Growth Curve Analysis System. The plate reader is operated in discontinuous mode, with absorbance readings performed in 30-minute intervals, and preceded by 30-second shaking intervals at maximum speed. Controls consist of inoculated medium lacking carbohydrate. Due to space limitations on the microtitre plate, the carbohydrates are divided into three separate groups: plate A (HMO precursors: glucose, galactose, lactose, NAG, fucose, fructose and sialic acid), plate B (Prebiotics: glucose, Purimune™ GOS, purified Purimune™ GOS, Vivinal® GOS, purified Vivinal® GOS, scFOS and PDX), and plate C (HMOs: glucose, 6'-SL, 3'-SL, 2'-FL, 3'-FL and LNnT). All three plates include a positive control (glucose) and negative control (no carbohydrate).

Table: Carbohydrates

Carbohydrate	Source
Dextrose (D-Glucose)	Fisher Scientific
D(+)-Galatose	ACROS-ORGANICS
α-Lactose	Fisher Scientific
L-(-) Fucose	SIGMA
D-Fructose	ALDRICH
Sialic acid (N-acetylneuraminic acid)	CALBIOCHEM
NAG (N-acetyl-D-glucosamine)	SIGMA
GOS (Purimune™ Galactooligosaccharide)	GTC Nutrition
Purified GOS (Purimune™ Galactooligosaccharide)	GTC Nutrition
Vivinal® GOS (Galactooligosaccharide)	Friesland Foods
Purified Vivinal® GOS (Galactooligosaccharide)	Friesland Foods
scFOS (Short-Chain Fructooligosaccharide)	Nutraflora® P-95 (GTC Nutrition)
PDX (Litesse® Polydextrose)	DANISCO

6'SL (6'-sialyllactose)	V-labs; SL 306 Lot#HGDX 21-163-1
3'SL (3'-sialyllactose)	V-labs; SL 302 Lot#HGDX 76-161-1
2'FL (2'-fucosyllactose)	V-labs; Lot# DX103
3'FL (3'-fucosyllactose)	V-labs; Lot# DX807
LNnT (Lacto-N-Neotetraose)	Abbott Nutrition

Bacterial growth curves

[0176] The OD600 data for each carbohydrate is corrected by subtracting the OD600 of the basal media (sMRS) + cysteine from the sample plate for each probiotic. Maximum OD is determined by inspection of the corrected growth data. OD is determined by subtracting the initial corrected OD (time point 0) from the maximum corrected OD. Samples are grown in biologically independent triplicates and the resulting growth kinetic data are expressed as the mean of these replicates.

[0177] For the growth curve plots, OD600 vs. time is first plotted for the bacteria grown on medium lacking carbohydrate (sMRS). For all other carbohydrates, the OD600 data is corrected by subtracting the OD600 of sMRS.

Purification of GOS

[0178] Purified GOS is obtained by purification of Purimune™ GOS (GTC Nutrition) and Vivinal® GOS (Friesland Foods Domo). Stock solutions of 1.5 g/100 mL are applied to a XK column (XK 50/100 column, 5.0 x 100 cm, GE healthcare) packed with Sephadex G25 medium (Sigma). The column is eluted with pure distilled water at a rate of 8 ml/min and is collected in 12-mL fractions by a Gilson FC 203B fraction collector.

[0179] Detection of carbohydrate in every 2-3 fractions is performed using the phenol–sulfuric acid assay. Briefly, 50 μL of sample (2 μL of fraction and 48 μL of distilled water in a well) is added to 150 μl of concentrated sulfuric acid rapidly in a 96-well microtitre plate. Immediately thereafter, 30 μl of 5% phenol is added and the plate is kept in a static water bath for 30 minutes at 80°C. After cooling to room temperature for 5 minutes, it is wiped dry and absorbance at 490 nm is measured by a SpectraMax Plus384 Spectrophotometer. Based on carbohydrate analysis, fractions containing minimal di- and monosaccharides are pooled and freeze dried (Freeze dry system/Freezezone 4.5/LABCONCO) for bacterial fermentation experiments. In addition, freeze dried GOS is

pooled from multiple runs in order to generate enough purified GOS for growth experiments (5 runs with Purimune™ GOS and 3 runs with Vivinal® GOS).

RESULTS & DISCUSSION:

GOS Purification

[0180] GOS is produced by the transgalactosylation of lactose and has been used as a prebiotic supplement in pediatric nutrition. Due to issues with GOS synthesis, commercial GOS products are a mixture of many different carbohydrates which may include mono- and disaccharides. In order to test the fermentation parameters of GOS and not the mono- and disaccharides which would not normally reach the colon, a purified GOS fraction, essentially free of mono- and disaccharides is obtained. Glucose (monosaccharide), lactose (disaccharide) and raffinose (trisaccharide) are used as standards. Consistent with information from the suppliers, Purimune™ GOS has less mono- and disaccharides than Vivinal® GOS. For example, the Purimune™ GOS peaks before the raffinose peak suggesting that Purimune™ GOS consists primarily of trisaccharides or larger. For Vivinal® GOS, the peak is observed at a similar fraction number as lactose. Since lactose begins to appear in fraction 55, fractions 30 through 55 are used as the purified GOS from both suppliers.

HMO Precursor Fermentation

[0181] All bifidobacteria tested grow very little in the basal media (sMRS + cysteine) (FIG. 5A), whereas they all grow well in glucose (FIG. 5B). In general, the bifidobacteria, which is not able to ferment galactose (FIG. 5C), also has reduced growth on lactose (FIG. 5D). None of the bifidobacteria are able to ferment L-fucose (FIG. 5E) or sialic acid (FIG. 5F), two key constituents of HMOs and mucin. Only *B. breve* ATCC 15700 is able to ferment NAG (FIG. 5G), a key component of HMOs and mucin. Lastly, the majority of bifidobacteria is able to ferment fructose (FIG. 5H).

Prebiotic Fermentation

[0182] Removal of mono- and disaccharides from Purimune™ GOS results in a decrease in growth for all bifidobacteria (FIG. 6A). In fact, *B. lactis* DSM 10140, *B. animalis* ATCC 25527, *B. bifidum* ATCC 29521, *B. lactis* Bf-6 and *B. longum* are not able to ferment the purified Purimune™ GOS (FIG.6D). A similar pattern is seen with purified Vivinal® GOS (FIG. 6F), except more growth is seen with Vivinal® GOS than

Purimune™ GOS. In order to mimic the colonic situation, the free mono- and disaccharides present in these products need to be removed. Also, it is clear that Purimune™ GOS has a higher relative concentration of oligosaccharides. Both *B. infantis* strains are among the best growers on purified GOS as determined by ΔOD, confirming that GOS is a reasonable prebiotic to add to infant formula if the goal is to increase *B. infantis*. All bifidobacteria tested, except for *B. animalis* ATCC 25527, are able to ferment scFOS (FIG. 6G), whereas no bifidobacteria are able to ferment polydextrose (PDX) (FIG. 6H).

HMO Fermentation

[0183] Only *B. infantis* ATCC 15697 and *B. infantis* M-63 are able to ferment 6'-SL, 3'-SL, 2'-FL and 3'-FL (FIG. 7C-7F). In all cases, *B. infantis* M-63 grows better than *B. infantis* ATCC 15697. On the more complex LNnT (FIG. 7G), *B. breve* ATCC 15700 and the two *B. infantis* strains grow well but not *B. breve* M16-V. In addition, the ability of the two *B. infantis* strains to ferment HMOs correlates with the abundance of *B. infantis* found in breast fed infants. Curiously, both *B. infantis* strains are not able to ferment fucose or sialic acid.

CONCLUSIONS:

[0184] There are significant differences amongst the tested bifidobacteria strains regarding their abilities to ferment HMO precursors, prebiotics and HMOs. Of the 12 bifidobacteria strains tested, none are able to ferment sialic acid. Regarding prebiotics, most of the bifidobacteria are able to ferment GOS and scFOS, but they are not able to ferment PDX. Amongst the bifidobacteria strains tested, only *B. infantis* ATCC 15697 and *B. infantis* M-63 are able to ferment 6'-SL, 3'-SL, 2'-FL and 3'-FL. *B. breve* ATCC 15700, *B. infantis* ATCC 15697 and *B. infantis* M-63 are able to ferment LNnT.

EXAMPLE 38

[0185] In this Example, the ability of Lacto-N-neotetraose (LNnT), 2'-Fucosyllactose (2'FL), and 6'-Sialyllactose (6'SL) to protect against feeding intolerance and necrotizing enterocolitis (NEC) by inducing epithelial cell differentiation and barrier function (cell resistance), promoting digestive function, and promoting antibacterial function are evaluated using cell culture models of the human small intestine. The ability of LNnT, 2'FL, and 6'SL to exert these protective and beneficial effects is evaluated using

in vitro cultures representing various phases of the differentiated intestinal epithelium. Epithelial cells are cultured in the presence of various concentrations of LNnT, 2'FL, 6'SL or a control oligosaccharide of each of these human milk oligosaccharides (HMOs) and the impact of the LNnT, 2'FL, 6'SL or controls on cell differentiation, barrier function, digestive function, and protection from bacteria is measured.

[0186] In a first experiment, HT-29 cells, which model the immature epithelial cells of the small intestine, were incubated in a humidified atmosphere of 5% carbon dioxide at 37°C in the presence of LNnT or 2'FL at concentrations of 0 mg/L ("0"), 20 mg/L ("20"), 200 mg/L ("200"), and 2000 mg/L ("2000") or in the presence of 6'SL at concentrations of 0 mg/mL ("0"), 40 mg/mL ("40"), 400 mg/mL ("400"), and 4000 mg/mL ("4000") for 72 hours. The culture medium utilized is Dulbecco's Modified Eagle Medium (Life Technologies, Foster City California) supplemented with 10% fetal calf serum and 2 mM glutamine. The controls ("energy") consisted of 91.5 mg lactose and 64.2 mg N-acetyllactosamine/L for LNnT; 133 mg lactose and 67 mg fucose/L for 2'FL; and 195 mg lactose and 205 mg/L sialic acid for 6'SL. The impact of the LNnT, 2'FL, and 6'SL at various levels and the controls on the alkaline phosphatase activity per milligram of protein for HT-29 cells is measured. Alkaline phosphatase activity is important for nutrient digestion, important for breakdown of the harmful bacteria lipopolysaccharide molecules that induce inflammation, and is a marker of cell differentiation. The results of the measurements are shown in FIGS. 8-10, which indicate that there is a significant increase in alkaline phosphatase activity (and thus an increase in cell differentiation, digestive function, and protection against harmful effects of bacteria) at the high dose of 2'FL, a trend toward an increase in cells treated with LNnT, and no apparent effect on cells treated with 6'SL.

[0187] FIGS. 11-13 illustrate the effect of LNnT, 2'FL, and 6'SL on cell resistance (transepithelial resistance), which is a marker for epithelial barrier function, wherein a higher resistance is associated with a higher barrier function. Epithelial cell resistance or barrier function is a measure of differentiated epithelial cell function. Specifically, as the cells mature, tighter junctions between the cells are formed resulting in a stronger epithelial cell barrier. This barrier prevents the movement of large molecules, bacteria, or viruses from one side of the barrier to the other, which could improve resistance to infection, sepsis, and NEC. Transepithelial resistance is measured using

Transwell Snapwell inserts containing the desired cell culture and are transferred to modified Ussing chambers and bathed in modified Kreb's solution at 37°C with 95% oxygen and 5% carbon dioxide. Transepithelial resistance is measured as the passive transport of ions across the monolayers.

[0188] In a second experiment, Caco-2 cells, which model more mature epithelial cells of the small intestine, were incubated in a humidified atmosphere of 5% carbon dioxide at 37°C in the presence of LNnT or 2'FL at concentrations of 0 mg/L ("0"), 20 mg/L ("20"), 200 mg/L ("200"), and 2000 mg/L ("2000") or in the presence of 6'SL at concentrations of 0 mg/mL ("0"), 40 mg/mL ("40"), 400 mg/mL ("400"), and 4000 mg/mL ("4000") for 72 hours. The culture medium utilized was Dulbecco's Modified Eagle Medium (Life Technologies, Foster City California) supplemented with 10% fetal calf serum and 2 mM glutamine. The controls ("energy") consisted of 91.5 mg lactose and 64.2 mg N-acetyllactosamine/L for LNnT; 133 mg lactose and 67 mg fucose/L for 2'FL; and 195 mg lactose and 205 mg sialic acid/L for 6'SL. The impact of the LNnT, 2'FL, and 6'SL at various levels and the controls on the alkaline phosphatase activity is important for nutrient digestion, for breakdown of the harmful bacterial lipopolysaccharide molecules that induce inflammation, and is a marker of cell differentiation. For these reasons, intestinal tissue with greater alkaline phosphatase activity would be expected to be more resistant to inflammation and NEC. The results of the measurements are shown in FIGS. 14-16, which indicate that there is a trend toward increased alkaline phosphatase activity (and thus an increase in cell differentiation, digestive function, and protection against harmful effects of bacteria) in 2'FL treat cultures, a trend toward an increase in cells treated with LNnT, and no apparent effect on cells treated with 6'SL.

[0189] The impact of LNnT, 2'FL, and 6'SL at various levels and the controls on the sucrase activity per milligram of protein for Caco-2 cells is measured as another indication of digestive function. The results of the measurements are shown in FIGS. 17-19, which indicate that there is a trend toward sucrase activity (and thus an increase in digestive function) in 2'FL treated cultures, and no apparent effect on cells treated with LNnT or 6'SL.

[0190] FIGS. 20-22 illustrate the effect of LNnT, 2'FL, and 6'SL on cell resistance (transepithelial resistance), which is a marker for epithelial barrier function, wherein a higher resistance is associated with a higher barrier function. Epithelial cell resistance or barrier function is a measure of differentiated epithelial cell function. Specifically, as the cells mature, tighter junctions between the cells are formed resulting in a stronger epithelial cell barrier. This barrier prevents the movement of large molecules, bacteria, or viruses from one side of the barrier to the other, which could improve resistance to infection, sepsis, and NEC. The results indicate that LNnT can have a positive effect on cell resistance for more mature Caco-2 cells. Transepithelial resistance was measured using Transwell Snapwell inserts containing the desired cell culture and were transferred to modified Ussing chambers and bathed in modified Kreb's solution at 37C with 95% oxygen and 5% carbon dioxide. Transepithelial resistance was measured as the passive transport of ions across the monolayers.

CONCLUSIONS:

[0191] The data reported in FIGS. 8-22 indicate that LNnT and 2'FL promote intestinal functions including digestion, barrier function, and protection against bacterial components that are known to induce inflammation. Promotion of digestive function, through increased digestive enzyme activities (sucrase and alkaline phosphatase) can help prevent or reduce the severity of feeding intolerance. Combined with effects on barrier function and protection against harmful bacterial metabolites, these data provide ample evidence that these HMOs could help protect against intestinal inflammation and NEC.

EXAMPLE 39

[0192] In this Example, the effect of HMOs, and the dose-dependency thereof, on increasing the expression of TFF3 and other goblet cell genes that promote feeding tolerance through enhanced gastrointestinal barrier function and healing by HMOs is analyzed.

[0193] Pooled HMOs are tested with respect to their ability to induce *MUC2*, *TFF3*, *RELMβ*, *CHST5*, and *GAL3ST2* expression in the human LS174T cell culture model of goblet cells. The human LS174T colorectal cancer cell line is obtained from the American Type Culture Collection (ATCC). LS174T cells are maintained in minimum

essential medium (MEM) supplemented with 10% Fetalplex (Gemini Biosciences), 1.5 g/L of Na_2CO_3, 10 ml/L penicillin G-streptomycin solution (Gemini Bio-products) at 37°C in 5% CO_2. Pooled HMOs are obtained from Lars Bode (University of California, San Diego) and dissolved in cell culture grade water to required concentration. The solution is subsequently filter sterilized and used for cell culture studies. LS174T cells are treated with the media described above containing 0, 1, or 5 mg HMO/mL.

[0194] LS174T cells are collected and suspended in Trizol reagent and total RNA is isolated using the RNeasy Plus Kit (Qiagen) according to the manufacturer's instructions. The quality and quantity of RNA isolates are determined by Nanodrop (Thermo Fisher Scientific). RNA isolates are reverse transcribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) to create cDNA, which is to assess gene expression via quantitative PCR.

[0195] For quantitative RT-PCR, specific TaqMAN gene expression assays are obtained from Applied Biosystems, which include expression assays for *MUC2* (Hs00159374_m1), *TFF3* (Hs00173625_m1), *RELMβ* (Hs00395669_m1), *CHST5* (Hs00375495_m1), *GAL3ST2* (Hs00223271_m1) and *GUSB* (Hs99999908_m1). Quantitative real-time PCR is performed using TaqMAN PCR Master Mix (Applied Biosystems). Reactions are run in duplicates in a 384-well plate using an Applied Biosystems 7900HT Fast Real-Time PCR System. The results are analyzed using SDS 2.3 software and calculated by delta delta Ct method. All samples are normalized to Gus-β expression and fold induction is calculated over untreated controls. Gene expression is expressed as fold increase compared to HMO-free control cells. The experiment is repeated three times. Data represent means+ SEM (n=3 plates per experiment). Statistical differences are indicated by different letters ($P < 0.05$).

[0196] Figures 23A-23E represent the combined results of three replicate experiments. Specifically, Figures 23A, 23B, and 23C illustrate that the treatment with HMOs at a level of at least 1 mg/mL increases the expression of the *MUC2*, *TFF3*, and *RELMβ* genes compared to control cultures. Increased expression of goblet cell genes is specific and not universal, as evidenced by the minimal induction or lack of induction of *CHST5* and *GAL3ST2*, respectively, by treatment with HMOs at either 1 mg/mL or 5 mg/mL.

[0197] In addition, Figures 23A and 23B indicate a dose dependent increase in expression of *MUC2* and *TFF3*, with a modest induction (~1.5 fold) noted at the 1 mg/mL treatment level and more pronounced increases (~2 fold) at 5 mg/mL. In addition, the expression levels of *RELMβ* (Figure 23C) were increased (~1.5 fold) at each of the 1 mg/mL treatment level and the 5 mg/mL treatment level. In contrast, gene expression of *CHST5* (Figure 23D) and *GAL3ST2* (Figure 23E) is not significantly impacted at any dose. As such, it can be concluded that the impact of HMOs on expression of several genes involved in the healing response of the gastrointestinal tract is dose-dependent.

[0198] These results indicate that HMOs promote the expression of several genes involved in GI barrier function and in the healing response of the GI tract. First, the protein products of the *MUC2, TFF3, and RELMβ* genes, each of which was induced by HMOs, work synergistically to create the mucus barrier that protects the GI tract from pathogens. Also, expression of *TFF3* has been positively associated with prevention and restitution of gastrointestinal damage to the epithelial cells in the intestine of mammals. Oral treatment with *TFF3* reduces the damage associated with different forms of colitis in animal models. Additionally, HMOs induce the expression of *MUC2*, which provides a barrier that protects the gastrointestinal tract from infection and other sources of injury. Further, HMOs induce the expression of *RELMβ*, which is a protein associated with resolution of inflammation. Because tissue damage is difficult to heal when inflammation is abundant, the inflammation resolving effects of *RELMβ* induced by HMOs also supports healing. The combined impact of HMOs on expression of *TFF3*, *MUC2*, and *RELMβ* enables a product to prevent necrotizing enterocolitis and support wound healing through its synergistic effects on cell healing, resolution of inflammation and promotion of barrier function.

WHAT IS CLAIMED IS:

1. A method of reducing the incidence of necrotizing enterocolitis in an infant, toddler or child, the method comprising administering to the infant, toddler, or child a composition comprising human milk oligosaccharides in a total amount of from about 0.001 mg/mL to about 20 mg/mL.

2. The method of claim 1, wherein the human milk oligosaccharides are selected from the group consisting of 6'-sialyllactose, 2'-fucosyllactose, and lacto-N-neotetraose, and combinations thereof.

3. The method of claim 2, wherein the composition comprises from 0.001 mg/mL to less than 0.25 mg/mL 6'-sialyllactose.

4. The method of claim 2, wherein the composition comprises from 0.001 mg/mL to less than 2 mg/mL 2'-fucosyllactose.

5. The method of claim 2, wherein the composition comprises from 0.001 mg/mL to less than 0.2 mg/mL lacto-N-neotetraose.

6. The method of claim 1, wherein the composition further comprises at least one of a prebiotic, a probiotic, a long chain polyunsaturated fatty acid, an antioxidant, and a milk protein of human and/or bovine origin.

7. A method of reducing the incidence of necrotizing enterocolitis in an infant, toddler, and child, the method comprising administering to the infant, toddler, or child a nutritional composition comprising 6'-sialyllactose, 2'-fucosyllactose, and lacto-N-neotetraose.

8. The method of claim 7, wherein the 6'-sialyllactose, 2'-fucosyllactose, and lacto-N-neotetraose are present in a total amount of from about 0.001 mg/mL to about 20 mg/mL.

9. The method of claim 7, wherein the nutritional composition comprises from 0.001 mg/mL to less than 0.25 mg/mL 6'-sialyllactose.

10. The method of claim 7, wherein the nutritional composition comprises from 0.001 mg/mL to less than 2 mg/mL 2'-fucosyllactose.

11. The method of claim 7, wherein the nutritional composition comprises from 0.001 mg/mL to less than 0.2 mg/mL lacto-N-neotetraose.

12. The method of claim 7, wherein the synthetic nutritional composition further comprises at least one of a prebiotic, a probiotic, a long chain polyunsaturated fatty acid, an antioxidant, and a milk protein of human and/or bovine origin.

13. A synthetic formula for improving the feeding tolerance of an infant, toddler, or child, the synthetic formula comprising from about 0.001 mg/mL to about 20 mg/mL of an oligosaccharide blend, wherein the oligosaccharide blend comprises a first oligosaccharide selected from the group consisting of a fructooligosaccharide, a galactooligosaccharide, lacto-N-neotetraose, 2'-fucosyllactose, and combinations thereof; a second oligosaccharide selected from the group consisting of 3'-sialyllactose, 6'-sialyllactose, and combinations thereof; and a third oligosaccharide selected from the group consisting of inulin, a gum, polydextrose, and combinations thereof.

14. The synthetic formula of claim 13, comprising from about 0.001 mg/mL to about 20 mg/mL of the first oligosaccharide blend, from about 0.001 mg/mL to about 20 mg/mL of the second oligosaccharide blend, and from about 0.05 mg/mL to about 20 mg/mL of the third oligosaccharide blend.

15. A synthetic pediatric formula for improving the feeding tolerance of an infant or toddler, the synthetic pediatric formula comprising from about 0.001 mg/mL to about 20 mg/mL of an oligosaccharide blend, wherein the oligosaccharide blend comprises a first oligosaccharide selected from the group consisting of a fructooligosaccharide, a galactooligosaccharide, lacto-N-neotetraose, 2'-fucosyllactose, and combinations thereof; and a second oligosaccharide selected from the group consisting of 3'-sialyllactose, 6'-sialyllactose, and combinations thereof.

16. A method of improving the feeding tolerance of an infant, toddler, or child, the method comprising administering to the infant, toddler, or child a nutritional composition comprising from about 0.001 mg/mL to about 20 mg/mL of an

oligosaccharide blend, wherein the oligosaccharide blend comprises a first oligosaccharide selected from the group consisting of a fructooligosaccharide, a galactooligosaccharide, lacto-N-neotetraose, 2'-fucosyllactose, and combinations thereof; a second oligosaccharide selected from the group consisting of 3'-sialyllactose, 6'-sialyllactose, and combinations thereof; and a third oligosaccharide selected from the group consisting of inulin, a gum, polydextrose, and combinations thereof.

17. A method of improving the feeding tolerance of an infant or toddler, the method comprising administering to the infant or toddler a synthetic pediatric formula comprising an oligosaccharide blend comprising a first oligosaccharide selected from the group consisting of fructooligosaccharide, galactooligosaccharide, lacto-N-neotetraose, 2'-fucosyllactose, and combinations thereof; a second oligosaccharide selected from the group consisting of 3'-sialyllactose, 6'-sialyllactose, and combinations thereof; and a third oligosaccharide selected from the group consisting of inulin, a gum, polydextrose, and combinations thereof.