HUMAN MILK OLIGOSACCHARIDES FOR MODULATING INFLAMMATION

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 61/428,860 filed on December 31, 2010, which disclosure is incorporated by reference in its entirety.

FIELD OF THE DISCLOSURE

[0002] The present disclosure relates to human milk oligosaccharides (HMOs) for modulating inflammation in an infant, toddler, or child. More particularly, the present disclosure relates to human milk fortifiers, preterm and term infant formulas and pediatric formulas comprising HMOs that can reduce inflammation and thereby prevent and/or treat inflammatory conditions and diseases.

BACKGROUND OF THE DISCLOSURE

[0003] The inflammatory response is an attempt by the body to restore and maintain homeostasis after invasion by an infectious agent, antigen challenge, or physical, chemical or traumatic damage. While the inflammatory response is generally considered a healthy response to injury, the immune system can present an undesirable physiological response if it is not appropriately regulated. Specifically, unregulated oxidation and associated inflammation are major causes of tissue damage and clinically significant disease in preterm and term infants. This is due in large part to the immaturity in function of the natural immune system of infants, and especially preterm infants.

[0004] Breastfeeding has been associated with enhanced development and balanced growth and maturation of the infant's respiratory, gastrointestinal and immune systems, thereby providing protection of the infant to infection and inflammatory diseases. Breast milk appears to contain endogenous antioxidants, such as superoxide dismutase, glutathione peroxidase and catalase, or other non-enzymatic antioxidants such as glutathione, lactoferrin and polyphenols, in addition to exogenous antioxidants, such as vitamins A, C, E and selenium. Further, breast milk includes HMOs that not only act as pathogen receptor analogues, but activate immune factors by infant intestinal epithelial

cells and/or associated immune cell populations. The function of these breast milk components, functioning as antioxidants and as immune modulators, includes not only the protection of breast milk lipids by peroxidation, but may also assist in the regulation of inflammatory responses to infection or other injury.

[0005] Not all infants receive human breast milk. Further, no vaccines are currently available for the prevention of inflammatory diseases. Therefore, development of safe and efficacious preventative or therapeutic methods would be beneficial, especially for infants.

[0006] It would therefore be desirable to provide nutritional compositions, and synthetic infant formulas in particular, that can produce nutritional benefits including improved immune system growth and development. It would additionally be beneficial if the nutritional compositions could modulate inflammation and enhance immunity against microbial infections, including bacterial and viral infections, and other inflammatory diseases.

SUMMARY OF THE DISCLOSURE

[0007] 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 one or more of long chain polyunsaturated fatty acids (LCPUFAs), antioxidants, and/or nucleotides, for reducing inflammation in an infant, toddler, or child, as well as methods of using the compositions.

[0008] One embodiment is a synthetic pediatric formula for modulating respiratory virus-induced inflammation. The synthetic pediatric formula comprises a mixture of 3'-sialyllactose and 6'-sialyllactose in a concentration of from about 0.001 mg/mL to about 20 mg/mL. The 3'-sialyllactose is present in an amount of from about 0.001 mg/mL to less than 0.15 mg/mL.

[0009] Another embodiment is a method of reducing inflammation in an infant, toddler, or child in need thereof. The method comprises administering to the infant, toddler, or child a composition comprising lacto-N-neotetraose in a concentration of from about 0.001 mg/mL to about 0.2 mg/mL.

[0009a] Another embodiment is the use of a synthetic pediatric formula in the manufacture of a medicament for reducing inflammation in an infant, toddler, or child, wherein the synthetic pediatric formula comprises lacto-N-neotetraose in a concentration of 0.01 mg/mL to 20 mg/mL and wherein the medicament is to be administered to the infant, toddler, or child.

[0010] Another embodiment is directed to a method of inhibiting the growth of respiratory viruses in an infant, toddler, or child. The method comprises identifying an infant, toddler, or child having at least one of respiratory syncytial virus, human parainfluenza virus type 3, and influenza A virus and administering to the infant, toddler, or child a composition comprising lacto-N-neotetraose in a concentration of from about 0.001 mg/mL to about 0.2 mg/mL.

[0011] It has been discovered that specific HMOs, such as 3'-sialyllactose, 6'-sialyllactose and others as noted herein, as well as specific combinations of HMOs as described herein, are highly effective in dampening inflammation generally in infants, toddlers, and children, and specifically in dampening virus-induced inflammation, including respiratory syncytial virus, human parainfluenza, and influenza A, in infants, toddlers, and children by reducing the production of some key cytokines from human immune cells without increasing viral load, which may lead to faster recovery from infections. Surprisingly, it was determined that the HMOs demonstrate the desirable dampening effects even at very low concentrations, including concentrations lower than those found in breast milk. Also, it was unexpectedly found that 6'-sialyllactose is immunomodulatory even in the absence of a virus, and induces the production of monocyte-derived cytokines. It has further been discovered that although biological reactions often occur within a 30 to 60 minute period, and thus a 30 to 60 minute incubation is generally used for *in vitro* procedures, a 24 hour pre-treatment of cells provides a closer reflection of the daily pre-exposure to HMOs that a breast-fed infant would receive from breast milk.

[0012] Additionally, it has been found that fucosyllated HMOs, including 3'-fucosyllactose, alone or in combination with sialic acid, are highly effective in inhibiting respiratory viruses. Even at low concentrations, the 3'-fucosyllactose and sialic acid are effective.

[0013] Moreover, it has been discovered that specific HMOs act in a synergistic manner against respiratory viruses, including RSV, when combined with a long chain polyunsaturated fatty acid and/or a carotenoid. These synergistic actions dampen virus-induced inflammatory cytokines, and specifically interferon-inducible protein 10 (IP-10). Additional components including antioxidants, such as vitamin A and vitamin E, or

nucleotides, may also be added to the HMO and long chain polyunsaturated fatty acid and/or carotenoid combinations.

[0014] It has further been found that a combination of HMOs including acidic/sialylated (e.g., 6'-sialyllactose) and/or neutral/fucosylated (e.g., 2'-fucosyllactose) and/or n-acetylglucosylated (e.g., LNnT) prevents the development of necrotizing entercolitis. Also, these HMOs have been found to decrease the oxidative stress in infants.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] FIG. 1 is a graph depicting H1N1 virus infectivity of MDCK cells in the presence of various HMOs as tested in Example 37.

[0016] FIG. 2 is a graph depicting blood plasma levels of glutathione from piglets as measured in Example 38.

[0017] FIG. 3 is a graph depicting IP-10 levels resulting from administration of 3'SL and 6'SL as measured in Example 39.

[0018] FIG. 4 is a graph depicting IP-10 levels resulting from administration of 3'SL and 6'SL as measured in Example 39.

[0019] FIG. 5 is a graph depicting IP-10 levels resulting from administration of LNnT as measured in Example 39.

[0020] FIG. 6 is a graph depicting IP-10 levels resulting from administration of LNnT as measured in Example 39.

[0021] FIG. 7 is a graph depicting IL-10 levels resulting from administration of 3'SL and 6'SL as measured in Example 39.

[0022] FIG. 8 is a graph depicting IL-10 levels resulting from administration of 3'SL and 6'SL as measured in Example 39.

[0023] FIG. 9 is a graph depicting IL-10 levels resulting from administration of LNnT as measured in Example 39.

[0024] FIG. 10 is a graph depicting IL-10 levels resulting from administration of LNnT as measured in Example 39.

[0025] FIG 11 is a graph depicting IL-8 levels resulting from administration of 3'SL and/or 6'SL as measured in Example 40.

[0026] FIG. 12A and FIG. 12B are graphs depicting chemiluminescence levels resulting from administration of 2'FL in the presence or absence of phorbal myristate acetate (PMA) as measured in Example 41.

[0027] FIG. 13 is a graph depicting the percent reduction in IP-10 resulting from administration of 2'FL and 6'SL alone or in combination as measured in Example 42.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0028] The nutritional compositions and methods described herein utilize HMOs alone or in combination with long chain polyunsaturated fatty acids, and/or antioxidants, and in particular carotenoids, and/or nucleotides for controlling and reducing a number of diseases and conditions related to inflammation. The nutritional compositions described herein include synthetic infant formulas that include one HMO or a combination of HMOs. 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.

[0029] 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.

[0030] 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.

[0031] 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.

[0032] The term "human milk oligosaccharide" or "HMO", as used herein, 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 include sialic acid and/or fucose.

[0033] 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.

[0034] 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.

[0035] The term "nutritional liquid" as used herein, unless otherwise specified, refers to nutritional products in ready-to-drink liquid form, concentrated form, and nutritional liquids made by reconstituting the nutritional powders described herein prior to use.

[0036] The term "nutritional powder" as used herein, unless otherwise specified, refers to nutritional products 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.

[0037] 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.

[0038] 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.

[0039] 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.

[0040] 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.

[0041] 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.

[0042] The term "newborn" as used herein, unless otherwise specified, refers to a person from birth up to four weeks of age.

[0043] The terms "infant formula" or "synthetic infant formula" as used herein, unless otherwise specified, are used interchangeably and refer to liquid and solid, and semi-liquid 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.

[0044] The term "synthetic pediatric formula" as used herein, unless otherwise specified, refers to liquid and solid and semi-liquid 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.

[0045] The term "synthetic child formula" as used herein, unless otherwise specified, refers to liquid and solid and semi-liquid 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.

[0046] 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.

[0047] 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.

[0048] The terms "absence of a virus" or "absent a virus" as used herein with respect to inducing production of monocyte-derived cytokines, unless otherwise specified, refer to an individual (e.g., an infant) without the virus or having the virus in an amount less than the amount required to illicit an immune response; that is, an amount that is less than required for the body's natural immune response to increase the production of cytokines and other immune factors.

[0049] The terms "inflammatory disease" or "inflammatory condition" as used herein, unless otherwise specified, refer to any disease, disorder, or condition characterized by inflammation. The term "infection-mediated inflammatory disease" as used herein, unless otherwise specified, refers to an inflammatory disease associated or induced by microbial infection, including viral and bacterial infection.

[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 terms "modulating" or "modulation" or "modulate" as used herein, unless otherwise specified, refer to the targeted movement of a selected characteristic.

[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 product 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, liquid, semi-solid, 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 as also defined herein.

[0059] The nutritional compositions of the present disclosure include one or more HMOs as described herein. The compositions may include one or more HMOs alone or in combination with other immune enhancing factors including, but not limited, to long chain polyunsaturated acids (LCPUFAs), nucleotides, and antioxidants, such as carotenoids and vitamins, as discussed below.

[0060] The nutritional compositions may be in any product form comprising the ingredients described herein, and which is safe and effective for oral administration. The nutritional compositions may be formulated to include only the ingredients described herein, or may be modified with optional ingredients to form a number of different product forms.

[0061] 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 HMOs, desirably in combination with at least one of protein, fat, vitamins, and minerals, to produce a nutritional composition.

[0062] The nutritional compositions 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 product for use in individuals afflicted with specific diseases or conditions or with a targeted nutritional benefit as described below.

[0063] 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, and liquid and powdered follow-on formulas suitable for use with infants and children.

Nutritional Liquids

[0064] 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.

[0065] 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.

[0066] 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%, of water by weight of the nutritional emulsions. 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.

[0067] 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.

[0068] 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.

[0069] 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 1 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

[0070] 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.

[0071] 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)

[0072] 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 composition of human milk oligosaccharides is very complex and more than 200 different oligosaccharide-like structures are known.

[0073] The HMOs may be included in the nutritional compositions alone, or in some embodiments, in combination with other immune enhancing factors (e.g., LCPUFAs, antioxidants, nucleotides, etc.) as described herein. 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.

[0074] Suitable HMOs for use in the nutritional compositions may include acidic oligosaccharides, neutral 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); 3'-Sialyllactose (3'SL); 6'-Sialyllactose (6'SL); 2'-Fucosyllactose (2'FL); 3'-Fucosyllactose (3'FL); Lacto-N-tetraose and Lacto-N-neotetraose (LNnT), and in particular, combinations of 6'SL and 3'SL; combinations of 3'FL and SA; combinations of 2'FL and 3'FL; combinations of

2'FL, 3'SL, and 6'SL; combinations of 3'SL, 3'FL, and LNnT; and combinations of 6'SL, 2'FL, and LNnT.

[0075] 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.

[0076] 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 0.01 mg/mL to about 10 mg/mL, including from about 0.01 mg/mL to about 5 mg/mL, including from about 0.001 mg/mL to about 1 mg/mL, including from about 0.001 mg/mL to about 0.23 mg/mL, including from about 0.01 mg/mL to about 0.23 mg/mL of total HMO in the nutritional composition. 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.

[0077] In one specific embodiment when the nutritional product 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).

[0078] In another specific embodiment, when the nutritional product 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).

[0079] In another specific embodiment when the nutritional product is a concentrated nutritional liquid, the total concentration of HMOs in the concentrated nutritional 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).

[0080] 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.

[0081] In some embodiments, the HMOs are used in combination to provide the desired immune enhancing effect. For example, in one embodiment, the nutritional composition includes 6'SL in combination with 3'SL in a total amount of HMO 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 0.23 mg/mL, including from about 0.01 mg/mL to less than 0.23 mg/mL, including from about 0.001 mg/mL to less than 0.15 mg/mL, and including from about 0.01 mg/mL to less than 0.15 mg/mL of the nutritional composition. In another embodiment, the nutritional composition includes 6'SL in combination with 3'SL in a total amount of HMO of from about 0.001 mg/mL to about 20 mg/mL, including from about 0.01 mg/mL to about 20 mg/mL and including greater than 0.65 mg/mL to about 20 mg/mL. In another embodiment, the nutritional composition includes 3'SL and 6'SL in a weight ratio of from about 1:20 to about 20:1, including from about 1:10 to about 10:1, and including from about 1:2 to about 2:1.

[0082] 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, including from about 0.01 mg/mL to less than 0.25 mg/mL, including from greater than 0.4 mg/mL to about 20 mg/mL, and including from about 0.1 mg/mL to about 0.5 mg/mL.

[0083] 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.

[0084] 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, and including from greater than 0.25 mg/mL to about 20 mg/mL.

[0085] 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 85% (by total weight HMOs) to about 100% (by total weight HMOs), and including about 100% (by total weight HMOs) 3'SL.

[0086] 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 about 0.001 mg/mL to about 0.1 mg/mL, and including from greater than 0.32 mg/mL to about 20 mg/mL.

[0087] In another specific embodiment, the nutritional composition includes 3'FL, 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 1 mg/mL, including from about 0.01 mg/mL to less than 1 mg/mL, and including from greater than 1.7 mg/mL to about 20 mg/mL.

[0088] In one specific embodiment, the nutritional composition includes 3'FL in combination with SA in a total amount of HMO of from about 0.001 mg/mL to about 20 mg/mL, including from about 0.01 mg/mL to about 20 mg/mL. In one embodiment, the nutritional composition includes 3'FL in an amount of from 0.001 mg/mL to less than 1 mg/mL, including from 0.01 mg/mL to less than 1 mg/mL and SA in an amount of about 1 mg/mL.

[0089] In another embodiment, the nutritional composition includes 2'FL, 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 2 mg/mL, including from about 0.01 mg/mL to less than 2 mg/mL, including from about 0.001 mg/mL to about 1 mg/mL, and including from about 0.01 mg/mL to about 0.001 mg/mL. In another embodiment, the nutritional composition includes 2'FL, 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 and including greater than 2.5 mg/mL to about 20 mg/mL.

[0090] In one specific embodiment, the nutritional composition includes 2'FL in combination with 3'FL in a total amount of HMO of from 0.001 mg/mL to about 20 mg/mL, including from about 0.01 mg/mL to about 20 mg/mL.

[0091] In yet another embodiment, the nutritional composition includes a combination of 6'SL, 2'FL, and LNnT in a total amount of HMO of from about 0.001 mg/mL to about 20 mg/mL, including from about 0.01 mg/mL to about 20 mg/mL.

Long Chain Polyunsaturated Fatty Acids (LCPUFAs)

[0092] In addition to the HMOs described above, the nutritional products of the present disclosure may include LCPUFAs. LCPUFAs are included in the nutritional compositions to provide nutritional support, as well as to reduce oxidative stress and enhance growth and functional development of the intestinal epithelium and associated immune cell populations. In some embodiments, the nutritional composition includes a

combination of one or more HMOs and one or more LCPUFAs such that the composition provides a synergistic benefit to the end user, such as a synergistic benefit in modulating anti-viral immune responses and dampening inflammation. In some embodiments, the HMO or HMOs used in combination with the LCPUFAs to provide the synergistic effect are acidic HMOs.

[0093] 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), acidarachidonic 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.

[0094] 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.

[0095] 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.

[0096] The nutritional compositions as described herein will typically comprise total concentrations of LCPUFA of from about 0.01 mM to about 10 mM and including from about 0.01 mM to about 1 mM. Alternatively, the nutritional compositions comprise total concentrations of LCPUFA of from about 0.001 g/L to about 1 g/L.

[0097] In one embodiment, the nutritional compositions include total long chain ω-6 fatty acids in a concentration of from about 100 to about 425 mg/L or from about 12 to about 53 mg per 100 kcals and/or further include total long chain ω-3 fatty acids in a concentration of from about 40 to about 185 mg/L or from about 5 to about 23 mg per 100 kcals. In one specific embodiment, the ratio of long chain ω-6 fatty acids to long chain ω-

3 fatty acids in the nutritional compositions ranges from about 2:1 to about 3:1, preferably about 2.5:1.

[0098] In one specific embodiment, the nutritional compositions include DHA in a concentration of from about 0.025 mg/mL to about 0.130 mg/mL or from about 3 to about 16 mg per 100 kcals. In another embodiment, the nutritional compositions include ARA in a concentration of from about 0.080 mg/mL to about 0.250 mg/mL or from about 10 to about 31 mg per 100 kcals. In yet another embodiment, the nutritional compositions include combinations of DHA and ARA such that the ratio of DHA to ARA ranges from about 1:4 to about 1:2.

Antioxidants

[0099] Additionally, the nutritional compositions may comprise one or more antioxidants in combination with the HMOs (and optionally LCPUFAs and/or nucleotides also) to provide nutritional support, as well as to reduce oxidative stress. In some embodiments, the nutritional composition includes a combination of HMOs and antioxidants such that the composition provides a synergistic benefit to the end user, such as a synergistic benefit in modulating anti-viral immune responses and dampening inflammation. In some embodiments, the HMO or HMOs is used in combination with carotenoids (and specifically lutein, beta-carotene, zeaxanthin and/or lycopene) to provide the synergistic effect.

[00100] 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, including lutein, beta-carotene, zeaxanthin, and lycopene, and combinations thereof, for example.

[00101] As noted, the antioxidants for use in the nutritional compositions may be used with the HMOs alone or in combination with HMOs and LCPUFAs and/or nucleotides. In one specific embodiment, the antioxidants for use in the nutritional compositions include carotenoids, and particularly, combinations of the carotenoids lutein, lycopene, zeaxanthin and/or 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.

[00102] It is generally preferable that the nutritional compositions comprise at least one of lutein, lycopene, zeaxanthin, and beta-carotene to provide a total amount of carotenoid of from about 0.001 μg/mL to about 10 μg/mL. More particularly, the nutritional compositions comprise lutein in an amount of from about 0.001 μg/mL to about 10 μg/mL, including from about 0.001 μg/mL to about 5 μg/mL, including from about 0.001 μg/mL to about 0.0190 μg/mL, including from about 0.001 μg/mL to about 0.0140 μg/mL, and also including from about 0.044 μg/mL to about 5 μg/mL of lutein. It is also generally preferable that the nutritional compositions comprise from about 0.001 μg/mL to about 10 μg/mL, including from about 0.001 μg/mL to about 5 μg/mL, from about 0.001 μg/mL to about 0.0130 μg/mL, including from about 0.001 μg/mL to about 0.0075 μg/mL, and also including from about 0.0185 μg/mL to about 5 μg/mL of lycopene. It is also generally preferable that the nutritional compositions comprise from about 1 μg/mL to about 10 μg/mL, including from about 1 μg/mL to about 5 μg/mL, including from about 0.001 μg/mL to about 0.025 μg/L, including from about 0.001 μg/mL to about 0.011 μg/mL, and also including from about 0.034 μg/mL to about 5 μg/mL of beta-carotene. It should be understood that any combination of these amounts of beta-carotene, lutein, zeaxanthin, and lycopene can be included in the nutritional compositions of the present disclosure. Other carotenoids may optionally be included in the nutritional compositions as described herein. Any one or all of the carotenoids included in the nutritional compositions described herein may be from a natural source, or artificially synthesized.

[00103] Each of the carotenoids in the selected combinations can be obtained from any known or otherwise suitable material source for use in nutritional compositions, and each can be provided individually, or all together, or in any combination and from any number of sources, including sources such as multivitamin premixes containing other vitamins or minerals in combination with one or more of the carotenoids as described herein. Non-limiting examples of some suitable sources of lutein, lycopene, beta-carotene, or combinations thereof include LycoVit® lycopene (available from BASF, Mount Olive, NJ), Lyc-O-Mato® tomato extract in oil, powder, or bead form (available from LycoRed Corp., Orange, NJ), beta-carotene, lutein, or lycopene (available from DSM Nutritional Products, Parsippany, NJ), FloraGLO® lutein (available from Kemin Health, Des Moines, IA), Xangold® Natural Lutein Esters (available from Cognis, Cincinnati, OH), and Lucarotin® beta-carotene (available from BASF, Mount Olive, N.J).

Nucleotides

[00104] In addition to the HMOs, the nutritional compositions of the present disclosure may additionally comprise nucleotides and/or nucleotide precursors selected from the group consisting of nucleosides, purine bases, pyrimidine bases, ribose and deoxyribose. 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. In some embodiments, the nutritional composition includes a combination of HMOs and nucleotides such that the composition provides a synergistic benefit to the end user, such as a synergistic benefit in modulating anti-viral immune responses and dampening inflammation and/or improving intestinal barrier integrity.

[00105] Incorporation of nucleotides in the nutritional compositions of the present disclosure improves intestinal barrier integrity and/or maturation, which is beneficial to preterm and term infants who have less developed intestinal flora and hence a slower maturing intestinal barrier.

[0106] 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.

[0107] The nucleotides are present in the nutritional products in total amounts of nucleotides of at least about 5 mg/L, including at least about 10 mg/L, including from about 10 mg/L to about 200 mg/L, including from about 42 mg/L to about 102 mg/L, and including at least about 72 mg/L of the nutritional product.

[0108] In one specific embodiment when the nutritional composition is a nutritional powder, the nucleotide may be present at a level of at least about 0.007%, including from about 0.0078% to about 0.1556%, and including about 0.056% (by weight of the nutritional powder), or at least about 0.007 grams, including from about 0.0078

grams to about 0.1556 grams, and including about 0.056 grams of nucleotide per 100 grams of nutritional powder.

[0109] In another specific embodiment, when the nutritional composition is a ready-to-feed nutritional liquid, the nucleotide is present at a level of at least about 0.001%, including from about 0.001% to about 0.0197%, and including about 0.0071% (by weight of the nutritional liquid), or at least about 0.001 grams, including from about 0.001 grams to about 0.0197 grams, and including about 0.0071 grams of nucleotide per 100 grams of ready-to-feed nutritional liquid.

[0110] In another specific embodiment when the nutritional composition is a concentrated nutritional liquid, the nucleotide is present at a level of at least about 0.0019%, including from about 0.0019% to about 0.0382%, and including about 0.0138% (by weight of the nutritional liquid), or at least about 0.0019 grams, including from about 0.0019 grams to about 0.0382 grams, and including about 0.0138 grams of nucleotide per 100 grams of concentrated nutritional liquid.

Macronutrients

[0111] 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.

[0112] 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, 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.

[0113] For the liquid preterm and term infant formulas, carbohydrate concentrations 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.

[0114] For the liquid human milk fortifiers, carbohydrate concentrations 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.

[0115] 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

[0116] 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 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

[0117] When the nutritional product 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 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.

[0118] 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 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.

[0119] 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 product. 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

[0120] The nutritional compositions of the present disclosure may, in addition to the LCPUFAs described above, comprise an additional source or sources of fat. Suitable additional sources of fat for use herein include any fat or fat source that is suitable for use in an oral nutritional product and is compatible with the essential elements and features of such products. For example, in one specific embodiment, the additional fat is derived from short chain fatty acids.

[0121] Additional non-limiting examples of suitable fats or sources thereof for use in the nutritional products 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

[0122] 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 products is suitable for use in the nutritional compositions.

[0123] Non-limiting examples of suitable proteins or sources thereof for use in the nutritional products 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.

Carbohydrate

[0124] The nutritional products of the present disclosure may further optionally comprise any carbohydrates that are suitable for use in an oral nutritional product and are compatible with the essential elements and features of such products.

[0125] Non-limiting examples of suitable carbohydrates or sources thereof for use in the nutritional products 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

[0126] 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 products 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.

[0127] Non-limiting examples of such optional ingredients include preservatives, emulsifying agents, buffers, fructooligosaccharides, galactooligosaccharides, polydextrose, and other prebiotics, probiotics, pharmaceutical actives, anti-inflammatory agents, additional nutrients as described herein, colorants, flavors, thickening agents and stabilizers, emulsifying agents, lubricants, and so forth.

[0128] 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 product may range from at least 0.01%, including from about 0.1% to about 10%, and also including from about 1% to about 6%, by weight of the nutritional product. 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 product.

[0129] 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.

[0130] 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.

[0131] The nutritional compositions may further comprise any of a variety of other vitamins or related nutrients, non-limiting examples of which include vitamin A, vitamin D, vitamin E, vitamin K, thiamine, riboflavin, pyridoxine, vitamin B_{12}, carotenoids (e.g., beta-carotene, zeaxanthin, lutein, lycopene), niacin, folic acid, pantothenic acid, biotin, vitamin C, choline, inositol, salts and derivatives thereof, and combinations thereof.

[0132] 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.

Methods of Manufacture

[0133] 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.

[0134] 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.

[0135] 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.

[0136] 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.

[0137] 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.

[0138] 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.

[0139] Other suitable methods for making nutritional products are described, for example, in U.S. Pat. No. 6,365,218 (Borschel, et al.), U.S. Patent 6,589,576 (Borschel, et al.), U.S. Pat. No. 6,306,908 (Carlson, et al.), U.S. Patent Application 20030118703 A1 (Nguyen, et al.), which descriptions are incorporated herein by reference to the extent that they are consistent herewith.

Methods of Use

[0140] The nutritional compositions as described herein can be used to address one or more of the diseases or conditions discussed herein, or can be used to provide one or more of the benefits described herein, to preterm infants, infants, toddlers, and children. The preterm infant, infant, toddler, or child 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, or child actually has the disease or condition, or is at risk or susceptible to the disease or condition, the preterm infant, infant, toddler, or child is classified herein as "in need of" assistance in dealing with and combating the disease or condition. For example, the preterm infant, infant, toddler, or child 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, or child 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, or child with the nutritional compositions described herein.

[0141] 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, and children will fall within the subset or subclass of preterm infants, infants, toddlers, and children as described herein for certain diseases or conditions.

[0142] The nutritional compositions as described herein comprise HMOs, alone or in combination with one or more additional components, to provide a nutritional source for reducing inflammation, such as respiratory inflammation (e.g., respiratory syncytial virus-induced inflammation), enteric inflammation, and nasopharyngeal inflammation. The nutritional compositions of the present disclosure comprising HMOs may also provide optimal development and balanced growth and maturation of the infant's gastrointestinal and immune systems, thereby enhancing the infant's ability to resist microbial infection and modulate inflammatory responses to infection (e.g., increased phagocytosis and increased production of reactive oxidative species).

[0143] The nutritional compositions also provide growth and maturation of the intestinal epithelial cells in an infant. In one specific embodiment, the administration of the nutritional compositions of the present disclosure including HMOs and nucleotides can further activate immune activity in or by the intestinal epithelial cells in a newborn.

[0144] Further, the use of HMOs in nutritional compositions can reduce the growth of respiratory viruses (e.g., RSV, human parainfluenza virus type 2, and influenza A virus), and thus, reduce viral-induced upper respiratory infections. As such, by utilizing HMOs, alone or in combination with other immune enhancing factors, in a nutritional product, such as an infant formula, it is now possible to provide infants with an alternative, or supplement, to breast milk that more closely mimics the benefits thereof.

[0145] Along with improved growth and maturation of the infant's immune system as described above, the use of the nutritional compositions of the present disclosure also functions as an immune modulator, thereby reducing inflammation induced by infection in infants, toddlers, and children such as respiratory virus-induced infection, and particularly, RSV-induced inflammation, and other infection-mediated inflammatory diseases.

[0146] The addition of HMOs can further increase glutathione levels in the body and blood of an infant, and in specific embodiments, of a preterm infant.

[0147] 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).

[0148] In addition to the benefits discussed above, it has been discovered that nutritional products including HMOs can modulate production of monocyte-derived cytokines in the infant, even in the absence of a virus. This production results in improved immunity to further prevent microbial infection and reduce the growth of viruses. In one specific embodiment, monocyte-derived cytokines produced by administration of the nutritional compositions of the present disclosure include, for example, interleukin-10, interleukin-8, interleukin-1α, interleukin-1β, interleukin-1ra, and combinations thereof.

[0149] Another benefit of utilizing HMOs in nutritional compositions is that it has been discovered that HMOs modulate the production of IP-10, which is a chemokine that plays an important role in the inflammatory response to viral infection. Specifically, a positive correlation exists between RSV clinical infection severity in children and serum IP-10 levels. Accordingly, a decrease in IP-10 signals a decrease in severity of RSV infection. In one specific embodiment, IP-10 production is reduced to the level found in uninfected controls.

[0150] Along with reducing IP-10, HMOs have been found to reduce platelet-neutrophil complex (PNC) formation, which is present in human blood and consists of up to 25% of unstimulated neutrophils. As PNCs are present in aggregates, they have a greater capacity to initiate inflammatory processes and can increase the production of reactive oxidative species. Accordingly, a decrease in PNC formation can lead to reduced oxidative stress and inflammation in the infant.

EXAMPLES

[0151] The following examples illustrate specific embodiments and/or features of the nutritional compositions 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.

[0152] 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.

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
3' sialylallactose (3'SL)	0.0948	0.090	0.085	9.479	9.005
Galactooligosaccharides (GOS)	8.63	8.63	8.63	8.63	8.63
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	47.4 g	47.4 g	47.4 g	47.4 g	47.4 g
Citric acid	29.77 g	29.77 g	29.77 g	29.77 g	29.77 g
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
6' sialylallactose (6'SL)	0.0948	0.0901	0.0853	9.479	9.0047
Galactooligosaccharides (GOS)	8.63	8.63	8.63	8.63	8.63
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
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	157.67	157.67	157.67	157.67	157.67
Lactose	108.66	108.66	108.66	108.66	108.66
High oleic safflower oil	26.82	26.82	26.82	26.82	26.82
Soybean oil	20.16	20.16	20.16	20.16	20.16
Coconut oil	19.24	19.24	19.24	19.24	19.24
3' sialylallactose (3'SL)	0.1896	0.1802	0.1706	18.958	18.009
Galactooligosaccharides (GOS)	17.67	17.67	17.67	17.67	17.67
Whey protein concentrate	12.20	12.20	12.20	12.20	12.20
Potassium citrate	1.277	1.277	1.277	1.277	1.277
Calcium carbonate	996.1 g	996.1 g	996.1 g	996.1 g	996.1 g
Soy lecithin	685.0 g	685.0 g	685.0 g	685.0 g	685.0 g
Monoglycerides	685.0 g	685.0 g	685.0 g	685.0 g	685.0 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	429.7 g	429.7 g	429.7 g	429.7 g	429.7 g
Ascorbic acid	293.8 g	293.8 g	293.8 g	293.8 g	293.8 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	173.3 g	173.3 g	173.3 g	173.3 g	173.3 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	57.50 g	57.50 g	57.50 g	57.50 g	57.50 g
Mixed carotenoid premix	41.90 g	41.90 g	41.90 g	41.90 g	41.90 g
Sodium chloride	23.50 g	23.50 g	23.50 g	23.50 g	23.50 g
L-carnitine	6.40 g	6.40 g	6.40 g	6.40 g	6.40 g
Tricalcium phosphate	AN	AN	AN	AN	AN
Potassium phosphate monobasic	AN	AN	AN	AN	AN
Potassium hydroxide	AN	AN	AN	AN	AN

AN = as needed

EXAMPLES 16-20

[0156] Examples 16-20 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. 16	Ex. 17	Ex. 18	Ex. 19	Ex. 20
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
HMO Mixture	0.0948	0.0901	0.0853	9.479	9.0047
6' sialyllactose (6'SL)	0.0316	0.0300	0.0284	3.159	3.002
2'fucosyllactose (2'FL)	0.0316	0.0300	0.0284	3.159	3.002
Lacto-N-neotetraose (LNnT)	0.0316	0.0300	0.0284	3.159	3.002
Galactooligosaccharides (GOS)	8.63	8.63	8.63	8.63	8.63
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
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 21-25

[0157] Examples 21-25 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. 21	Ex. 22	Ex. 23	Ex. 24	Ex. 25
Water	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.
Condensed Skim Milk	157.67	157.67	157.67	157.67	157.67
Lactose	108.66	108.66	108.66	108.66	108.66
High oleic safflower oil	26.82	26.82	26.82	26.82	26.82
Soybean oil	20.16	20.16	20.16	20.16	20.16
Coconut oil	19.24	19.24	19.24	19.24	19.24
HMO Mixture	18.957	18.009	17.061	19.905	20.853
6' sialyllactose (6'SL)	6.319	6.003	5.687	6.635	6.951
2'fucosyllactose (2'FL)	6.319	6.003	5.687	6.635	6.951
Lacto-N-neotetraose (LNnT)	6.319	6.003	5.687	6.635	6.951
Galactooligosaccharides (GOS)	17.67	17.67	17.67	17.67	17.67
Whey protein concentrate	12.20	12.20	12.20	12.20	12.20
Potassium citrate	1.277	1.277	1.277	1.277	1.277
Calcium carbonate	996.1 g	996.1 g	996.1 g	996.1 g	996.1 g
Soy lecithin	685.0 g	685.0 g	685.0 g	685.0 g	685.0 g
Monoglycerides	685.0 g	685.0 g	685.0 g	685.0 g	685.0 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	429.7 g	429.7 g	429.7 g	429.7 g	429.7 g
Ascorbic acid	293.8 g	293.8 g	293.8 g	293.8 g	293.8 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	173.3 g	173.3 g	173.3 g	173.3 g	173.3 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	57.50 g	57.50 g	57.50 g	57.50 g	57.50 g
Mixed carotenoid premix	41.90 g	41.90 g	41.90 g	41.90 g	41.90 g
Sodium chloride	23.50 g	23.50 g	23.50 g	23.50 g	23.50 g
L-carnitine	6.40 g	6.40 g	6.40 g	6.40 g	6.40 g
Tricalcium phosphate	AN	AN	AN	AN	AN
Potassium phosphate monobasic	AN	AN	AN	AN	AN
Potassium hydroxide	AN	AN	AN	AN	AN

AN = as needed

EXAMPLES 26-30

[0158] Examples 26-30 illustrate human milk fortifier liquids 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.
Non-fat milk	353	353	353	353	353
Corn Syrup Solids	85.3	85.3	85.3	85.3	85.3
Medium Chain Triglycerides	53.2	53.2	53.2	53.2	53.2
Whey Protein Concentrate	47.2	47.2	47.2	47.2	47.2
HMO Mixture	18.957	18.009	17.061	19.905	20.853
6' sialyllactose (6'SL)	6.319	6.003	5.687	6.635	6.951
2'fucosyllactose (2'FL)	6.319	6.003	5.687	6.635	6.951
Lacto-N-neotetraose (LNnT)	6.319	6.003	5.687	6.635	6.951
Calcium Phosphate	25.5	25.5	25.5	25.5	25.5
Ascorbic Acid	5.6	5.6	5.6	5.6	5.6
Potassium Citrate	3.1	3.1	3.1	3.1	3.1
Magnesium Chloride	2.8	2.8	2.8	2.8	2.8
Sodium Citrate	1.4	1.4	1.4	1.4	1.4
Sodium Chloride	1.4	1.4	1.4	1.4	1.4
Soy Lecithin	609 g	609 g	609 g	609 g	609 g
M-Inositol	500 g	500 g	500 g	500 g	500 g
Niacinamide	400 g	400 g	400 g	400 g	400 g
ARA Oil	313 g	313 g	313 g	313 g	313 g
Tocopherol Acetate	310 g	310 g	310 g	310 g	310 g
Zinc Sulfate	300 g	300 g	300 g	300 g	300 g
Calcium Pantothenate	182 g	182 g	182 g	182 g	182 g
Ferrous Sulfate	133 g	133 g	133 g	133 g	133 g
DHA Oil	116 g	116 g	116 g	116 g	116 g
Vitamin A Palmitate	100 g	100 g	100 g	100 g	100 g
Cupric Sulfate	51.0 g	51.0 g	51.0 g	51.0 g	51.0 g
Thiamine Hydrochloride	50.0 g	50.0 g	50.0 g	50.0 g	50.0 g
Riboflavin	47.0 g	47.0 g	47.0 g	47.0 g	47.0 g
Pyridoxine Hydrochloride	27.0 g	27.0 g	27.0 g	27.0 g	27.0 g
Vitamin D_3	20.0 g	20.0 g	20.0 g	20.0 g	20.0 g
Folic Acid	3.5 g	3.5 g	3.5 g	3.5 g	3.5 g
Biotin	3.4 g	3.4 g	3.4 g	3.4 g	3.4 g
Manganous Sulfate	1.5 g	1.5 g	1.5 g	1.5 g	1.5 g
Phylloquinone	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g
Cyanocobalamin	100 mg	100 mg	100 mg	100 mg	100 mg
Sodium Selenate	43.0 mg	43.0 mg	43.0 mg	43.0 mg	43.0 mg

EXAMPLES 31-35

[0159] Examples 31-35 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. 31	Ex. 32	Ex. 33	Ex. 34	Ex. 35
Condensed Skim Milk	698.5	698.5	698.5	698.5	698.5
Lactose	386.0	386.0	386.0	386.0	386.0
High oleic safflower oil	114.4	114.4	114.4	114.4	114.4
Soybean oil	85.51	85.51	85.51	85.51	85.51
Coconut oil	78.76	78.76	78.76	78.76	78.76

3' sialylallactose (3'SL)	0.3792	0.3604	0.3412	37.916	36.0188
Galactooligosaccharides (GOS)	69.50	69.50	69.50	69.50	69.50
Whey protein concentrate	51.08	51.08	51.08	51.08	51.08
Potassium citrate	9.168	9.168	9.168	9.168	9.168
Calcium carbonate	4.054	4.054	4.054	4.054	4.054
Soy lecithin	1.120	1.120	1.120	1.120	1.120
ARA oil	2.949	2.949	2.949	2.949	2.949
Nucleotide/chloride premix	2.347	2.347	2.347	2.347	2.347
Potassium chloride	1.295	1.295	1.295	1.295	1.295
Ascorbic acid	1.275	1.275	1.275	1.275	1.275
Vitamin mineral premix	1.116	1.116	1.116	1.116	1.116
DHA oil	1.113	1.113	1.113	1.113	1.113
Magnesium chloride	1.038	1.038	1.038	1.038	1.038
Sodium chloride	579.4 g	579.4 g	579.4 g	579.4 g	579.4 g
Ferrous sulfate	453.6 g	453.6 g	453.6 g	453.6 g	453.6 g
Choline chloride	432.1 g	432.1 g	432.1 g	432.1 g	432.1 g
Vitamin A, D_3, E, K_1 premix	377.2 g	377.2 g	377.2 g	377.2 g	377.2 g
Ascorbyl Palmitate	361.3 g	361.3 g	361.3 g	361.3 g	361.3 g
Mixed carotenoid premix	350.1 g	350.1 g	350.1 g	350.1 g	350.1 g
Mixed Tocopherols	159.2 g	159.2 g	159.2 g	159.2 g	159.2 g
L-carnitine	26.30 g	26.30 g	26.30 g	26.30 g	26.30 g
Riboflavin	3.181 g	3.181 g	3.181 g	3.181 g	3.181 g
Tricalcium phosphate	0-5.23	0-5.23	0-5.23	0-5.23	0-5.23
Potassium phosphate monobasic	0-5.23	0-5.23	0-5.23	0-5.23	0-5.23
Potassium hydroxide	AN	AN	AN	AN	AN

AN = as needed

EXAMPLE 36

[0160] In this Example, the effect of purified human milk oligosaccharides (HMO) on *in vitro* inhibition of viral infectivity is analyzed.

[0161] Samples are prepared by co-incubation of a uniform virus dose of from about 500 units/mL to about 1,000 units/mL of one of three respiratory viruses: (1) respiratory syncytial virus (RSV); (2) human parainfluenza virus (HPIV3); or (3) H1N1 influenza virus with one of the following HMOs: (1) 3'-sialyllactose (3'SL); (2) 6'-sialyllactose (6'SL); (3) 3'-fucosyllactose (3'FL); (4) 2'-fucosyllactose (2'FL); (5) lacto-N-neotetraose (LNnT); or (6) sialic acid (SA). The HMOs are added at concentrations of either 1 mg/mL or 10 mg/mL. The antiviral activities of the various HMOs on the respiratory viruses are evaluated, and the results are shown in the table below:

HMO	**IC50 (mg HMO/mL)**		
	RSV	**HPIV3**	**H1N1 Influenza**
3'SL	>10	>10	~5

6'SL	>10	>10	~10
3'FL	~5	~2	~5
2'FL	>10	>10	~10
LNnT	>10	NT	>10
SA	NT	~2	~5

NT = Not Tested

[0162] The results show that 3'FL, at a concentration of 1 mg/ML (IC50 ~2-5 mg/ML), has anti-viral activity for all three respiratory viruses. This result is unexpected as previous published reports show only sialylated oligo forms providing antiviral activity. SA significantly inhibits HPIV3 and H1N1 viruses at a concentration of 1 mg/mL. H1N1 influenza virus is also inhibited by 3'SL at a concentration of 1 mg/mL.

EXAMPLE 37

[0163] In this Example, the ability of various HMOs to block H1N1 influenza virus infectivity *in vitro* is analyzed.

[0164] Virus infectivity is assessed by observing cytopathic effect (CPE) and quantifying virus focus forming units. To create virus stocks, H1N1 influenza virus is purchased from ATCC (VR 1469) and expanded in Madin-Darby Canine Kidney (MDCK) epithelial cells (ATCC CCL-34). Cell-free supernatants are frozen in aliquots to maintain stock virus. During initial virus culture and expansion to create virus stocks, cell CPE is observed.

[0165] To quantify virus infectivity, an immunocytochemical focus forming unit (FFU) assay is developed using commercially purchased mouse monoclonal antibodies against the virus nucleoprotein coupled with a biotinylated anti-mouse IgG secondary antibody. To visualize virus-infected cell foci, color development is performed using Strepavidin HRP (ABC from Vector Laboratories, Inc.). Although the total number of virus foci appear proportional to the infecting virus concentration, the foci are quite large, disperse, and there are numerous individually infected cells that do not form foci, especially at higher virus concentrations. As this makes quantifying of virus infectivity difficult and time-consuming, the FFU assay is further refined by varying virus

concentration and by applying an overlay medium of Tragacanth gum to help reduce Brownian movement spread of the virus throughout the cell layer.

[0166] The use of Tragacanth gum improves the assay by reducing the number of individually infected cells while still allowing for the formation of readily observable foci. While the foci vary in size, with some being quite large, they are still easily quantified and directly proportional to virus concentration or titer by using a grid technique during the enumeration.

[0167] Once verified, the assay is used with various HMOs for the ability to block H1N1 virus infectivity. Specifically, the HMOs are added, at concentrations of 0.01 mg/mL, 0.1 mg/mL, 1.0 mg/mL, and 10 mg/mL, to the inoculating virus suspension, incubated at 37°C for one hour, and then added to MDCK monolayer cells. This mixture is allowed to bind to the cell layer for thirty minutes at 37°C. The cell layer is then washed, and the cells are further incubated for approximately 18-24 hours before fixing and processing for immunocytochemical staining. The results are shown in FIG. 1.

[0168] As shown in FIG. 1, 3'FL, 3'SL, and SA each inhibit virus infectivity by greater than 90% when used at a concentration of 10 mg/mL. 2'FL and 6'SL inhibit infectivity by approximately 60% at 10 mg/mL.

EXAMPLE 38

[0169] In this Example, nutritional compositions including various HMOs are evaluated for their effects on reducing oxidative stress in preterm piglets.

[0170] Preterm piglets are harvested by caesarian section (CS) at 92% of gestation. Piglets receive total parenteral nutrition (TPN) for 48 hours. After 48 hours, TPN is ceased and the piglets are randomized into three groups: a formula group (n=7) that is fed Enfamil® Lacto-Free, commercially available from Mead Johnson, Evansville, IN; a treatment group (n=9) that is fed Enfamil® Lacto-Free with the addition of a combination of 400 mg/L 6'SL, 1500 mg/L 2'FL, and 200 mg/L LNnT; and a colostrum group (n=5) that is fed bovine colostrum. Piglets are fed their respective feeding enterally at a rate of 120 mL formula per kg body weight for the next 48 hours. Piglets are then euthanized after 48 hours of enteral nutrition (EN), or earlier if a piglet develops signs of necrotizing

enterocolitis. Blood is collected via an umbilical artery catheter, and plasma is separated from the blood and stored at -70°C until analyzed.

[0171] Glutathione (GSH) concentrations are measured in plasma taken from the piglets just prior to feeding time (time 0), and at 6 hours, 12 hours, 24 hours, 36 hours, and 48 hours after feeding using a commercially available assay (NWLSS Glutathione Assay #NWK-GSH01, Northwest Life Science Specialties, Vancouver, WA). The results are shown in FIG. 2.

[0172] As shown in FIG. 2, the concentration of GSH in blood plasma from the control group declines from time 0 to 6 hours after feeding. GSH remains lower in the control group 24 hours after EN. In contrast, piglets fed the composition with a combination of HMOs have a pattern of blood plasma GSH levels that are comparable to the colostrum piglets.

EXAMPLE 39

[0173] In this Example, the abilities of 3'SL, 6'SL, and LNnT to reduce virus-induced inflammation *in vitro* are demonstrated.

[0174] Specifically, either 3'SL or 6'SL is added, at concentrations of 0.1 mg/mL, 0.2 mg/mL, or 0.5 mg/mL to fresh peripheral blood mononuclear cells and incubated at 37°C in 5% CO_2 to pretreat the cells for approximately 24 hours. LNnT is added, at concentrations of 0.1 mg/mL, 0.2 mg/mL, or 1 mg/mL to fresh peripheral blood mononuclear cells and incubated at 37°C in 5% CO_2 to pretreat the cells for approximately 24 hours. Lactose is included as a carbohydrate control. Matched endotoxin unit concentration controls are included to allow differentiation of ingredient effects from inherent low levels of endotoxin. Some variables are then incubated with RSV at a multiplicity of infection (MOI) of 0.1 for approximately 1 hour at 37°C in 5% in CO_2. Uninfected control variables are incubated with medium for approximately 1 hour at 37°C in 5% CO_2. After approximately one hour, fresh medium alone, or fresh medium containing the appropriate concentration of 3'SL, 6'SL, LNnT, lactose, or endotoxin is added to the appropriate tubes and the cells are incubated for 48 hours at 37°C in 5% CO_2. Supernatants are collected at 24 and 48 hours post-infection.

[0175] Cytokines are measured in supernatants for each variable at 24 and 48 hours to assess the effects of HMOs on the early immune response to RSV. Cytokines are measured using custom Bio-Plex Human cytokine kits from Bio-Rad. Results for interferon-inducible protein 10 (IP-10, also known as CXCL 10) are shown in FIGS. 3 and 4 for 3'SL and 6'SL, and in FIGS. 5 and 6 for LNnT. IP-10 is a CXC chemokine that attracts, binds to and activates the CXCR3 receptor on natural killer cells and memory T cells. IP-10 is expressed by monocytes and a number of other cells, and is induced by interferon. A positive correlation exists between RSV clinical disease severity in children (as measured by: length of hospital stay, fever, and number of days supplemental O_2 is required) and serum IP-10. Therefore, a decrease in IP-10 signals a decrease in severity of RSV disease experienced.

[0176] IP-10 results for 3'SL and 6'SL are detailed in FIGS. 3 and 4 and show some variability in donor response, but surprisingly, 6'SL clearly downregulates IP-10 in virus-infected variables in both donors. Note that 6'SL is able to reduce IP-10 to levels found in uninfected controls. 3'SL is not effective in Donor B, but downregulates RSV-induced IP-10 in Donor E. These data show both 3'SL and 6'SL dampen RSV-induced IP-10, but that 6'SL is more effective at downregulation of IP-10. Results also suggest that levels below 0.1 mg/mL of 6'SL as well as levels greater than 0.5 mg/mL may be effective at reducing IP-10 in some individuals.

[0177] IP-10 results for LNnT are detailed in FIGS. 5 and 6 and show some variability in donor response, but surprisingly, LNnT clearly downregulates IP-10 in virus-infected variables in both donors. Note that LNnT is able to reduce IP-10 to levels found in uninfected controls. Results also suggest that levels between 0.2 and 1 mg LNnT/mL as well as greater than 1 mg/mL may be effective at reducing IP-10 in some individuals. Inclusion of matched endotoxin unit concentration controls clearly indicates that the decrease in IP-10 is not due to the presence of very low levels of endotoxin in the LNnT.

[0178] In FIGS. 7 and 8, cytokine results also surprisingly show 6'SL increases interleukin 10 (IL-10) concentration in a dose-dependent manner in the presence or absence of RSV. IL-10 results for LNnT are shown in FIGS. 9 and 10. Surprisingly, LNnT increases IL-10 concentration in a dose-dependent manner in the presence or absence of RSV. IL-10 is produced by activated CD8+ T-cells, by CD4+ T-cells after both antigen-

specific and polyclonal activation, and by monocytes following cell activation by bacterial lipopolysaccharides. Inclusion of matched endotoxin unit concentration controls clearly differentiates that the increase in IL-10 is not due to the presence of very low levels of endotoxin in the 6'SL or the LNnT.

[0179] Surprisingly, it is found that pretreatment for 24 hours by 6'SL, 3'SL, or LNnT is effective in reducing inflammation caused by RSV. Moreover, 6'SL and LNnT are shown to be more effective than 3'SL at dampening virus-induced inflammation as measured by a decrease in IP-10. Further, it is shown that 6'SL is immunomodulatory in the absence of the virus, as the inclusion of 6'SL induces and/or modifies the production of monocyte-derived cytokines such as IL-10, MIP-1β, Interferon-γ, IL-8, IL-1α, IL-1β, and IL-1ra. Surprisingly, 3'SL is also immunomodulatory in the presence or absence of the virus, as the inclusion of 3'SL induces and/or modifies the production of monocyte-derived cytokines such as MIP-1β, Interferon-γ, IL-8, and IL-1ra. Surprisingly, LNnT is also immunomodulatory in the presence or absence of the virus, as the inclusion of LNnT induces and/or modifies the production of monocyte-derived cytokines such as IL-10, MIP-1β, Interferon-γ, IL-8, IL-1α, IL-1β, and IL-1ra.

EXAMPLE 40

[0180] In this example, the combination of 3'SL and 6'SL to reduce the inflammatory cytokine IL-8 *in vitro* is analyzed.

[0181] Specifically, 3'SL and 6'SL are added individually at concentrations of 0.1 mg/mL, 0.2 mg/mL, or 0.5 mg/mL or in combination (Combo 1 = 1 part 3'SL to 1 part 6'SL; Combo 2 = 1 part 3'SL to 2 parts 6'SL), at total concentrations of 0.2 mg/mL, 0.4 mg/mL, or 1.0 mg/mL to fresh peripheral blood mononuclear cells (PBMCs) and incubated at 37°C in 5% CO_2 to pretreat the cells for approximately 24 hours. Lactose is included as a carbohydrate control. Matched endotoxin unit concentration controls are included to allow differentiation of ingredient effects from inherent low levels of endotoxin. After approximately 24 hours, some variables are then incubated with RSV at a multiplicity of infection (MOI) of 0.1 for approximately 1 hour at 37°C in 5% in CO_2. Uninfected control variables are incubated with medium for approximately 1 hour at 37°C in 5% CO_2. After approximately 1 hour, fresh medium alone or containing the appropriate concentrations of

3'SL or 6'SL, individually or in combination, is added to the appropriate variables, and the PBMCs are incubated for 48 hours at 37°C in 5% CO_2. Supernatants are collected at 24 and 48 hours post-infection, aliquotted and stored frozen at -70°C for later analysis. Cytokines are measured in supernatants for each variable at 24 and 48 hours to assess the effects of the 3'SL and/or the 6'SL on the early immune response to RSV. Cytokines are measured using custom Bio-Plex Human cytokine kits from Bio-Rad.

[0182] Interleukin 8 (IL-8) results are shown in FIG. 11. IL-8 is produced by stimulated monocytes, macrophages and fibroblasts and is a chemotactic factor for all migratory immune cells. IL-8 activates neutrophils and enhances metabolism of reactive oxygen species. *In vivo*, IL-8 strongly binds to erythrocytes, and once bound, no longer activates neutrophils. IL-8 is an inflammatory cytokine, and as such, it is generally desired to decrease the level of IL-8.

[0183] As shown in FIG. 11, the combination of 3'SL and 6'SL modifies the dynamics of the IL-8 response in both the presence or absence of RSV. Individually, in the presence of RSV, increasing concentrations of 3'SL and 6SL increase IL-8. Combo 2 and to some extent Combo 1 show decreasing IL-8 concentration with increasing HMO concentration. As such, it can be concluded that by administering a combination of 3'SL and 6'SL, inflammation may be reduced.

EXAMPLE 41

[0184] In this example, the ability of 2'FL to reduce extracellular reactive oxygen species (ROS) in both uninfected and RSV infected peripheral blood mononuclear cells (PBMC) in the presence or absence of phorbal myristate acetate (PMA) is demonstrated.

[0185] On day 0, fresh human PBMCs are isolated from whole blood. 2'FL is added to PBMCs at concentrations of 0.1 mg/mL, 0.2 mg/mL, or 1 mg/mL and incubated for approximately 24 hours at 37°C in 5% CO_2. On day 1, the cell supernatants are removed and the PBMCs are incubated with medium alone or medium plus Respiratory Syncytial Virus (RSV) for approximately 1 hour at 37°C in 5% CO_2 at a multiplicity of infection (MOI) of 1. After approximately 1 hour, fresh medium alone or containing the appropriate concentration of 2'FL is added to the appropriate tubes, and the cells are incubated for 48 hours at 37°C in 5% CO_2.

[0186] On day 3, 20% (3.5 x 105 cells) of the PBMC culture is removed for analysis of NADPH oxidase derived reactive oxygen production using the chemiluminescent probe isoluminol. The isolated cells are divided evenly into two tubes with the reaction mixture containing isoluminol and horseradish peroxidase. One tube is treated with phorbol 12-myristate 13-acetate to induce activation of the NADPH oxidase enzyme complex. Reactive oxygen derived chemiluminescence is monitored in a luminometer over a 60 minute period. Reactive oxygen production is quantified by integrating the area under the curve over the 60 minute period. The remaining PBMC supernatants and cell lysates are collected separately, aliquotted and stored frozen at -70°C for later analysis.

[0187] The results as shown in FIG. 12B indicate that 2'FL dampens release of extracellular reactive oxygen species (ROS) in uninfected (0.1- 0.2 mg 2'FL/mL) and infected cells (0.1-1.0 mg 2'FL/mL) in the absence of phorbol myristate acetate (PMA). Further, the results as shown in FIG. 12A indicate that 2'FL dampens ROS in uninfected (0.1-1.0 mg 2'FL/mL) and infected (0.1- 0.2 mg 2'FL/mL) PBMCs in the presence of PMA. Dampening ROS may decrease RSV induced oxidative stress damage to lungs and other tissues.

EXAMPLE 42

[0188] In this example, the ability of the combination of 2'FL and 6'SL to reduce IP-10, a marker of viral inflammation in vitro is demonstrated.

[0189] Specifically, on day 0, fresh human peripheral blood mononuclear cells (PBMCs) are isolated from whole blood. 2'FL (at concentrations of 0.1 mg/mL, 0.2 mg/mL, 0.5 mg/mL or 1 mg/mL) alone or in combination with 6'SL (at concentrations of 0.05 mg/mL, 0.1 mg/mL, 0.2 mg/mL or 1 mg/mL) is added to the PBMCs and incubated for approximately 24 hours at 37°C in 5% CO_2. On day 1, the cell supernatants are removed and the PBMCs are incubated with medium alone or medium plus Respiratory Syncytial Virus (RSV) for approximately 1 hour at 37°C in 5% CO_2 at a multiplicity of infection (MOI) of 1. After approximately 1 hour, fresh medium alone or containing the appropriate concentration of 2'FL and or 6'SL is added to the appropriate tubes, and the cells are incubated for 48 hours at 37°C in 5% CO_2. On day 3, supernatants are collected (48 hours post-infection). Cytokines are measured in supernatants for each variable at 48

hours using Luminex human cytokine kits to assess the effects of HMOs on the early immune response to RSV.

[0190] Surprisingly, as shown in FIG. 13, the combination of 2'FL and 6'SL synergistically reduces production of IP-10 by 54%. Interferon-inducible Protein 10 (IP-10, also known as CXCL10) is a CXC chemokine that attracts, binds to and activates the CXCR3 receptor on Natural Killer Cells and Memory T cells. IP-10 is expressed by monocytes and a number of other cells, and is induced by interferon. A positive correlation exists between RSV clinical disease severity in children (as measured by: length of hospital stay, fever, and number of days supplemental O_2 was required) and serum IP-10. Therefore, a decrease in IP-10 may signal a decrease in severity of RSV disease experienced. As such, it can be concluded from the results that by administering the combination of 2'FL and 6'SL, the severity of RSV disease experienced may be reduced.

WHAT IS CLAIMED IS:

1. Use of a synthetic pediatric formula in the manufacture of a medicament for reducing inflammation in an infant, toddler, or child, wherein the synthetic pediatric formula comprises lacto-N-neotetraose in a concentration of 0.01 mg/mL to 20 mg/mL and wherein the medicament is to be administered to the infant, toddler, or child.

2. The use of claim 1, wherein the inflammation is respiratory inflammation.

3. The use of claim 1, wherein the inflammation is selected from the group consisting of respiratory syncytial virus-induced inflammation, enteric inflammation, nasopharyngeal inflammation, and combinations thereof.

4. The use of claim 1, wherein the synthetic pediatric formula further comprises from 0.001 mg/mL to less than 0.25 mg/mL 6'-sialylallactose.

5. The use of claim 1, wherein the synthetic pediatric formula further comprises from 0.001 mg/mL to less than 0.15 mg/mL 3'-sialylallactose.

6. The use of claim 1, wherein the synthetic pediatric formula further comprises 3'-sialyllactose and 6'-sialyllactose in a weight ratio of 3'-sialyllactose to 6'-sialyllactose of from 1:20 to 20:1.

7. The use of claim 1, wherein the synthetic pediatric formula further comprises 3'-sialyllactose and 6'-sialyllactose in a weight ratio of 3'-sialyllactose to 6'-sialyllactose of from 1:2 to 2:1.