formulas (rather than nursing), the extensive use of antibiotics, sanitation practices in neonatal facilities/settings, and the microbial environments of homes and hospitals where that infant is raised.

[0006] Dysbiosis can also occur in humans of all age groups, and in other domesticated mammalian species such as, but not limited to, agriculturally-relevant mammals (e.g., cows, pigs, rabbits, goats, and sheep), mammalian companion animals (e.g., cats, dogs, and horses), and performance mammals (e.g., thoroughbred race horses, racing camels, and working dogs) for similar reasons of hygiene, extensive use of antibiotics, and the industrialization of foods and feeds for those humans and animals.

[0007] Previous treatment protocols for a dysbiotic mammal include the administration of an antibiotic that eradicated all, or the majority of, bacteria in the microbiome. For example, Necrotizing Enterocolitis (NEC), a condition that occasionally develops in very small preterm infants, is a severe condition which often requires major surgery to resect certain parts of the necrotic bowel having life-long sequelae, and can often lead to the death of the infant, is universally treated with antibiotics.

[0008] Other dysbiotic gut microbial community compositions can exist within adult or young mammals (e.g., piglets, foals, and calves). Under intensive agricultural production of pigs and horses, antibiotics are frequently used prophylactically, and the microbial diversity of the animals under these conditions is lowered and a dysbiotic gut microbial community ensues. Ironically, this can often lead to pathology (e.g., scours in piglets, or outbreaks of pathogenic bacteria such as *Clostridium difficile* or *C. perfringens* in foals) that are treated by yet more powerful antibiotics to prevent the life threatening diarrhea and possibly death. Presently, the only choice for the elimination of these pathogenic bacteria in such situations is the continued and extensive use of antibiotics and supportive or palliative care. Thus, there is a need for an effective method to reduce dysbiosis and prevent disease in mammals of all ages (including humans as well as companion, performance, and production animals) that does not involve the additional administration of antibiotics.

SUMMARY

[0009] The instant disclosure relates in part to the inventors' discovery that mammalian milks, and especially the glycan components of milk, have evolved to feed two consumers: the immediate offspring; and the offspring's appropriate gut bacteria. The inventors have discovered that in the absence of the evolutionary-associated bacteria (or the presence of a dysbiotic gut), the indigestible

glycans of mammalian milk become susceptible to hydrolysis by other bacteria. This releases Free Sugar Monomers (FSMs), which are capable of enabling the growth of opportunistic or highly destructive pathogens that would not have flourished otherwise. The term “dietary glycans”, as used herein, refers to those indigestible glycans, sometimes referred to as “dietary fiber”, or the carbohydrate polymers which are not hydrolyzed by the endogenous enzymes in the digestive tract (e.g., the small intestine) of the mammal.

[0009a] In one aspect, the present disclosure refers to use of at least two non-pathogenic microbes in the manufacture of a composition for improving the health of an infant mammal, wherein one of the at least two non-pathogenic microbes is from a first species capable of internalizing and/or metabolizing dietary glycans, which is *Bifidobacterium longum* subsp. *infantis*, and wherein one of the at least two non-pathogenic microbes is from a second species capable of consuming and metabolizing free sugar monomers, which is *Lactobacillus crispatus*.

[0009b] In another aspect, the present disclosure refers to use of at least two non-pathogenic microbes in the manufacture of a composition for treating a dysbiotic subject, wherein one of the at least two non-pathogenic microbes is from a first species capable of internalizing and/or metabolizing dietary glycans, which is *Bifidobacterium longum* subsp. *infantis* and wherein one of the at least two non-pathogenic microbes is from a second species capable of consuming and metabolizing free sugar monomers, which is *Lactobacillus crispatus*.

[0010] Some examples of the disclosure involve compositions and methods of delivering to the gut of a dysbiotic mammal, compositions that include components capable of consuming the dietary glycans. Such compositions can reduce the concentration of FSMs in the mammal. The reduction of FSMs and dietary glycans can minimize the likelihood of an overpopulation of pathogenic bacteria that can harm that mammal. In some examples the compositions comprise certain bacteria (alive or dead) or other orally provided compounds that bind and/or metabolize the FSMs, thereby preventing them from being used as an energy source by the pathogenic bacteria.

[0011] Some of the examples of the present disclosure provide diagnostics for the presence of substrates enabling growth of pathogenic bacteria within mammalian neonates. Specifically, some examples provide diagnostics to determine the presence of FSMs.

[0012] Some of the examples of the present disclosure deliver a suite of a) microorganisms (*e.g.*, bacteria or yeast) that act as probiotics to actively remove substrates including intact dietary glycans and FSMs; b) enzymes capable of inactivating or eliminating dietary glycans and/or sugars; and/or c) binding agents that physically bind and render free sugars monomers unavailable as substrates supporting the growth of pathogenic microorganisms.

[0013] Some examples of the disclosure provide a composition administered to reduce the concentration of FSMs that may be the consequence of the use of antibiotics to treat the pathogenic bacterial overgrowth in mammals including humans.

Brief Description of the Drawings

[0014] FIG. 1: Graph showing typical piglet E. coli isolate on pig milk sugar constituent sugars vs. conjugated glycans.

CLAIMS

1. Use of at least two non-pathogenic microbes in the manufacture of a composition for improving the health of an infant mammal, wherein one of the at least two non-pathogenic microbes is from a first species capable of internalizing and/or metabolizing dietary glycans, which is *Bifidobacterium longum* subsp. *infantis*, and wherein one of the at least two non-pathogenic microbes is from a second species capable of consuming and metabolizing free sugar monomers, which is *Lactobacillus crispatus*.

2. The use according to claim 1, wherein the free sugar monomers are fragments cleaved from complex carbohydrate oligomers, which are indigestible milk oligosaccharides.

3. The use according to claim 1 or claim 2, wherein the second species is selected based on the free sugar monomers that are present or predicted to be present in an infant mammal.

4. The use according to any one of claims 1-3, wherein the free sugar monomers are present in the infant mammal, and wherein the presence is determined by measuring the free sugar monomers in a fecal sample of the infant mammal.

5. The use according to claim 4, wherein the free sugar monomer is present in an amount of at least from 1 μg/g feces to 100 μg/g feces, or at least 5 μg of free sugar monomer per gram of dry weight of feces.

6. The use according to any one of claims 4 or 5, wherein the presence of free sugar monomers in feces is measured by using an assay to determine the presence of free sugar monomers in a fecal sample.

7. The use according to claim 3, wherein the free sugar monomers are predicted to be present in an infant mammal, and wherein the presence is predicted based on a pathogenic bloom of the infant mammal.

8. The use according to claims 7, wherein the second species is selected based on the free sugar monomers that are preferred by a pathogen, and wherein the infant mammal has an increased likelihood of a pathogenic overpopulation of the pathogen.

9. The use according to claim 8, wherein the increased likelihood of a pathogenic overpopulation of the pathogen is due to an outbreak in the surroundings of the infant mammal.

10. The use according to any one of claim 7-9, wherein the pathogen is a member of the Class Clostridia or Phylum Proteobacteria.

11. The use according to claim 10, wherein the Clostridia is *C. difficile* or *C. perfringens*.

12. Use of at least two non-pathogenic microbes in the manufacture of a composition for treating a dysbiotic subject, wherein one of the at least two non-pathogenic microbes is from a first species capable of internalizing and/or metabolizing dietary glycans, which is *Bifidobacterium longum* subsp. *infantis* and wherein one of the at least two non-pathogenic microbes is from a second species capable of consuming and metabolizing free sugar monomers, which is *Lactobacillus crispatus*.

13. The use according to any one of claims 1-11, wherein the subject is an infant that is receiving dietary glycans contemporaneously with the administration of the composition, preferably a nursing infant.

14. The use according to any one of claims 1-13, wherein the *B. longum* subsp. *infantis* of the first species of non-pathogenic microbe is activated.

15. The use according to any one of claims 1-14, wherein the composition is in the form of a dry powder or a dry powder suspended in an oil.

16. The use according to claim 15, wherein the composition is spray dried or freeze-dried.

17. The use according to claim 16, wherein the composition is freeze-dried in the presence of a suitable cryoprotectant.

18. The use according to claim 17, wherein the suitable cryoprotectant is glucose, lactose, raffinose, sucrose, trehalose, adonitol, glycerol, mannitol, methanol, polyethylene glycol, propylene glycol, ribitol, alginate, bovine serum albumin, carnitine, citrate, cysteine, dextran, dimethyl sulphoxide, sodium glutamate, glycine betaine, glycogen, hypotaurine, peptone, polyvinyl pyrrolidone, or taurine.

19. The use according to any one of claims 1-18, wherein the total count of non-pathogenic microbes is from about 10 million to 100 billion cfu per gram.

20. The use according to any one of claims 1-19, further comprising about 5 to 90 % by weight dietary glycans.

21. The use according to claim 20, wherein the dietary glycans may be derived from a human, swine, or bovine source.

22. The use according to any one of claims 1-21, wherein the composition is capable of growing on dietary glycans, wherein the dietary glycans comprises sialic acid and/or fucose, and from 1-10 % of sialic acid and/or from 1-10 % of the fucose remains as free sugar monomers after a culture of the composition has ceased to grow.

23. The use according to any one of claims 1-22, wherein at least one of the non-pathogenic microbes comprises a gene coding for a sialidase or a fucosidase, preferably wherein the sialidase or fucosidase is the first species of the non-pathogenic microbe.

24. The use according to any one of claims 1-23, wherein at least one of the non-pathogenic microbes comprises a gene coding for a sialic acid or a fucose transporter, preferably wherein the sialic acid or a fucose transporter is the second species of the non-pathogenic microbe.

25. The use according to any one of claims 1-24, wherein at least one of the non-pathogenic microbes comprises a gene coding for a complex oligosaccharide transporter.