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.

**[0010]** Some embodiments 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 embodiments 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 embodiments of the present disclosure provide diagnostics for the presence of substrates enabling growth of pathogenic bacteria within mammalian neonates. Specifically, some embodiments provide diagnostics to determine the presence of FSMs.

**[0012]** Some of the embodiments 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 embodiments 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.

**[0013a]** Disclosed is a composition comprising at least two non-pathogenic microbes, wherein one of the at least two non-pathogenic microbes is from a first species capable of internalizing and/or metabolizing dietary glycans, and wherein one of the at least two non-pathogenic microbes is from a second species capable of consuming and metabolizing free sugar monomers. In one aspect, there is provided use of at least two non-pathogenic microbes in the manufacture of a composition for treating or preventing infection of an infant mammal by a pathogenic microbe, wherein one of the at least two non-pathogenic microbes is from a first species capable of internalizing and/or metabolizing dietary glycans, and wherein one of the at least two non-pathogenic microbes is from a second species capable of consuming and metabolizing free sugar monomers.

**[0013b]** Disclosed is the use of at least two non-pathogenic microbes in the manufacture of a medicament for treating or 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, and wherein one of the at least two non-pathogenic microbes is from a second species capable of consuming and metabolizing free sugar monomers, is accomplished.

**[0013c]** Disclosed is a method of detecting a dysbiotic subject comprising determining a presence of free sugar monomers in the subject's feces and reporting the presence of free sugar monomers.

**[0013d]** Disclosed is the use of non-pathogenic microbes in the manufacture of a medicament for treating a dysbiotic subject, wherein the non-pathogenic microbes are capable of metabolizing and/or sequestering the free sugar monomers detected by the method as disclosed herein.

## 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 treating or preventing infection of an infant mammal by a pathogenic microbe, wherein one of the at least two non-pathogenic microbes is from a first species capable of internalizing and/or metabolizing dietary glycans, and wherein one of the at least two non-pathogenic microbes is from a second species capable of consuming and metabolizing free sugar monomers.

2. The use according to claim 1, wherein the pathogenic microbe preferentially consumes sialic acid.

3. The use according to claim 2, wherein the pathogenic microbe is *Clostridium difficile*, or *C. perfringens*.

4. The use according to any one of claims 1-3, wherein the second non-pathogenic species is Lactobacillus plantarum, or L. crispatus, or L. gasseri, or L. salivarius.

5. The use according to any one of claims 1-4, wherein the first non-pathogenic species is *Bifidobacterium breve*, or *B. longum* subspecies. *infantis*, or *B. pseudocatenulatum*.

6. The use according to any one of claims 1-5, wherein the infant mammal is a nursing human, or a nursing foal, or a nursing piglet.

7. The use according to any one of claims 1-6, wherein the composition is to be administered to the infant mammal at a period within 96 hours of the birth of the infant mammal.

8. The use according to any one of claims 1-7, wherein the first non-pathogenic microbe is activated *B. longum* subspecies *infantis*.