[0006] The non-infant mammalian microbiome contains a complexity and diversity of species of bacteria, which develops only after the cessation of milk consumption as a sole source of nutrition. Conventional teaching with regards to the non-infant mammalian microbiome is that complexity provides stability. To be able to effectively consume the complex non-infant diet, maintaining a diversity of microorganisms in the microbiome is thought to be the key to promoting gut health. Lozupone, Nature, Vol. 489, pp. 220–230 (2012).

[0007] Treatment of any animal, including all mammals, with antibiotics has an immediate effect of altering the absolute amount and complexity of that animal's microbiome. At the cessation of the course of antibiotic treatment, the rebuilding of the microbiome may be affected by the food being eaten by that mammal and the presence of, or inoculation with, specific bacteria in the intestine. Similar wholesale changes in the microbiome are seen with the use of many chemotherapeutic drugs and therapies, such as fecal microbial transplants.

[0008] The transition from a lower diversity unstable, dysbiotic intestinal ecosystem (caused by the use of antibiotics, chemotherapeutic drugs, a change in diet, blooms of pathogenic bacteria, or the like) and the subsequent re-establishment of a complex microbiome of the gastrointestinal tract (GI tract) is a major medical and physiological challenge. This transition often results in the sporadic and damaging proliferation of normally minor bacteria, termed 'local blooms' including specific strains of bacteria such as *Salmonella*, *E. coli, Enterobacteria,* and *Clostridium* spp. These blooms of bacteria are in turn detrimental themselves to the host by causing inflammation and direct damage to mucosal cells of the GI tract (Stecher et al 2013). Such bacterial blooms in mammals, depending on the nature of the strains involved, can be reflected in symptoms such as colitis, diarrhea, colic and scours and, under some circumstances, may lead to necrosis, sepsis, and even death.

SUMMARY

[0009] The inventors have discovered that one cause of the bacterial blooms responsible for inflammation and dysbiosis that occur in the microbiome transitional stages is the direct result of the combination of indigestible components ("dietary fiber") of specific foods consumed by the 'host' that reach the large intestine and the pattern by which those food components are broken down by commensal and opportunistic bacteria present in the host's GI tract. Moreover, the inventors have discovered that a critical mechanism underlying these microbial blooms, inflammation, and the resulting dysbiosis, is the breakdown and incomplete

absorption of the complex undigested carbohydrates, proteins and peptides by resident colonic bacteria, resulting in the release of large amounts of Free Sugar Monomers (FSMs), Free Amino Acids (FAA's) and small peptides. Access to FSMs, FAA's, and peptides released into the large intestine become an enabling food source which opportunistic bacteria use as a growth substrate. This represents a mismatch between the diet and the bacteria that use those substrates. Not all bacteria in the gut are equal; they have different potentials to use and survive in a complex intestinal nutrient environment. The inventors have discovered that there are better choices for pairing bacteria and dietary fibers during weaning (transitions between new states) to minimize access to any free sugars released as a result of gut activities and any subsequent pathology.

[0010] The inventors have further discovered that different FSMs are released from the intact dietary fibers of different food sources in the colon by the action of colonic microbes. These dietary fibers are generally broken down into FSMs by the action of extracellular enzymes produced by various colonic microbes, but these microbes may or may not have the ability to utilize all of the FSMs produced by this enzymatic digestion of the complex oligosaccharides. Indeed, the inventors have discovered that different types of commensal and pathogenic bacteria in the lower GI tract, and particularly in the colon, have different and specific abilities to import and metabolize these FSMs to provide cellular energy. Finally, the inventors have discovered that, by providing specific commensal bacteria as probiotics to an individual who is adding a new source of dietary fiber to their diet, one can minimize the risk of producing blooms of pathogenic microbes that can lead to gut pain, discomfort, or changes in fecal transit times. One mechanism is through controlling the access to FSMs.

[0011] This disclosure provides a composition comprising: (i) a non-milk food, (ii) mammalian milk oligosaccharides (MMO), and (iii) a bacterial culture comprising one or more commensal bacterial species. The bacterial culture is preferably provided in a dose from 10^7 – 10^{12} cfu. Preferably, the bacterial culture is selected from *Lactobacillus*, *Pediococcus*, and/or *Bifidobacterium* species. The bifidobacteria may be selected from *B. longum* subsp. *longum, B. longum* subsp. *infantis, B. breve, Bacterium pseudocatanulatum, B. bifidum, B. adolescentis, B. pseudolongum, B. animalis (e.g., B. animalis* subsp. *animalis*, *B. animalis* subsp. *lactis*), *B. catenulatum*, and combinations thereof, the Lactobacillus may be selected from is *L. crispatus, L. casei, L. silivarius, L. antri, L. coleohominis, L. pentosus, L. sakei, L. plantarum*, and combinations thereof, and Pediococcus may be selected from *P. pentosaceus, P. stilesii, P.*

acidilacti, P. argentenicus, P. claussenii or a combinations thereof.

[0011a] In one aspect, there is provided a composition of a non-milk food comprising mammalian milk oligosaccharides (MMO) from a human, bovine or caprine source, or the MMO comprises HMO, BMO, BCO, GMO, GOS, FOS, or combinations thereof.

[0011b] In another aspect, there is provided a composition described herein, comprising a non-milk food and a bacterial culture, wherein the bacterial culture comprises commensal bacteria, the commensal bacteria optionally selected from the species of *Lactobacillus*, *Bifidobacterium*, *Pediococcus* and combinations thereof.

[0012] Non-milk food, if it contains any dietary fiber, does not provide MMO as the majority of the dietary fiber. Infant formula as marketed today would be considered a non-milk food for the purposes of this disclosure, since it does not include MMO. Preferably, the non-milk food composition contributes a controlled portion of dietary fiber to adapt to the bacterial culture. For example, the non-milk food can contribute about 50%, less than 50%, less than 40%, less than 30%, less than 20%, less than 15%, less than 10%, less than 5%, less than 2.5%, less than 1%, less than 0.5% by weight of the total dietary fiber in the diet, depending on the phase of weaning. In some embodiments, the non-milk food can contribute more than 50%, more than 55%, more than 60%, more than 70%, more than 75%, more than 80%, more than 85%, more than 90%, more than 95%, more than 99% of the dietary fiber by weight in the composition. Fiber is described herein in grams and their percentages are described herein as percent by weight.

[0013] Preferably, the milk source of the MMO is from a human, bovine, ovine, equine, or caprine source. Preferably, the MMO contributes a controlled portion of dietary fiber. For example, the MMO can contribute more than 50%, more than 55%, more than 60%, more than 70%, more than 75%, more than 80%, more than 85%, more than 90%, more than 95%, more than 99% by weight of the dietary fiber in the composition, depending on the phase of weaning. In some embodiments, the non-milk food can contribute about 50%, less than 50%, less than 40%, less than 30%, less than 20%, less than 15%, less than 10%, less than 5%, less than 2.5%, less than 1%, less than 0.5% by weight of the dietary fiber in the composition, depending on the phase of weaning.

[0014] In one embodiment, the non-milk food can contribute about 50%, less than 50%, less than 40%, less than 30%, less than 20%, less than 15%, less than 10%, less than 5%, less than 2.5%, less than 1%, less than 0.5% by weight of the dietary fiber in the composition, and the MMO can contribute more than 50%, more than 55%, more than 60%, more than 70%, more than 75%, more than 80%, more than 85%, more than 90%, more than 95%, more than 99% by weight of the dietary fiber in the composition. In another embodiment, the non-milk food can contribute more than 50%, more than 55%, more than 60%, more than 70%, more than 75%, more than 80%, more than 85%, more than 90%, more than 95%, more than 99% by weight of the dietary fiber in the composition, and the non-milk food can contribute about 50%, less than

50%, less than 40%, less than 30%, less than 20%, less than 15%, less than 10%, less than 5%, less than 2.5%, less than 1%, less than 0.5% by weight of the dietary fiber in the composition.

[0015] In one embodiment, the non-milk food may be a feed for a non-human mammal. The non-human mammal may be a buffalo, camel, rabbit, mouse, rat, pig, cow, goat, sheep, horse, dog, or cat. In some embodiments, the non-human mammal is a laboratory animal. Alternatively, the non-milk food may be a food for a human. In one embodiment, the human may be a baby in need of weaning. The mammal may be on or have just completed a course of oral antibiotics. In one embodiment, the mammal (e.g., human) is on or has just completed a course of chemotherapy, or the mammal (e.g., human) is preparing for, or has just completed, a fecal microbial transplant.

[0015a] In another aspect, there is provided a composition described herein for use in treating a subject receiving antibiotic therapy or a subject who has recently finished antibiotic therapy, or a subject receiving or who has recently received a fecal microbial transplant, wherein upon administration of said composition, the gut microbiome complexity in the subject is increased, preferably wherein the subject in need thereof is a human, and said human may not be an infant or said human may be an adult.

[0015b] In another aspect, there is provided a composition for use in treating a subject receiving antibiotic therapy or a subject who has recently finished antibiotic therapy, or a subject receiving or who has recently received a fecal microbial transplant, said composition comprising a non-milk food, MMO, and a bacterial culture, wherein the MMO may be derived from or substantially identical to human milk glycans, bovine milk glycans, or goat milk glycans or the MMO may comprise HMO, BMO, BCO, GMO, GOS, FOS, or combinations thereof, the milk glycans optionally representing at least 20% of the total daily dietary fiber of the diet, and wherein the bacterial culture may comprise bacteria from *Bifidobacteria*, *Lactobacillus*, *Pediococcus*, or combinations thereof, preferably wherein the bacterial culture comprising *B. infantis, B. breve, L. plantarium, L. reuteri,* or combinations thereof, preferably comprising *Bifidobacteria longum* subsp. *infantis*, the bacterial culture optionally being provided in a daily dose of from $10^7 - 10^{12}$ cfu, optionally wherein the subject is a human, preferably an adult human.

[0016] In another embodiment, this disclosure provides a composition comprising a non-milk baby food and MMO from a human, bovine, equine, or caprine source. Such compositions may be administered for a period to accommodate progressive change in the microbiome, with or without concurrent administration of probiotic bacteria. In a typical embodiment of this disclosure, the mammal has a microbiome in need of increasing its complexity by at least 10%, preferably by at least 20%, more preferably by at least 30% of the total bacterial species present in the gut. The phrase "increase complexity" when used herein means increasing the complexity based on taxonomic classification of the bacteria in the microbiome of the mammal, and/or increasing the complexity based on the proportional number of bacteria by classification in the microbiome of the mammal, which may be generally calculated from the amount of DNA with sequences specific to a particular genus, species, or strain normalized against the total amount of DNA sequences in stool.

[0017] In one embodiment, gut microbiome complexity of a mammal (e.g., a human infant) is increased by providing a dietary composition comprising a non-milk food, MMO, and a bacterial culture, where the MMO are from human, bovine, ovine, equine or caprine milk or the MMO are non-milk oligosaccharides substantially identical to human milk oligosaccharides, bovine milk oligosaccharides (BMO), caprine milk oligosaccharides (CMO), porcine milk oligosaccharides (PMO), equine milk oligosaccharides (EMO), and/or ovine milk oligosaccharides (OMO). The bacterial culture can be chosen from *Bifidobacterium*, *Pediococcus*, and *Lactobacillus*, which may be provided in a daily dose of from $10^7 - 10^{12}$ cfu.

[0018] In another mode, this disclosure provides methods of increasing the gut

microbiome complexity in a mammal by administering any of the compositions described herein to the mammal. In one embodiment, gut microbiome complexity of a mammal (e.g., a human infant) is increased by providing a dietary composition comprising a non-milk food and a bacterial culture, to the mammal (e.g., a human infant), where the mammal is contemporaneously receiving MMO from another source (e.g., mother's milk). The bacterial culture can be chosen from *Bifidobacterium*, *Pediococcus*, and *Lactobacillus*, which may be provided in a daily dose of from $10^7 - 10^{12}$ cfu.

[0019] In another embodiment, gut microbiome complexity is increased in a mammal during or following antibiotic therapy by providing a dietary composition comprising a non-milk food, MMO, and a bacterial culture, where the MMO are from human, bovine, ovine, equine, or caprine milk or the MMO are non-milk oligosaccharides substantially identical to HMO, BMO, CMO, EMO, and/or OMO, and the bacterial culture provides probiotic bacteria chosen from *Bifidobacterium, Pediococcus,* and *Lactobacillus*, or combinations thereof. The bacterial culture may be provided in a daily dose of from $10^7 - 10^{12}$ cfu. In any of these embodiments, at least one of the bacterial species is preferably *Bifidobacteria longum* subsp. *infantis*.

[0020] Typically, feeding the controlled diet to the mammal is continued for a period of days to weeks, for example, following the reduction in breastmilk, an increase in formula feeding, an increase in complementary foods, administration (and/or cessation) of antibiotics, administration (and/or cessation) of chemotherapy, and infusion of the fecal microbial transplant composition. Any of the embodiments described herein may include the administration of compositions of varying MMO and non-milk food dietary fibers. For example, the initial stage of administration may include a composition where the MMO provides more than 50% of the dietary fiber of the composition, and where the non-milk food provides less than 50% of the dietary fiber of the composition. A later stage of administration may include a composition where the MMO provides less than 50% of the dietary fiber of the composition, and where the non-milk food provides more than 50% of the dietary fiber of the composition.

[0020a] In another aspect, there is provided a fecal microbial transplant composition comprising a fecal microbiome from a healthy individual, milk glycan, and a bacterial culture, preferably wherein the fecal microbial transplant composition comprises 5 g of the milk glycan, and wherein the bacterial culture preferably comprises bacteria of the genus *Bifidobacteria*, *Lactobacillus*, *Pediococcus*, or combinations thereof, more preferably *Bifidobacterium infantis* subsp *infantis*, said bacterial culture optionally comprising from 1 x 10^6 to 100 x 10^9 cfu of the bacteria.

[0021] In still another mode, this disclosure provides a method of increasing the gut microbiome complexity in a human in need thereof by: (a) initiating a controlled diet comprising low fiber food and 5-40 g/day of MMO for said human for from 2-7 days prior to a fecal microbial transplant (FMT); (b) preparing a modified FMT composition comprising the fecal microbiome from a healthy individual, at least 5 g/day of MMO, and from 1-1000 x 10^8 cfu of

Bifidobacterium longum subsp. *infantis*; (c) infusing the colon of said human with the modified FMT composition; and (d) following the FMT with the controlled diet of step (a) for from 0 to 7 days. Preferably, the MMO comprise from at least from 20% to at least 70% of the total dietary oligosaccharides of the controlled diet.

[0022] In yet another mode, this disclosure provides a method of increasing the gut microbiome complexity in a human in need thereof consisting of (a) preparing a dry composition of a weaning food by cooking the food, drying the cooked food and milling the dried food to a powder usable as a weaning food; (b) growing a culture of *Bifidobacterium* and/or *Lactobacillus* which is selected from a group that consumes FSMs found in the feces of an infant fed a similar weaning food, harvesting the culture and drying the cell mass in the presence of a preservative; and (c) combining the dry composition of weaning food with the dry composition of bacterial culture in a ratio of from $10^8 - 10^{12}$ cfu of bacterial culture to 100 g of weaning food.

[0022a] In another aspect, there is provided a composition for use in treating or preventing colitis, diarrhea, colic, and/or scours in a mammal in need of weaning from a diet of mammalian milk and/or infant formula, wherein the composition comprises weaning food and a bacterial culture, and wherein the composition is in a dry powder form, wherein the bacterial culture preferably comprises bacteria from the genus *Bifidobacterium*, *Lactobacillus*, or combinations thereof, more preferably the bacterial culture comprises *B. infantis, B. breve, L. planatrum, L. reuteri*, or combinations thereof, wherein the bacterial culture may further comprise a preservative, said composition optionally comprising bacterial culture and weaning food in a ratio of from $10^6 - 10^{11}$ cfu of bacterial culture to 100 g of weaning food.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] FIG. 1: Chart showing the base 10 log change in *B. infantis* levels by day during mucosal healing diet including BMO, GOS, and *B. infantis*. The data are reported as CFU *B. infantis* per ug DNA divided by CFU total bacteria per ug DNA.

[0024] FIG. 2A: A plot showing the addition of *B. infantis* to a breast-fed infant. *B. infantis* was provided contemporaneously with breast milk to establish the dominance of *B. infantis* in the infant gut. The *B. infantis* levels were maintained by keeping the majority of fiber coming from human milk. Complementary foods were introduced at low levels during this time period.

[0025] FIG. 2B: A plot showing the addition of *B. infantis* to a breast-fed infant. *B. infantis* was provided contemporaneously with breast milk to establish the dominance of *B. infantis* in the infant gut. The *B. infantis* levels were maintained by keeping the majority of fiber coming from human milk. Complementary foods were introduced at low levels during this time period.

[0026] FIG. 3A: A plot showing the same introduction of *B. infantis* to the infant as in Figures 2A-B. *B. infantis* was provided contemporaneously with breast milk to establish the dominance of *B. infantis* in the infant gut. However, this infant switched their diet to a non-milk food, infant formula with and without low-level complementary feeding. The overall effect was a net reduction in MMO and a decrease in the abundance of *B. infantis* at later time points.

CLAIMS

1. A composition of a non-milk food comprising mammalian milk oligosaccharides (MMO) from a human, bovine or caprine source, or the MMO comprises HMO, BMO, BCO, GMO, GOS, FOS, or combinations thereof.

2. The composition of claim 1, wherein the dietary fiber in the composition comprises at least 20% of MMO, at least 30% of MMO, at least 50% of MMO, at least 60% of MMO, at least 70% of MMO, or wherein less than 10% of the dietary fiber in the composition is provided by the non-milk food.

3. The composition of any one of claims 1–2, wherein the non-milk food is a feed for a non-human mammal, preferably wherein the non-human mammal is a pig, cow, goat, sheep, horse, dog, or cat.

4. The composition of any one of claims 1–2, wherein the non-milk food is a food for a human, preferably wherein said human is a baby, optionally wherein the human has a microbiome in need of increasing its complexity by at least 10%, by at least 20%, or by at least 30%.

5. The composition of claim 4, wherein the human is receiving or has just completed a course of oral antibiotics, or wherein the human is receiving or has just completed a course of chemotherapy, or wherein the human is receiving or has just completed a fecal microbial transplant.

6. The composition of any one of claims 1–5 wherein the non-milk food comprises complex oligosaccharides from meat, fish, eggs, shellfish, fruits, vegetables, grains, nuts, seeds, or combinations thereof, and/or the non-milk food comprises complex oligosaccharides from barley, corn, hummus, lentils, oats, rice, wheat, avocado, beetroot, broccoli, squash, carrots, green beans, peas, potatoes, cassava, sweet potatoes, pumpkin, apples, bananas, plantains, blueberries, mango, peach, pear, papaya, watermelon, or combinations thereof.

7. A composition of any one of claims 1-6, comprising a non-milk food and a bacterial culture, wherein the bacterial culture comprises commensal bacteria, the commensal bacteria optionally selected from the species of *Lactobacillus*, *Bifidobacterium*, *Pediococcus* and combinations thereof.

8. The composition of any one of claims 1-7, wherein the *Bifidobacteria* is selected from *B. longum* subsp *longum, B. longum* subsp *infantis, B. breve, Bacterium pseudocatanulatum, B. bifidum, B. adolescentis, B. pseudolongum, and B. animalis*, and/or the *Lactobacillus* is selected from *L. crispatus, L. casei, L. silivarius, L. reuteri*, and *L. plantarum*, and/or the *Pediococcus* is selected from *P. pentosaceus, P. stilesii, P. acidilacti, P. argentenicus,* and *P. claussenii*.

9. The composition of any one of claims 7-8, wherein the bacterial culture is provided in a dose from $10^7 - 10^{12}$ cfu.

10. A composition of any one of claims 1–9 for use in treating a subject receiving antibiotic therapy or a subject who has recently finished antibiotic therapy, or a subject receiving or who has recently received a fecal microbial transplant, wherein upon administration of said composition, the gut microbiome complexity in the subject is increased, preferably wherein the subject in need thereof is a human, and said human may not be an infant or said human may be an adult.

11. A composition for use in treating a subject receiving antibiotic therapy or a subject who has recently finished antibiotic therapy, or a subject receiving or who has recently received a fecal microbial transplant, said composition comprising a non-milk food, MMO, and a bacterial culture, wherein the MMO may be derived from or substantially identical to human milk glycans, bovine milk glycans, or goat milk glycans or the MMO may comprise HMO, BMO, BCO, GMO, GOS, FOS, or combinations thereof, the milk glycans optionally representing at least 20% of the total daily dietary fiber of the diet, and wherein the bacterial culture may comprise bacteria from *Bifidobacteria*, *Lactobacillus*, *Pediococcus*, or combinations thereof, preferably wherein the bacterial culture comprising *B. infantis, B. breve, L. plantarium, L. reuteri,* or combinations thereof, preferably comprising *Bifidobacteria longum* subsp. *infantis*, the bacterial culture optionally being provided in a daily dose of from $10^7 - 10^{12}$ cfu, optionally wherein the subject is a human, preferably an adult human.

12. A fecal microbial transplant composition comprising a fecal microbiome from a healthy individual, milk glycan, and a bacterial culture, preferably wherein the fecal microbial transplant composition comprises 5 g of the milk glycan, and wherein the bacterial culture preferably comprises bacteria of the genus *Bifidobacteria*, *Lactobacillus*, *Pediococcus*, or

combinations thereof, more preferably *Bifidobacterium infantis* subsp *infantis*, said bacterial culture optionally comprising from 1 x 10^6 to 100 x 10^9 cfu of the bacteria.

13. The composition of claim 12, wherein the subject in need thereof has been given a controlled diet prior to the fecal microbial transplant, wherein the controlled diet may comprise low-fiber food and milk glycan, preferably wherein the controlled diet comprises 1–30 g per day of the milk glycan, optionally wherein the controlled diet has been administered to said subject for a period of from at least 1 to 7 days prior to the fecal microbial transplant, preferably wherein the milk glycan comprises from at least 20% to at least 70% of the total dietary glycans of the controlled diet.

14. A composition for use in treating or preventing colitis, diarrhea, colic, and/or scours in a mammal in need of weaning from a diet of mammalian milk and/or infant formula, wherein the composition comprises weaning food and a bacterial culture, and wherein the composition is in a dry powder form, wherein the bacterial culture preferably comprises bacteria from the genus *Bifidobacterium*, *Lactobacillus*, or combinations thereof, more preferably the bacterial culture comprises *B. infantis, B. breve, L. planatrum, L. reuteri*, or combinations thereof, wherein the bacterial culture may further comprise a preservative, said composition optionally comprising bacterial culture and weaning food in a ratio of from $10^6 - 10^{11}$ cfu of bacterial culture to 100 g of weaning food.

15. The composition of claim 14, wherein the weaning food comprises meat, fish, eggs, shellfish, fruits, vegetables, grains, nuts, seeds, dairy, or combinations thereof, preferably wherein the weaning food comprises barley, corn, hummus, lentils, oats, rice, wheat, avocado, beetroot, broccoli, squash, carrots, green beans, peas, potatoes, cassava, sweet potatoes, pumpkin, apples, bananas, plantains, blueberries, mango, peach, pear, papaya, watermelon, cheese, or combinations thereof.