Title: MILK OLIGOSACCHARIDE COMPOSITIONS AND USE THEREOF IN TREATING INFECTION IN ANIMALS

Abstract: Animal food composition or veterinary pharmaceutical composition containing one or more oligosaccharides derived from milk, or a glycoconjugate including the oligosaccharide(s). Also disclosed are uses thereof for treating infected animals.
Milk Oligosaccharide Compositions and
Use thereof in Treating Infection in Animals

RELATED APPLICATION

This application claims priority to U.S. Provisional Application No. 61/168,674, filed on April 13, 2009, the content of which is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

Human infants are vulnerable to pathogens due to their immature immune systems. They rely heavily on human milk for protection against infections. It is known that human milk contains various components conferring immunologic benefits. Young animals, on the other hand, often do not receive the same kinds of immune protection from the milk of their mothers. Indeed, non-mammals do not normally consume milk during their infancy.

Like in humans, infection is a serious health problem in animals (especially livestock), resulting in enormous economic consequences. There is a need to develop a method for controlling infection in animals.

SUMMARY OF THE INVENTION

In one aspect, the present invention features a food composition for animal consumption, which includes an animal foodstuff and a milk-derived oligosaccharide or a glycoconjugate containing the oligosaccharide. The oligosaccharide, preferably derived from human milk, contains a first sugar unit (i.e., a fucose, a galactose, a mannose, or a sialic acid) linked to a second sugar unit (i.e., a galactose, a glucose, a mannose, or an N-acetylglucosamine). The first sugar unit is located at a non-reducing end of the oligosaccharide. In one example, the oligosaccharide is a linear molecule having one non-reducing end and one reducing end. In another example, it is a branched molecule having multiple non-reducing ends and one reducing end. When the oligosaccharide has two non-reducing ends, the sugar unit at one non-reducing end can be fucose and that at the other non-reducing end can be fucose, galactose, or sialic acid, or alternatively, the sugar unit at one non-reducing end is sialic acid and that at the other non-reducing end is galactose or sialic acid. The sugar unit at the reducing end can be a glucose or an N-acetylglucosamine.

In one example, the composition of this invention contains two or more different
milk-derived oligosaccharides as described above. In one example, all of the oligosaccharides are attached to a backbone molecule (e.g., a lipid, a peptide, or a carbohydrate) to form a glycoconjugate.

In another aspect, this invention features a veterinary pharmaceutical composition (i.e., a pharmaceutical composition formulated for animal use) containing any of the above mentioned milk-derived oligosaccharides or glycoconjugates and a pharmaceutically acceptable carrier.

In yet another aspect, the present invention features a method for treating infection in an animal that needs the treatment by administering to the animal an effective amount of one or more of the above-described milk-derived oligosaccharides or glycoconjugates. The infection to be treated by this method can be caused by a bacterium, a fungus (e.g., yeast), a protozoan, or a virus. Examples of infectious microbes include, but are not limited to, *Campylobacter, Clostridium, Escherichia, Streptococcus, Helicobacter, Mycobacterium*, pathogenic *Bacteriodes, Vibrio, Candida, Astrovirus, Herpesvirus, Influenza, Norovirus*, and *Rotavirus*. The term "animal" refers to non-human vertebrates (e.g., mammals, birds, fishes, reptiles, and amphibians), including both young and adult ones. Examples include, but are not limited to, cat, cattle, cow, dog, goat, horse, pig, rabbit, rodent, mink, sheep, chicken, duck, goose, turkey, ostrich, emu, swan, peafowl, pheasant, partridge, and guineafowl.

The details of one or more examples of the invention are set forth in the description below. Other features or advantages of the present invention will be apparent from the following detailed description of several embodiments, and also from the appended claims.

**BRIEF DESCRIPTION OF THE DRAWINGS**

The drawing is first described:

Fig. 1 is a chart showing the inhibitory effects of human milk (5 g/L), skim human milk (5 g/L), total proteins from human milk (5 g/L), and oligosaccharides from human milk (HMO, 5 g/L) on *Clostridium perfringens* growth.
Detailed Description of the Invention

Disclosed herein is a milk-derived oligosaccharide effective in treating infection in an animal. A milk-derived oligosaccharide, i.e., having at least three sugar units, is either a naturally-occurring oligosaccharide found in milk, a fragment of the naturally-occurring oligosaccharide, or a variant thereof that contains a modified (e.g., a sulfated, acetylated, or phosphorylated) sugar unit. This oligosaccharide includes a non-reducing end motif SiS₂, in which Si is fucose, galactose, mannose, or sialic acid (N-acetyl or N-glycolyl) and S₂ is galactose, glucose, mannose, or N-acetylglucosamine. Si is linked to S₂ via an α or β glycosidic bond. When Si is fucose, the glycosidic bond between Si and S₂ preferably is an α1,2, an α1,3, or an α1,4 bond. When it is sialic acid, the glycosidic bond preferably is an α2,3 or an α2,6 bond.

The following tables list exemplary oligosaccharides that naturally occur in human milk:

Table 1. Fucosyl oligosaccharides

<table>
<thead>
<tr>
<th>Oligosaccharide</th>
<th>Structural Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>2'FL</td>
<td>Fucα1,2Galβ1,4Glc</td>
</tr>
<tr>
<td>LNF-I</td>
<td>Fucα1,2Galβ1,3GlcNAcβ1,3Galβ1,4Glc</td>
</tr>
<tr>
<td>LNF-II</td>
<td>Galβ1,3,4GalNAcβ1,3Galβ1,4Glc</td>
</tr>
<tr>
<td>3FL</td>
<td>Galβ1,4,6Glc</td>
</tr>
<tr>
<td>LNF-III</td>
<td>Galβ1,4,6GlcNAcβ1,3Galβ1,4Glc</td>
</tr>
<tr>
<td>LDFH-I</td>
<td>Fucα1,2Galβ1,3,4GalNAcβ1,3Galβ1,4Glc</td>
</tr>
<tr>
<td>LDFT</td>
<td>Fucα1,2Galβ1,4Glc</td>
</tr>
</tbody>
</table>

- 3 -
Table 2. Nonfucosylated, nonsialylated oligosaccharides

<table>
<thead>
<tr>
<th>LNT</th>
<th>Lacto-(\text{-}N)-tetraose</th>
<th>(\text{Gal}\beta1,3\text{GlcNAc}\beta1,3\text{Gal}\beta1,4\text{Glc})</th>
</tr>
</thead>
<tbody>
<tr>
<td>LNneoT</td>
<td>Lacto-(\text{-}N)-neotetraose</td>
<td>(\text{Gal}\beta1,4\text{GlcNAc}\beta1,3\text{Gal}\beta1,4\text{Glc})</td>
</tr>
</tbody>
</table>

Table 3. Sialyl milk oligosaccharide structures

<table>
<thead>
<tr>
<th>3'-'SL</th>
<th>3'-Sialyllactose</th>
<th>NANA(\alpha),2Gal(\beta),3Gal(\beta),14Glc</th>
</tr>
</thead>
<tbody>
<tr>
<td>6'-'SL</td>
<td>6'-Sialyllactose</td>
<td>NANA(\alpha),2,6Gal(\beta),1,4Glc</td>
</tr>
<tr>
<td>SLNT-c</td>
<td>Sialyllacto-(\text{-}N)-neotetraose c</td>
<td>NANA(\alpha),2,6Gal(\beta),1,4GlcNAC(\beta),1,3Gal(\beta),1,4Glc</td>
</tr>
<tr>
<td>MSLNH</td>
<td>Monosialyllacto-(\text{-}N)-hexaose</td>
<td>NANA(\alpha),2,6Gal(\beta),1,4GlcNAC(1,6)Gal(\beta),1,4Glc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gal(\beta),1,3GlcNAC(1,3)Gal(\beta),1,4Glc</td>
</tr>
<tr>
<td>DSLNH-I</td>
<td>Disialyllacto-(\text{-}N)-hexaose I</td>
<td>NANA(\alpha),2,3Gal(\beta),1,3GlcNAC(1,3)Gal(\beta),1,4Glc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NANA(\alpha),2,6Gal(\beta),1,4GlcNAC(1,6)Gal(\beta),1,4Glc</td>
</tr>
<tr>
<td>MSLNh-I</td>
<td>Monosialyllacto-(\text{-}N)-neohexaose I</td>
<td>NANA(\alpha),2,6Gal(\beta),1,3GlcNAC(1,3)Gal(\beta),1,4Glc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gal(\beta),1,4GlcNAC(1,6)Gal(\beta),1,4Glc</td>
</tr>
<tr>
<td>SLNh-II</td>
<td>Monosialyllacto-(\text{-}N)-neohexaose II</td>
<td>Gal(\beta),1,4GlcNAC(1,3)Gal(\beta),1,4Glc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NANA(\alpha),2,6Gal(\beta),1,4GlcNAC(1,6)Gal(\beta),1,4Glc</td>
</tr>
<tr>
<td>DSLNh</td>
<td>Disialyllacto-(\text{-}N)-neohexaose</td>
<td>NANA(\alpha),2,6Gal(\beta),1,4GlcNAC(1,3)Gal(\beta),1,4Glc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NANA(\alpha),2,6Gal(\beta),1,4GlcNAC(1,6)Gal(\beta),1,4Glc</td>
</tr>
<tr>
<td>DSLNT</td>
<td>Disialyllacto-(\text{-}N)-tetraose</td>
<td>NANA(\alpha),2,6Gal(\beta),1,4GlcNAC(1,3)Gal(\beta),1,4Glc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NANA(\alpha),2,3Gal(\beta),1,3Gal(\beta),1,4Glc</td>
</tr>
<tr>
<td>DSLNH-II</td>
<td>Disialyllacto-(\text{-}N)-hexaose II</td>
<td>NANA(\alpha),2,6Gal(\beta),1,4GlcNAC(1,3)NANA(\alpha),2,3Gal(\beta),1,3Gal(\beta),1,4Glc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gal(\beta),1,4GlcNAC(1,6)Gal(\beta),1,4Glc</td>
</tr>
<tr>
<td>SLNT-a</td>
<td>Sialyllacto-(\text{-}N)-tetraose a</td>
<td>NANA(\alpha),2,3Gal(\beta),1,3GlcNAC(1,3)Gal(\beta),1,4Glc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gal(\beta),1,4GlcNAC(1,6)Gal(\beta),1,4Glc</td>
</tr>
<tr>
<td>DSLNH-I</td>
<td>Disialyllacto-(\text{-}N)-hexaose I</td>
<td>NANA(\alpha),2,3Gal(\beta),1,3GlcNAC(1,3)Gal(\beta),1,4Glc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NANA(\alpha),2,6Gal(\beta),1,4GlcNAC(1,6)Gal(\beta),1,4Glc</td>
</tr>
<tr>
<td>SLNT-b</td>
<td>Sialyllacto-(\text{-}N)-tetraose b</td>
<td>NANA(\alpha),2,6Gal(\beta),1,4GlcNAC(1,3)Gal(\beta),1,4Glc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gal(\beta),1,3)Gal(\beta),1,4Glc</td>
</tr>
</tbody>
</table>
Table 4. Sialyl fucosyl oligosaccharides

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>3'-S-3FL</td>
<td>3'-Sialyl-3-fucosyllactose</td>
<td>NANA(\alpha)2,3Gal(\beta)1,4Glc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fuc(\alpha)1,3(\alpha)</td>
</tr>
<tr>
<td>DSFLNH</td>
<td>Disialomonofucosyllacto-N-neohexaose</td>
<td>NANA(\alpha)2,6Gal(\beta)1,4Glc(\alpha)Acβ1,6(\beta)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fuc(\alpha)1,3(\alpha)</td>
</tr>
<tr>
<td>MFMSLNO</td>
<td>Monofucosylymonosialyllacto-N-octaose (sialyl Lea)</td>
<td>Gal(\beta)1,4Glc(\alpha)Acβ1,3Gal(\beta)1,4Glc(\alpha)Acβ1,6(\beta)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gal(\beta)1,4Glc (\alpha)AcN(\beta)1,3(\alpha)Acβ1,3(\alpha)</td>
</tr>
<tr>
<td>SLNFH-II</td>
<td>Sialyllacto-N-fucohexaose II</td>
<td>NANA(\alpha)2,3Gal(\beta)1,3Glc(\alpha)Acβ1,3Gal(\beta)1,4Glc</td>
</tr>
<tr>
<td>DSNFII</td>
<td>Disialyllacto-N-fucopentaose II</td>
<td>NANA(\alpha)2,6(\alpha)Acβ1,3Gal(\beta)1,3Glc(\alpha)Acβ1,3Gal(\beta)1,4Glc</td>
</tr>
<tr>
<td>MFNLNT</td>
<td>Monofucosylsialyllacto-N-tetraose</td>
<td>NANA(\alpha)2,6(\alpha)Acβ1,3Gal(\beta)1,3Glc(\alpha)Acβ1,3Gal(\beta)1,4Glc</td>
</tr>
</tbody>
</table>

The milk-derived oligosaccharide described herein can be prepared by conventional methods, e.g., synthesized chemically, purified from milk, or produced in a microorganism. See WO2005/055944. Their anti-infection properties can be confirmed by methods also known in the art. See, e.g., WO2005/055944.

The milk-derived oligosaccharide can be linked to a backbone molecule (e.g., a carbohydrate, a lipid, a nucleic acid, or a peptide) directly or via a linker to form a glycoconjugate. As used herein, "glycoconjugate" refers to a complex containing a sugar moiety associated with a backbone moiety. The sugar and the backbone moieties can be associated via a covalent or noncovalent bond, or via other forms of association, such as entrapment (e.g., of one moiety on or within the other, or of either or both entities on or within a third moiety). The glycoconjugate described herein can contain one type of milk-derived oligosaccharide (i.e., one or more copies of a milk-derived oligosaccharide attached to one backbone molecule). Alternatively, the glycoconjugate contains multiple types of milk-derived oligosaccharides. In one example, the milk-derived oligosaccharide (e.g., lacto-N-fucopentaose I, 2-fucosyllactose, lacto-N-difucohexaose I, lactodifucotetraose, or an acetylated variant thereof) is covalently linked via its reducing end sugar unit to a lipid.
a protein, a nucleic acid, or a polysaccharide. Preferably, the reducing end sugar unit is N-acetylglucosamine.

Peptide backbones suitable for making the glycoconjugate described above include those having multiple glycosylation sites (e.g., asparagine, lysine, serine, or threonine residue) and low allergenic potential. Examples include, but are not limited to, amylase, bile salt-stimulated lipase, casein, folate-binding protein, globulin, gluten, haptocorrin, lactalbumin, lactoferrin, lactoperoxidase, lipoprotein lipase, lysozyme, mucin, ovalbumin, and serum albumin. Typically, a milk-derived oligosaccharide can be covalently attached to a serine or threonine residue via an 0-linkage or attached to an asparagine residue via an N-linkage. To form these linkages, the sugar unit at the reducing end of the oligosaccharide is preferably an acetylated sugar unit, e.g., N-acetylagalactosamine, N-acetylgalactosamine, and N-acetylmannosamine. An oligosaccharide can be attached to a peptide (e.g., a protein) using standard methods. See, e.g., McBroom et al, Complex Carbohydrates, Part B, 28:212-219, 1972; Yariv et al, Biochem J, 85:383-388, 1962; Rosenfeld et al, Carbohydr. Res., 46:155-158, 1976; and Pazur, Adv. Carbohydr. Chem, Biochem., 39:405-447, 1981.

In one example, a milk-derived oligosaccharide is linked to a backbone molecule via a linker. Exemplary linkers are described in WO2005/055944. The oligosaccharide can be bonded to a linker by an enzymatic reaction, e.g., a glycosyltransferase reaction. A number of glycosyltransferases, including fucosyltransferases, galactosyltransferases, glucosyltransferases, mannosyltransferases, galactosaminyltransferases, sialyltransferases and N-acetylglucosaminyltransferases, can be used to make the glycoconjugate described herein. More details about these glycosyltransferases can be found in U.S. Patent Nos: 6,291,219; 6,270,987; 6,238,894; 6,204,431; 6,143,868; 6,087,143; 6,054,309; 6,027,928; 6,025,174; 6,025,173; 5,955,282; 5,945,322; 5,922,540; 5,892,070; 5,876,714; 5,874,261; 5,871,983; 5,861,293; 5,859,334; 5,858,752; 5,856,159; and 5,545,553.

One or more of the above described milk-derived oligosaccharides and/or glycoconjugates can be mixed with an animal foodstuff to form a food composition (which is not naturally occurring) for animal consumption. The term "an animal foodstuff" used herein refers to a food product or food supplement for animal consumption, not for human consumption. In one example, the animal foodstuff is a commercially available animal food, e.g., those provided by Purina Mills. The animal food composition, which may be free of a
milk product or milk by-product, can be in any suitable form, such as a biscuit, a cracker, a
gel, a granule, a kibble, a liquid, a nugget, a paste, a pellet, a powder, or a syrup.

One or more of the milk-derived oligosaccharides and/or glycoconjugates described
herein can also be mixed with a pharmaceutically acceptable carrier to form a veterinary
pharmaceutical composition. Optionally, the pharmaceutical composition further contains
one or more additional therapeutic agents, e.g., an analgesic, an antibiotic, an
anti-inflammatory agent, or a probiotic. An "acceptable carrier" is compatible with the
active ingredient(s) of the composition, i.e., the milk-derived oligosaccharide(s) or the
glycoconjugate(s), preferably capable of stabilizing the active ingredient(s), and not
deleterious to the animal to be treated. Suitable carriers include microcrystalline cellulose,
mannitol, glucose, polyvinylpyrrolidone, and starch, or a combination thereof. Methods for
preparing a veterinary pharmaceutical composition are known in the art. See e.g., US Patent
No. 5,958,464.

A veterinary pharmaceutical composition can be administered buccally, nasally,
oraly, parenterally, rectally, topically, vaginally, or via an implanted reservoir, inhalation
spray, or direct infusion into the GI tract or stomach.

A veterinary pharmaceutical composition for oral administration can be any orally
acceptable dosage form including, but not limited to bolus, capsule, dispersion, electuary,
emulsion and aqueous suspension, gel, granule, paste, pellet, powder, slurry, solution, syrup,
and tablet. An orally administered veterinary composition can include binders, lubricants,
inert diluents, lubricating, surface active or dispersing agents, flavoring agents, and
humectants. In the case of a tablets/capsules, carriers which are commonly used include
lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically
added. Tablets/capsules may optionally be coated or formulated so as to provide sustained,
delayed, or controlled release of the active ingredient therein. When aqueous suspensions or
emulsions are administered orally, the active ingredient can be suspended or dissolved in an
oily phase combined with emulsifying or suspending agents. A nasal aerosol or inhalation
composition can be prepared according to techniques well known in the art of pharmaceutical
formulation.

The particular formulation and dosage depends on the animal species; the
administration route; the characteristics of the formulation; age/size of the animal; and the
nature of the animal's infection, if any. In some cases, the dosage will be at a concentration
similar to that found for similar oligosaccharides present in human breast milk.

Also disclosed herein is a method of using the milk-derived oligosaccharide and the glycoconjugate for treating animal infection, i.e., a detrimental colonization of a microbe in a host animal, resulting in an illness in the animal. The term "treating" used herein refers to the application or administration of a composition including one or more active agents to an animal, who has infection, a symptom of the infection, or a predisposition toward the infection, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the infection, the symptoms of the infection, or the predisposition toward the infection. To treat infection, an effective amount of the milk-derived oligosaccharide or the glycoconjugate can be administered to an animal that is infected by a pathogenic microbe or at risk for infection via conventional routes. An "effective amount" is the amount of each active agent required to confer therapeutic effect on the animal, either alone or in combination with one or more other active agents. Effective amounts vary, as recognized by those skilled in the art, depending on route of administration, excipient usage, and co-usage with other active agents.

The method of this invention can be used for treating infections caused by various pathogens in various types of animals. Exemplary pathogens include, but are not limited to, *Actinobacillus*, *Adenovirus*, *Aeromonas*, *Arenavirus* (e.g., *Lassa* virus), *Aspergillus*, *Astrovirus*, *Bacillus*, pathogenic *Bacteroides*, *Balantium*, *Bovine* virus diarrhea-mucosal disease virus, *Bovine* viral diarrhea virus 2, *Brucella*, *Caliciviruses* (including *Vesivirus*, *Lagovirus*, and *Norovirus*, e.g. Norwalk Virus), *Campylobacter* (e.g., *C. jejuni*, *C. pylori*, *C. coli*, *C. lari*, and *C. upsaliensis*), *Candida*, Classical Swine Fever Virus, *Chlamydia*, *Clostridium*, *Coccidia*, *Coronavirus*, *Cryptosporidium*, *Echoviruses*, *Eimeria*, *Enterococcus*, *Enterovirus*, *Escherichia* (e.g., *EHEC*, *EPEC*, *ETEC*, and *STEC* strains), Foot-and-mouth disease virus, *Francisella*, *Giardia*, *Haemophilus* (e.g., *H. influenzae*), *Helicobacter*, *Hepatitis virus* (A and E), *Herpesvirus* (e.g., *Herpes* spp.), *Histoplasma*, *Influenza*, *Leptospira*, *Listeria*, *Lymphocytic Choriomeningitis Virus*, *Microsporidia*, *Mycobacterium* (e.g., *M. tuberculosis*), *Parvovirus*, *Polyomavirus*, *Poxvirus*, *Proteus*, *Prototheca*, *Psuedomonas*, *Reovirus*, *Rinderpest virus*, *Rotavirus*, *Salmonella*, *Sarcocystis*, *Shigella*, *SIV*, *Streptococcus* (e.g, Group B *Streptococcus*), *Toxoplasma*, *Vesicular stomatitis virus*, *Vibrio* (e.g., *V. cholerae*), and *Yersinia*.

Animals that can be treated by the method include cat, dog, marsupial, primate, rabbit,
rodent (e.g., chinchilla, gerbil, guinea pig, mouse, and rat), ungulate (for example, bovid (e.g., African Buffalo, bison, cow, goat, ox, sheep, water buffalo, or yak), camelid (e.g., alpaca, camel, and llama), deer, equine (e.g., donkey, horse, and mule), and pig), weasel (e.g., ermine, ferret, mink, and weasel), chicken, dove, duck, finch, goose, guinea fowl, hummingbird, parrot (e.g., cockatiel, cockatoo, lorikeet, lovebird, macaw, parakeet), pheasant (e.g., partridge, pheasant, peafowl, and quail), pigeon, raptor, ratite (e.g., emu, kiwi, ostrich, and rhea), swan, turkey, anchovy, barramundi, bass, catfish, cod, cyprinid (e.g., carp, goldfish, koi, and minnow), eel, flounder, herring, mackerel, mullet, perch, pollack, salmon, sardine, sturgeon, tilapia, trout, tuna, frog, toad, and reptile (e.g., crocodilian, lizard, snake, and turtle).

An animal in need of the treatment can be fed on the oligosaccharide/glycoconjugate together with water or its daily food (e.g., fruit, raw meat, hay, or a commercial animal food). For example, when a livestock animal (e.g., cow, goat, horse, pig, and sheep) is to be treated, the oligosaccharide can be mixed with fodder (e.g., alfalfa, barley, corn, grain, grass, hay, legume, millet, nut, oat, rice, rye, seaweed, seed, sorghum, soybean, straw, and wheat) and provided during regular feedings. Alternatively, the animal is fed with the animal food compositions described above or administered with the pharmaceutical composition also described above via conventional routes.

To reduce the risk of contracting an infection in an animal, the milk-derived oligosaccharide or glycoconjugate is preferably co-used with a probiotic (i.e., a dietary supplements of a live bacterium or yeast that benefits the animal to be treated) or a prebiotic (i.e., a non-digestible food ingredient that beneficially affects an animal to be treated by selectively stimulating the growth and/or activity of beneficial bacteria in the digestive tract). Prebiotics are typically oligosaccharides, including fructooligosaccharides, xylooligosaccharides, polydextrose, galactooligosaccharides, and mannooligosaccharides.

Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present invention to its fullest extent. The example provided below, showing the inhibitory effect of oligosaccharides of human milk against C. perfringens proliferation, is therefore to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All publications cited herein are incorporated by reference.

Anaerobic batch cultures were used to study the effect of human milk
oligosaccharides on growth of *C. perfringens*, a pathogen capable of causing severe infections in farm animals (e.g., cow, pig, horse, poultry such as chicken, ostrich, and emu, sheep, rabbit, goat, hog, cattle, mink), birds, dogs, and cats, etc..

Briefly, *C. perfringens* cells were cultured in a medium (10 ml) in the presence of (a) whole human milk, (b) skim human milk, (c) total proteins isolated from human milk, or (d) oligosaccharides purified from human milk. The oligosaccharides and other milk fractions were prepared following the method described in Newburg *et al.*, *Journal of Infectious Diseases* 1990; 162: 1075-1080, and Yolken *et al.*, *Journal of Clinical Investigation* 1992; 90: 1984-1991.

*C. perfringens* cells, cultured in the same medium without any carbohydrate addition, were used as a blank control.

Twenty-four hours later, the cells in each group were collected and subjected to a routine procedure (see, e.g., Henriksen, *et al.*, *Avian Dis* 2009; 53: 441-8 and Wise *et al.*, *Appl Environ Microbiol* 2005; 71: 391 1-6) to determine the copy number of the 16S rRNA gene, which is used as a measure of the number of *C. perfringens* cells. As shown in Fig. 1, the copy number of the 16S rRNA gene in the *C. perfringens* cells incubated with whole human milk, skim human milk, or human milk oligosaccharides were much lower than that in the *C. perfringens* cells incubated with human milk proteins or in the control cells. This indicates that the *C. perfringens* cells treated with whole human milk, skim human milk, or human milk oligosaccharides multiplied much less than those untreated or treated with human milk proteins. In sum, the result obtained from this study demonstrates that human milk fractions containing oligosaccharides and the isolated oligosaccharides per se are effective in suppressing *C. perfringens* growth. Thus, human milk oligosaccharides are effective in treating infections caused by *C. perfringens*. 
OTHER EMBODIMENTS

All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, other embodiments are also within the claims.
What is claimed is:

1. A food composition for animal consumption, comprising an animal foodstuff, and a first milk-derived oligosaccharide or a glycoconjugate containing the first oligosaccharide, the first oligosaccharide including a first sugar unit linked to a second sugar unit, wherein the first sugar unit, located at a first non-reducing end, is fucose, galactose, mannose, or sialic acid and the second sugar unit is galactose, glucose, mannose, or N-acetylglucosamine.

2. A veterinary pharmaceutical composition, comprising a first milk-derived oligosaccharide or a glycoconjugate containing the first oligosaccharide, the first oligosaccharide including a first sugar unit linked to a second sugar unit, wherein the first sugar unit, located at a non-reducing end, is fucose, galactose, mannose, or sialic acid and the second sugar unit is galactose, glucose, mannose, or N-acetylglucosamine and a pharmaceutically acceptable carrier; wherein the pharmaceutical composition is formulated for animal use.

3. The composition of claim 1 or 2, wherein the first oligosaccharide is derived from human milk.

4. The composition of claim 3, wherein the first sugar unit is fucose or sialic acid.

5. The composition of claim 4, wherein the first oligosaccharide is 2'-fucosyllactose, lacto-iV-fucopentaose I, 3'-sialyllactose, 6'-sialyllactose, sialyllacto-N-neotetraose c, sialyllacto-iV-tetraose a, or a variant thereof that is identical to one of the oligosaccharides except that the glucose at the reducing end is replaced with N-acetylglucosamine.
6. The composition of claim 3 or claim 4, wherein the first oligosaccharide includes a second non-reducing end.

7. The composition of claim 6, wherein the sugar unit at the second non-reducing end is selected from the group consisting of fucose, galactose, and sialic acid.

8. The composition of claim 7, wherein the first oligosaccharide is lacto-N-difucohexaose I, lactodifucoetraose, lacto-TV-fucopentaose II, 3-fucosyllactose, lacto-N-fucopentaose III, 3'-sialyl-3-fucosyllactose, disialomonofucosyllacto-iV-neohexaose, monofucosylmonosialyllacto-iV-octaose, sialyllacto-iV-fucohexaose II, disialyllacto-iV-fucopentose II, monofucosyl disialyllacto-iV-tetraose, monosialyllacto-iV-hexaose, monosialyllacto-iV-neohexaose I, monosialyllacto-iV-neohexaose II, sialyllacto-iV-tetraose b, disialyllacto-iV-hexaose I, disialyllacto-iV-neohexaose, disialyllacto-iV-tetraose, disialyllacto-iV-hexaose II, or a variant thereof that is identical to one of the oligosaccharides except that the glucose at the reducing end is replaced with N-acetylglucosamine.

9. The composition of claim 1 or 2, further comprising a second milk-derived oligosaccharide including a first sugar unit linked to a second sugar unit, the first sugar unit, located at a first non-reducing end, being fucose, galactose, mannose, or sialic acid and the second sugar unit being galactose, glucose, mannose, or N-acetylglucosamine, wherein the second oligosaccharide is different from the first oligosaccharide.

10. The composition of claim 9, wherein the second oligosaccharide is 2'-fucosyllactose, lacto-JV-fucopentaose I, 3'-sialyllactose, 6'-sialyllactose, sialyllacto-iV-neotetraose e, sialyllacto-iV-tetraose a, lacto-N-difucohexaose I, lactodifucoetraose, lacto-iV-fucopentaose II, 3-fucosyllactose, lacto-iV-fucopentaose III, 3'-sialyl-3-fucosyllactose, disialomonofucosyllacto-iV-neohexaose, monofucosylmonosialyllacto-iV-octaose, sialyllacto-iV-fucohexaose II, disialyllacto-iV-fucopentose II, monofucosyl disialyllacto-iV-tetraose, monosialyllacto-iV-hexaose, monosialyllacto-iV-neohexaose I, monosialyllacto-iV-neohexaose II, sialyllacto-iV-tetraose b, disialyllacto-iV-hexaose I, disialyllacto-iV-neohexaose,
disialyllacto-iV-tetraose, disialyllacto-iV-hexaose II, or a variant thereof that is identical to one of the oligosaccharides except that the glucose at the reducing end is replaced with N-acetylglucosamine.

11. The composition of any of claims 3-10, comprising the glycoconjugate to which the first oligosaccharide and the second oligosaccharide, if any, are attached.

12. The composition of claim 11, wherein in the glycoconjugate, the oligosaccharide(s) is conjugated with a carbohydrate, a lipid, or a peptide.

13. The composition of any of claims 1-12, wherein the composition is free of a milk product or milk by-product.

14. The composition of any of claims 1-13, wherein the composition is for use in treating an infection in an animal.

15. The composition of claim 14, wherein the infection is caused by a bacterium, a fungus, a protozoan, or a virus.

16. The composition of claim 15, wherein the infection is caused by Campylobacter, Clostridium, Escherichia coli, Group B Streptococcus, Helicobacter, Mycobacterium tuberculosis, pathogenic Bacteriodes, Vibrio, Candida, Astrovirus, Herpes spp., Influenza, Norovirus, or Rotavirus.

17. The composition of claim 14, wherein the animal is a mammal or a bird.

18. The composition of claim 17, wherein the mammal is selected from the group consisting of cat, cattle, cow, dog, goat, horse, pig, rabbit, rodent, mink, and sheep and the bird is selected from the group consisting of chicken, duck, goose, turkey, ostrich, emu, swan, peafowl, pheasant, partridge, and guineafowl.
Fig. 1

Log 10 (genome equivalent/ml)

- Control
- Whole milk
- Skim milk
- Total milk proteins
- Total milk oligosaccharides
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A23C 9/1.2; A01 N 43/04; A61 K 31/70 (201 0.01 )
USPC - 424/34; 514/23

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
USPC-424/34; 514/23

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC-424/34; 514/23 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWest, USPC, Google Scholar
oligosaccharide compositions and animal supplement or animal feeds

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>US 6,126,961 A (Kross) 3 October 2000 (03. 10. 2010) col 9, lns 8-10; col 10, lns 9-12</td>
<td>1</td>
</tr>
</tbody>
</table>

D

Further documents are listed in the continuation of Box C.

- Special categories of cited documents
  "A" document defining the general state of the art which is not considered to be of particular relevance
  "E" earlier application or patent but published on or after the international filing date
  "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  "O" document referring to an oral disclosure, use, exhibition or other means
  "P" document published prior to the international filing date but later than the priority date claimed
  "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
  "X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
  "Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
  "&" document member of the same patent family

Date of the actual completion of the international search
13 May 2010 (13.05.2010)

Date of mailing of the international search report
21 MAY 2010

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-320 1

Authorized officer
Lee W. Young
PCT H(i)/pt/111. 571-272-4380
PCT OSP- 571-272-774

Form PCT/ISA/2 10 (second sheet) (July 2009)
This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. **Claims Nos.**
   - Because they relate to subject matter not required to be searched by this Authority, namely

2. **Claims Nos.**
   - Because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically

3. **Claims Nos.**
   - 6-8 and 11-18
   - Because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 64(a)

This International Searching Authority found multiple inventions in this international application, as follows:

1. **As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.**
2. **As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.**
3. **As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.**
4. **No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims, it is covered by claims Nos.**

**Remark on Protest**
- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation
- No protest accompanied the payment of additional search fees