Abstract:

A composition comprising oligofructose (OF) for use in the improvement of short term memory and other cognitive benefits.

The invention relates to a nutritional composition comprising oligofructose (fructose oligosaccharide; OF; FOS) having an effect on the promoting, enhancement or improvement of the short term memory of the subjects, in mammals, preferably young mammals. The composition can be an infant formula.
COMPOSITION COMPRISING OLIGOFRUCTOSE (OF) FOR USE IN THE IMPROVEMENT OF SHORT TERM MEMORY AND OTHER COGNITIVE BENEFITS

BACKGROUND

[0001] This invention relates to a composition for use in the improvement of short term memory and/or the development of optimum short term memory and other related cognitive benefits, especially, in infants and young children.

[0002] The present invention also relates generally to the field of neuronal health, neuronal protection and neuronal development. The invention specifically relates to administration of fructo-oligosaccharides, especially, or oligofructose (OF) for inducing the development of an optimum short term memory, and/or improving the short term memory and/or promoting the healthy establishment of cognitive functions in infants and young children. The invention may be of particular use for fragile or preterm infants.

[0003] The central nervous system (CNS), and in particular the brain, drives the cognitive functions. The cerebral cortex, which is a sheet of neural tissue that is outermost to the cerebrum of the mammalian brain, plays a key role in attention, perceptual awareness, higher order cognition (executive function) and information integration of sensory input.

[0004] Central nervous system development and maturation is a highly complex biological phenomenon that involves a number of physiological processes including, for example, neuron and glial cell growth and differentiation, neuronal pathfinding and branching, and establishment of inter neuronal communication (nerve signals) via axon growth and synaptogenesis.
Neuronal plasticity, which is defined as the ability of the brain to continuously adapt its functions and structural organization to changing requirements is important in nervous system maturation and adult function. It is essential for the correct functioning of the brain and necessary for cognition, learning and memory. Some of the neuronal markers, including proteins and neurotrophic factors, like Brain Derived Neurotrophic Factor (BDNF) required for, or at least, associated with these physiological processes, have been identified in the literature and studied [Huang, E.J. and Reichardt, L. F. (2001); Neurotrophins: Roles in Neuronal Development and Function, *Annu. Rev. Neurosci.*, 24: 677-736]; [Musumeci, G. and Minichiello, L. (2011); BDNF-TrkB signalling in fear learning: from genetics to neural networks, *Rev. Neurosci.*, 22(3):303-15]; [Xiao, J. et al. (2009); The role of neurotrophins in the regulation of myelin development, *Neurosignals*, 17: 265-276] and [Von Bohlen and Halbach, O. (2011); Immunohistological markers for proliferative events, gliogenesis, and neurogenesis within the adult hippocampus, *Cell Tissue Res.*, 345(1):1-19].

The central nervous system develops starting early after conception, throughout gestation and continues to mature until early adulthood. In particular, structural maturation is mostly pre-natal while functional network maturation is mostly post-natal. In human fetuses, the cerebral cortex develops quite late and over a protracted period of time. In utero, there is a peak of neuronal/brain maturation and growth from week 30 of gestation in humans.

The development of the capacity to temporarily storing and managing information (such as visual signals), referred as short term memory, is a crucial step in the development of the cognitive functions in infants and young children. The development
and/or improvement of short term memory is closely linked to the neuronal plasticity. Although such development and improvement of short term memory is of particular importance during the first months / years of life (where the neuronal plasticity is the highest), it also affects older subjects, teenagers and adults. Short-term memory decline in aging population, both healthy elderly as well as elderly suffering from diseases.

[0009] Premature babies by definition enter the world with a still primitive brain, indeed they exhibit very basic electrical activity in the primary sensory regions of the cerebral cortex-those areas that perceive touch, vision, and hearing, as well as in primary motor regions of the cerebral cortex. For these babies the post-natal gradual maturation of the brain is essential to compensate for their lower brain maturation status at birth, this compensatory maturation is particularly important for the more complex part of the brain that mediates much of their emotional, social and cognitive maturation in the first few years of life [Lubsen, J. et al. (2011); Microstructural and functional connectivity in the developing preterm brain, Seminars in Perinatology, 35, 34-43].

[0010] Preterm babies are born at a time that is crucial for structural and functional brain development and maturation and, so, they miss out on in utero brain development. They are at risk for medical conditions after birth, including hemorrhagic and hypoxic-ischemic brain injuries, as well as for development problems later in life, including cognitive deficits. This risk seems to be higher the younger the babies are delivered and the lower their birth weight is. Cognitive deficits in terms of lower IQ, lower attention and working memory abilities, and problems in executive functions may persist into school-age and adolescence [Talge, N. et al. (2010). Late-Preterm Birth and its Association with Cognitive and Socioemotional Outcomes at 6 Years of Age. Pediatrics, 126, 1124-1 131; van Baar,

More generally CNS immaturity or delayed maturation of the CNS, can be observed in infants such as:

- Preterm infants, low birth weight (<2500 g), very low and extremely low birth weight infants (<1500 g), extremely low birth weight (<1000g) and in small for gestational age infants [Allen, M.C. (2008); Neurodevelopmental outcomes of preterm infants, Curr. Opin Neurol., 21(2): 123-8].

- Premature or term-born infants having experienced an intrauterine growth retardation (IUGR) that occurred following any adverse events during the gestation (smoking of the mother, medication of the mother, low placenta quality, abnormal placenta positioning, malnutrition of the mother and the foetus, excessive stress/anxiety of the mother, etc); [Gregory, A. et al. (2008); Intrauterine Growth Restriction Affects the Preterm Infant's Hippocampus, Pediatric Research, 63(4): 438-443].

- Any neonate and young infant showing nervous system growth retardation following, for example, hypoxemia-ischemia at birth, postnatal complications, postnatal steroid treatments or any other adverse event [Barrett, R.D. et al. (2007); Destruction and reconstruction: hypoxia and the developing brain, Birth Defects Res. C. Embryo Today, 81: 163-76].
Cognitive dysfunctions are reported in these infants, along with dysfunction in their growth and development, indicating that an optimal "catch-up" of the neurodevelopmental process is not achieved. Immaturity or delayed maturation of the cerebral cortex can lead to delayed and/or impaired learning ability, information integration, processing of sensory input, loss of, or poor development of higher reasoning, executive functions, concentration, attention, motor skills and language. This may lead to behavioral problems abnormally low intelligence, and thus, abnormally low mental performance.

It has been generally observed that that breastfeeding preterm infants can result in improved neurodevelopment compared to formula feeding. (See for example: Roze et al. The apparent breastfeeding paradox in very preterm infants: relationship between breast feeding, early weight gain and neurodevelopment based on results from two cohorts, EPIPAGE and LIFT. BMJ Open 2012;2:e000834. doi: 10.1136/bmjopen-2012-000834).

According to the inventors, this tends to indicate that some nutrients present in the human breast milk may be missing from synthetic formula or delivered in a sub-optimal amount. There is a need to identify the key differences between conventional formula and human breast milk and adapt the synthetic formula accordingly.

Behavioral and neurodevelopmental disorders associated with delayed maturation of the cerebral cortex include attention deficit/hyperactivity disorders and autism spectrum disorders.

Cognitive function may be measured in humans with clinical tests that depend on age; many such tests known to pediatricians and child development experts. For babies and infants, development screening and neurodevelopment tests exist such as for...
example, BSID - Bayley Scales of Infant Development, Brazelton Neonatal Behavioral Assessment Scale, NEPSY - A Developmental NEuroPSYchological Assessment and Griffiths Mental Development Scales. For pre-school and/or school children tests for cognitive abilities include PPVT (Peabody Picture Vocabulary Test), TONI-2 (Test of Nonverbal Intelligence-2), WPPSI (Wechsler Preschool and Primary Scales of Intelligence), and CPM (Raven's Coloured Progressive Matrices).

One aspect of the cognitive development can be tested through testing the short term memory. Such tests are known in the art. They may provide strict reading of the ability to recognize signals (short term) and are also indicators of the general cognitive development of the subject. These tests, known in the art, are able to distinguish the short term memory from other form of memory such as the long-term memory. Testing for memory are conventionally available and are described in particular in Ross-Sheehy, S. et al. (2003); The development of visual short-term memory capacity in infants. Child Dev, 74(6):1807-22 and Gathercole (1999), Cognitive approaches to the development of short-term memory TICS, 3(1):410-419

It is known that nutrition plays an important role in neuronal maturation in the brain (reviewed in Huppi, P.S. (2008); Nutrition for the Brain, Pediatric Research, 63(3): 229-231 and Cusik and Georgieff (2016); The Role of Nutrition in Brain Development: The Golden Opportunity of the "First 1000 Days", Journal of Pediatrics, 175:16-21).

The consequences of malnutrition can be irreversible and may include poor cognitive development, poor memory, educability, and thus future economic productivity.

While it is known that breast milk of mothers provides the best nutritional support to the developing brain, when breastfeeding is not possible, there is a need to provide synthetic nutritional compositions (such as infant formula or follow on formula) that induce an improvement or promote the development of optimal cognitive functions.

Thus, oral interventions are an appropriate way to positively impact on the development of the nervous system, so as to ensure optimal development of cognitive function, memory and mental performance in the preterm or term born neonates, infants, toddlers, children or young adults or young animals.

However little is known and has so far been proven, on the capacity of nutritional diets or nutritional compositions to influence the development or the promotion of memory, in particular the short memory, and especially in infants and young children.

There is a need to promote and support the healthy establishment of cognitive function in general and of short term and long term memory in particular.

There is a need to improve such memory functions in young subjects, particularly infants and young children.

There is a need to promote the development of such memory functions in infants and young children.

There is a need to provide nutritional compositions to infants and young children that improve short term memory and/or enhance and promote the development of short term memory.
There is a need to promote and/or improve the short term memory performance independently from the long term memory.

There is a need to provide such promotion, improvement, enhancement of short term memory and/or working memory and/or declarative memory, via a nutritional intervention, especially among infants and young children.

There is a need to provide such nutritional intervention and/or prophylactic nutritional intervention in a form that is well accepted by the subject population, in particular those of in these populations that are the most fragile or the most in need. There is a further need to not induce disadvantages, side-effects or negatives in such population.

There is a need to provide such solutions to the subject populations in the most simple and most cost-effective way, preferably not through the use of actual ingredients considered as drugs or medicaments, and preferably as part of the diet.

The present invention applies to all mammals, including animals and humans and in particular in infants and young children for which the brain plasticity is highest.

SUMMARY OF THE INVENTION

The present inventors have found surprisingly that the administration of a specific oligosaccharide or a mixture of specific oligosaccharides, especially comprising oligofructose [and/or specific human milk oligosaccharides] is particularly effective in the enhancement and/or promotion and/or improvement of the short term memory and/or the declarative memory and/or the working memory, in particular in young subjects such as infants and/or young children. The administration can be done as part of a nutritional intervention.
BRIEF DESCRIPTION OF THE DRAWINGS

[0033] Figure 1. shows the effect of various diets (OF, OF+HMO) in piglets on the brain volume. An effect of dietary treatment was observed for relative volume of the olfactory bulb ($P = 0.02$). Means without a common superscript letter differ ($P < 0.05$). CON: control; OF: oligofructose; OF+ HMO: oligofructose + human milk oligosaccharides (2FL).

[0034] Figure 2: shows the effect of various diets (OF, OF+HMO) in piglets in a recognition test indicative of the short term memory - Recognition index using 1h or 2 days inter-trial interval induced by various diets (OF, OF+HMO (2FL), control CON). OF alone increases recognition memory with 1h inter-trial interval. OF+HMO increases recognition memory with 24h inter-trial interval.

DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS

r00351DEFINITIONS:

[0036] As used herein, the following terms have the following meanings.

[0037] The term "infant" means a child under the age of 12 months.

[0038] The term "young child" means a child aged between one and three years.

[0039] The term "oligofructose" (abbreviated OF) as used herein refers to a fructose oligomer (i.e. a fructose oligosaccharide) having a degree of polymerization of from 2 to 10, for example a degree of polymerization of from 2 to 8. Oligofructose can also be referred as Fructo-Oligo-Saccharides (abbreviated FOS) or short-chain Fructo-Oligo-Saccharides (abbreviated scFOS). In the present document the terms oligofructose (OF), fructose oligosaccharide (FOS), Fructo-Oligo-saccharide (FOS), short-chain-fructo-oligosaccharide (scFOS) have the same meaning and can be used interchangeably.
The Inulin, being polymers of long chains are specifically excluded from the present definition of OF. Oligofructose is distinguishable from Inulin by its degree of polymerization (Inulin having much longer chains).

JFOS / scFOS / Oligofructose is typically commercially available, for example under the commercial name ORAFTI Oligofructose by Beneo GmbH (Mannheim, Germany) (for example ingredient Orafti® P95).

The term "sn-2 palmitate" as used herein refers to palmitic acid in the sn-2 position of the triglyceride to which it is bonded.

The term "short term memory" (STM) refers to a system for temporarily storing and managing information required to carry out complex cognitive tasks such as learning, reasoning, and comprehension. The short term memory is essential for the recognition of signals such as visual signals.

Short-term memory (STM) allows recalling something for a period of several seconds to an hour without rehearsal. Short-term memory encodes e.g. acoustical information, is supported by transient patterns of neuronal communication, and depends on regions of the frontal lobe (especially dorsolateral prefrontal cortex) and the parietal lobe, which stores items transiently.

The term "infant formula" means a foodstuff intended for particular nutritional use by infants during the first four to six months of life and satisfying by itself the nutritional requirements of this category of person (Article 1.2 of the European Commission Directive 91/321/EEC of May 14, 1991 on infant formulae and follow-on formulae).
[0046] The term "follow-on formula" means a foodstuff intended for particular nutritional use by infants aged over four months and constituting the principal liquid element in the progressively diversified diet of this category of person.

[0047] The term "starter infant formula" means a foodstuff intended for particular nutritional use by infants during the first four months of life.

[0048] Infant formula follow on formula and starter infant formula can either be in the form of a liquid, ready-to-consumer or concentrated, or in the form of a dry powder that may be reconstituted to form a formula upon addition of water. Such formulae are well-known in the art.

[0049] The term "baby food" means a foodstuff intended for particular nutritional use by infants during the first years of life.

[0050] The term "infant cereal composition" means a foodstuff intended for particular nutritional use by infants during the first years of life.

[0051] The term "growing-up milk" means a milk-based beverage adapted for the specific nutritional needs of young children.

[0052] The term "weaning period" means the period during which the mother's milk is substituted by other food in the diet of an infant.

[0053] The term "nutritional composition" means a composition which nourishes a subject. This nutritional composition is usually to be taken orally or intravenously, and it usually includes a lipid or fat source and a protein source. Preferably the nutritional composition is a complete nutrition mix that fulfils all or most of the nutritional needs of a subject (for example an infant formula).
The term "synthetic mixture" means a mixture obtained by chemical and/or biological means, which can be chemically identical to the mixture naturally occurring in mammalian milks.

The term "sialylated oligosaccharide" means an oligosaccharide having a sialic acid residue.

The term "fucosylated oligosaccharide" means an oligosaccharide having a fucose residue.

The term "prebiotic" means non-digestible carbohydrates that beneficially affect the host by selectively stimulating the growth and/or the activity of healthy bacteria such as bifidobacteria in the colon of humans (Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr. 1995;125:1401-12).

The term "probiotic" means microbial cell preparations or components of microbial cells with a beneficial effect on the health or well-being of the host. (Salminen S, Ouwehand A. Benno Y. et al. "Probiotics: how should they be defined" Trends Food Sci. Technol. 1999:10 107-10).

All percentages are by weight unless otherwise stated.

When the ingredients amounts are provided for as weight of ingredient / weight of powder nutritional composition is also intended that the invention comprises also the corresponding amount by litre taking in to account a dilution factor of the dry powder nutritional composition of 130 g/L (or a specified otherwise in the dilution instructions).

Human Milk Oligosaccharides:
Human milk oligosaccharides (HMOs) are, collectively, the third largest solid constituents in human milk, after lactose and fat. HMO usually consists of lactose at the reducing end with a carbohydrate core that often contains a fucose or a sialic acid at the non-reducing end. There are approximately one hundred milk oligosaccharides that have been isolated and characterized, however these represent only a very small portion of the total number remaining to be characterized.

In the past, infant formulae were developed using HMO ingredients, such as fucosylated oligosaccharides, lacto-N-tetraose, lacto-N-neotetraose, or sialylated oligosaccharides, for different purposes.

EP0975235B1 from Abbott Laboratories describes a synthetic nutritional composition comprising one or more human milk oligosaccharides, wherein the HMOs in the composition are chosen among a group of eight HMOs (3-fucosyllactose, lacto-N-fucopentaose III, lacto-N-fucopentaose II, difucosyllactose, 2'-fucosyllactose, lacto-N-fucopentaose I, lacto-N-neotetraose and lacto-N-fucopentaose V) wherein said composition is intended for cases of normal, healthy infants, children, adults or individuals having specialized needs such as those that accompany certain pathological conditions. This European patent states that, generally speaking, oligosaccharides protect infants from viral and bacterial infections of the respiratory, gastrointestinal and uro-genital tracts.

The composition of the invention may contain 2'-fucosyllactose (2FL) and/or a N-acetyl-lactosamine such as lacto-N-neotetraose (LNnT) or lacto-N-tetraose (LNT).

In one embodiment the nutritional composition according the invention comprises human milk oligosaccharide selected from the list consisting of N-acetyl-lactosamine,
sialylated oligosaccharides, fucosylated oligosaccharides, 2FL, LNN, LNT or a combination thereof.

N-acetyllactosamine

In some embodiments the composition of the invention contains at least one N-acetyllactosamine. That is to say that the composition according to the invention contains N-acetyllactosamine and/or an oligosaccharide containing N-acetyllactosamine. Suitable oligosaccharides containing N-acetyllactosamine include lacto-N-tetraose (LNT) and lacto-N-neotetraose (LNN).

Thus, according to the invention, the N-acetyllactosamine is preferably selected from the group comprising lacto-N-tetraose (LNT) and lacto-N-neotetraose (LNN).

LNT and LNN may be synthesised chemically by enzymatic transfer of saccharide units from donor moieties to acceptor moieties using glycosyltransferases as described for example in US patent No. 5,288,637 and WO 96/10086. Alternatively, LNT and LNN may be prepared by chemical conversion of keto-hexoses (e.g. fructose) either free or bound to an oligosaccharide (e.g. lactulose) into N-acetylhexasamine or an N-acetylhexasamine-containing oligosaccharide as described in Wrodnigg, T.M.; Stutz, A.E. (1999) Angew. Chem. Int. Ed. 38:827-828. N-acetyllactosamine produced in this way may then be transferred to lactose as the acceptor moiety.

Preferably the composition according to the invention contains from 0.1 to 3g N-acetyllactosamine per 100g of composition on a dry weight basis. Preferably it contains 0.1 to 3g of LNN per 100g of composition on a dry weight basis.
In one embodiment the nutritional composition according the invention comprises a N-acetyl-lactosamine, preferably selected from the group comprising lacto-N-tetraose (LNT) and lacto-N-neotetraose (LNnT).

Sialylated Oligosaccharides

The composition according to the invention, in some embodiments, can comprise one or more sialylated oligosaccharides.

The sialylated oligosaccharides may be selected from the group comprising 3'-sialyllactose and 6'-sialyllactose. Preferably, both 3'-sialyllactose and 6'-sialyllactose are present in said composition. In this embodiment, the ratio between 3'-sialyllactose and 6'-sialyllactose lies preferably in the range between 5:1 and 1:2.

The 3'- and 6'- forms of sialyllactose may be isolated by chromatographic or filtration technology from a natural source such as animal milks. Alternatively, they may be produced by biotechnological means using specific sialyltransferases or sialidases, neuraminidases, either by an enzyme based fermentation technology (recombinant or natural enzymes), by chemical synthesis or by a microbial fermentation technology. In the latter case microbes may either express their natural enzymes and substrates or may be engineered to produce respective substrates and enzymes. Single microbial cultures or mixed cultures may be used. Sialyl-oligosaccharide formation can be initiated by acceptor substrates starting from any degree of polymerisation (DP), from DP=1 onwards. Alternatively, sialyllactoses may be produced by chemical synthesis from lactose and free N'-acetyleneuraminic acid (sialic acid). Sialyllactoses are also commercially available for example from Kyowa Hakko Kogyo of Japan.
Preferably the composition according to the invention contains from 0.05 to 2 g, more preferably 0.1 to 2 g, of sialylated oligosaccharide(s) per 100 g of composition on a dry weight basis.

In one embodiment the nutritional composition according to the invention comprises sialylated oligosaccharide, preferably selected from the group comprising 3'-sialyllactose and 6'-sialyllactose. More preferably said composition comprises both 3'-sialyllactose and 6'-sialyllactose, the ratio between 3'-sialyllactose and 6'-sialyllactose lying preferably in the range between 5:1 and 1:2.

Fucosylated oligosaccharide

The composition according to the invention may comprise one or more fucosylated oligosaccharides. Preferably the fucosylated oligosaccharides consist or comprises 2'-fucosyllactose (2-FL).

The fucosylated oligosaccharide may be selected from the group comprising 2'-fucosyllactose, 3-fucosyllactose, difucosyllactose (DiFL), lacto-N-fucopentaoses (that is to say lacto-N-fucopentaose I, lacto-N-fucopentaose II, lacto-N-fucopentaose III and lacto-N-fucopentaose V), lacto-N-difucohexaose I, fucosyllacto-N-hexaose, Difucosyllacto-N-hexaose I and Difucosyllacto-N-neohexaose II. A particularly preferred fucosylated oligosaccharide is 2'-fucosyllactose (2-FL) or DiFL.

The fucosylated oligosaccharide may be isolated by chromatography or filtration technology from a natural source such as animal milks. Alternatively, it may be produced by biotechnological means using specific fucosyltransferases and/or fucosidase either through the use of enzyme-based fermentation technology (recombinant or natural enzymes) or microbial fermentation technology. In the latter case, microbes may either
express their natural enzymes and substrates or may be engineered to produce respective substrates and enzymes. Single microbial cultures and/or mixed cultures may be used. Fucosylated oligosaccharide formation can be initiated by acceptor substrates starting from any degree of polymerization (DP), from $\text{DP} = 1$ onwards. Alternatively, fucosylated oligosaccharides may be produced by chemical synthesis from lactose and free fucose. Fucosylated oligosaccharides are also available for example from Kyowa Hakko Kogyo of Japan.

[0083] Preferably, the composition according to the invention contains from 0.1 to 3g of fucosylated oligosaccharide(s) per 100g of composition on a dry weight basis, most preferably being 2FL.

[0084] In one embodiment the nutritional composition according the invention comprises a fucosylated oligosaccharide, preferably selected from the group comprising 2'-fucosyllactose, 3-fucosyllactose, difucosyllactose, lacto-N-fucopentaoses (that is to say lacto-N-fucopentaose I, lacto-N-fucopentaose II, lacto-N-fucopentaose III and lacto-N-fucopentaose V), lacto-N-difucohexaose I, fucosyllacto-N-hexaose, Difucosyllacto-N-hexaose I and Difucosyllacto-N-neohexaose II, and preferably the fucosylated oligosaccharide is 2'-fucosyllactose (2-FL).

[0085] **Further Prebiotics**

[0086] Additional to the essential oligosaccharides of the present patent claims, the composition of the invention can further comprise at least one or one further prebiotic, usually in an amount between 0.3 and 10% by weight of composition.
Prebiotics are usually non-digestible in the sense that they are not broken down and absorbed in the stomach or small intestine and thus remain intact when they pass into the colon where they are selectively fermented by the beneficial bacteria.

The composition according to the invention can comprise, in some embodiments, Oligofructose (OF). An example of such OF is the commercial ingredient ORAFTI® by Beneo GmbH (Mannheim, Germany).

In some embodiments the prebiotics of the composition of the invention, comprise other fructooligosaccharides (FOS) or/and galactooligosaccharides (GOS). A combination of prebiotics may be used such as 90% GOS with 10% short chain fructooligosaccharides such as in the product by BENE-Orafti sold under the trademark "Orafti® oligofructose" (see http://www.beneo-rafti.com/Our-Products/Oligofructose) (previously Raftilose®) or 10% inulin such as in the product sold by BENE-Orafti under the trademark "Orafti® inulin" (see http://www.beneo-rafti.com/Our-Products/Inulin) (previously Raftiline®). Another combination of prebiotics is 70% short chain fructooligosaccharides and 30% inulin, which is a product sold by BENE-Orafti® under the trademark "Prebio 1".

In one embodiment the nutritional composition according the invention comprises a prebiotic selected from the list bovine milk oligosaccharides, inulin, xylooligosaccharides, polydextrose or any combination thereof.

In one embodiment the nutritional composition according the invention comprises a bovine milk oligosaccharide, said bovine milk oligosaccharides being an N-acetylated oligosaccharide, a galacto-oligosaccharide, a sialylated oligosaccharide, a fucosylated oligosaccharide or a combination thereof.
Probiotics

The composition of the invention can further comprise at least one probiotic bacterial strain, said probiotic bacterial strain preferably being *Bifidobacteria* and/or *Lactobacilli*.

Suitable probiotic bacterial strains include *Lactobacillus rhamnosus* ATCC 53103 available from Valio Oy of Finland under the trademark LGG, *Lactobacillus rhamnosus* CGMCC 1.3724, *Lactobacillus paracasei* CNCM 1-2116, *Lactobacillus johnsonii* CNCM 1-1225, *Streptococcus salivarius* DSM 13084 sold by BLIS Technologies Limited of New Zealand under the designation KI2, *Bifidobacterium lactis* CNCM 1-3446 sold *inter alia* by the Christian Hansen company of Denmark under the trademark Bb 12, *Bifidobacterium longum* ATCC BAA-999 sold by Morinaga Milk Industry Co. Ltd. of Japan under the trademark BB536, *Bifidobacterium breve* sold by Danisco under the trademark Bb-03, *Bifidobacterium breve* sold by Morinaga under the trade mark M-16V, *Bifidobacterium infantis* sold by Procter & Gamble Co. under the trademark Bifantis and *Bifidobacterium breve* sold by Institut Rosell (Lallemand) under the trademark R0070.

Preferably, the composition according to the invention contains from $10^3$ to $10^{12}$ cfu of probiotic bacterial strain, more preferably between $10^7$ and $10^{12}$ cfu, per g of composition on a dry weight basis.

In one embodiment the nutritional composition of the comprises a probiotic bacterial strain selected from the list consisting of *Lactobacillus acidophilus*, *Lactobacillus salivarius*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, *Lactobacillus casei*, *Lactobacillus johnsonii*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus lactis*, *Lactobacillus delbrueckii*, *Lactobacillus helveticus*, *Lactobacillus bulgari*,
Lactococcus lactis, Lactococcus diacetylactis, Lactococcus cremoris, Streptococcus salivarius, Streptococcus thermophilus, Bifidobacterium lactis, Bifidobacterium animalis, Bifidobacterium longum, Bifidobacterium breve, Bifidobacterium infantis, or Bifidobacterium adolescentis or any mixture thereof.

**Target population**

[0097] The present invention targets mammals. Preferably the mammals are human, dog or cat.

[0098] In one embodiment the present invention is for young mammals, such as infants (for example 0 to 6 months or 0 to 12 months), young children (for example 1 to 3 years or 1 to 7 years), or young dogs (for example puppies) or young cats. Without to be bound by the theory the young mammals have a high brain plasticity and brain (or brain connectivity) development and benefit most of the present invention. It is also contemplated that the present invention may target particular windows of nutritional intervention (for example such as children 6 months to 3 years, 3 months to 18 months), subpopulations having particular need (fragile young mammals; senile or semi-senile mammals) or in a recovery phase. In one embodiment the present invention is especially targeted at populations suffering from a disease, preferably a disease related to the old age or related to a degeneration of the cognitive or brain functions, most preferably Alzheimer or related diseases.

[0099] In particular, it is contemplated that the present invention can also benefit old mammals or senior populations such are old god, old cat or human elderly or senior adults (for example +60 years, +65 years +70 years, +75 years or +80 years adults), especially for preventing a decline in their short term memory, or those having already
experienced a decline of the short term memory or those having already experience any
sign of brain or cognitive degeneration.

[00101] According to a preferred embodiment, the composition according to the
invention is for use in healthy infants and/or healthy young children. In one embodiment
the invention is of particular relevance in fragile infants, preterm infants, and/or infant born
with a subnormal birth weight and/or infants subject of intrauterine growth retardation.
The preferred period of use is during the period(s) of most rapid development of the
memory and/or development of the brain connectivity.

[00102] The composition of the invention is targeted to infants and/or young children,
of age 7 years or less, preferably of age 3 years or less, most preferably age 1 year of
less. In one embodiment the composition is intended for infants of 6 months or less. In
embodiments of the invention the composition is used during the first 6 months of life, first
1 year of life, first 3 years of life, first 7 years of life, and/or during a period of recovery
after sickness or of low development.

[00103] **Nutritional composition**

[00104] The composition according to the invention is preferably a synthetic
nutritional composition. The composition of the invention can for example be a starter
infant formula, an infant formula, a baby food, an infant cereal composition, a follow-on
formula or a growing-up milk, and said composition is preferably a starter infant formula.
The composition according to the invention can also be for use before and/or during a
weaning period. In one embodiment the nutritional composition may be a complete
nutritional composition or a supplement for aging, elderly or fragile persons.
The composition according to the invention can be completed composition provide 100% or a majority of the nutritional needs of the target populations (for example in term of caloric needs; or in terms of vitamin or minerals needs, in in term of protein, lipids or carbohydrate needs). Alternatively the composition of the invention can be a supplement to be consumer in addition to a regular diet). In that case however the dosage and overall consumption of the composition is adapted to provide the claimed benefit on the short term memory (for example proportionally to the caloric load and to the subject caloric needs).

The use of composition of the invention can encompass the cases where the composition is a supplement, preferably provided in the form of unit doses. In one embodiment the composition is a supplement to human breast feeding.

The composition can be in the form of a powder composition for example intended to be diluted with water or mixed with milk (for example human breast milk), or ingested as a powder. In one embodiment the composition of the invention is in liquid form; either ready-to-drink or to be diluted in water or mixed with milk (for example human breast milk).

The composition according to the invention can also contain a protein source, preferably in an amount below 2.5 g/100kcal or below 2.0g per 100 kcal, even more preferably in an amount below 1.8g per 100 kcal. In one embodiment the protein content is below 1.6 g/100kcal. The type of protein is not believed to be critical to the present invention provided that the minimum requirements for essential amino acid content are met and satisfactory growth is ensured. Thus, protein sources based on whey, casein and mixtures thereof may be used as well as protein sources based on soy. As far
as whey proteins are concerned, the protein source may be based on acid whey or sweet whey or mixtures thereof and may include alpha-lactalbumin and beta-lactoglobulin in any desired proportions.

[00109] The composition according to the present invention generally contains a carbohydrate source. This is particularly preferable in the case where the nutritional composition of the invention is an infant formula. In this case, any carbohydrate source conventionally found in infant formulae such as lactose, saccharose, maltodextrin, starch and mixtures thereof may be used although the preferred source of carbohydrates is lactose.

[00110] The composition according to the present invention generally contains a source of lipids. This is particularly relevant if the nutritional composition of the invention is an infant formula. In this case, the lipid source may be any lipid or fat which is suitable for use in infant formulae. Preferred fat sources include palm oleic, high oleic sunflower oil and high oleic safflower oil. The essential fatty acids linoleic and α-linolenic acid may also be added as may small amounts of oils containing high quantities of preformed arachidonic acid and docosahexaenoic acid such as fish oils or microbial oils. The fat source preferably has a ratio of n-6 to n-3 fatty acids of about 5:1 to about 15:1; for example about 8:1 to about 10:1.

[00111] The composition of the invention also contains preferably all vitamins and minerals understood to be essential in the daily diet and in nutritionally significant amounts. Minimum requirements have been established for certain vitamins and minerals. Examples of minerals, vitamins and other nutrients optionally present in the composition of the invention include vitamin A, vitamin B1, vitamin B2, vitamin B6, vitamin
B12, vitamin E, vitamin K, vitamin C, vitamin D, folic acid, inositol, niacin, biotin, pantothenic acid, choline, calcium, phosphorous, iodine, iron, magnesium, copper, zinc, manganese, chlorine, potassium, sodium, selenium, chromium, molybdenum, taurine, and L-carnitine. Minerals are usually added in salt form. The presence and amounts of specific minerals and other vitamins will vary depending on the intended population.

If necessary, the composition of the invention may contain emulsifiers and stabilisers such as soy, lecithin, citric acid esters of mono- and di-glycerides, and the like.

The composition of the invention may also contain other substances which may have a beneficial effect such as lactoferrin, nucleotides, nucleosides, and the like.

In one embodiment, the composition of the invention, especially in the form of an infant formula, comprises from about 1.8 to about 2.2 g of total protein per 100 kcal, for example, about from 1.8 to about 2.1 g or from about 1.9 to about 2.1 g protein per 100 kcal, optionally wherein from about 0.3 to about 0.4 g/100 kcal of protein is alpha-lactalbumin. The infant formula and follow-on formula of this invention may be in the form of a ready-to-feed liquid, or may be a liquid concentrate or powdered formula that can be reconstituted into a ready-to-feed liquid by adding an amount of water that results and follow-on formula of this invention includes all the ingredients that are required by law in the US or EU, including but not limited to certain vitamins, minerals, and essential amino acids. It may also include nucleotides, such as CMP, UMP, AMP, GMP and IMP, lutein, zeaxanthin, and other ingredients known in the art.

In one embodiment of the invention the nutritional composition is a pet food (for example for dogs or cats or young dogs or young cats).

Effect(s) and use of the composition of the invention
The present invention relates to the enhancement and/or promotion and/or improvement of the short term memory function of a subject. This can include enhancement and/or promotion and/or improvement of the working memory and/or the declarative memory. By enhancing the short term memory function the composition of the invention can also positively impact the subject’s ability of acquire and process new knowledge (learning skills).

One embodiment the invention also enhances long-term memory function. One embodiment selectively enhances short term memory without enhancing long term memory.

Without to be bound by the theory, in one aspect of the invention such improvement, enhancement and/or promotion is believed to be linked to the effect of the oligosaccharide of the invention on the gut microbiota. OF, 2FL or LNnT are only slightly digested in the small intestine, thus the majority becomes available for fermentation by microbiota in the colon. Indeed, OF or 2FL or LNnT have been shown to promote the growth of intestinal bifidobacteria as reflected by an increase in this family of bacteria in the stools in infants fed Oligosaccharides-supplemented formulas (with OF, or2FL and/or LNnT). In short, the present nutritional composition may be used in enhancing memory function via its effect on gut microbiota and gut-brain axis which has been shown in influencing many aspects of brain functioning. One could also contemplate that the improvement in the memory function may be related to an increase the sialic acid (Neu5Ac) concentration in the brain of said individual, mediated by differential metabolic pathways influenced by the gut microbiota.
In another aspect the effect of the invention can be linked to the enhancement of the neuroplasticity in the brain of the subject, and/or by enhancing neurodevelopment, neurogenesis, axonal sprouting and/or maturation in the brain of said subject (without to be bound by the theory).

The declarative memory, working memory and, more generally, the short-term memory represents essential aspects of the brain function and development and are crucial for the overall cognitive development of the subject. It influences in particular the processing, classification and management of external signals, the learning ability as well as spatial orientation.

In one embodiment of the present invention, the declarative memory is recognition memory. Recognition memory is of capital importance in the social and cognitive development, especially of young subjects.

In one embodiment the invention present relates to (or is defined by) the use of a nutritional composition comprising a fructose oligosaccharide (FOS) to improve, enhance or promote declarative memory, short term memory and/or working memory in a mammal.

In one embodiment the invention present relates to (or is defined by) the use of the herein described nutritional composition wherein said composition is an infant formula, follow on formula, human milk fortifier, growing up milk, or pet food.

In one embodiment the invention present relates to (or is defined by) a fructose oligosaccharide for use in improving, enhancing or promoting short term memory in a mammal wherein said mammal is preferably a human, cat or dog and wherein said human is preferably an infant or a young child.
In one embodiment the invention present relates to (or is defined by) a nutritional composition comprising a fructose oligosaccharide for use in improving, enhancing or promoting declarative memory, short term memory and/or working memory in a mammal, preferably said mammal being a young mammal, a human, a dog or a cat, more preferably a young human, dog or cat, most preferably an infant or young children. The nutritional composition can also comprise one or more of the optional ingredients described herein.

In one embodiment the invention present relates to (or is defined by) the use of fructose oligosaccharide in the manufacture of a nutritional composition for improving, enhancing or promoting declarative memory, short term memory and/or working memory in a mammal, preferably said mammal being a young mammal, a human, a dog or a cat, more preferably a young human, dog or cat, most preferably an infant or young children. In one embodiment the subject is an elderly mammal, preferably suffering or at risk of suffering from a cognitive dysfunction (such as Alzheimer disease).

**Dosage**

Fructo oligosaccharide / FOS / OF: The nutritional composition of the present invention may contain from 0.1 to 20 g of oligofructose (OF) per 100 g of composition on a dry weight basis, e.g. from 1 to 6 g or from 3 to 5 g of oligofructose (OF) per 100 g of composition on a dry weight basis.

The one embodiment of the invention the nutritional composition comprises an amount of fructose oligosaccharide in the following ranges or amount:

0.1 to 20 g/L or 0.5 to 10 g/L or 1 to 8 g/L or 2 to 6 g/L or 1.5 g/L or 3 g/L or 5 g/L of nutritional composition, when the composition is in a ready-to-feed liquid form, or
[00132] 0.1 to 20g/L or 0.5 to 10 g/L or 1 to 8 g/L or 2 to 6 g/L or 1.5G/L or 3g/L or 5 g/L (of the liquid diluted form) when the composition is in powder form and intended to be recomposed into a diluted liquid form, or

[00133] the same as above multiplied by 2, 5, 10, 20, 50 or 100 when the nutritional composition is in the form of a concentrated composition intended to be diluted (respectively 2, 5, 10, 20, 50, or 100 times) into water or human breast milk or intended to be used directly as a concentrated form, or

[00134] 0.4 g to 15 g/100g of nutrition composition powder, or 0.8 to 10 g/100g, or 1 to 6 g/100g, or 2 to 5 g/100g or 2.1 to 4 g/100g or 1.2 g/100g or 2.3 g/100g or 3.8 g/100g or 4 g/100g or 6 g/100g of nutrition composition powder, when the nutritional composition is in the form of a dry powder.

[00135] In one embodiment the OF content may be 0.07 g to 3 g/100kcal of nutrition composition powder, or 0.1 to 2 g/100kcal, or 0.4 to 1.5 g/100kcal, or 0.45 to 1 g/100kcal or 0.45 to 0.75 g/100kcal or 0.3 g/100kcal or 0.4 g/100kcal or 0.5 g/100kcal or 0.75 g/100kcal or 1 g/100kcal of nutrition composition powder, when the nutritional composition is in the form of a dry powder.

[00136] 2FL: The nutritional composition of the present invention may contain from 0.02 to 10 g of 2FL per 100g of composition on a dry weight basis, e.g. from 0.2 to 0.5 g or from 0.3 to 1 g of 2FL per 100g of composition on a dry weight basis.

[00137] The one embodiment of the invention the nutritional composition comprises an amount of 2FL in the following ranges or amount:
0.05 to 20 g/L or 0.1 to 5 g/L or 0.2 to 3 g/L or 0.1 to 2 g/L or 0.25 g/L to 1 g/L or 0.25 g/L or 1 g/L of nutritional composition, when the composition is in a ready-to-feed liquid form, or

0.05 to 20 g/L or 0.1 to 5 g/L or 0.2 to 3 g/L or 0.1 to 2 g/L or 0.25 g/L to 1 g/L or 0.25 g/L or 1 g/L (of the liquid diluted form) when the composition is in powder form and intended to be recomposed into a diluted liquid form, or

the same as above multiplied by 2, 5, 10, 20, 50 or 100 when the nutritional composition is in the form of a concentrated composition intended to be diluted (respectively 2, 5, 10, 20, 50, or 100 times) into water or human breast milk or intended to be used directly as a concentrated form, or

0.04 g to 1.5 g/100 g of nutrition composition powder, or 0.08 to 1.2 g/100 g, or 0.1 to 1 g/100 g, or 0.2 to 0.8 g/100 g or 0.2 g/100 g or 0.4 g/100 g or 0.8 g/100 g or 1 g/100 g or 1 g/100 g of nutrition composition powder, when the nutritional composition is in the form of a dry powder.

In one embodiment the 2FL content may be 0.01 g to 0.3 g/100 kcal of nutrition composition powder, or 0.02 to 0.2 g/100 kcal, or 0.04 to 0.15 g/100 kcal, or 0.02 g/100 kcal or 0.04 g/100 kcal or 0.07 g/100 kcal or 0.15 g/100 kcal or 0.3 g/100 kcal of nutrition composition powder, when the nutritional composition is in the form of a dry powder.

LNNnT: The nutritional composition of the present invention may contain from 0.01 to 1 g of LNNnT per 100 g of composition on a dry weight basis, e.g. from 0.1 to 0.25 g or from 0.15 to 0.5 g of LNNnT per 100 g of composition on a dry weight basis.
The one embodiment of the invention the nutritional composition comprises an amount of LNnT in the following ranges or amount:

0.02 to 5g/L or 0.05 to 2.5 g/L or 0.1 to 1.5 g/L or 0.05 to 1 g/L or 0.12g/L to 0.5 g/L or 0.12g/L or 0.5 g/L or 1 g/L of nutritional composition, when the composition is in a ready-to-feed liquid form, or

0.02 to 5g/L or 0.05 to 2.5 g/L or 0.1 to 1.5 g/L or 0.05 to 1 g/L or 0.12g/L to 0.5 g/L or 0.12g/L or 0.5 g/L or 1g/L (of the liquid diluted form) when the composition is in powder form and intended to be recomposed into a diluted liquid form, or

the same as above multiplied by 2, 5, 10, 20, 50 or 100 when the nutritional composition is in the form of a concentrated composition intended to be diluted (respectively 2, 5,10, 20, 50, or 100 times) into water or human breast milk or intended to be used directly as a concentrated form, or

0.02 g to 0.75 g/100g of nutrition composition powder, or 0.04 to 0.6 g/100g, or 0.05 to 0.5 g/100g, or 0.1 to 0.4 g/100g or 0.1 g/100g or 0.2 g/100g or 0.25 g/100g or 0.5 g/100g or 1 g/100g or 3 g/100g of nutrition composition powder, when the nutritional composition is in the form of a dry powder.

In one embodiment the LNnT content may be 0.01 g to 0.3 g/100kcal of nutrition composition powder, or 0.02 to 0.2 g/100kcal, or 0.04 to 0.15 g/100kcal, or 0.02 g/100kcal or 0.04 g/100kcal or 0.07 g/100kcal or 0.15 g/100kcal or 0.3 g/100kcal of nutrition composition powder, when the nutritional composition is in the form of a dry powder.

The composition of the invention, in particular when in the form of an infant formula, can contain at least about 0.4 g of oligofructose of oligofructose per 100 kcal. In
some embodiments, it contains from about 0.4 to about 0.9 g, from about 0.4 to about 0.7 g, from about 0.4 to about 0.5 g, from about 0.7 to about 0.8 g, or from about 0.7 to about 0.9 g, oligofructose per 100 kcal. The oligofructose has a degree of polymerization of from 2 to 10. In one embodiment, at least 90% of the oligofructose has a degree of polymerization of from 2 to 8.

[00151] **Method for manufacturing the nutritional composition**

[00152] The nutritional composition may be prepared in any suitable manner known in the art. For example commercial infant formula or follow-on formula can serve as a base composition to which is added the required amount of oligosaccharides (e.g. OF, 2FL, LNnT or else), preferably in a dry form. Alternatively the oligosaccharide can be added as dry ingredient or liquid ingredient into a liquid premix that will serve as a base to manufacture the nutritional composition of the invention. The liquid mix can then be dried by any conventional means.

[00153] For example, it may be prepared by blending together a protein source, a carbohydrate source (different from the oligosaccharide combination of the present invention), and a fat source in appropriate proportions. If used, the emulsifiers may be included at this point. The vitamins and minerals may be added at this point but are usually added later to avoid thermal degradation. Any lipophilic vitamins, emulsifiers and the like may be dissolved into the fat source prior to blending. Water, preferably water which has been subjected to reverse osmosis, may then be mixed in to form a liquid mixture. The temperature of the water is conveniently in the range between about 50[deg.]C and about 80[deg.]C to aid dispersal of the ingredients. Commercially available liquefiers may be used to form the liquid mixture. The 3’-Siallylactose (3’-SL) and 6’-
Siallylactose (6'-SL) will be added at this stage if the final product is to have a liquid form. If the final product is to be a powder, the 3'-Siallylactose (3'-SL) and 6'-Siallylactose (6'-SL) may likewise be added at this stage if desired. The liquid mixture is then homogenized, for example in two stages.

The liquid mixture may then be thermally treated to reduce bacterial loads, by rapidly heating the liquid mixture to a temperature in the range between about 80[deg.]C and about 150[deg.]C for a duration between about 5 seconds and about 5 minutes, for example. This may be carried out by means of steam injection, an autoclave or a heat exchanger, for example a plate heat exchanger. Then, the liquid mixture may be cooled to between about 60[deg.]C and about 85[deg.]C, for example by flash cooling. The liquid mixture may then be again homogenized, for example in two stages between about 10 MPa and about 30 MPa in the first stage and between about 2 MPa and about 10 MPa in the second stage. The homogenized mixture may then be further cooled to add any heat sensitive components, such as vitamins and minerals. The pH and solids content of the homogenized mixture are conveniently adjusted at this point. The homogenized mixture is transferred to a suitable drying apparatus such as a spray dryer or freeze dryer and converted to powder. The powder should have a moisture content of less than about 5% by weight.

If a liquid composition is preferred, the homogenized mixture may be sterilized then aseptically filled into suitable containers or may be first filled into the containers and then retorted.

Particular lipids
The composition of the invention can comprise selected lipids have particular effects.

Those lipids can in particular include DHA, ARA, linoleic acid or Sphingomyelin, preferably in an amount suitable to deliver actual brain health benefits and within the general regulatory requirements for the type of products (for example WHO recommendations for infant formula; CODEX or European directives on infant formula).

In some embodiments the composition of the invention comprises a relatively high level of sn2 palmitate or sphingomyelin. Those have been linked to optimum brain performance and development and can act synergistically with essential compounds of the composition of the invention.

Although feeding an infant a formula containing a high percentage of sn-2 palmitate (in absence of OF) helps to promote the growth of bifidobacteria in the colon, the combination of high sn-2 palmitate with oligofructose is thought to provide significantly superior growth of bifidobacteria in the colon of formula-fed infants. A significant reduction in the amount of potentially pathogenic bacteria can also be achieved. It has been discovered that feeding an infant a high sn-2 palmitate-containing infant formula containing from about 3 to about 5 g/L, or from about 0.4 to about 0.7 g/100 kcal, of oligofructose is more beneficial than feeding the infant the same formula without oligofructose. Without being bound by the theory such synergistic effect between OF and sn2 palmitate can also promote short term memory though in particular (but most probably not only) its effect on the microbiome of the subject and the population of bifidobacteria. "High Sn2 Palmitate" is to be understood as ingredients having a high % of the fatty acids (preferably more than 33% of the fatty acids) as palmitate in the sn2
position of the Triglycerides. Such ingredients are commercially available under the trade name BETAPOL® (Loders Croklaan, Wormerveer, Netherlands) or INFAT® (Advanced Lipids AB, Karlshamn, Sweden, joint venture of AAK B.V. (Zaandijk, Netherlands) and Enzymotec Inc, Morristown, USA)

[00161] Recent infant clinical studies have shown that nutritional formulas containing at least one omega 6 fatty acid and at least one omega 3 fatty acid in a ratio of from about 6 to about 1 increased DHA accretion in erythrocytes and plasma. A balanced ratio of about 6:1 of omega 6 fatty acid to omega 3 fatty acid may also provide long term health benefits including optimum brain and neurological development. Such balance will be achieved by formulating the present invention with vegetable oil fat sources that have omega 6 fatty acid content, such as, for example, soybean oil and sunflower oil, and omega 3 fatty acid content, for example, rapeseed, canola, flaxseed, chia, perlla or walnuts. A unique fat blend with 5 different oils will be used to achieve the modified fat blend.

[00162] The following examples are presented to illustrate certain embodiments and features of the present invention, but should not be construed as limiting the scope of this invention.

[00163] EXAMPLES

[00164] Example 1: Nutritional composition comprising Oligofructose (OF) and/or 2FL and/or LNnT

[00165] A nutritional composition of the invention comprising Oligofructose (OF) and/or 2FL and/or LNnT is given in Table 1 below. This composition is given by way of
The composition of Table 1 can be an infant formula. Alternatively is can be adapted to be a follow-on formula.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>per 100kcal</th>
<th>per litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>100</td>
<td>670</td>
</tr>
<tr>
<td>Proteins (g)</td>
<td>1.83</td>
<td>12.3</td>
</tr>
<tr>
<td>Fats (g)</td>
<td>5.3</td>
<td>35.7</td>
</tr>
<tr>
<td>Linoleic acid (g)</td>
<td>0.79</td>
<td>5.3</td>
</tr>
<tr>
<td>α-Linolenic acid (mg)</td>
<td>101</td>
<td>675</td>
</tr>
<tr>
<td>Lactose (g)</td>
<td>11.2</td>
<td>74.7</td>
</tr>
<tr>
<td>Minerals (g)</td>
<td>0.37</td>
<td>2.5</td>
</tr>
<tr>
<td>Na (mg)</td>
<td>23</td>
<td>150</td>
</tr>
<tr>
<td>K (mg)</td>
<td>89</td>
<td>590</td>
</tr>
<tr>
<td>Cl (mg)</td>
<td>64</td>
<td>430</td>
</tr>
<tr>
<td>Ca (mg)</td>
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<td>410</td>
</tr>
<tr>
<td>P (mg)</td>
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<td>210</td>
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<tr>
<td>Mg (mg)</td>
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</tr>
<tr>
<td>Mn (μg)</td>
<td>8</td>
<td>50</td>
</tr>
<tr>
<td>Se (μg)</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>Vitamin A (μg RE)</td>
<td>105</td>
<td>700</td>
</tr>
<tr>
<td>Vitamin D (μg)</td>
<td>1.5</td>
<td>10</td>
</tr>
<tr>
<td>Vitamin E (mg TE)</td>
<td>0.8</td>
<td>5.4</td>
</tr>
<tr>
<td>Vitamin K1 (μg)</td>
<td>8</td>
<td>54</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>10</td>
<td>67</td>
</tr>
<tr>
<td>Vitamin B1 (mg)</td>
<td>0.07</td>
<td>0.47</td>
</tr>
<tr>
<td>Vitamin B2 (mg)</td>
<td>0.15</td>
<td>1.0</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>1</td>
<td>6.7</td>
</tr>
<tr>
<td>Vitamin B6 (mg)</td>
<td>0.075</td>
<td>0.50</td>
</tr>
<tr>
<td>Folic acid (μg)</td>
<td>9</td>
<td>60</td>
</tr>
<tr>
<td>Pantothenic acid (mg)</td>
<td>0.45</td>
<td>3</td>
</tr>
<tr>
<td>Vitamin B12 (μg)</td>
<td>0.3</td>
<td>2</td>
</tr>
<tr>
<td>Nutrient</td>
<td>per 100kcal</td>
<td>per litre</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------------</td>
<td>-----------</td>
</tr>
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<td>35.7</td>
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<tr>
<td>Nutrient</td>
<td>Amount</td>
<td>Upper Limit</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>--------</td>
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</tr>
<tr>
<td>Linoleic acid (g)</td>
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<td>oLinolenic acid (mg)</td>
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<td>675</td>
</tr>
<tr>
<td>Lactose (g)</td>
<td>11.2</td>
<td>74.7</td>
</tr>
<tr>
<td>Prebiotic (70%OF, 30% insulin) (g)</td>
<td>0.64</td>
<td>4.3</td>
</tr>
<tr>
<td>(For example Beneo-Orafti® P95 and Inulin Beneo-Orafti®) and/or 2FL (g)</td>
<td>0.3</td>
<td>2</td>
</tr>
<tr>
<td>and/or LNNt (mg)</td>
<td>30</td>
<td>200</td>
</tr>
<tr>
<td>Minerals (g)</td>
<td>0.37</td>
<td>2.5</td>
</tr>
<tr>
<td>Na (mg)</td>
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</tr>
<tr>
<td>Vitamin B12 (μg)</td>
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[00171] Experimental data

[00172] In a piglet study, we investigated the impact of OF with or without 2'FL on recognition memory. Thirty-six male piglet (12 per treatment group) were treated from 48h post-farrowing until 33 days of life with either control (CON, Purina ProNurse Livestock Milk Replacer), oligofructose (OF) [CON + 5 g/L OF], or OF + human milk oligosaccharide HMO [CON + 5 g/L OF + 1.0 g/L HMO (2'FL)]. All diets contained a formulation space of 8.0 g/L that was reserved for addition of dietary test articles. Piglets were fed at a rate of 285 mL and 325 mL of reconstituted diet per kg BW from PND 2-6 and PND 7-33, respectively. Piglet were tested in the recognition memory between PND 22 and 31, and were scanned in the MRI on day 32 or 33.

[00173] Magnetic Resonance Imaging

[00174] All piglets underwent MRI procedures at PND 32 at the Beckman Institute Biomedical Imaging Center using a Siemens MAGNETOM Trio 3T MRI, with a Siemens 32-channel head coil. Each piglet underwent imaging protocols only once, and scans for each replicate were completed all on the same day. The piglet neuroimaging protocol included three magnetization prepared rapid gradient-echo (MPRAGE) sequences and diffusion tensor imaging (DTI) to assess brain macrostructure and microstructure, respectively, as well as magnetic resonance spectroscopy (MRS) to obtain brain metabolite concentrations. In preparation for MRI procedures, anesthesia was induced using an intramuscular injection of telazol (50.0 mg of tiletamine plus 50.0 mg of
zolazepam reconstituted with 5.0 Dl water; Zoetis, Florham Park, NJ) administered at 0.07 mL/kg BW, and maintained with inhalation of isoflurane (98% O₂, 2% isoflurane). Piglets were immobilized during all MRI procedures. Visual observation of each piglet's well-being, as well as observations of heart rate, PO2 and percent of isoflurane were recorded every 5 minutes during the procedure, and every 10 minutes post-procedure until animals recovered. Total scan time for each pig was approximately 60 minutes. Imaging techniques are briefly described below.

**Structural MRI Acquisition and Analysis**

A T₁-weighted MPRAGE sequence was used to obtain anatomic images of the piglet brain, with a 0.7 isotropic voxel size. Three repetitions were acquired and averaged using SPM8 in Matlab 8.3, and brains were manually extracted using FMRIB Software Library (FSL) (FMRIB Centre, Oxford, UK). The following sequence specific parameters were used to acquire T₁-weighted MPRAGE data: TR = 1900 ms; TE = 2.49 ms; 224 slices; FOV = 180 mm; flip angle = 9°. Methods for MPRAGE averaging, manual brain extraction were previously described (Mudd et al., 2016). All data generated used a publicly-available population-averaged piglet brain atlas (http://piqmri.illinois.edu) (Conrad et al., 2014).

For volumetric assessments, individual brains were segmented into 19 different regions of interest (ROI) using the piglet brain atlas. Total brain and individual region volume analysis was performed in which an inverse warp file for each ROI was generated from the DARTEL-generated warp files for each region using the using the SPM software. Generation of region-specific warp files was previously described (Mudd et al., 2016a; Radlowski et al., 2014). In order to account absolute whole-brain volume,
all regions of interest were also expressed as a percent of total brain volume (%TBV),
using the following equation: (region of interest absolute volume)/(total brain absolute
volume) x 100, within subject.

[00178] Voxel-based morphometry (VBM) analysis was performed, to assess gray
and white matter tissue concentrations using SPM8 software (Wellcome Department of
Clinical Neurology, London, UK). Manually extracted brains were aligned to piglet brain
atlas space using a 12-parameter affine transformation. The "Segment" function of SPM
and piglet-specific prior probability tissue maps were then used to segment the brains into
gray matter and white matter. The DARTEL toolbox was used with piglet-specific
specifications that included changing the bounding box of -30.1 to 30.1, -35 to 44.8, -28
to 31.5; and a voxel size of 0.7 mm³. After the nonlinear transformation of the data in the
DARTEL procedure, flow fields were created and converted to warp files. The warp files
generated were then applied to the subject's gray and white matter. The modulated data
were smoothed with a 4 mm full-width half maximum (FWHM), and were subjected to
VBM procedures using the SPM8 toolbox. For voxel-based morphometry analyses, two-
sample permutation t-tests were performed on a voxel-by-voxel basis for gray and white
matter volume differences between all AR and SR animals with an uncorrected $P < 0.001$.
An additional threshold criterion of at least 20-edge connected voxels was used.

[00179] Statistical Analysis

[00180] Data analysis was conducted using the MIXED procedure of SAS 9.4 (SAS
Institute, Cary, NC, USA). All data (i.e., brain volumes, DTI measures, and MRS
metabolites) were subjected to a one-way analysis of variance (1 way-ANOVA) to assess
the effect of dietary treatment. Study replicate was included in the model as a random
variable. Statistical significance was defined as $P \leq 0.05$ and trends defined as $0.05 < P \leq 0.10$. Data are presented as means ± SEM.

For individual brain region volume assessment, total brain volume was expressed in both absolute (i.e., mm$^3$) and relative units (i.e., regional volume as a proportion of total brain volume, within subject).

**Behavioral Testing**

**Novel Object Recognition (NOR):** NOR, a perirhinal dependent task, was used to assess recognition memory. Testing consisted of a habituation phase, a sample phase, and a test phase. During the habituation phase, pigs were placed in an empty testing arena for 10 minutes each day for two days leading up to the sample phase. In the sample phase, the pig was placed in the arena containing two identical objects and was given 5 minutes for exploration. After a delay of 1 hour or 2 days (48 hours), the pig was returned to the arena for the test phase. During the test phase, the pig was placed in the arena containing one object from the sample phase as well as a novel object and allowed to explore for 5 minutes. Between trials objects were removed, immersed in hot water with detergent, and rubbed with a towel to mitigate odor, and the arena was sprayed with water and mopped to remove urine and feces. Objects chosen had a range of characteristics (i.e., color, texture, shape, and size), however the novel and sample objects only differed in shape and size. Only objects previously shown to elicit a null preference were used for testing.

Task order was counterbalanced between replicates. Habituation trials began at 22 days of age, and samples trials on the NOR task began at 25 days of age. The amount of time exploring objects and distance moved was measured using a combination of automated procedures using Ethovision and manual tracking. Exploratory behavior was broken down into 20 behavioral endpoints to assess effects of diet on behavioral performance. Recognition index was
used to measure recognition memory (time spent exploring the new object)/(time spent exploring the new object + time spent exploring the known object).

[00185] Statistical Analysis

[00186] All data generated as part of this study were subjected to an Analysis of Variance (ANOVA) using the MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC). All statistical models employed included replicate as a random effect and data collected at a single time-point (habituation and sample trials) were analyzed by a 2-way ANOVA, and 2) data collected from the same animal on more than one occasion (e.g., multiple test trials etc.) were analyzed as a 3-way repeated-measures ANOVA. Post-hoc comparisons were analyzed for significant interaction effects via adjusted Tukey Test. For some behavioral outcomes (Table 2) a one sample t-test was conducted. In all instances, statistical significant was considered at $P < 0.05$, with trending significance considered at $P < 0.10$

[00187] Results

[00188] Volumetric Assessment

[00189] An effect of dietary treatment was observed for relative volume of the olfactory bulb ($P = 0.02$), Figure 1

[00190] The nutritional intervention with OF shows a positive effect on the volume of the olfactory bulb. This may be related to the increase in short term memory.

[00191] Recognition index:

[00192] The influence of the diets on the recognition index is shown in Table 3 and Figure 2. Treatment of piglet with OF resulted in a significant increase of short-term recognition memory with 1h inter-trial interval but not with 2d inter-trial interval (figure 2, table 3). There was a non-significant effect going in the same direction on number of visits to the novel object. While such effects of HMO and/or OF could be expected with a
2d inter-trial interval, the inventors found that while OF treatment has no effect on long term memory (2d inter-trial interval) it has a positive effect on short term memory (1h inter-trial interval).

[00193] Treatment of piglet with OF+HMO resulted in a significant increase of long term memory with 2d inter-trial interval (figure 2, table 3), as expected. There was a similar effect observed on number of visits to the novel object.

[00194] In other words, OF group has statistically significant higher recognition index compared to CON; OF group exhibited numerically higher number of novel visits compared to CON. The OF+HMO group had statistically significant higher recognition index and number of novel visits compared to CON, as shown in Table 3.

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<td><strong>Measure</strong></td>
<td><strong>Control</strong></td>
<td><strong>OF</strong></td>
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<tr>
<td>Recognition index</td>
<td>0.515(^a)</td>
<td>0.693(^b)</td>
</tr>
<tr>
<td>Number of Novel Visits</td>
<td>5.00(^a)</td>
<td>6.75(^ab)</td>
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\(^a\): Significantly different from control group.\(^b\): Significantly different from other group.

Table 3. Effects of oligofructose and HMO supplementation on exploratory behaviour during the test trial of the NOR task.
Exemplary Claims:

Below are non-limiting examples of claims that may be pursued in a non-provisional application that claims benefit of this provisional application. However, these claims are to be understood to be presented for the purposes of illustration only and do not in any way limit the scope of the inventive concept(s) described or otherwise contemplated herein.

1. Use of a nutritional composition comprising a fructose oligosaccharide (FOS) to improve, enhance or promote declarative memory, short term memory and/or working memory in a mammal.

2. Use of a nutritional composition comprising a fructose oligosaccharide according to claim 1 wherein said declarative memory is recognition memory.

3. Use of a nutritional composition comprising a fructose oligosaccharide according to claim 1 or 2 wherein the mammal is a human, cat or dog and preferably wherein the mammal is a young mammal, an infant or young child.

4. Use of a nutritional composition comprising a fructose oligosaccharide according to claim 1, wherein the fructose oligosaccharide is present in an amount of from 0.1 to 10g/L or 0.3 to 6 g/L or 1 to 3 g/L or 1.25 to 2 g/L or 1.5g/L.

5. Use of a nutritional composition according to any one of the preceding claims which further comprises a prebiotic, said prebiotic being selected from the list bovine milk oligosaccharides, inulin, xylooligosaccharides, polydextrose or any combination thereof.

6. Use of a nutritional composition according to claim 5 wherein said composition further comprises a bovine milk oligosaccharide, said bovine milk oligosaccharides being an N-acetylated oligosaccharide, a galacto-oligosaccharide, a sialylated oligosaccharide, a fucosylated oligosaccharide or a combination thereof.
7. Use of a nutritional composition according to any of claims 1 to 4, in infants and young children, wherein said composition further comprises a human milk oligosaccharide selected from the list consisting of N-acetyl-lactosamine, sialylated oligosaccharides, fucosylated oligosaccharides, 2FL, LNnT, LNT or a combination thereof, and wherein said composition is targeted to infants and young children, such as an infant formula, a follow-on formula and a growing-up milk.

8. Use of a nutritional composition according to claim 7, wherein the N-acetyl-lactosamine is selected from the group comprising lacto-N-tetraose and lacto-N-neotetraose.

9. Use of a nutritional composition comprising according to claim 6 or 7, wherein the sialylated oligosaccharide is selected from the group comprising 3'-sialyllactose and 6'-sialyllactose, and preferably said composition comprises both 3'-sialyllactose and 6'-sialyllactose, the ratio between 3'-sialyllactose and 6'-sialyllactose lying preferably in the range between 5:1 and 1:2.

10. Use of a nutritional composition comprising a fructose oligosaccharide according to claim 6 or 7, wherein the fucosylated oligosaccharide is selected from the group comprising 2'-fucosyllactose, 3-fucosyllactose, difucosyllactose, lacto-N-fucopentaoses (that is to say lacto-N-fucopentaose I, lacto-N-fucopentaose II, lacto-N-fucopentaose III and lacto-N-fucopentaose V), lacto-N-difucohexaose I, fucosyllacto-N-hexaose, Difucosyllacto-N-hexaose I and Difucosyllacto-N-neohexaose II, and preferably the fucosylated oligosaccharide is 2'-fucosyllactose (2-FL).

11. Use of a nutritional composition according to any one of the preceding claims which further comprises a probiotic, wherein the probiotic is a probiotic bacterial strain selected from the list consisting of Lactobacillus acidophilus, Lactobacillus salivarius, Lactobacillus rhamnosus, Lactobacillus paracasei, Lactobacillus casei, Lactobacillus johnsonii, Lactobacillus plantarum, Lactobacillus fermentum,
Lactobacillus lactis, Lactobacillus delbrueckii, Lactobacillus helveticus, Lactobacillus bulgari, Lactococcus lactis, Lactococcus diacetylactis, Lactococcus cremoris, Streptococcus salivarius, Streptococcus thermophilus, Bifidobacterium lactis, Bifidobacterium animalis, Bifidobacterium longum, Bifidobacterium breve, Bifidobacterium infantis, or Bifidobacterium adolescentis or any mixture thereof.

12. Use of a nutritional composition according to any one of the preceding claims wherein said composition is an infant formula, follow on formula, human milk fortifier, growing up milk, or pet food.

13. Fructose oligosaccharide for use in improving, enhancing or promoting short term memory in a mammal wherein said mammal is preferably a human, cat or dog and wherein said human is preferably an infant or a young child or an elderly subject.

14. A nutritional composition comprising a fructose oligosaccharide for use in improving, enhancing or promoting declarative memory, short term memory and/or working memory in a mammal, preferably said mammal being a young mammals, a human, a dog or a cat, more preferably a young human, dog or cat, most preferably an infant or young children.

15. The use of fructose oligosaccharide in the manufacture of a nutritional composition for improving, enhancing or promoting declarative memory, short term memory and/or working memory in a mammal, preferably said mammal being a young mammals, a human, a dog or a cat, more preferably a young human, dog or cat, most preferably an infant or young children.
An effect of dietary treatment was observed for relative volume of the olfactory bulb ($P = 0.02$). $^{ab}$Means without a common superscript letter differ ($P < 0.05$). CON: control; OF: oligofructose; OF+ HMO: oligofructose + human milk oligosaccharides (2FL).
FIG. 2

Recognition index using 1h or 2 days inter-trial interval induced by various diets [OF, OF+HMO (2FL), control (CON)]
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. A23L33/00 A61K31/702 A23L33/21

ADD.

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)

A23L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal , BIOSIS, EMBASE, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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Note: Further documents are listed in the continuation of Box C.

See patent family annex.

* 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
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  - **P** document published prior to the international filing date but later than the priority date claimed

**Date of the actual completion of the international search**

26 June 2018

**Date of mailing of the international search report**

05/07/2018

**Name and mailing address of the ISA/ISA/**

European Patent Office, P.B. 5818 Patentlaan 2
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Fax: (+31-70) 340-3016

**Authorized officer**

Smeets, Dieter

Form PCT/ISA/210 (second sheet) (April 2005)
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