

NUTRITIONAL COMPOSITIONS SHOWING PHASE SEPARATION UNDER GASTRIC CONDITIONS COMPRISING PHOSPHOLIPIDS, METHODS FOR PREPARING THE SAME, AND USES THEREOF

5 The invention relates to the field of nutritional compositions, methods for providing them and uses thereof. More in particular, it relates to ready-to-feed and powdered infant and growing up milk formulas showing desirable gastric digestion properties.

10 It has been considered for a long time by nutritionists that the best food or nutrition supplied to an infant is its own mother's milk; i.e. fresh human milk. It is recognized, however, that many situations arise wherein the infant cannot be fed mother's milk and as a result cows' milk based formulas have been prepared and used to nourish an infant. These formulas contain a mixture of casein and whey proteins to provide an amino acid profile as close as possible to that of
15 mother's milk. Much effort has been made to improve infant milk formulas to more closely mimic mother's milk. This resemblance of mother's milk may reside in the composition of the infant formula. Additionally and/or alternatively it may reside in the growth and development of the infant being more similar to infants exclusively being fed with mother's milk.

20 The protein systems of human milk and cows' milk differ substantially, both quantitatively and qualitatively. Prominent quantitative differences include a lower total protein content, often expressed as the total nitrogen content multiplied by 6.25 or 6.38, of human milk (11 g/L) compared to cows' milk (33-35 g/L). "Increasing evidence indicates that, relative to breastfed infants, a higher
25 protein intake in formula fed infants causes an early rapid weight gain during the first year of life which may affect body composition later in life. Another difference is that human milk does not comprise β -lactoglobulin (bLac).

 Total nitrogen containing components in milk can be divided into true protein and non-protein nitrogen (NPN), with caseins and serum proteins (the
30 latter also called whey proteins) as the main classes of proteins. Caseins are the proteins from milk that precipitate at pH 4.6, whereas whey proteins remain soluble at this pH. In mature human milk, the ratio of whey protein to casein is

about 60:40 to 50:50, whereas the whey protein to casein ratio is approximately 18:82 in cows' milk. The casein fraction in cows' milk comprises α_{a1} -, α_{s2} -, β - and κ -casein, whereas α_{s2} -casein seems to be absent in human milk. Also the composition of the whey protein fraction differs between human and cows' milk. The most
5 abundant whey proteins in human milk are α -lactalbumin, lactoferrin and immunoglobulins, whereas the whey protein fraction of cows' milk comprises approximately ~50% β -lactoglobulin and ~15% α -lactalbumin.

The fat content of human milk increases throughout expression, which means that the composition of the product entering the infant's stomach is dynamic
10 in composition. Foremilk usually contains only 2% fat, whereas the fat content of hindmilk can be 8% or even higher. This results in initial ingestion of breastmilk with a lower caloric value, followed by an increase in caloric value with increasing fat content. As a consequence, the gastric emptying that is mostly regulated by caloric value, follows a biphasic, "phased" pattern with an initial fast emptying of
15 the watery phase and a delayed emptying of fat. In contrast, conventional infant formulas are typically of a homogeneous nature, and therefore empty with a constant caloric value at a more constant and slower rate when compared to breastmilk.

Hence, it is not only important to prepare an infant milk formula by the
20 appropriate blending of nutritional ingredients, including varying proportions of selected vegetable fats optionally combined with milk fat to yield a composition approximating that of human milk, but also to assure an optimal absorption of the nutrients by phasing the digestion and stomach emptying. This will help to prevent an overload of protein and fat in the intestine and hence will lead to a beneficial
25 health impact with respect to gastrointestinal comfort, energy regulation and metabolic health. Overall, optimized digestion and absorption-kinetics of formula may further contribute to a normal healthy growth as observed with breastfed infants.

In an attempt to develop a method of feeding infants that provides a
30 gradual increase of fat content that is similar to that of the breast feeding, US2006/188614 discloses a method comprising the steps of (a) feeding the infant by foremilk equivalent having a volume of, for example, 30-60% of a total meal and

a fat percentage of for example, 2.5-3.5%; and (b) feeding the infant by hindmilk equivalent having a volume of, for example, 40-70% of the total meal and a percentage of fat of, for example, 3.7-5.5%. To that end, it is proposed that the feeding is performed with a bottle which is divided into two compartments, one
5 being filled with the foremilk equivalent and the other with the hindmilk equivalent, the bottle being designed in such a manner enabling the infant to consume at first the foremilk equivalent and thereafter the hindmilk equivalent gradually admixed with the foremilk equivalent. A drawback of this approach is that multiple compositions need to be prepared and administered, each in the
10 desired amounts.

EP2296494B1 aims at providing a single nutritional composition that can mimic the concentration differences in fat of human milk. Disclosed is an infant formula having a sufficient amount of fat droplets with an increased diameter (5-25 μm) compared to standard infant milk formula. Due to the differences in
15 densities between the fat and water, the fat droplets will "cream" to result in an increased fat concentration in the upper part of the container e.g. a drinking bottle compared to the lower part of the container. However, it can take a considerable time before such a fat gradient is established in the bottle. E.g. even when droplets have a size of 15 micron, one can calculate that the creaming will take a long time.
20 (e.g. approximately 1 hour for a droplet of this size to cream a few (e.g. 3-6) centimeters). Moreover, such an infant milk will be inhomogeneous, which is usually not perceived as very attractive.

Infant formulae are often based on cow's milk. Cow's milk comprises more protein and less nonprotein nitrogen (NPN) compared to human milk. The most
25 abundant NPN in human milk is urea. In human milk the amount of urea can approximately be 10-13 % of total nitrogen, resulting in a concentration of about 15 to 35 mg urea per 100 ml. Methods are known in the art to determine NPN and urea. Urea is also known as carbamide, $\text{CO}(\text{NH}_2)_2$. Urea is commercially available from various sources in food grade quality, for example from NuGenTec, CA USA,
30 Spectrum chemical, USA, or it can be enriched from cow's milk.

Human milk (HM, also referred to as human breast milk, HBM) provides infants with macro- and micronutrients needed for growth and development. Milk

phospholipids (PL) are important sources of bioactive components, such as long-chain polyunsaturated fatty acids (LC-PUFA) and choline, crucial for neural and visual development. Phospholipids in milk may be characterized using methods known in the art e.g. using LC-MS or ³¹P-NMR. Different classes of milk PL have been identified: PE: Phosphatidylethanolamine, PC: Phosphatidylcholine, SM: Sphingomyelin.

Sphingomyelin or ceramide 1-phosphocholine is a sphingophospholipid that consists of a ceramide unit with a phosphorylcholine moiety attached to position 1 of the sphingoid base component. It is thus the sphingolipid analogue of phosphatidylcholine, and like that lipid it is zwitterionic, although the similarities between the two do not extend much further. However, they are related metabolically. Sphingomyelin is primarily of animal origin and is a ubiquitous component of all animal cell membranes. Phospholipids, sphingomyelin, sialic acid, gangliosides are all nutrients which are recognized as important nutrients for the infant. The challenge has been to be able to incorporate suitable sources of these nutrients into the composition of the formula without negative effect on other important nutrients and the general composition of the product (EP3175719 A1). Due to the amphiphilic character, as well as their interfacial properties and their natural sources of production, the use of phospholipids as emulsifiers has been investigated since the late 1960s. A consequent number of papers have been published dealing with potential applications of PLs-stabilized emulsions for the pharmaceutical industry, and/or for the food or cosmetic industry.

WO2020200989 relates to ready-to- feed and powdered infant or growing up milk formulas showing desirable gastric digestion properties based on *in vitro* experiments. WO2020200989 discloses a milk formula comprising fat droplets that are coated predominantly with the milk-derived caseins which induce fat creaming in the stomach. In other words the oil-in-water stability of WO2020200989 is balanced and specifically tailored in such a way that it is stable and homogenous, and does not induce creaming or the formation of visible "fat eyes" in the drinking bottle. Once in an acidic environment e.g. in the stomach, the oil in water emulsion rapidly loses its stability resulting in fat droplet flocculation and separation of fat

from the water layer. It discloses that such a balanced emulsion-stability is ingredient and pH dependent. It further discloses that that it is important that the emulsion rapidly loses its stability once in an acidic environment in order to mimic the phased release of mother's milk. It also discloses how such formula may be
5 obtained.

The present inventors therefore aimed at developing a nutritional composition based on bovine milk that mimics the *in-vivo* phased release of breastmilk from the stomach into the small intestine, without having to rely on establishing a fat gradient and/or other inhomogeneities prior to administering the
10 composition to an infant. Preferably, the nutritional composition has an earlier onset of fat layer volume formation and/or a smaller maximum fat layer volume and/or short time to maximum fat layer volume. The inventors further aimed at developing a nutritional composition that mimics human breast milk, for example in the presence of sphingomyelin and phospholipids. Additionally, these benefits
15 should be obtained without affecting the absorption of nutrients present in the composition, as represented by free fatty acid (FFA), insulin, glucose and further metabolomics' levels *in vivo*. Ideally, the composition is stable and homogenous, and does not induce creaming or the formation of visible "fat eyes" in the drinking bottle. Furthermore, such 'phased release' composition can preferably be
20 manufactured in a manner that minimally affects biological activity provided by bioactive milk protein e.g. milk immunoglobulins (Igs).

At least some of these goals were met by the surprising finding that fat creaming in the stomach can be maintained in the presence of sphingomyelin, and phospholipids. The fat creaming in the stomach being induced by a milk formula
25 comprising fat droplets that are coated predominantly with the milk-derived caseins. Caseins are hydrolyzed in the stomach very fast (even at pH 6) by the gastric protease pepsin. Since hydrolyzed caseins on the interface of the fat droplet and the remaining aqueous composition are unstable, the droplets start to flocculate and large fat containing particles are formed which start to cream.
30 Normally, fat droplets in infant formula have a mode diameter of between 0.1-2 micrometer such as approximately 0.5 micrometer. Fat droplets of this size, coated with mainly caseins, will flocculate in "early" gastric digestion (pH 6.0 to pH 5.5),

to result in phase separation into a bottom layer low in fat and an upper layer enriched in fat. This was particularly surprising as the compositions of the invention were comprising significant higher levels of sphingomyelin and phospholipids which are known to stabilize oil in water emulsions. With
5 compositions of the invention gastric emptying of such a nutritional composition involves an initial low-fat phase that is followed by a (delayed) phase of fat entry into the intestines while maintaining the benefit of phospholipids.

Accordingly, the invention relates to a nutritional composition comprising
10 casein, whey proteins comprising α -lactalbumin (aLac) and β -lactoglobulin (bLac), and protein-coated fat droplets, wherein

- (i) the protein content of the composition is 5 to 20% (w/w), preferably 7-16 w%, based on total solids;
- (ii) the bLac content is at least 3% (w/w) based on total protein level and the
15 aLac + bLac content is at least 25% (w/w) based on the total protein level;
- (iii) the fat content is in the range of 10% to 50% (w/w) based on total solids;
- (iv) the fat droplets are coated with an average protein load of 2-10 mg/m² and wherein the weight ratio of casein: (aLac + bLac) on the fat droplets is at least 4 times higher, preferably at least 4.5 times higher, than the weight ratio of casein:
20 (aLac + bLac) in the total composition;
- (v) optionally the amount of urea per 100 g of composition is between 30 mg and 140 mg based on total solids;
- (vi) the level of sphingomyelin per 100 g of composition is between 70 mg and 200 mg based on total solids, preferably between 75 mg and 150 mg; more
25 preferably between 75 mg and 130 mg; and
- (vii) the level of phospholipids per 100 g of composition is between 280 mg and 950 mg based on total solids, preferably between 285 mg and 850 mg, more preferably between 285 mg and 700 mg, most preferably between 290 mg and 650 mg; and wherein the amount of casein macropeptide (CMP) per 100 g of
30 composition being between 100 mg and 500 mg, based on total solids.

Preferably, the invention relates to a nutritional composition comprising casein, whey proteins comprising α -lactalbumin (aLac) and β -lactoglobulin (bLac), and protein-coated fat droplets, wherein

- (i) the protein content of the composition is 5 to 20% (w/w), preferably 7-16 w%,
5 based on total solids;
 - (ii) the bLac content is at least 3% (w/w) based on total protein level and the aLac + bLac content is at least 25% (w/w) based on the total protein level;
 - (iii) the fat content is in the range of 10% to 50% (w/w) based on total solids;
 - (iv) the fat droplets are coated with an average protein load of 2-10 mg/m² and
10 wherein the weight ratio of casein: (aLac + bLac) on the fat droplets is at least 4 times higher, preferably at least 4.5 times higher, than the weight ratio of casein: (aLac + bLac) in the total composition;
 - (v) the amount of urea per 100 g of composition is between 30 mg and 140 mg based on total solids, preferably between 35 mg and 125 mg, more preferably
15 between 40 mg and 110 mg, most preferably between 40 mg and 60 mg;
 - (vi) the level of sphingomyelin per 100 g of composition is between 70 mg and 200 mg based on total solids, preferably between 75 mg and 150 mg; more preferably between 75 mg and 130 mg; and
 - (vii) the level of phospholipids per 100 g of composition is between 280 mg and
20 950 mg based on total solids, preferably between 285 mg and 850 mg, more preferably between 285 mg and 700 mg, most preferably between 290 mg and 650 mg;
- and wherein the amount of casein macropeptide (CMP) per 100 g of composition being between 100 mg and 500 mg based on total solids.

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The concept of covering/coating fat droplets with a protein load that is enriched in caseins in the presence of specific enriched levels of sphingomyelin, and phospholipids disclosed in the present invention is not taught or suggested in the art. It has been reported that modification of fat droplets (also referred to in
30 the art as 'lipid globules') can be used to modulate the rate and extent of protein aggregate formation in the stomach and/or to increase the gastric emptying rate. However, these approaches focused on coating the fat droplets with defined lipid

components. Use of phospholipids and sphingomyelin in infant formula has also been reported, however these products are known for their stabilization of oil-in-water emulsions such as infant formula. As such it was expected that higher levels of these components would stabilize the composition of the invention and prevent fat creaming of the composition in the acid environment of the stomach.

For example, WO2010/027259 relates to the field of infant milk formula and growing up milks for preventing obesity later in life. It discloses a nutritional composition comprising 10 to 50 wt.% vegetable lipids based on dry weight of the composition, and i) 0.5 to 20 wt.% phospholipids based on total lipids or ii) 0.6 to 25 wt.% of polar lipids based on total lipids, wherein polar lipids are the sum of phospholipids, glycosphingolipids and cholesterol, and said composition comprising lipid globules with a core comprising said vegetable lipids and a coating comprising said phospholipids or polar lipids.

WO2016/163882 relates to a method for reducing the rate and extent of protein aggregate formation in the stomach and/or increasing the gastric emptying rate in a subject, comprising administering to the subject a nutritional composition comprising carbohydrates, protein, and lipid globules, wherein the protein comprises casein and the lipid globules comprise triglycerides derived from vegetable fat and phospholipids derived from non-human mammalian milk. The lipid globules have a mode diameter from 2 to 6 μm and/or a specific surface area from 0.5 to 15 m^2/g lipid, and the lipid globules comprise a coating comprising the phospholipids.

A nutritional composition of the present invention comprises casein, whey proteins, in particular α -lactalbumin and β -lactoglobulin, and protein-coated fat droplets in the presence of sphingomyelin and phospholipids.

The total protein content of the composition is 5 to 20% (w/w), preferably 7-16 w%, based on total solids. The total amount of α -lactalbumin and β -lactoglobulin is at least 25% (w/w) based on the total protein level.

In one embodiment, the composition of the invention has a low protein content, i.e. the protein content in the composition of the invention is less than 4 g per 100 kcal, e.g. less than 3.8 g per 100 kcal, preferably less than 3.0 g / 100 kcal, more preferably less than 2 g / 100 kcal. Even more preferably, the protein content

in the composition of the invention is between 0.95 g and 3.8 g per 100 kcal, particularly preferably between 1.0 g and 2.5 g per 100 kcal, most preferably between 1.6 and 1.8 g per 100 kcal.

The α -lactalbumin and β -lactoglobulin for use in the present invention
5 can be derived from any suitable whey protein source. For example, either one or both of α -lactalbumin and β -lactoglobulin is derived from milk, from cheese whey, from acid casein whey or from milk serum or from concentrated, diluted, demineralized or powdered variants thereof. In addition to α -lactalbumin and β -lactoglobulin, a composition of the invention may contain further whey proteins,
10 such as serum albumin, lactoferrin and/or immunoglobulins. In a specific aspect, it contains all "non-casein" proteins as found in bovine milk.

To allow for optimal protein digestion, the α -lactalbumin and β -lactoglobulin in a composition of the invention are predominantly in a native state e.g. from the total amount of α -lactalbumin and β -lactoglobulin >50% is
15 soluble at pH 4.6. Preferably, at least 60% of the α -lactalbumin and β -lactoglobulin is native, more preferably at least 80%, most preferably at least 90%, or at least 95%. This is suitably achieved by using proteins that have not been exposed to temperatures above 85 °C (e.g. UHT treatment) at which > 50% of the whey proteins denature. Preferably, sources of aLac and bLac are used that have
20 undergone at least one heat treatment of 15 s at 72°C or an equivalent "mild" heat treatment.

Likewise, the casein can be obtained from conventional sources. In one embodiment, the casein is selected from the group consisting of micellar casein, non-micellar casein, acid casein, calcium caseinate, magnesium caseinate, sodium
25 caseinate, potassium caseinate and ammonium caseinate, or any combination thereof. The casein can for example be obtained from whole milk, skimmed milk and/or milk protein concentrate.

Typically, the weight ratio of caseins to total whey proteins (i.e. α -lactalbumin, β -lactoglobulin, and optional further whey proteins) in a nutritional
30 composition is in the range of 70:30-20:80, for example 60:40-20:80, 55:45-25:75 or 50:50-20:80. Preferably, the weight ratio of caseins to total whey proteins is in the range of 50:50-30:70.

Urea is abundant in human milk, representing a large part of the non-protein nitrogen (NPN). The role of urea in infant nutrition is not fully understood and urea levels in infant formula are generally lower than the levels in human breast milk. Preferably, the urea content in the composition of the invention is in the range of 30 mg to 140 mg per 100 g of composition, based on total solids. In one embodiment, it is between 35 mg and 125 mg, more preferably between 40 mg and 110 mg, most preferably between 40 mg and 60 mg per 100 g of composition, based on total solids. Urea may be obtained from any source, such as synthetic sources. Alternatively, bovine-milk fractions rich in urea may be used.

The level of sphingomyelin per 100 g of composition, based on solids, is between 70 mg and 200 mg. In one embodiment it preferably is between 75 mg and 150 mg; more preferably between 75 mg and 130 mg per 100 g of composition, based on solids.

The level of phospholipids per 100 g of composition, based on solids, is between 280 mg; and 950 mg. In one embodiment it preferably is between 285 mg and 850 mg, more preferably between 285 mg and 700 mg, most preferably between 290 mg and 650 mg.

Phospholipids and sphingomyelin may be obtained from milk fractions enriched in such components. Such milk fractions are well-known to the skilled person. For example is specific milk fat fractions being enriched in Milk Fat Globular Membrane (MFGM).

The nutritional composition of the invention may further comprise one or more selected from the group consisting of:

- (viii) fat droplets having an average diameter in the range of 0.2 to 1.0 micron;
- (ix) the amount of sialic acid per 100 g of composition (on total solids) being between 75 mg and 120 mg, preferably between 80 mg and 110 mg;
- (x) the amount of casein macropeptide (CMP) per 100 g of composition being between 100 mg and 400 mg (on total solids), preferably between 100 mg and 300 mg, more preferably between 105 mg and 250 mg, most preferably between 105 mg and 225 mg; and

- (xi) the sphingomyelin content per 100 g of fat in the composition being between 300 mg and 950 mg, preferably between 320 mg and 850 mg, more preferably between 330 mg and 750 mg, most preferably between 330 mg and 700 mg.

In one preferred embodiment the composition of the invention comprises
5 two or more of group consisting of viii, ix, x, and xi as defined above, such as ix and x, or as ix and xi, or as x and xi. More preferably the composition of the invention comprises all of ix, x, and xi. Even more preferably the composition of the invention comprises all of viii, ix, x, and xi.

The "average diameter" of the fat droplets as used herein may also be
10 referred to as "mode diameter" and relates to the diameter which is the most present based on volume of total lipid, i.e. the peak value in a graphic representation, having on the X-axis the diameter and on the Y-axis the volume(%). The volume distribution of the particle diameter of the lipid globules is determined using "Laser Diffraction Particle Sizing", for example using a Malvern
15 Mastersizer apparatus.

Sialic acid (N-acetylneuraminic acid, NANA) is an essential component of mucins, glycoproteins and gangliosides and therefore important for the function of cell membranes, membrane receptors and the normal development of the brain. The amount of sialic acid as used herein, is referring to the total amount of sialic
20 acid in the composition i.e. free sialic acid and bound sialic acid. Bound sialic acid may for example be part of a glycan chain in a glycoprotein or in gangliosides. The amount of sialic acid may be determined using methods known in the art such as for example HPLC-based determination methods.

In yet another embodiment the nutritional composition of the invention
25 further comprises one or more selected from the group consisting of:

- (xii) fat droplets having an average diameter in the range of 0.2 to 1.0 micron;
(xiii) the amount of sialic acid per 100 g of composition (total solids) being between 80 mg and 110 mg;
(xiv) the amount of casein macropeptide (CMP) per 100 g of composition being
30 between 105 mg and 225 mg based on total solids; and
(xv) the sphingomyelin content per 100 g of fat in the composition being between 330 mg and 700 mg.

In one preferred embodiment the composition of the invention comprises two or more of group consisting of xii, xiii, xiv, and xv as defined above, such as xiii and xiv, or as xiii and xv, or as xiv and xv. More preferably the composition of the invention comprises all of xiii, xiv, and xv. Even more preferably the composition
5 of the invention comprises all of xii, xiii, xiv, and xv.

The composition of the invention may further have a relative low amount of casein macropeptide (CMP), which may also be referred to as glycomacropeptide (GMP). The low level of CMP per 100 g of composition (total solids) being between 100 mg and 500 mg, preferably between 100 mg and 300 mg, more preferably
10 between 105 mg and 250 mg, most preferably between 105 mg and 225 mg. Lower levels of CMP are beneficial in the prevention of hyperthreonina in formula fed infants.

The fat content of a nutritional composition is in the range of 10% to 50% (w/w) based on total solids. In one embodiment, the fat content is 15-40% (w/w),
15 preferably 25-35 w%. Whereas the weight ratio of total fat to total protein is not critical, it is preferably in the range of 3.5:1-1:1.5.

Preferably, the fat is homogenized in order to obtain a stable and homogeneous product wherein the fat is predominantly present in the composition in the form of protein-coated fat droplets. In one aspect, the fat droplets have a
20 core that consists of at least 90w%, preferably at least 95w% of triglycerides. The fat droplets typically have an average diameter in the range of 0.2 to 1.0 micron, preferably 0.3 to 0.8 micron, i.e. much smaller than that of human milk or the synthetic composition of EP2296494B1. Besides caseins and whey proteins, the fat droplet surface may contain milk fat globule membrane (MFGM) material.

25 Any type of fat source commonly used in nutritional (infant) formulas can be used. The fat component of infant formulas has traditionally been considered the most important energy source for the infant as well as a necessary requirement for normal growth and development. The Codex Standard established for the amount of fat in an infant formula is not less than 3.3 grams and not more than
30 6.0 grams per 100 available kilocalories. Fat provides approximately 9 kilocalories per gram. Consequently, fat contributes between 30 percent and 54 percent of

available kilocalories in an infant formula. In most commercial infant formulas, fat provides about half of the food energy.

The nutritional composition of the invention is a synthetic nutritional composition, i.e. it is produced by humans from different ingredients. The nutritional composition of the invention is not milk from a mammal, such as human milk.

In one embodiment, the fat source is a dairy milk fat, a vegetable oil, a vegetable fat, a hydrogenated vegetable oil, a marine oil, an algae oil, single cell oil or a mixture of any of the foregoing. 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. In a preferred aspect, the composition comprises a dairy milk fat, more preferably a dairy milk fat selected from the group consisting of whole milk, cream, anhydrous milk fat and fractions from milk fat. In one embodiment, a combination of a dairy milk fat and a vegetable fatblend is used. For example, the composition comprises a mixture of milk fat and vegetable oils. Preferred fat sources include milk fat, sunflower oil, coconut oil and rapeseed oil. In a specific aspect, the fat in the composition consists of at least 20 w%, preferably at least 30w%, dairy milk fat.

Preferably, a composition according to the present invention also comprises a source of long-chain polyunsaturated fatty acids, preferably selected from docosahexaenoic acid (DHA), arachidonic acid (ARA), eicosapentaenoic acid (EPA) and/or dihomo-gamma-linolenic acid (DGLA).

The invention also provides a method for providing a nutritional composition according to the invention. In one embodiment, such method comprises the steps of:

- a. Blending skimmed milk and a whey protein source comprising α -lactalbumin and β -lactoglobulin and /or other whey proteins and optionally of urea;
- b. Pasteurization of the blend at a temperature of less than 85 °C;
- c. Evaporation at a temperature of less than 68 °C;
- d. Addition of a fat source;
- e. Homogenization of the composition obtained in step d);
- f. Drying the composition obtained in step e. to obtain a powdered composition preferably using spray drying, and;

g. Optionally reconstituting the powdered composition with a liquid;
wherein a source of sphingomyelin, of phospholipid, and/or of sialic acid is
blended into i) the blended skimmed milk and whey protein source of step a.
and/or into ii) the powdered composition of f).

5 Preferably wherein the source of sphingomyelin, of phospholipid, and/or of sialic
acid is blended into the powdered composition of f). Drying step f. may be done
using methods known in the art such as using freeze drying, especially when a
more hygroscopic dried product is required.

Prior to blending with the whey proteins, the skimmed milk may be
10 treated with ceramic membrane filtration to reduce the bacterial count. To ensure
optimal digestibility of the milk proteins, the method preferably does not comprise
exposing α -lactalbumin and β -lactoglobulin to conditions that induce denaturation
and/or aggregation of α -lactalbumin and β -lactoglobulin. In a specific aspect, at
least 95% of α -lactalbumin and β -lactoglobulin are native.

15 Preferably the pasteurization step is done at a temperature of less than 80°C,
more preferably between 70 and 80°C.

The source of α -lactalbumin and β -lactoglobulin can be cheese whey, acid whey or
milk serum obtained via membrane filtration, optionally wherein the whey is
treated with ceramic membrane filtration to reduce the bacterial count. For
20 example, the composition comprises demineralized whey, a whey protein
concentrate or a milk serum concentrate, fractions, or combinations thereof. For
example, an aLac- or milk phospholipid-enriched WPC can be used.

In an alternative embodiment, a method for providing a nutritional
25 composition according to the invention comprises the steps of

- a. Preparing a first base powder comprising or consisting of a caseinate-
stabilized emulsion with a carbohydrate as a carrier.
- b. Preparing a second base powder comprising α -lactalbumin and
 β -lactoglobulin (and possibly other whey proteins), and optionally other milk
30 proteins, minerals and vitamins.

- c. Dry blending the powders obtained in steps a and b, optionally together with one or more additives such as nucleotides, oligosaccharides, trace elements, etc.

Preferably, the carbohydrate in step a. is lactose.

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A composition of the invention is among others characterized in that the fat droplets are coated predominantly with caseins and whey proteins comprising α -lactalbumin and β -lactoglobulin, at an average protein load of 2-10 mg/m², like 2-6 mg/m², preferably 3-10 m/m², like 3-8. In one embodiment, it is below 8 mg/m²,
10 for example in the range of 3-7 mg/m². In a specific aspect, the protein load is between 3 and 5.5 mg/m².

Furthermore, the weight ratio of casein: (aLac + bLac) on the fat droplets is at least 4 times higher than the weight ratio of casein: (aLac + bLac) in the total
15 composition. In other words, the caseins in the composition are not distributed evenly across the entire composition but they are enriched/concentrated at the surface of the fat droplets. Since caseins are hydrolyzed very fast under gastric conditions, the fat droplets become instable and start to flocculate to form large fat-containing particles. This creaming process induces a phase separation into an
20 upper fat-enriched phase and a lower low-fat phase. Preferably, the weight ratio of casein: (aLac + bLac) on the fat droplets is at least 4.5 times higher, e.g. at least 4.6, 4.8, 5.0, 5.2 or 5.5 times higher than the weight ratio of casein: (aLac + bLac) in the total composition. In one embodiment, the ratio between (a) the weight ratio of casein: (aLac + bLac) on the fat droplets and (b) the weight ratio of casein: (aLac
25 + bLac) in the total composition is in the range of 4.2-8.0, preferably 4.5-7.5.

The protein load on fat droplets and the ratio of casein: whey protein (aLac+bLac) in a composition and fractions thereof can be determined by methods known in the art using fractionation and taking into account the fat droplet
30 particle size, the protein content and the fat content. If the composition is in a dry form, it is first reconstituted e.g. using demineralized water, to a fat content in the range of 2-10% (w/w). The size distribution of the fat droplets can be determined

using a Malvern Mastersizer. The fat droplets are typically separated from the remainder of the composition using density centrifugation. Separation of the protein-coated fat droplets from the remainder of the composition advantageously comprises increasing the density of the liquid phase surrounding the fat droplets.

5 This is suitably done by adding sucrose to the reconstituted composition.

The ratios between caseins and the whey proteins α -lactalbumin + β -lactoglobulin on the fat droplets and in the total nutritional composition are suitably determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions. Therefore, the ratio based on mass ratios
10 would even be slightly higher than ratio based on intensities. The amount of milk protein in the protein bands corresponding to major caseins (e.g. β -casein and α -s-casein), aLac and bLac can be quantified by methods known in the art, for example by a stain free imaging methodology (see also Example 5 herein below). In one embodiment, the protein ratios are determined using a stain free enabled Bio-Rad
15 ChemiDoc XRS+ Documentation System Bio-Rad unit provided with ImageLab software.

Typically, the ratio between the band intensities of caseins and the whey proteins α -lactalbumin and β -lactoglobulin in a total composition of the invention is below 1, and for example ranges from about 0.35 to about 0.95. In contrast, said
20 ratio in the fat fraction comprising protein-coated lipid droplets is generally above 1.5, preferably at least 1.8, like 2.0 or higher, or 3.0 or higher. As a result, the ratio of casein: (aLac + bLac) on the fat droplets is at least 4 times higher, preferably at least 4.5 times higher, than the weight ratio of casein: (aLac + bLac) in the total composition.

25 A nutritional composition according to the invention can be in the form of a dry, semi-dry or liquid composition. For example, it is a powdered composition which is suitable for making a liquid composition after reconstitution with an aqueous solution, preferably with water.

In another embodiment, it is a liquid composition, for instance a ready-
30 to-consume drinkable or spoonable composition. In a specific aspect, the (semi-) liquid composition has a total protein content up to 30 grams per liter. The relative amounts of casein and whey proteins can vary. In one embodiment, the casein

content of composition is up to 20 grams per liter, preferably up to 18 grams per liter.

A nutritional composition according to the invention can be any type of product for use in mammalian nutrition, in particular human nutrition. In view of its unique digestibility properties mimicking the gastric behavior of human milk, it is advantageously an infant formula, a follow-on formula or a growing up milk.

A person skilled in the art will recognize and appreciate that a composition of the invention finds various interesting applications. For example, it is suitably used in a method of controlled release of protein and fat into the intestine of a subject, preferably a human subject. Further uses, which can be either therapeutic or non-therapeutic, include a method to improve gastrointestinal health, a method to improve energy regulation such as in one embodiment the prevention and/or treatment of obesity and/or a method to improve metabolic health in a subject. In one embodiment, gastrointestinal health includes gastrointestinal comfort such as less cramps in the gastrointestinal tract or less regurgitation. In one embodiment, the invention provides the use of a nutritional composition (obtainable by a method) as herein disclosed in a method of controlled release of protein and fat into the intestine of a subject. Also provided is the use of a nutritional composition in a method to maintain or improve gastrointestinal health, energy regulation and/or metabolic health in a subject. In another embodiment, the invention provides the use of a nutritional composition according to the invention and / or as obtainable in accordance with a method of the invention, in a method to control overall formula digestion kinetics in subject. As used herein, "control overall formula digestion" as used herein is defined as a digestion of a formula wherein the initial amount of fat and protein is lower as compared to the final amount of protein and fat being released to the small intestine. It is believed that such a controlled overall formula digestion helps in preventing accelerated growth of the subject as is normally observed with formula fed subjects when compared to breast fed subjects. The subject is preferably a human subject, more preferably a human subject with an age between 0 and 36 months.

The whey protein fraction of mature bovine milk contains about 10-15% immunoglobulins or antibodies. Immunoglobulins are a protective antibody family found in whey. A further application of a "low heat processed" composition of the invention relates to the fact that it contains a relatively high level of intact and functionally active cow's milk immunoglobulins, which are known to have health-promoting effects in humans. More in particular, milk immunoglobulins can prevent the attachment of pathogen to the epithelial lining that is a critical step in the establishment of infection. It has been reported that orally administered bovine colostrum or milk immunoglobulins have proven effective in the prevention of orally mediated infections. Clinical studies have been undertaken to evaluate the potential of immune milk products as preventative treatment for various hospital infections, especially those caused by antibiotic resistant bacteria and *Helicobacter pylori*, the causative agent of chronic gastritis. El-Loly (Int. J. of Dairy Science, Volume 2 (3): 183-195, 2007) reviewed the properties of bovine immunoglobulins, their isolation from colostrum and utilization in the preparation of bovine immune milk for prevention and treatment of microbial infections in humans and animals. So, preferably the composition of the invention additionally comprises at least 2.0 g of bovine immunoglobulin (g Ig) per 100 g of composition (dry weight), more preferably at least 2.5 g, even more preferably at least 3.0 g. The level of b Ig normally is less than 5.0 g per 100 g of composition.

Accordingly, the present invention also provides a nutritional composition for use as immune milk product, among others in a method for the prevention or treatment of microbial infection in a subject, preferably a human subject, more preferably a human subject with an age between 0 and 36 months. Preferably, the nutritional composition is pasteurized at a temperature between 70 and 80°C In one embodiment, the microbial infection is a gastrointestinal infection.

In another aspect, the invention relates to the use of the composition of the invention or to a composition obtainable by the method of the invention, in the manufacture of a medicament for to maintain or improve gastrointestinal health, gastrointestinal comfort, energy regulation and/or metabolic health in a subject, preferably a human subject, more preferably a human subject with an age between 0 and 36 months. Preferably the metabolic health is a prevention of obesity.

In another aspect, the invention relates to the use of the composition of the invention or to a composition obtainable by the method of the invention, in the manufacture of a medicament for the prevention or treatment of microbial infection in a subject, preferably a human subject, more preferably a human
5 subject with an age between 0 and 36 months. Preferably the microbial infection is a gastrointestinal infection.

When referring to amounts to 100 ml this relates to packed ready-to-drink liquid products, but also to products that are ready to drink after they have been reconstituted with water from a powder or a concentrate according to instructions.
10 Normally 13-14 of dry product is reconstituted with water to obtain 100 mL of reconstituted product.

LEGENDS TO THE FIGURES

In Figure 1 a schematic overview of test session for participants is shown.
15 After a fasting period of minimally 12 hours, they were allowed to drink water up to 2 hours prior to their visit. Upon arrival at the hospital, a cannula was placed in an antecubital vein, a blood sample was taken, a baseline MRI scan was performed, and appetite ratings were obtained (Baseline). Subsequently, participants consumed one of the two IFs with a mean ($\pm$ SE) ingestion time of 2.1
20 ($\pm$ 0.1) min (minimally processed, EF IF: 2.0 ± 0.2 min, control IF: 2.3 ± 0.2 min); T=0. Subsequently, gastric MRI scans were performed at 5-minute intervals during the first 30 minutes. After that, scans were made every 10 minutes, up until 2 hours post-prandially. Blood samples were taken at t = 15, 30, 45, 60, 75, 90, 120 min.

25 In Figure 2 an illustration of *in vivo* onset of fat layer formation in the stomach as determined using MRI is shown. Treatment "A" is the EF-IF according to the invention as defined in the examples. Treatment "B" is the control IF. A straight line between treatment "A" and "B" represents the difference in onset of fat layer formation within one subject depending on the composition consumed.
30 Statistics: Paired t-test: P = 0.0167. Average time increase going from EF-IF to control IF is 13.4 minutes with a standard error of 5.1 minutes. Corrected for

baseline: $P=0.0014$ and the Average time increase going from EF-IF to control IF is 13.6 minutes with a standard error of 3.6 minutes.

In Figure 3 the time to peak of fat layer formation is shown. Illustration of *in vivo* time to peak fat layer in the stomach as determined using MRI. Treatment Treatment “A” is the EF-IF according to the invention as defined in the examples. Treatment “B” is the control IF. Statistics: Paired t-test $P = 0.0222$. Average time difference between EF-IF and control IF is 13.9 min

EXPERIMENTAL SECTION

EXAMPLE 1:

Methods

In vitro digestion

In vitro gastric digestion was performed using a semi-dynamic digestion model simulating infant and adult gastric conditions. In short, digestion units contained 2.5 ml (infant) or 6 ml (adult) simulated gastric fluid (SGF) pH 1.5, containing 30 mM HCl (Sigma-Aldrich) and 300 U/ml pepsin (Sigma-Aldrich, P6887), at the start of the experiment to simulate the fasting state. 60 ml formula was added immediately (adult) or with a feed flow of 3 ml/min (simulating a typical infant feeding time of 20 min) and SGF with a flow of 0.39 ml/min (infant) or 0.72 ml/min (adult) was added until sampling pH using a preprogrammed DAS-box scripts. Subsequently, protease inhibitors (Pepstatin A, 5 μ M, Sigma-Aldrich) were added to stop the enzymatic reactions before analyses of creaming behavior by analyzing layer formation over time.

In vivo trial

Design

This study was a randomized crossover trial in which healthy men underwent gastric MRI scans and blood sampling at baseline and after consumption of Infant Formula (IF) at predetermined timepoints. The primary outcome is gastric top layer formation. Secondary outcomes were gastric emptying, gastric emptying half time and blood parameters related to metabolic responses,

including free fatty acids, glucose, insulin and (NMR-based) metabolomics (data not shown). In addition, subjective appetite ratings (hunger, fullness, thirst, desire to eat, prospective consumption and nausea) were collected (data not shown). The procedures followed were approved by the Medical Ethical Committee of Wageningen University in accordance with the Helsinki Declaration of 1975 as revised in 2013. This study was registered with clinicaltrials.gov under number NCT05224947. All participants signed informed consent.

Participants

Twenty healthy (self-reported) males aged 18-45 y were included. Participants were excluded if they reported an allergy or intolerance for cow milk, lactose, soy and/or fish, gastric disorders or regular gastric complaints, used medication that affects gastric behavior, smoked more than 2 cigarettes per week, had an alcohol intake >14 glasses per week, or had contra-indication to MRI scanning (including but not limited to pacemakers and defibrillators, ferromagnetic implants and claustrophobia). Participants were recruited via digital advertisement (e-mail and social media). In total, 20 men participated in the study (age: 25.5 ± 5.8 y, BMI: 21.9 ± 1.5 kg/m²).

Treatments

A routine cow's milk based formula (control IF) and an experimental formula (EF-IF) comprising a native whey protein concentrate (Hiprotal® Milkserum 60 Liquid, FrieslandCampina) and a whey protein concentrate rich in phospholipids (Vivinal® MFGM, FrieslandCampina) were used in the study. Both products were designed to meet the nutritional requirements of infants (0 to 6 months). The nutritional composition of the formulae is presented in Table 1 A and B. The participants consumed 600 ml of the IFs at approximately 37 °C.

Table 1 A. Nutrient composition of the two IFs per 100 ml of ready to drink product

Composition per 100 ml	Control IF	EF-IF
Energy (kcal)	65	66
Protein ¹ (g)	1.4	1.4
- Casein ¹ (g)	0.5	0.5
- Whey protein ¹ (g)	0.9	0.9
Fat (g)	3.5	3.5
Carbohydrates (g)	6.7	7.0
Galacto-oligosaccharides (g)	0.4	0.4
bLac (g)	0.4	0.4
Urea (mg)	19	6
Sphingomyelin (mg)	7.4	13
Phospholipids (mg)	29	49
Sialic acid (mg)	19	14
Casein MacroPeptide (CMP) (mg)	143	29
Sphingomyelin per 100g of fat (mg)	211	368

1: N*6.25

5 Table 1 B. Nutrient composition of the two IFs per 100 gram powder

Composition per 100 g powder	Control IF	EF-IF
Energy (kcal)	501	499
Protein ¹ (g)	11.0	10.1
- Casein ¹ (g)	3.8	3.7
- Whey protein ¹ (g)	7.2	6.4
Fat (g)	27.0	26.4
Carbohydrates (g)	52	53
Galacto-oligosaccharides (g)	3	3
bLac (g)	3.0	3.1
Urea (mg)	148	47
Sphingomyelin (mg)	57	97
Phospholipids (mg)	223	369
Sialic acid (mg)	151	107
Casein MacroPeptide (CMP) (mg)	1097	217

1: N*6.25

The fat droplets of both the Control IF and the EF IF had an average diameter of about 0.4 micron.

Study Procedures

5 The participants (20) were instructed to consume the same meal the evening before their overnight fast. During the fasting period of minimally 12 hours, they were allowed to drink water up to 2 hours prior to their visit. The study took place at a local hospital. Upon arrival at the hospital, a cannula was placed in an antecubital vein, a blood sample was taken, a baseline MRI scan was
10 performed, and appetite ratings were obtained. Subsequently, participants consumed one of the two IFs with a mean ($\pm$ SE) ingestion time of 2.1 ($\pm$ 0.1) min (minimally processed, MFGM IF: 2.0 ± 0.2 min, control: 2.3 ± 0.2 min).

Subsequently, gastric MRI scans were performed at 5-minute intervals during the first 30 minutes. After that, scans were made every 10 minutes, up until
15 2 hours post-prandially. Blood samples were taken at $t = 15, 30, 45, 60, 75, 90, 120$ min. (Figure 1).

MRI

Participants were scanned in a supine position with the use of a 3-Tesla
20 Philips Ingenia Elition X MRI scanner (Philips, Eindhoven, The Netherlands). A 2-D Turbo Spin Echo sequence (37 4-mm slices, 1.4 mm gap, 1 x 1 mm in-plane resolution, TR: 550 ms, TE 80 ms, flip angle: 90 degrees) was used with breath hold command on expiration to fixate the position of the diaphragm and the stomach. The scan lasted approximately 20 seconds.

25

Gastric volume

Fat layer volume and total gastric content volume were manually delineated on every slice with the use of the program MIPAV (Medical Image Processing, Analysis and Visualization Version 7.4.0, 2016). Fat layer volume and
30 total gastric volume were calculated for each time point with the use of MATLAB (2021b).

The stomach contents were delineated by two researchers. To ensure that both researchers delineated total gastric volume and fat layer in the same manner, several scans (n = 3) were analyzed by both researchers. The results were then discussed and agreements were made regarding the delineation. After reaching
5 consensus, all scans were delineated by either one of the two researchers. 15% of the scans were analyzed in duplicate for validation. For fat layer volume this resulted in a interclass correlation coefficient of 0.903 (95% CI: 0.849 – 0.938) which indicates good reliability. For total gastric content volume this was 0.997 (95% CI: 0.993-0.998), indicating excellent reliability.

Analysis

Differences in fat layer volume and total gastric volume over time were tested by using linear mixed models, testing for main effects of time, treatment and treatment*time interactions, with baseline gastric volume as a covariate.
15 Tukey corrected post-hoc tests were used to compare individual time points.

For fat layer volume, the time at which the fat layer appeared (onset) was compared between treatments. In addition, the maximum fat layer volume and time to maximum fat layer volume were calculated. All three parameters were compared with a paired t-test. AUC of the fat layer and total gastric volume over
20 time were calculated using the trapezoidal rule and compared with paired t-tests. Pearson correlation coefficients were calculated for baseline gastric volume and fat layer characteristics. One person was excluded from the analyses because no fat-layer formation was visible for both treatments.

Onset fat layer

As shown in Figure 2 the *in vivo* onset of fat layer formation in the stomach as determined using MRI after consumption of the EF-IF is shorter as compared to the control. Paired t-test: P = 0.0167. Average time increase going from EF-IF to control IF is 13.4 minutes with a standard error of 5.1 minutes.
30 Corrected for baseline: P=0.0014 and the Average time increase going from EF-IF to control IF is 13.6 minutes with a standard error of 3.6 minutes. In other words, the EF showed a faster fat layer formation as compared to the control IF. These

results confirm the phased-release behavior of the composition according to the invention *in vivo*.

As shown in Figure 3, the time to reach the peak of the fat layer thickness i.e. maximum fat layer thickness which corresponds to the maximum fat layer volume, is also significantly shorter for the EF-IF vs control -IF. After consumption of the EF-IF the maximum fat layer thickness is reached 13.9 minutes faster as compared to the control formula (P=0.0222). Likewise the peak volume of the fat layer after consumption of the EF-IF is 46.3 mL smaller than after consumption of the control IF (paired t test P = 0.03) . The area under the curve (AUC) of the fat layer volume over time was the same for both treatments which was expected as both compositions had a similar fat concentration.

The results of the MRI experiment are summarized in Table 2 below:

Table 2. Nutrient composition of the two IFs per 100 ml

	EF-IF	Control IF	Mean difference (95%CI)	P-value
Mean time of onset (min)	26.1 (± 14.5)	39.5(± 19.4)	13.4 (2.6 – 24.1) (SE: 5.1)	0.0167
-- corrected for baseline gastric volume			13.6 (SE: 3.6)	0.0014
Time to peak (min)	27.4 (± 15.1)	41.3 (± 20.5)	13.9 (2.2 – 25.7) (SE: 5.6)	0.0222
-- corrected for baseline gastric volume			14.1 (SE: 4.1)	0.0030
Maximum peak (mL)	154.6 (± 46.7)	203.4 (± 102.5)	48.8 (4.2 – 93.4) (SE: 21.2)	0.0339

	EF-IF	Control IF	Mean difference (95%CI)	P-value
-- corrected for baseline gastric volume			48.3 (SE 16.9)	0.0111
AUC (mL*min)	5085.3 (± 1938.3)	5160.7 (± 2798.1)	75.4 (-1339.0 – 1489.8)	0.9121

Claims

1. A nutritional composition comprising casein, whey proteins comprising α -lactalbumin (aLac) and β -lactoglobulin (bLac), and protein-coated fat droplets,
5 wherein
- (i) the protein content of the composition is 5 to 20% (w/w), preferably 7-16 w%, based on total solids;
- (ii) the bLac content is at least 3% (w/w) based on total protein level and the aLac + bLac content is at least 25% (w/w) based on the total protein level;
- 10 (iii) the fat content is in the range of 10% to 50% (w/w) based on total solids;
- (iv) the fat droplets are coated with an average protein load of 2-10 mg/m² and wherein the weight ratio of casein: (aLac + bLac) on the fat droplets is at least 4 times higher, preferably at least 4.5 times higher, than the weight ratio of casein: (aLac + bLac) in the total composition;
- 15 (v) optionally the amount of urea per 100 g of composition is between 30 mg and 140 mg based on total solids;
- (vi) the level of sphingomyelin per 100 g of composition is between 70 mg and 200 mg based on total solids, preferably between 75 mg and 150 mg; more preferably between 75 mg and 130 mg;
- 20 (vii) the level of phospholipids per 100 g of composition is between 280 mg and 950 mg based on total solids, preferably between 285 mg and 850 mg, more preferably between 285 mg and 700 mg, most preferably between 290 mg and 650 mg; and wherein the amount of casein macropeptide (CMP) per 100 g of composition being between 100 mg and 500 mg based on total solids).
- 25
2. The nutritional composition of claim 1, in addition comprising one or more selected from the group consisting of:
- (viii) fat droplets having an average diameter in the range of 0.2 to 1.0 micron;
- (ix) the amount of sialic acid per 100 g of composition being between 75 mg and
30 120 mg based on total solids, preferably between 80 mg and 110 mg;
- (x) the amount of casein macropeptide (CMP) per 100 g of composition being between 100 mg and 400 mg based on total solids, preferably between 100 mg

and 300 mg, more preferably between 105 mg and 250 mg, most preferably between 105 mg and 225 mg; and

(xi) the sphingomyelin content per 100 g of fat in the composition being between 300 mg and 950 mg, preferably between 320 mg and 850 mg, more preferably between 330 mg and 750 mg, most preferably between 330 mg and 700 mg.

3. The composition according to claim 1 or 2, wherein at least 70% of the aLac and bLac, preferably at least 80%, more preferably at least 90%, most preferably 95%, are in a native state; and / or wherein the weight ratio of caseins to total whey proteins in the composition is in the range of 70:30-20:80, preferably 50:50-30:70.

4. The nutritional composition according to any one of the preceding claims, wherein aLac and bLac are derived from milk, from cheese whey, from acid casein whey or from milk serum or from concentrated, diluted, demineralized and/or powdered variants thereof; and/or wherein the casein is selected from the group consisting of micellar casein, non-micellar casein, acid casein, calcium caseinate, magnesium caseinate, sodium caseinate, potassium caseinate and ammonium caseinate.

5. The nutritional composition according to any one of the preceding claims, wherein the fat source is a dairy milk fat, a vegetable oil, a vegetable fat, an, a hydrogenated vegetable oil, a marine oil, an algae oil, single cell oil or a mixture of any of the foregoing, preferably a dairy milk fat, more preferably a dairy milk fat selected from whole milk, cream, anhydrous milk fat and fractions from milk fat.

6. The nutritional composition according to anyone of the preceding claims, wherein the weight ratio of fat to protein is in the range of 3.5:1-1:1.5.

7. The nutritional composition according to anyone of the preceding claims, being a powdered composition.
8. The nutritional composition according to anyone of claims 1 to 8, being
5 a liquid composition, preferably a liquid composition having a protein content up to 30 gram per liter.
9. The nutritional composition according to anyone of the preceding claims, being an infant formula, follow-on formula or growing up milk.
- 10
10. A method for providing a nutritional composition according to any one of claims 1-9 comprising the steps of:
- a. Blending skimmed milk and a whey protein source comprising α -lactalbumin and β -lactoglobulin and /or other whey proteins and optionally
15 of urea;
 - b. Pasteurizing the blend at a temperature of less than 85 °C;
 - c. Evaporating at a temperature of less than 68 °C ;
 - d. Addition of a fat source;
 - e. Homogenization of the composition obtained in step d;
 - 20 f. Drying the composition obtained in step e to obtain a powdered composition, preferably using spray drying;
- wherein a source of sphingomyelin, of phospholipid, and/or of sialic acid is blended into
- i) the blended skimmed milk and whey protein source of step a. and/or into
25 ii) the powdered composition of f).
11. The method of claim 10 further comprising a step of reconstituting the powdered composition of step f with a liquid.
- 30 12. The method according to anyone of claim 10 or 11, which does not comprise exposing α -lactalbumin and β -lactoglobulin to conditions that induce denaturation and/or aggregation of α -lactalbumin and β -lactoglobulin.

13. The use of a nutritional composition according to any one of claims 1 to 9, or obtainable by a method according to claim 10, 11, or 12, in a method of controlled release of protein and fat into the intestine of a subject, preferably a human subject, more preferably a human subject with an age between 0 and 36 months.

14. The use of a nutritional composition according to any one of claims 1 to 9, or obtainable by a method according to claim 10, 11, or 12, in a method to maintain or improve gastrointestinal health, energy regulation and/or metabolic health in a subject, preferably a human subject, more preferably a human subject with an age between 0 and 36 months.

15. A nutritional composition according to any one of claims 1 to 9, or obtainable by a method according to claim 10, 11, or 12, for use in a method for the prevention or treatment of microbial infection in a subject, preferably a human subject, more preferably a human subject with an age between 0 and 36 months; preferably wherein the microbial infection is a gastrointestinal infection.