Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Department of Molecular Sciences

Macronutrients in human milk and infant formula – bioactivity and health effects

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Bachelor Thesis • 15 credits • First cycle, G2E
Agriculture programme - Food Science
Moleculra Sciences, 2019:6
Swedish University of Agricultural Sciences
Uppsala, 2019

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Credits: 15 credits
Level: First cycle, G2E
Course title: Independent project in Food Science
Course code: EX0876
Programme/education: The agriculture programme - Food Science
Course coordinating department: Department of Molecular Sciences

Place of publication: Uppsala
Year of publication: 2019
Title of series: Molecular Sciences
Part number: 2019:6
Online publication: https://stud.epsilon.slu.se

Keywords: macronutrients, human milk, infant formula, bioactive compounds, health effects

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Abstract

Human milk has been considered the ultimate food for infants to optimize their growth and development, but it has lately also shown to impact long-term health. Infant formula is the complementary food to neonates who are not breastfed, and its nutrients should mimic human milk as close as possible.

Since infant formula mainly is based on cow's milk, there are several important differences e.g. protein content, and bioactive compounds, that need to be modulated in infant formula. The protein levels in human milk are significantly lower than in cow's milk. Bioactive compounds found in human milk that are not present in cow's milk have shown to improve metabolic pathways in the neonate through several mechanism e.g. protection against infections, improving nutrient absorption and benefiting neurological function.

Galactooligossacharides (GOS) and fructooligossacharides (FOS) are supplemented to infant formula to compensate human milks high levels of oligosaccharides. Milk fat global membrane (MFGM) and docosahexaenoic acid (DHA) are other constituents that are usually added in infant formulas to benefit infant health.

The different outcome of health effects between formula fed and breastfed infants highlight the importance of prevention strategies. The nutrient composition of human milk is complex, however our knowledge expands rapidly and thereby increases the nutritional requirements of infant formula. Long-term studies will have to confirm the beneficial effect of receiving certain nutrients early in life. Future studies on how infant formula should be supplemented to promote health in neonates are also necessary.

Key words: macronutrients, human milk, infant formula, bioactive compounds, health effects

Sammanfattning

Modersmjölk har ansetts vara den ultimata födan för spädbarn som optimerar deras tillväxt och utveckling, men har nyligen också visat sig påverka den långsiktiga hälsan. Modersmjölksersättning är det komplementära livsmedlet för nyfödda som inte ammas, och dess näringsämnen ska imitera modersmjölken så likt som möjligt.

Eftersom modersmjölksersättning främst är baserad på komjölk, finns det flera viktiga skillnader t.ex. protein innehåll och bioaktiva föreningar, som behöver moduleras i modersmjölksersättning. Proteinhalten i modersmjölk är signifikant lägre än i komjölk. Bioaktiva ämnen i modersmjölk som inte finns i komjölk har visat sig förbättra nedbrytningsmekanismer i nyfödda genom åtskilliga mekanismer t.ex. skydd mot infektioner, förbättrad näringsabsorption och främja neurologiska funktionen.

Galaktooligosackarider (GOS) och fructooligosackarider (FOS) är berikade till modersmjölksersättning för att kompensera modersmjölkens höga halt av oligosackarider. Mjölkfettkulmembran (MFGM) och dokosahexaensyra (DHA) är andra komponenter som vanligen adderas i modersmjölksersättning som befrämjar spädbarnshälsa.

Olika erhållna hälsoeffekter mellan flaskmatade och ammande spädbarn markerar betydelsen av preventionsstrategier. Näringssammansättningen av modersmjölk är komplex, likväl vår kunskap som expanderar snabbt och som därmed leder till att de näringsmässiga kraven i modersmjölksersättning ökar. Långtidsstudier bör vidare bekräfta den främjande effekten av särskilt erhållna näringsämnen tidigt i livet. Framtida studier om hur modersmjölksersättning skall vara berikad för att gynna hälsan hos nyfödda är också nödvändigt.

Nyckelord: makronutrienter, modersmjölk, modersmjölksersättning, bioaktiva föreningar, hälsoeffekter

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Abbreviations

β-palmitate	Sn-2 palmitic acid
BBSL	Bile salt-stimulated lipase
DHA	Docosahexaenoic
FOS	Fructooligosaccharides
GOS	Galactooligosaccharides
HMO	Human milk oligosaccharide
MFGM	Milk fat globule membrane
SIgA	Secretory immunoglobulin A
SLV	Livsmedelsverket

1 Introduction

The importance of early nutrition is of particular interest as recent findings has highlighted its impact on adult health. Referring to the concept “nutritional programming” which lately has received attention in human health, certain nutrients will have long-term effects on metabolic health and immune status (Agostoni *et al.* 2019). For example, a high protein diet has been linked to a rapid early child growth in formula fed infants. In contrast, breastfed infants have a slower growth rate explained by the lower protein content in human milk compared to bovine milk. Infants with rapid weight gain have shown to be more likely to develop conditions e.g. metabolic syndrome and cardiovascular disease in adultescence. Formula fed infants have also shown a negatively influence on their neurological development compared to breastfed infants (Agostoni *et al.* 2013).

According to World Health Organization, human milk is a complete nutrient source for infants as it ensures optimal growth and health. It protects against infectious and chronic diseases as well as promotes sensory and cognitive development. Because of this, exclusively breastfeeding i.e. no additional foods, is recommended for infants during the first 6 months postpartum and breastfeeding is considered an important nutrient source for up to 2 years old of life (Gaggero 2017). It is undeniable that human milk reduces the risk for both infant morbidity and mortality since this has been reported by researchers during the last decades (Lamberti *et al.* 2011).

Furthermore, as the knowledge of human milk composition increases, the role of human milk for infant health has become more apparent (Ballard & Morrow 2013). The main objective of infant formula is to imitate the nutritional composition of human milk as close as possible. The manufacture of infant formula has progressively been modernized and is today considered as a nutritional, healthy and safe food for new-born’s (Happe & Gambelli 2015).

There are several formula options such as cow, goat, soy and hypoallergenic varieties with additives like fibres to serve different nutritional demands. There is however no specific formula that meets the nutritional requirements for all neonates. As scientific evaluation reveals that enriched formulas have significant impact on long-term health, continuous research is necessary for understanding the complexity of human milk and for the improvement of infant formula. Increased awareness on how specific nutrients effects human health rises opportunities for expanding prevention strategies (Ahern *et al.* 2019).

1.1 Aim

The aim of this thesis is to review the composition of human milk and today's infant formula based on bovine milk. Nutritional components of human and cow's milk as well as today's infant formula will be presented. The text will focus on the health effects of macronutrients and bioactive compounds.

The questions to be answered include how the nutritional composition differ between human milk and infant formula and how the quality of infant formulas can be improved in relations to long-term health effects. In order to answer these questions and since infant formula usually is based on cow's milk, human milk will be compared to cow's milk with respect to selected nutrients.

1.2 Method

The literature used for this study was mainly scientific articles found in the databases Web of Science, PubMed, Google Scholar and Advances in Nutrition. The following words were used in different combinations to conduct the search for relevant literature: *human milk*, infant formula*, macronutrients*, bioactive compounds*, proteins, lipid, lactose, oligosaccharides, health.*

The research field of human milk has taken place for decades although analysing the nutritional components has not been performed in detail. Thus, bioactive proteins have not until recently been highlighted and understood for their important health effects. The discussion will be limited to the area

of valuable components that are considered to impact long-term health. The aspect of micronutrients i.e. minerals and vitamins are beyond the scope of this thesis and will briefly be mentioned in relation to the health effects of macronutrients.

2 Human milk

Human milk is an extremely complex biological fluid that is the fundamental source of food for infant nutrition. It contains a unique mixture of different proteins, lipids and carbohydrates of which thousands are multiple biologically active components (Su *et al.* 2017).

The nutritional composition changes over lactation stage to meet the infant's needs, and depending on lactation stage, milk can be categorised into colostrum, transitional milk and mature milk (Andreas *et al.* 2015). Colostrum is a thick, yellowish fluid secreted a few days pre- and postpartum, while transitional milk refers to 7-14 days postpartum and mature milk >30 days postpartum (Erick 2018).

Human milk is further affected by diet, stored nutrients and by nutrient synthesis in the lactocyte. In general, the nutritional quality of human milk is solid, although variations in fatty acid composition and some vitamins exist (Ballard & Morrow 2013).

2.1 Macronutrients

The macronutrient composition of mature human milk is 0.9-1.2 g/100 mL for protein, 3.8 g/100 mL for fat and 7.0 g/100 mL for lactose as seen in Table 1. The concentration of protein is significantly higher in colostrum compared to mature milk (Ahern *et al.* 2019), illustrating changes in the composition over lactation, with a protein content that gradually decreases (Ballard & Morrow 2013). Colostrum will also have lower contents of both lactose and fat compared to mature milk. The lactose content in milk is highest between 4-7 months postpartum, thereafter decreasing, whereas the concentration of lipids remain at the same level throughout lactation (Andreas *et al.* 2015).

2.1.1 Proteins

There are over 400 hundred different proteins present in human milk, contributing to varies functions important for survival and health of the infant. The proteins are typically divided into the three groups i.e. caseins, whey and mucin proteins. In colostrum milk the concentration of whey protein is significantly higher compared to mature milk (Andreas et al., 2015).

Whey proteins are in general the most dominating protein in human milk, including α-lactalbumin, lactoferrin and lysozyme. These bioactive compounds are recognized as important factors to gastrointestinal and immunological functions. Mucins and osteopontin are glycoproteins in human milk that also have received attention for these effects. Moreover, human milk is particularly rich in the important antibody secretory immunoglobulin A (SIgA) (Ahern *et al.*, 2019).

Casein

Human milk mainly contains κ-casein and β-casein with only a small amount of αs1-casein. Interestingly, 40% of the κ-casein in human milk consist of carbohydrates; oligosaccharides with bioactive properties e.g. the glycomacropeptide, a proteolytic fragment that is well known to be a prebiotic (Lönnerdal 2016). One of its known functions is to prevent pathogens e.g. *Salmonella enteritidis, Escherichia coli, Vibro cholera* and *Helico pylori* from infecting intestinal cells (Feeney *et al.* 2017).

The casein concentration is approximately 13% of the total protein content in human milk. This is the lowest amount of casein among any studied specie (Andreas *et al.* 2015). A low concentration of casein is favourable for humans as it makes the curd gentler on digestion in the infant stomach (Ahern *et al.* 2019).

Whey proteins

Human milk whey proteins are consisted mainly of α-lactalbumin and the glycoproteins lactoferrin and lysozyme (Andreas et al., 2015) which are further described below. They comprise more than twice the amount of caseins in human milk and also exist in larger concentrations compared to bovine milk. Concentrations of whey proteins are particularly high in colostrum when compared to mature milk (Castellote *et al.* 2011). The levels are as high as 90% of the total protein in colostrum with decreased level to 60% in

mature milk. The bioactivity of these proteins has been studied and highlighted for their protective properties against infections (Lönnerdal 2016).

Biologically active peptides are formed from **α-lactalbumin** during digestion of human milk. These peptides have the ability to bind essential micronutrients, e.g. calcium, iron and zinc, thereby enhancing their absorption (Lönnerdal 2016). The concentration of α-lactalbumin is approximately 22% of total true protein and is an optimal source of essential amino acids e.g. lysine, cysteine, tryptophan, leucine, isoleucine and valine (Layman *et al.* 2018).

Lactoferrin is an iron-binding glycoprotein that has the ability to kill pathogens such as *Vibrio cholera* (Acosta-Smith *et al.* 2018) and *Streptococcus mutans* (Velusamy *et al.* 2014). Because of its highly positively net charge, it can easily bind and kill gram-negative bacteria that generally are more resistant to bactericidal activity (Lönnerdal 2016). Lactoferrin in human milk is also well-known for its bacteriostatic activity against *Escherichia coli*. In addition, the protein has reported to modulate immune functions through several mechanism which thereby affects the health outcome (Lepanto *et al.* 2019). Supplementation of lactoferrin in bovine based formula has for example shown to improve infant growth (Lönnerdal, 2016). In addition, lactoferrin has also shown anti-inflammatory and antioxidant properties on neurological function (van de Looij *et al.* 2014).

Lysozyme is a glycoprotein and enzyme that can hydrolyse the 1-4 linkage between N-acetylmuramic acid and N-acetylglucosamine in the cell walls of certain bacteria. It is specifically known to degrade Enterobacteriaceae and Gram-positive bacteria (Yang *et al.* 2011).

The levels of lysozyme in human milk are 300 times higher than in cow's milk i.e. 50 mg/100 ml in human milk compared to traces in bovine milk (Ahern *et al.* 2019). Its bacteriostatic activity clearly affects the microflora of the intestinal tract in breastfed infants. For example, in a 2-week followed up study on infants, lysozyme together with lactoferrin showed a beneficial effect on reducing diarrheal problems (Lönnerdal 2016).

SIgA is one of the five antibody isotypes that exists among immunoglobulins. In human milk, immunoglobulins IgG and IgM are found but in particularly IgA. The concentration of SIgA is a 100-fold greater in human milk compared to levels in cow's milk (Ahern *et al.* 2019). The quantity of SIgA is predominantly high in colostrum, providing passive immunological protection while the immune system of the neonate matures. SIgA has an effect

on pathogens via several mechanisms, e.g. via proteolysis and by blocking adhesion to epithelial cell surfaces but also neutralizing toxins and virulence factors (Andreas *et al.* 2015).

Mucin

Mucins are the largest glycoprotein in human milk. Its molecular size ranges between 200-2000 kDa and is dominating in extracellular matrix of the gastrointestinal tract. The large size and hydrophobic properties of mucins makes them more challenging to isolate and purify, but researchers are beginning to understand their protective function in infants (Liu & Newburg 2013)

Mucins has been noticed for its ability to block infections caused by viruses and bacteria. They have shown to act as potential prophylactic and therapeutic agents that inhibit infant from e.g. salmonella (Liu et al., 2012). Furthermore, scientific evidence also suggest that one type of mucin, MUC1, may function in cancer protection (Hattrup & Gendler 2008). This has been confirmed by its ability to inhibit the growth of tumour cells via inducing apoptotic pathways (Yuan *et al.* 2015).

Osteopontin

Osteopontin is another glycoprotein present in human milk that is biologically active in biomineralization, bone remodeling, cell proliferation and immune modulatory functions. It is acidic phosphorylated and post-translationally modified with significantly higher levels in human milk compared to bovine milk (Ahern *et al.* 2019). Studies has shown that osteopontin regulates inflammation diseases including obesity, diabetes and cardiovascular disease, based on its ability to modulate the immune cell response (Kahles *et al.* 2014).

The concentration of osteopontin varies over lactation stage where colostrum milk contains higher amounts and mature milk lower amounts. Analyzed human milk received to breastfed infants at 1-, 4-, and 6-months of age showed that the concentration significantly decreased between 1 to 4 months but actually increased slightly from 4 to 6 months (Jiang & Lönnerdal 2019). Importantly, this study found that a high level of osteopontin stimulated cell proliferation while a lower level instead enhanced cell differentiation. This highlights the impact of how different quantities of a nutrient can impact biological functions.

2.1.2 Non-protein molecules

The total nitrogen content in human milk are consisted of 25% non-protein fragments, e.g. free amino acids, peptides, nucleotides, creatinine and urea which also contributes to bioactive molecules important from a health perspective. For example, nucleotides are essential nutrients in early life, with key functions in enzymatic and metabolic processes as well as beneficial effects on development of the gut microbiota (Andreas *et al.* 2015).

2.1.3 Carbohydrates

Human milk carbohydrates mainly consist of lactose and comprises 7 g/100 mL as seen in Table 1. Among the macronutrients, lactose concentration is the least variable during breastfeeding. In addition, mothers that produces higher quantities of milk also produces a higher amount of lactose (Ballard & Morrow 2013).

The concentration of lactose in human milk is the largest compared to any other specie which reflects the huge energy requirements of the brain. Another type of carbohydrate present are human milk oligosaccharides (HMO). This distinctive segments is not digestible but function as nutrients in the intestinal microbiota of the infant, and thus act as prebiotics (Andreas *et al.* 2015).

Oligosaccharides

The third largest component in breast milk, after lactose and fat, consists of HMO as seen in Table 1. HMO has a unique composition when compared to oligosaccharides in milk from any other mammal (Bode 2012). The size of the HMOs ranges from 3 to 32 units per molecule, sugars appearing with different sequences and orientations. The composition differs between mother's in a similar way as blood group do (Andreas *et al.* 2015).

Human colostrum contains 2-2.5 g/100 mL of HMOs. These levels decrease to 0.5-2 g/100 mL in mature milk which can be seen in Table 1. Since this amount still exceeds the concentration of total protein content in human milk, it highlights the nutritional importance for infants. HMOs consists of five monosaccharides: glucose, galactose, fucose, N-acetylglucosamine, and sialic acid (Bode 2012). According to Andreas *et al.* (2015), more than 200 types of oligosaccharides are discovered which all have lactose at the reducing end.

The main nutritional function of HMOs is to support the establishment and growth of prebiotics in the gastrointestinal tract of the infant, i.e. encouraging beneficial bacteria protecting against intestinal colonization by pathogenic bacteria (Cheng *et al.* 2017). HMOs also have a defensive ability to competitively bind to pathogens. In addition, it has been shown that they can modulate the immune response by reducing cell growth and inducing differentiation and apoptosis (Andreas *et al.* 2015).

Since HMOs can provide important components like fucose and sialic acid, they are valuable to both immune system and neurodevelopmental respectively (Underwood 2013). The concentration of HMOs is approximately twice as high in colostrum milk compared to mature milk, emphasizing the immunologic role of colostrum in the neonate (Andreas *et al.* 2015). More specifically, sialic acid, predominately present as N-acetylneuraminic acid in human milk, is an essential nutrient in brain development, with the ability to modify synaptic connectivity, cell-to-cell interactions, neuronal outgrowth and memory formation. (Wang 2009). This is also confirmed by studies that shows that breastfed infants have significantly higher levels of sialic acid compared to formula fed infants (Bode 2012).

2.1.4 Lipids

The main source of energy for infants are lipids, providing approximately 50% of the total energy. Milk lipids consist of 98% triglycerides that originate from the core of the milk fat globules. The remaining lipids that surrounds the fat and making up the so called milk fat membrane are residues of diacylglycerides, monoacylglycerides, phospholipids, cholesterol and free fatty acids (Andreas *et al.* 2015).

The fat composition is the most variable macronutrient of human milk. During breastfeeding, the concentration of fat in the milk will increase with time since the amount of fat is up to three times larger in hindmilk i.e. the last milk during breastfeeding (Ballard & Morrow 2013). Moreover, lipid digestion is very efficient for breastfed infants because of the unusual high levels of bile salt-stimulated lipase (BBSL) in human milk that actively catalyses the breakdown of triglycerides (Lönnerdal 2016).

Palmitic acid

The most common saturated fatty acid present in the human body is palmitic acid

(Carta *et al.* 2017). It contributes to approximately 25% of the total fatty acids in

human milk (Demmelmair & Koletzko 2018). Notably, palmitic acid is relatively constant at 20-25% of total fatty acid content in human milk and will not be affected by maternal diet. The amount of other fatty acids in human milk like oleic-, linoleic and linolenic acid has shown to widely differ depending on fat composition (Innis 2011).

During digestion, palmitic acid is weakly absorbed by the infant and as a result forms indigestible soaps which causes constipation but also reduces the uptake of dietary fat and calcium (Ahern *et al.* 2019). Providentially, human milk contains BBSL which breaks the bond between palmitic acid and glycerol in the sn-2 position. This is an unique ability among lipases which stabilizes the absorption facility and comforts the stomach of the infant (Lönnerdal 2016). This is confirmed by Litmanovitz *et al.* (2013) who explains that palmitic acid in the sn-2 position (β-palmitate) is better absorbed by infants than palmitic acid originating from the sn-1 and sn-3 position.

Human milk phospholipids and DHA

The phospholipids present in human milk are considered to have bioactive properties that affect neurobehavioral development. They contributes to the presence of long-chain polyunsaturated fatty acids such as arachidonic acid and docosahexaenoic acid DHA (Ahern *et al.* 2019). There are several health benefits of DHA, which is not surprising, considering their role in fetal-, brain- and retina development and their significant presence in the cell membrane. Sufficient amounts of DHA support healthy infants but also health throughout life and optimizes healthy aging. Studies also show that it prevents cardiovascular and Alzheimer's disease (Swanson *et al.* 2012).

3 Principle differences between human and bovine milk composition and its impact on health

There are two major differences between human milk and bovine milk. One is that human milk contains a significantly lower amount of protein compared to cow's milk. Also, the protein composition between human milk and cow's milk are largely distinct from each other. To compensate for this, infant formula must undergo several modification processes to resemble the composition of human milk. The other clear difference is that human milk has a much higher concentration of lactose compared to cow's milk. Fat content is similar in human milk and cow's milk but the fatty acid composition also differs to a larger extent (Ahern *et al.* 2019).

3.1 Proteins

The protein content in bovine milk is approximately 3.3 g/100 mL seen in Table 2. Like in human milk, it also includes whey, casein and mucin proteins. In contrast to human milk, the most abundant protein in bovine milk is casein. The caseins in bovine milk are comprised of αs1-casein, αs2-casein, β-casein and κ-casein (Ahern *et al.* 2019). The casein micelle size varies between 100-200 nm (Bouchoux *et al.* 2010) which is significantly larger than the caseins present in human milk. The higher casein content in bovine milk has shown to affect infant digestion (Thompkinson & Kharb 2007). Caseins in bovine milk results in a large, strong and rough curd which is more difficult to digest. A high casein level reduces pH in the infant's stomach which can cause abdominal pain and swelling. The small amount of casein

results in a softer and stringy curd that stays in the stomach for a shorter time, and thus a faster and easier digestion (Thompkinson & Kharb 2007).

The dominating whey protein in bovine milk is β-lactoglobulin. In human milk there is only traces of this compound as it instead comprises a higher amount of α-lactalbumin (Hochwallner *et al.* 2014). This difference can also have an unfavourable effect on infant's as β-lactoglobulin has been linked to cow's milk allergy. Since the amount of α-lactalbumin is considerably low in bovine milk, it can beneficially be supplemented to infant formulas (Layman *et al.* 2018). That would not only provide sufficient amount of essential amino acids that are lacking in bovine milk, but also promote gut health and stimulate neurological development (Thompkinson & Kharb 2007). Furthermore, the content of lactoferrin, lysozyme and SIgA is significantly different as seen in Table 1.

3.2 Carbohydrates

The lactose content in bovine milk is lower than in human milk and is estimated to 4.8 g/100 mL as seen in Table 2. There is also a higher amount of mono- and oligosaccharides present in human milk which are undetectable in bovine milk (Thompkinson & Kharb 2007). These differences appear as bovine milk containing only digestible carbohydrates while human milk contains both digestible and indigestible ones.

3.2.1 Oligosaccharides

Bovine milk consists of up to 1000-fold lower levels of oligosaccharides than human milk (Bode 2012). Taken together and with the significant low amounts of oligosaccharides in bovine milk as stated in Table 1, it suggests major differences in the health outcome between formula fed and breastfed infants.

3.3 Lipids

Cow's milk has approximately twice as high amount of palmitic acid found in human milk as seen in Table 1. The palmitic acid is esterified to β-palmitate between 60-70% in human milk and only about 40% in cow's milk (Innis 2011). A review article by Miles & Calder (2017) concludes that infant formula high in β-palmitate improved the gut microbiota in human infants. Since

there is no BSSL present in cow's milk and the absorption of palmitic acid is inefficient, the lipid digestion might be weakened and negatively impact the gastrointestinal function of formula-fed infants (Lönnerdal 2016).

Moreover, only a third of the amount of oleic acid is present in cow's milk compared to human milk and the amount of myristic acid in cow's milk is approximately 3 times higher compared to human milk (Ahern *et al.* 2019).

Table 1. Overview of major nutrients in human- and bovine milk (per 100 mL)

Nutrient component	**Human milk**	**Bovine milk**
Energy (kcal)	71	60-70
Protein (g)	0.9-1.2	3.3
Whey protein (g)	0.72	0.6
Casein (g)	0.31	2.6
Casein:Whey	40:60	80:20
Lactoferrin (g)	0.15	Trace
Lysozyme (g)	0.05	Trace
Immunoglobulin A (g)	$26.2*10^{-6}$	$0.1*10^{-6}$
Lactose (g)	7.0	4.8
Oligosaccharides (g)	0.5-2.0	0.005
Fats (g)	3.8	3.7
Fatty acids (% total fat)		
Palmitic acid (C16:0)	20.2%	42.9%
Oleic acid (C18:1)	46.4%	16.7%
Linoleic acid (C18:2)	13.0%	1.6%
Linolenic acid (C18:3)	1.4%	1.8%

Adapted from (Ahern *et al.* 2019) *and (Thompkinson & Kharb 2007)*

4 Infant formula based on bovine milk

4.1 Today's formulas

Infant formula is considered to be among the most complex foods that exist worldwide. It is composed of over 50 components combined in probably thousands of different recipes. Yet, it has a basic combination of fat, protein, carbohydrates, vitamins and minerals. Formulas are divided into stages from 1 to 5. Stage 1 aims for new-born infants up to 6 months of age whereas stage 2 targets babies from 6 to 12 months. Stage 2 differs from stage 1 mainly in being less energy dense due to that babies is expected to start eating other foods. Stage 3-5 is the least energy dense formula and produced as growing up milk for children from 1 to 6 years old (Happe & Gambelli 2015).

In Sweden, legislation related to the ingredients included in infant formula is the responsibility of the National food agency, Livsmedelsverket (SLV). There are specific and mandatory criteria regarding the general composition of protein, carbohydrates, fat, vitamins and minerals. Infant formulas must also contain choline and inositol in certain amounts that are present in human milk. Choline has shown to be an essential nutrient crucial for early brain development explained by Wiedeman *et al.* (*2*018) while inositol is a key factor in biological functions especially for neonates (Brown *et al.* 2009). Furthermore, there are maximum levels related to pesticide and foreign substances to ensure that they will not contribute to health risk for infants (Livsmedelsverket 2019).

Table 2. Overview of selected nutritional components per 100 mL in Babysemp 1 Lemolac sensipro infant formula

Nutrient component	Infant formula
Energy (kcal)	66
Protein (g)	1.3-2.0
Casein:whey (g)	40:60
Lactose (g)	6.9
Galactooligossacharides (g)	$0.3*10^{-6}$
Nucleotides (g)	$3.3*10^{-3}$
Fat (g)	3.5
Fatty acids (% total fat)	
Saturated fat	37%
Monounsaturated fat	40%
Polyunsaturated fat	17%

Adapted from (Semper 2016b) and (Lundin 2016)

4.2 Basic composition of infant formulas

4.2.1 Protein content

The protein content is allowed to be based on either cow's or goatmilk but also hydrolysed proteins or isolated soy protein. Further, SLV highlights the importance that the amount of essential amino acids should be at least the same as in human breast milk (Livsmedelsverket 2019). To achieve this in bovine milk based infant formula, the protein content is significantly higher and is considered to compensate for the differences between human milk and cow's milk e.g., the low amount of α-lactalbumin (Layman *et al.* 2018). A higher level of protein is also to make up for the ratio of casein to whey proteins as well as their amino acid profiles (Traves 2015).

The protein content should be between 1.8-3g/100 according to SLV (Lundin 2016). Converting this value per 71 kcal, comparable to those in Table 1, it equals 1.3-2.1 g/100 mL. This protein value is consistent with an infant formula from the company Semper (Sweden) at stage 1 based on cow's milk protein, where the protein content is 1.3 g/100 mL/66 kcal seen in Table 2 (Semper 2016a).

As seen in Table 1, the ratio between casein and whey protein in human milk is 40:60 while bovine milk has a high ratio of 80:20 (Ahern *et al.* 2019). According to Happe & Gambelli (2015), the casein to whey protein ratio in infant formula has been adapted to that in human milk since 1962. This is confirmed in the infant formula in Table 2, modified to exactly the same casein to whey ratio as human milk (Semper 2016b).

4.2.2 Carbohydrate content

The food authority in Sweden has determined that infant formula must have a minimum carbohydrate content of 9g/100 kcal and maximum 14 g/100 kcal. Adapting these values to 71 kcal in 100 mL milk, equal to numbers in Table 1, the minimum carbohydrate content should be 6.4g/100 mL and maximum 9.9g/100 mL (Lundin 2016 The lactose content is 6.9g/100 mL in the Swedish infant formula manufactured by the company Semper, which is comparable to that in human milk (7 g) seen in Table 1. Notably, although lactose is an important energy source in milk from both humans and cows, constituting about 40% of the daily energy intake in infants, its nutrient role is not established in detail (Grenov *et al.* 2016). This is also described by Thompkinson & Kharb (2007), who states that there is no evidence for a certain need of lactose for infants. Although, they propose that the lactose content should be at least half of the total minimum carbohydrate content (4.5 g/100 kcal). This is a considerably lower amount comparing with the lactose content of human milk (7 g) as seen in Table 1. However, several formulas have adjusted the lactose content similar to human milk (HiPP 2019; Oksnes 2017; Semper 2016a)

4.2.3 Fat content

There is no general recipe for the fat composition in infant formula although its production should aim to be similar to human milk composition (Happe &

Gambelli 2015). The fat content in the infant formula in Table 3 is 3.5 g/100 mL. This is in line with the obligated values from SLV of fat content that are minimum 2.9 g/100 mL and maximum 4 g/100 mL (Lundin 2016). Further, according to SLV, there are standard values for the amount of some specific fatty acids that can be included in infant formula.

The saturated fat content in the form of lauric- and myristic acid are approved to maximum 20% of the total fat. This could be considered as equivalent to the approximately 20% of saturated fat as palmitic acid in human milk seen in Table 1. However, SLV does not seem to have any other obligations in regard to other saturated fatty acids. The saturated fat content in infant formula is almost twice as high compared to human milk (Table 1 & 2), which is then more comparable to the saturated fat content in bovine milk.

Polyunsaturated fatty acids are allowed to be supplemented as long as the amount does not exceed 1% for linolenic acid (omega 3) and 2% for linoleic acid (omega 6) out of the total fat content (Lundin 2016). There are maximum levels regarding other fatty acids e.g., trans-fatty acids should not exceed 3% of the total fat. Furthermore, obligations concerning monounsaturated fatty acids are not prescribed to any maximum levels, probably because that the higher amount is associated with health benefits and decreased risk of cardiovascular disease (Livsmedelsverket 2018).

The level of monounsaturated fat in human milk and infant formula are similar (Table 2). Moreover, the presence of specific fatty acids e.g., β-palmitic acid, is not stated by SLV or declared in the studied infant formulas (HiPP 2019; Lundin 2016; Oksnes 2017; Semper 2016a). Nevertheless, Happe & Gambelli (2015) describe that Betapol® is an available product with the unique fat composition high in β-palmitic acid that is similar to the fat profile in human milk. Surprisingly, it has been commercially added to formulas since 1995 in countries like China and the US. However, it seems to be an approved product in EU as well (Betapol 2016).

4.3 Enriched bioactive components in formulas

A randomized control trial on formula-fed infants that were supplemented with nucleotides confirmed that the gut microbiota was improved (Singhal *et al.* 2008). As seen in Table 2, the selected infant formula is supplemented with 3.3 g/100 mL nucleotides. Milk fat globule membrane (MFGM) and DHA

are also beneficially added in today's infant formula. Clinical trials on humans have emphasized that the supplementation with MFGM has a positive impact on neurocognitive development (Huërou-Luron *et al.* 2018). This theory was tested in a randomized study by He *et al.* (2019) where infants fed MFGM supplemented formulas showed an induced circulation of lysophospholipids that are an important substrate for optimal phospholipid synthesis during brain development. The study also showed that supplementation of MFGM improved the metabolic profile in formula fed infants similar to breastfed infants. Therefore, researches warrant future studies on the metabolic pathway of breastfed infants.

EU regulations suggest obligatory addition of DHA in infant formula, given the well-known and beneficial effects on neurological and behavioural functions. However, there is a conflict between researches whether DHA alone is behind the effect on cognitive development (Ahern *et al.* 2019). At the same time, it would be a risk to not include DHA when several studies show a clear and beneficial effect on neurodevelopment. Additional studies are therefore necessary in this area. The infant formula from Semper (2016) contains 6.9 mg/100 mL DHA and is also declared with MFGM supplementation. Infant formula from the food company Nestlé has 8.3 mg/100 mL supplemented DHA (Oksnes 2017) while the brand HiPP contains 7 mg/100 mL (HiPP 2019).

4.3.1 Oligosaccharides

To resemble the prebiotic function of HMOs, a mixed concentration of galactooligosaccharides (GOS) and fructooligosaccharides (FOS) was supplemented to preterm formulas in a study from 2002. In this study, it was shown that the growth of Bifidobacteria in preterm infants was stimulated, protecting against infections and stabilizing the intestinal microflora similarly to the effect of Bifidobacteria in breastfed infants (Boehm *et al.* 2002). This was more recently also confirmed in another study where supplementation of a GOS/FOS mixture in infant formula increased growth of Bifidobacteria and Lactobacilli to levels comparable to levels in breastfed infants. One significant effect was that supplemented formula infants and breastfed infants had less allergic reactions to food products compared to formula fed infants with no supplementation (Ivakhnenko & Nyankovskyy 2013).

The infant formula from HiPP and Semper has 0.3 mg GOS supplemented whereas Nestlé contains a mixture of GOS/FOS (HiPP 2019;

Oksnes 2017; Semper 2016a). Notably, in a review article by Bode (2012), studies highlight that even though the addition of GOS and FOS has shown to be beneficial, these oligomers are not found in human milk. There is also a lack of evidence regarding long-term health benefits of this supplementation, i.e. how it will affect the infant in adulthood.

Another attempt to resemble HMOs in formulas is by adding pectin hydrolysate consisting of galacturonic acid oligomers. This introduces a negative charge like the sialylated oligosaccharides in human milk responsible for several HMOs effects. Nevertheless, further investigations on its impact on health are also necessary (Bode 2012).

5 Discussion

To make cow's milk resemble human milk with respect to macronutrient content and composition as well as bioactive compounds, many challenges must be solved, not to mention their essential micronutrients and amino acids required for infants (Thompkinson & Kharb 2007). The result shows that the macronutrient composition of infant formula is similar to human milk. However, the specific proteins in human milk e.g. lysozyme, lactoferrin and SIgA as well as other bioactive components e.g. HMOs are not compensated in today's infant formula.

According to Andreas *et al.* (2015) about 400 proteins exists in human milk while other researchers has identified over a thousand proteins using a small-volume proteomic method (Beck *et al.* 2015). This suggests that a significant amount of human milk proteins is beyond our current knowledge and should be analyzed further with the right methods and techniques.

The protein content of infant formulas in Sweden are obligated to 1.3-2.1 g/100 mL which highly exceeds the protein quantity in human milk (Lundin 2016). As noted, infants that receive a high protein formula are at potential risk for unfavorable health effects later in life (Agostoni *et al.* 2013). This is supported by a double-blind randomized clinical trial with long-term follow-up, which showed that reduced protein content in infant formula highly prevented obesity in children up to 6-year-old. The protein content in formulas with 1.25 g/100 mL resulted in slower weight gain compare to formulas with 2.0 g/100 mL protein, connected to a significant rapid weight gain (Weber *et al.* 2014). Another randomized clinical trials also support the fact that infants fed a higher protein formula, increased the risk of overweight and obesity up to 2 years of age. The study showed that there was a decreased health risk for breastfed infants and those fed a low protein formula (Koletzko *et al.*

2009). These results clearly show how a high protein formula increases weight gain and risk factors for developing cardiovascular disease. Therefore, it should be prioritized retaining it at a low level as long as the essential amino acids in sufficient amounts are included.

The question on whether these effects will be applicable in adultscence are unclear. Although some studies show few consistent results where low protein diet early in life decreases the risk of cardiovascular mortality (Martin *et al.* 2004, 2005). However, the responsible mechanisms are remained uncertain and promotes further long-term interventions. Nevertheless, a review article has proposed that nutritional programming could be caused by endocrine mechanisms, appetite regulation, epigenetic programming and accelerated biologic ageing (Singhal 2016). This further highlight the necessity of further investigations in this huge nutritional field.

The results further show that the positioning of fatty acids impacts the effectiveness of lipid absorption. This is currently not declared in the selected formulas and not mentioned by SLV. Since β-palmitate is easier digested for the infant it should be considered when modifying the formula (Ahern *et al.* 2019). In several studies described by Miles & Calder (2017), the presence of β-palmitate has shown to impact infants gastrointestinal tolerance by reducing fat soaps and increased fecal bifidobacteria. The role of β-palmitate has been investigated further in a randomized clinical trial, where a high content of β-palmitate in infant formula reduced the indigestible soaps in infants (Nowacki *et al.* 2014). Also, in a study by Litmanovitz *et al.* (2013), infants fed high β-palmitate formulas had an increased bone strength explained by a better absorption of calcium, compared with infants fed low β-palmitate formulas. Since calcium is an essential nutrient to infants for optimal bone growth and mineralization, one can suggest that supplemented β-palmitin can help to maintain calcium homeostasis and decrease the risk from osteoporosis in adolescence (Koo & Warren 2003).

Regarding the HMOs, they are well recognized for their prebiotic effects e.g. limiting pathogens indirectly by promoting growth of beneficial bacteria in the infant's intestine (Bode 2012). In addition, studies show that HMOs directly reduces bacterial colonization that protects the infant from microbial infections. This has been confirmed by Ruiz-Palacios *et al.* (2003) whereby HMOs prevented *Campylobacter jejuni* infections, one of the most responsible causes for infant diarrhoea and mortality, by serving as an antiadhesive antimicrobial. Furthermore, HMOs has shown to modulate immune- and intestinal epithelial responses (Eiwegger *et al.* 2004; Kuntz *et al.* 2009). They

are also explained by Wang (2009) to provide nutrients for brain function and suggested by Stefanutti *et al.* (2005) to protect against necrotizing enterocolitis. This highlight the necessity of receiving HMOs for all infants.

GOS and FOS are not optimal supplementations to compensate the HMO and there seems to be no other resources available today (Bode 2012). However, there are some potential alternatives that might be considered. The sialylated and fucosylated oligosaccharides are suggested to be responsible for the key roles in bacterial binding explained by Newburg *et al.* (2005). Interestingly, milk from chimpanzees contains 50% fucosylated oligosaccharides which can be compared to HMOs with 50-80% (Tao *et al.* 2011). Their oligosaccharides are also sialylated with 10-30% exactly like human milk. This shows that there is milk from other species that possesses oligosaccharides that could be functional to human infants, and it would be intriguing to study these similarities further. Finally, a quantitative analysis of sialylated oligosaccharides found in milk from different cow breeds showed significant variations (Kelly *et al.* 2013). Therefore, it is reasonable to propose breeding as a possibility to increase the important oligosaccharides in infant formulas, as future research could explore this further.

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