Bioactive Components of Human Milk: Similarities and Differences between Human Milk and Infant Formula

Esmat Aly, Aliaa Ali Darwish, Ruben Lopez-Nicolas, Carmen Frontela-Saseta and Gaspar Ros-Berruezo

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http://dx.doi.org/10.5772/intechopen.73074

Abstract

Nowadays, there is an increasing awareness regarding the relationship between food, nutrition, and health. It is obvious that this relation starts from the birth. In the early stage of life, breastfeeding is considered the preferred choice for infant feeding and human milk is the optimal food for an infant to keep its nutritional and health status. Because it contains a large group of bioactive compounds such as proteins, vitamins, nucleotides, oligosaccharides, immunoglobulins, and some of the bioavailable minerals beyond its content of the essential nutrients, human milk is classified as the first functional food in the infant life. The various bioactive components of human milk play a pivotal role in preventing the gastrointestinal and respiratory infections, anemia, and bone-related problems as well as it enhances the immune function and helps in the maturation of the digestive system. The exclusive breastfeeding pattern during the first 6 months of infant life and introducing complementary foods after this period have a potential role in protecting against certain diseases in the adult stage of life. This chapter is underlying the great potential of breastfeeding for mothers and babies. Moreover, it discusses the functionality of some components of human milk and its similarities and differences between human milk and infant formulas.

Keywords: breastfeeding, human milk, bioactive components, lactoferrin, oligosaccharides, infant formulas

1. Introduction

Milk and dairy product are considered a main part of the healthy and balanced diet [1]. In this context, human milk is the most appropriate choice for feeding newborns and provides all the energy and nutrients needed to ensure proper growth and development [2]. Furthermore,
human milk provides a large group of functional components which improve the newborn health, increase the immunity, and protect against the gastrointestinal and respiratory infections [3]. So, it was noted that breastfed infants suffer less gastrointestinal disorders and respiratory infections rather than formula-fed infants [4]. The exclusive breastfeeding pattern during the first 6 months of life was recommended by various health and breastfeeding organizations such as the American Academy of Pediatricians (AAP) and the American Academy of Family Physicians (AAFP) [5].

It is scientifically accepted that using the nutritional factors may decrease or prevent the extension of these diseases and its implications to the adult life. Nowadays, researchers and health and breastfeeding organizations are trying to discover the precise substances in human milk that seem to supply physiological benefits beyond its normal nutritional value which contribute earlier in delay, treatment or prevent some diseases [6]. Thus, these functional ingredients hold a great promise for future trends in human nutrition. Additionally, the relationship between milk consumption and human health requires a deeper understanding to uncover the protective role of some bioactive compounds, which naturally present in human milk.

Although human milk is considered the optimal food and supplies all the nutrient and some bioactive components, it is no longer sufficient to meet all the necessary nutritional requirements after 4–6 months of infant life [7]. In this case, infant formulas play an indispensable role in infant feeding. The formulas should be similar to mature human milk regarding its micronutrient and macronutrient contents. During the evolution of infant formulas, the manufacturers should take into account the necessary nutritional requirements of the newborns and infants and enrich the formulas with the functional ingredients which are naturally present in human milk [8]. The aim of this review is to present current knowledge regarding evidence on the importance of breastfeeding, the functionality of selected human milk ingredients, infant formulas as a human milk alternative, and the similarities and differences between human milk and infant formulas.

2. Meaning of breastfeeding for mothers and babies

It is well established that breastfeeding pattern of newborn provides more benefits for both mothers and their babies. It is well documented that breastfeeding not only provides the optimal nutrition [9] but also has many health benefits for both children and their mothers [10]. Breastfeeding pattern is positively associated with maternal sensitivity and with bonding between mother and infant [11]. It helps to build up a safe and full-of-feeling relationship between the mother and her infant and offers numerous other positive advantages. Breastfeeding mothers suffer less from certain serious diseases: reduced risk of certain cancers, including ovarian and breast cancer, type 2 diabetes, delayed resumption of menses, and more rapid postpartum weight loss. So, breastfeeding mothers are likely to be more health conscious, and, therefore, to promote healthy habits, which are likely to prevent overweight and obesity later in childhood [12].
On the other hand, breastfeeding pattern provides the protection against childhood-related diseases. In this regard, breastfed infants showed higher resistance to infectious disease and stronger immune systems, leading to lower rates of chronic diseases as compared to formula-fed infants. The ideal composition of human milk provides nutritional, growth, and developmental advantages to the child [13]. By the way, lower risk of gastrointestinal infection, otitis media, asthma, allergies, respiratory tract infection, type 1 diabetes, and sudden infant death syndrome are observed in breastfed infants. Other benefits include evidence of protection against childhood obesity, which may persist into adulthood, and less cognitive development and behavioral problems in breastfed children compared with children not breastfed [14]. Based on the abovementioned, breastfeeding should be actively recommended and supported as the most preferred method of infant feeding at both nutritionally and healthy states.

3. Nutritional significance of breastfeeding

It is well established that infancy is the most important stage of human life where newborns are growing with high rate allowing to duplicate the infant weight in only 4–5 months. So, an adequate supply of nutrients represents paramount importance in this early stage of infant life. The breastfeeding of infants is obviously important to provide the needed nutrients and energy for the synthesis and deposition of new tissues from birth until 4–6 months of infant life. The high metabolic requirements contrast with the limited ability of young infants to compensate for an inadequate supply of nutrients due to the diminished body reserves of nutrients and the immature homeostatic mechanisms. For example, the activity of some metabolic pathways and the kidney’s ability to concentrate the urine are still low during the first 3–4 months of life. Young children may not be able to synthesize sufficient amounts of certain substrates considered as nonessential or dispensable since the limited capacity of the specific metabolic synthesis is not always sufficient to achieve the high requirements. Some nutrients considered nonessential in adults and older children can become indispensable for young infants that would be supplied in the diet [15].

In addition to the immediate consequences of infant feeding on growth, body composition, health, and wellness, a number of recent studies have also provided indications that the quantity and quality in the supply of nutrients during childhood has important long-term consequences in the development and function of the organs, health, and risk of disease as well as in the cognitive ability [16]. Human milk is nowadays universally recognized as the optimal feeding choice for every infant [17] where it contains many nutritional components that are able to conserve his development.

The nutritional components of human milk are classified into two categories: macronutrients and micronutrients [18]. Macronutrients of human milk include protein, fat, and sugar. The concentration of human milk macronutrients differed during the course of lactation and between the mothers as well as differed between term and preterm milk. The latter has a higher content of protein and fat. Generally, the mean macronutrient composition of term
mature human milk is presented in Table 1 as compared with cow milk, the most common milk type used in infant formula manufacturing.

Proteins provide amino acids for growth as well as are presented in the form of polypeptides that facilitate digestion [19], the defense of the guest [20], and other functions [21]. Fats provide energy, but some have antiviral properties [22]. Carbohydrates provide energy and can also stimulate the absorption of minerals [23], and various human milk oligosaccharides (HMOs) play a pivotal role in the microbial intestinal balance. Energy estimates range from 65 to 70 kcal/dL and are highly correlated with the fat content of human milk. Butte et al. [24] also clearly showed that intakes of energy, protein, fat, and carbohydrate were lower in breastfed than in formula-fed infants at 3 and 6 months. The differences in composition between human milk and infant formulas seem to affect the growth pattern between breastfed infants and formula-fed infants [25]. However, no apparent consequences were associated with the lower intake and slower weight gain of breastfed infants where they do not differ in activity level, and they suffer less gastrointestinal and respiratory infections and have higher cognitive development [26].

Because human milk is considered the optimal and first functional food for infant feeding, nowadays, especially in the USA, pasteurized donor milk represents the suitable alternative provided for an infant that is in high risk [28]. However, infant formulas become necessary for infant feeding when human milk is unavailable or the mother cannot breastfeed her infant. So, special efforts are needed to ensure an adequate diet composition in young infants [25].

Various negative consequences are noted with very low- or very high-specific nutrients [29]. For example, cow’s milk is not an ideal food during the first year of life. The ingestion of protein for the infants fed with cow’s milk is higher than that for those fed with human milk, and this leads to overload renal solutes [30]; in addition, a high-protein intake can cause hypercalciuria [31]. On the other hand, high consumption of cow’s milk below the first year of life is one of the most important risk factors for the development of iron deficiency anemia. Cow’s milk is low in iron, and much of that iron is attached to the casein micelles, which interferes with its absorption. Additionally, its low content in vitamin C does not favor the absorption of the little iron that contains [32].

Overall, the breastfeeding pattern is the preferred choice of infant nutrition and human milk provides all the nutritional components during 4–6 months of life. It also provides a large group of bioactive components, which play an indispensable role in protecting the infant health.

<table>
<thead>
<tr>
<th>Components</th>
<th>Content (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Human milk</td>
</tr>
<tr>
<td>Protein</td>
<td>1.2</td>
</tr>
<tr>
<td>Fat</td>
<td>3.7</td>
</tr>
<tr>
<td>Sugar</td>
<td>7</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>65</td>
</tr>
</tbody>
</table>

Adapted from [27].

Table 1. Macronutrient concentration of human milk and cow milk.
4. The bioactive components of human milk

Increasing evidence currently shows that short- and long-term benefits of human milk feeding are resulted by its content of various components named functional or bioactive components. These functional components involved a large group of several compounds such as protein (such as lactoferrin (Lf)), carbohydrates (especially human milk oligosaccharides), fats (polyunsaturated fatty acids), vitamins, nucleotides, minerals, and immunoglobulins. In this section, the occurrence, variation, and functionality of selected components of human milk are discussed.

4.1. Lactoferrin: for anemia fighting

Lactoferrin (Lf) is the second most abundant protein in human milk belonging to the transferrin family [33]. It is a glycoprotein first isolated from cow’s milk and second from human milk [34]. It is well known as the principal iron-binding protein in mammals’ milk [35] and the first-line defense molecule against infections [33]. The highest content of Lf is found in human colostrum (7 g/L), and this content declined after 2 weeks after birth reaching 2–4 g/L of mature human milk [36]. While Lf content in cow colostrum and milk is 10-fold lower [37]. Structurally, Lf is an iron-binding glycoprotein consisting of a single polypeptide chain distributed to two lobes (N and C lobes). Both human Lf and bovine Lf are sharing a sequence homology of about 70%, and their 3D structures (Figure 1) are very similar but not identical [38]. Each lobe of Lf contains an iron-binding site with a high affinity and a glycan-binding site. N and C lobes have very similar conformations but show slight differences in their affinity for iron [39]. Table 2 presents the differences between human and bovine Lf.

Due to its distribution in several parts of the body and its involvement in several physiological processes, Lf is considered as a multifunctional protein. Moreover, numerous studies have been carried out to uncover the wide range of activities of Lf and its peptides [42, 43]. Iron absorption enhancement by Lf is one of the most observed activities especially in breastfed as compared to formula-fed infants. In this context, the high affinity of Lf to bind iron is a key characteristic of this beneficial role. Although iron is the main cation bound by Lf, other metals such as Cu\(^{2+}\), Zn\(^{2+}\), and Mn\(^{2+}\) ion can be bound by Lf [44]. Lf exists in three forms, according

![Figure 1. Protein structure of human Lf. Source: Ref. [41].](http://dx.doi.org/10.5772/intechopen.73074)
to its saturation degree with iron: apo-lactoferrin (iron free), mono-ferric form (one ferric iron), and holo-lactoferrin (binds two Fe$^{3+}$ ions) [45]. Apo-lactoferrin is the secreted form of Lf in human milk where its saturation degree does not exceed 10%, whereas its saturation degree in cow milk is about 20% [46].

The apo-Lf molecule is an open molecule, whereas the holo-Lf is a closed molecule [47]. Thus, apo-Lf is less stable than holo-Lf against gastrointestinal enzymes [48]. In view of this, the stability of Lf against gut enzymes is determined by its degree of saturation with iron. Interestingly, media pH plays a key role in iron release from Lf. So, bovine Lf retains the metal over a wide range of pH and starts to release its iron below pH 4 and at pH 2 iron is completely released, while it starts to release at pH 3 in human Lf [42].

Among the principal factors that influence the iron bioavailability is its distribution in milk where 20–45% of iron in human milk is mainly bound to Lf, while 24% of iron in cow milk is bound to casein micelles [49]. This distribution resulted in a high iron bioavailability from human milk. Moreover, the high iron absorption from human milk was attributed to its high content of Lf. This hypothesis was supported by the discovery of species-specific receptors with high affinity for Lf (Lf receptors) in the enterocytes. This would explain the high bio-availability of iron from human milk, as only human lactoferrin releases iron to the enterocyte by this mechanism [48]. Additionally, Lf can increase the gene expression of divalent metal transporter 1 (DMT1) receptors that may play a central role in enhancing Fe uptake via proton-coupled mechanism [50]. It was also reported that Lf may be useful as a natural solubilizer of iron for food products, and it was suggested that Lf, orally administered, could solubilize ferric Fe in the intestine [51]. The endocytosis, another possible mechanism, was speculated to explain the role of Lf in iron absorption. The enterocytes catch Lf-iron complex through the endocytosis and then release its iron, through Lf degradation, at the intracellular level [52]. The released iron inside the cell is quickly complexed, forming another protein named ferritin, and then, apo-form of Lf comes back again to mucosa surface to catch another iron to start another transport process [53]. Figure 2 shows the possible mechanisms of iron absorption enhancement by Lf.

Overall, Lf is a multifunctional glycoprotein and has a central role in decreasing the gastrointestinal and respiratory infections and protecting the newborn from anemia.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Human Lf</th>
<th>Bovine Lf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight (kDa)</td>
<td>80</td>
<td>77</td>
</tr>
<tr>
<td>Amino acids</td>
<td>711</td>
<td>689</td>
</tr>
<tr>
<td>N lobe</td>
<td>1–332</td>
<td>1–233</td>
</tr>
<tr>
<td>C lobe</td>
<td>344–703</td>
<td>345–689</td>
</tr>
<tr>
<td>α-Helix</td>
<td>333–344</td>
<td>334–344</td>
</tr>
</tbody>
</table>

Table 2. Structure of human Lf and bovine Lf.

Adapted from [38, 40].
4.2. Oligosaccharides

Human milk oligosaccharides (HMOs), the third most abundant component of human milk, are another multifunctional milk ingredient. Its content is higher in colostrum (15–23 g/L) than mature milk, which contains 8–12 g/L [54]. Structurally, human milk contains more than one hundred oligosaccharides with diverse structure and functions. A wide range of activities were reported for HMOs [55]. The prebiotic activity of HMOs has been observed by various studies [54] where it acts as a bifidogenic molecule that improves the beneficial microflora growth. It also provides functional capacity including anti-adhesive and immunomodulators [56].

HMOs are nondigestible substances, and this property is the main key to its physiological role. HMOs can survive against the gastrointestinal conditions, digestive enzymes, and pH and thus reach the colon in an intact form where these serve as fermentable substances, leading to improvement in the beneficial bacteria growth and activity [57], preferably Bifidobacteria [58]. The fermentation of prebiotics is accompanied by organic acid production and pH decrease. Hence, prebiotic fermentation may create an environment in the colon that inhibits the growth and activity of pathogens. In addition, prebiotics fermentation may enhance the beneficial bacteria in the colon that can produce various antibacterial factors, leading to pathogen growth inhibition. Additionally, HMOs possess direct activities resulted in pathogen inhibition where it has anti-adhesive effects that reduce or prevent the pathogen biofilm formation through its ability to reduce pathogens binding to colonocytes [59]. Similarly, HMOs also act as receptor analogues to inhibit the adhesion of pathogens on the epithelial surface, and this evidence is seen as a passive defense of the host [60].

HMO’s structure and diversity represent another difference among human milk, cow milk, and infant formulas. As well known that human milk is structurally very complex and has huge diversity [61], identical structures are not available for use in infant formulas [62]. Thus, several researchers proposed using oligosaccharides much simpler such as GOS and FOS or that derived from cow milk [63]. Thus, breastfed infants have less gastrointestinal infections and their stools contain more beneficial bacteria, Lactobacilli and Bifidobacteria, as compared
to formula-fed infants. The positive microbial intestinal balance partially attributed to HMOs plays a pivotal role in improving the gut health.

4.3. Nucleotides

Nucleotides, another bioactive ingredient of human milk, are nitrogenous compounds which play a main role in various metabolism processes, such as energy transfer, nucleic acid synthesis (DNA and RNA), and carbohydrates, lipids, and proteins synthesis. Nucleotides are found in human milk in free form as ribonucleotides and ribonucleosides accounting 2–5% of non-protein nitrogen and participate in protein utilization by breastfed infants [35]. Free nucleotide content is higher in human milk than cow milk. Additionally, some related components such as nucleosides, purine and pyrimidine bases, nucleic acids, and products derived from them (such as uridine diphosphate galactose) have been found in human milk [64]. Human milk contains a higher content of free nucleotides than cow milk. Thus, it is recommended to enrich cow milk-based formulas with the nucleotide level similar to that found in human milk [65]. Recently, legislation allows the addition to infant formulas and follow-on formula, nucleotides in quantities of: 1.5 mg adenosine-5-phosphate/100 kcal, 2.5 cytosine-5-phosphate/100 kcal, 0.5 kcal guanosine-5-phosphate/100 mg, 1.75 mg uridine-5-phosphate/100 kcal, 1 mg inosine-5-phosphate/100 kcal, until a total concentration of 5 mg/100 kcal, which is similar to the amounts of free ribonucleotides in milk (4–6 mg/100 kcal) [25]. Also in this context, Koletzko et al. [15] reported that ESPGHAN supports the optional addition of nucleotides in amounts not to exceed 5 mg/100 kcal as adverse effects have been seen with higher concentrations.

Addition of nucleotides to infant formulas have been found to increase the probiotic bacteria counts and reduce the pathogen counts in stool samples in infants fed on nucleotide-supplemented formula as compared to whose fed standard infant formula, but probiotic counts in the stool of breastfed infants were still higher. The intestinal microflora modulation attributed to nucleotides due to that nucleotides serve as an energy source of intestinal microflora. Because probiotic bacteria are characterized by a higher growth rate than pathogenic bacteria, they limit the growth of pathogens. Thus, supplementation with nucleotides able to positively modulate the intestinal microbial balance, leading to increase probiotic growth and limit the growth of the pathogens [66].

5. Infant formula: looking for the best alternative

Although human milk contains all nutrients and provides diverse bioactive ingredients and considered the first functional food in infant life, it is not generally attractive, adequate, or acceptable or it is not available; in some cases, the infants cannot be breastfed. Thus, looking for a suitable alternative is of importance.

In these cases, infant formulas play an indispensable role in infant nutrition. All efforts of industry are aimed to resemble human milk composition [67]. The accumulated knowledge about human milk composition highly assists in infant formula development. However, the human milk composition is not stable, since it changes along breastfeeding period [68], as well
as depending on different factors such as environment, mother’s diet, and so on. Nowadays, companies and research centers are devoted to prepare these formulas focused on enhancing the quality of infant formulas, not only adapting the concentration of macronutrients and micronutrients but also the composition of bioactive compounds to make it as similar as possible to human milk [69] where the final aim of infant formula development is not necessarily to mimic the composition of human milk in every respect but to achieve physiological effects as in breastfed infants [70].

Nowadays, there are numerous infant formulas adapted to special physiological state and infant formula based on soy or without lactose, among others. But, in this chapter, we are focused on those formula based on supplemented cow milk with functional ingredients. The current trend of infant formula manufacturing is to enrich it with the functional ingredients that naturally found in human milk. Thus, these ingredients such as probiotics, prebiotics (oligosaccharides), proteins such as lactoferrin and α-lactalbumin, nucleotides, and polyunsaturated fatty acids (mainly docosahexaenoic and arachidonic acids) among others are incorporated in infant formulas to make them more functional [71]. In fact, many studies revealed the higher efficacy of infant formulas supplemented with certain bioactive ingredients than the unsupplemented ones [72].

5.1. Nutritional components of infant formula

Human milk must be always selected as the first option for the best infant nutrition. However, when it is impossible, an adequate substitute should be found. Historically, milk from different animals was studied, obtaining the best results for the cow. However, some problems have been found after using cow milk as a substitute, since the high-protein content, the different protein composition, and the sodium content, among others, could induce some metabolic problems to the not fully developed gastrointestinal system of newborns. From last decades until now, the infant formula has been developed trying to mimic to human milk in macronutrients and energy density, but it is in the most recent past when the functional ingredients are included in the infant formulation to simulate the beneficial health effects of breast milk. 

Table 3 shows the composition of infant formula supplemented or not including legal limits according to the European Commission [73].

As can be seen, different compounds are included in supplemented infant formulas in different concentrations. The caloric values have also been considered to establish a minimum or maximum legal limit for each one. Prebiotics (FOS and GOS) are considered as key compounds in human milk in order to promote an adequate intestinal microbiota; for this reason, infant formulas should be adequately supplemented. Beneficial bacteria of human milk should be also included in infant formulas; however, it is very difficult that added bacteria achieve colon as live microorganisms with beneficial effects on health.

5.2. Functional components of infant formula: resembling the standard model

One of the functional ingredients added to infant formula is oligosaccharides (fructo- and/or galactooligosaccharides) since they are in human breast milk providing a beneficial effect
on newborns due to its prebiotic activity. Furthermore, these compounds show anti-adhesive properties, protecting the intestinal epithelium against pathogens as well as enhancing the immune system that, in infants younger than 6 months, is not still fully developed.

Polyunsaturated fatty acids can be produced by newborns using its precursors (linolenic and linoleic acid), but this transformation pathway is not fully developed at this age. For that, most infant formulas are enriched with arachidonic and docosahexaenoic acids (ARA and DHA, respectively), since they play an important role in neuronal function and cognitive and visual development [74].

Other important bioactive compounds present in human milk are polyamines that are nitrogen compounds and are present in a very low concentration in cow’s milk. Nowadays, based on different studies, polyamines should be added to infant formula; however, due to the scarce information found in the literature about its specific function and the effect on human health on medium-long term, more studies should be developed. Studies performed show an interesting role of polyamines in the maturation of intestinal and immune systems of infants [75].

Studies in infant formula composition must be studied in detail. Related to this, there is no doubt that the proteins in human milk provide an important source of amino acids to rapidly growing breastfed infants. However, this is not the only one role of milk proteins since many of them also play a role in facilitating the digestion and uptake of other nutrients in breast milk. Included in this group of functional proteins are lactoferrin and haptocorrin, which may assist in the absorption of

<table>
<thead>
<tr>
<th></th>
<th>Standard infant formula</th>
<th>Supplemented infant formula</th>
<th>Supplemented infant formula</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Minimum limit</td>
<td>Maximum limit</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>68</td>
<td>60</td>
<td>70</td>
</tr>
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<td>Protein (g)</td>
<td>1.3</td>
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<tr>
<td>Fat (g)</td>
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<tr>
<td>α-Linoleic (mg)</td>
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<td>840</td>
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<td>α-Linolenic (mg)</td>
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</tr>
<tr>
<td>AA (mg)</td>
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<td>—</td>
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<tr>
<td>DHA (mg)</td>
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<td>35</td>
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<tr>
<td>Carbohydrate (g)</td>
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<td>l-Carnitin (mg)</td>
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<td>8.4</td>
</tr>
<tr>
<td>Nucleotides (mg)</td>
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<td>3.2</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Source: Ref. [73].

Table 3. The composition of different infant formulas. Values are expressed as grams per 100 mL of reconstituted formula.
iron and vitamin B12, respectively [76]. Vitamin B12 in human milk is mostly bound to haptocorrin, and these proteins have also been suggested to inhibit pathogenic bacterial growth by tightly binding and withholding vitamins and minerals from the bacteria [77].

Another important human milk protein is alpha-lactalbumin (15% of the total protein content). This protein is responsible, in the mammary gland, for lactose synthesis, but it is also secreted in the milk. This functional protein is easily digested, forming peptides that may provide different bioactivities in the upper gastrointestinal tract (duodenum and jejunum) [78]. Alpha-lactalbumin, after gastrointestinal digestion, serves as a good source of essential amino acids.

6. Conclusion

Breastfeeding during the first 6 months of infant life represents the cornerstone of building his body and maintaining his health status. Feeding of an infant during this critical period of life has several short-term and long-term effects. So, breastfeeding is recommended exclusively after birth till 6 months; then, the complementary food must be included to meet the increasing nutrients needed. As we know, human milk is the optimal food during this early stage of life. In some cases, infant formulas represent the best alternative for infant feeding which must resemble human milk composition not only in its macronutrients but also in its functionality.

Author details

Esmat Aly1, Aliaa Ali Darwish2, Ruben Lopez-Nicolas3, Carmen Frontela-Saset3 and Gaspar Ros-Berruezo*1

*Address all correspondence to: gros@um.es

1 Special Foods and Nutrition Department, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt
2 Dairy Technology Research Department, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt
3 Food Science and Nutrition Department, Faculty of Veterinary Sciences, University of Murcia, Espinardo, Spain

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