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Functional Aspect of Colostrum and Whey Proteins in Human Milk

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Abstract

The colostrum serum in the human milk has a variety of proteins that characterize and contribute exclusively to the quality of nutrition. In addition to being an important source of special amino acids required for rapid growth of the baby, many of these proteins have specific functions; such as assisting in the absorption of the fluids, defending against viral and bacterial infections, and stimulating the development of the intestinal mucosa by increasing the immunocompetence of the neonate. Some proteins such as the bile, a salt-stimulated lipase, the α-amylase and α1-antitrypsin, exert their function in the gastrointestinal tract, helping the absorption of the micronutrients and macronutrients milk, because they are relatively resistant to digestive enzymes. Furthermore, other proteins such as lactoferrin, secretory immunoglobulin A, lactoperoxidase, haptocorrin, and lactaderina bioactive peptides are formed during digestion, human milk proteins may inhibit the growth of pathogens such as bacteria and viruses, or promote probiotic activity, favoring the growth of beneficial bacteria such as strains of the genera Lactobacillus and Bifidobacterium. There are proteins, such as cytokines, that modulate the immune response, regulate the inflammatory response and the development and growth of the newborn. Currently it is possible to reproduce the recombinant proteins of human milk in transgenic plants and animals on a large scale at a low cost. Thus, some components found in human milk can be added to other diets, subsituting some qualities of the food, however it is unlikely that all original components in human milk can be reproduced and used, by employing the available technology.

INTRODUCTION

Human milk is commonly considered the best form of nutrition for newborns. It has bioactive properties that facilitate the transition from the intra-uterine state to extra- uterine state, which stimulates the development of the brain, the digestive and the immune system. Milk is the only food that contains all the necessary nutrients for the newborns during their first weeks of life. These include the energy sources (fats, proteins and carbohydrates), water, and other substances that serve as raw materials for tissue such as fatty acids, amino acids, minerals, vitamins and trace elements, for the perfect growth and development of the newborn [1,2].

Among the major components of human milk are proteins that account for approximately 75% of the nitrogen compounds present in the milk [3]. The nitrogen that is not incorporated into the protein is present in the form of urea, peptides, amino acids (such as cysteine and taurine, which are fundamental for the development of the central nervous system), nucleotides and DNA [4].

Usually, the milk is fractionated by precipitation/ sedimentation procedures into three main fractions: fat, casein and whey [5].The proteins found in the lipid fraction are membrane proteins that flood the fat globules, as mucins, and contribute a small percentage of the total protein content of milk [6]. Casein is the only group of proteins that can be pelleted by centrifugation, allowing its separation from the fraction of the whey proteins. The β-casein is the predominant casein in human milk, which forms micelles in a relatively small volume, which produces a lighter gastric curd with digestible floccules and reduces gastric emptying time. The major whey proteins are α-lactalbumin, lactoferrin, immunoglobulin A (IgA), and serum albumin, and a large number of other proteins present at low concentrations [7]. The micellar casein and whey proteins are present in human milk at a ratio of 40:60 [4].The numerous

components of milk are distributed in these fractions by forces directed by biological and physico-chemical properties, which act during the synthesis of milk secretion and after excretion [8].

These proteins are the major constituents of protoplasm and therefore during the growth of the organism there should be supply of exogenous proteins, or of their constituent amino acids [9]. The human body has a considerable ability to interconvert amino acids, however, some of them are considered more vital: arginine, lysine, leucine, isoleucine, valine, methionine, phenylalanine, threonine and tryptophan. Moreover, the newborn has a specific temporary requirement for histidine and cystine, and for a period, may be unable to convert phenylalanine to tyrosine [10]. In addition to being an excellent source of amino acids for body, whey proteins in colostrum and breast milk play an important role for the optimal development of the newborn. They can be classified according to their functions in enzymes, binding proteins, defense proteins (acting against pathogenic microorganisms) and protein nutritional reserves, as described in Table 1. There is no strict separation in this classification, as there are the same proteins, for example, an enzyme may also act in defense of the body of the newborn (such as lysozyme), therefore, each class includes a series of proteins [11].

Several enzymes are also present in the human milk. Some are specific for the biosynthesis of components in the mammary gland of milk (lactose synthetase, fatty acid synthetase, thioesterase), while others are specific for digestion of proteins, carbohydrates and fatty acids, which facilitates the breakdown and absorption of food substances present in milk by nursing mothers [12]. There are also other proteins which act as carriers for ions such as zinc, selenium and magnesium [13].

There are two sources of proteins in the milk, whey proteins such as α- lactalbumin and lactoferrin which are synthesized in the mammary gland, and other proteins that include serum albumin and various enzymes and protein hormones that are transfered to the milk from the plasma [8]. Furthermore, there is a peculiar dimer of the secretory IgA protein, the main immunoglobulin in milk, which is synthesized by epithelial cells of the mammary gland from the connection of two IgA molecules produced locally by resident lymphocytes in breast tissue with the other two proteins, the J chain and secretory component-specific, through which the dimer is transported to the milk [14].

The use of proteomics as a tool to identify the whey protein in human milk and colostrum

Considered the future of molecular biology, proteomics emerged in the mid- 90s. It designates the set of proteins that can be found in a cell or tissue. The term “proteome” was originally coined to describe the set of proteins encoded by the genome. The concept was readily absorbed by the academic community as a component of ‘post- genomic era’. Proteins are complex molecules responsible for almost everything that happens in living organisms, from the formation and composition to the regulation and operation. They are assembled inside the body on the basis of information contained in the genes, and are specifically built to determine how and whether to act on the cell [15].

At present, two general strategies for proteome analysis are being used. The first strategy, two dimensional polyacrylamide gel electrophoresis (2D-PAGE): it constitutes of an analytical methodology capable of separating thousands of proteins in a single analytical run. In this case, the gel applied to the sample is subjected to an electric field for two-dimensional separation. In the first dimension, the separation occurs according to the isoelectric point of proteins (isoelectric focusing). In the second dimension, the separation takes place according to their molecular masses. The identification of spots in the 2D gels is typically performed by cutting protein spots from the gel, digesting the gel protein with

Table 1: Examples of proteins present in whey and colostrum and their functions in the human newborn.

CLASSIFICATION	EXAMPLES	FUNCTION IN NEWBORN	BIBLIOGRAPHIC SOURCE
ENZYMES	Lysozyme	Bacterial	Hamosh [85]
	Lipase	Hydrolysis of fats	Chen *et al.* [65]
	Sulfhydryl Oxidase	Oxidation of Sulfhydryl groups Regulation of Enzyme activity	Hamosh [86]
	Glutathion Peroxidase	Selenium alloy facilitating its release for infant	Mannan & Picciano, [87]
BINDERS	Lactoferrin	Bactericidal Possible role in intestinal iron absorption	Tomita *et al,;* Rosa & Trugo, [88,89]
	Haptcorrina	Possible bacteriostatic Possible role in the absorption of Vitamin B12	Gullberg,Trugo *et al.* Trugo [90-92]
	Folate binding protein	Possible role in the uptake of this vitamin serum Possible role in the intestinal absorption	Ford, 1974; Verwei *et al.* 2005 [93,94]
NUTRITIONAL	Lactalbumina	Rich source of amino acids Lactose synthesis	Forsum, Brew & Hill [95,96]
PROTECTION	Immunoglobul ins	Act as antibodies, such as IgA, IgG, etc.	Goldman & Goldblum, [97]
	Fibronectin	Facilitating the training of particles by phagocytic cells	Friss *et al.*, [76]
	Lactoferrin	Bacteriostatic - competes with siderophilic bacteria by ferric ion	Tomita et al., 1991 [88]

trypsin, peptides extracting and identifying these fragments by mass spectrometry [16].

To date there are a very few articles that discuss the identification of whey protein in human milk. Murakami *et al.*[7], used two-dimensional electrophoresis and Edman sequencing to identify components of whey after removal of the three major whey proteins with specific antibodies, and identified 22 protein isoforms mainly casein, albumin and immunoglobulins. Conte-Junior *et al* [17] showed that the major proteins present both in the serum of colostrum and in the serum of human mature milk could be removed using Cibacron Blue resin. These authors also compared the protein- dimensional map of the serum proteome, colostrum whey and whey of mature human milk, demonstrating that there were great qualitative differences with respect to at least minority proteins.

CHANGES IN THE COMPOSITION OF PROTEINS IN HUMAN MILK DURING THE FIRST MONTHS OF LACTATION

Colostrum

From the second trimester of pregnancy, a woman's mammary gland is sufficiently active to produce breast milk. Throughout the milk production period, constant variations in milk composition, and differences are observed from one woman to another, and in between samples taken from the same woman during the same day and even during the same feeding session [18]. Even during these changes, human milk has always maintained a balanced chemical composition regarding the demand for the necessary nutrients required for rapid growth and maturation of tissue, and at the same time, for the organs involved in the regulation of endogenous metabolism [19]. Still, given this large variation it is very important to know how the milk samples are collected. The idea is to collect a sample of all the milk produced within 24 hours and at different times of lactation [17].

During the last trimester of pregnancy, the mammary gland accumulates in the lumen of the alveoli pre-colostrum, chiefly composed of plasma exudate, cells, immunoglobulins, lactoferrin, serum albumin, ions such as sodium and chlorine, and a small amount of lactose [20]. On average, the first 7 days after delivery, colostrum is produced, it is the first product of the of lactic nurse secretion. It is a yellow, high denisty, low volume fluid. The early days produce 220ml per dose, which is sufficient to meet the requirements of a newborn [21]. Colostrum has a lower energy content, lactose, lipids, glucose, urea, water soluble vitamins and nucleotides than mature milk, however, it has a greater protein content (Table 2), sialic acid, lipid-soluble vitamins E, A, K and carotenes than mature milk [22,23]. The concentrations of minerals such as zinc, sodium, iron, sulfur, selenium, manganese and potassium are also higher in colostrum.

The ratio of whey proteins/casein ratio is 80/20 in colostrum, whereas in mature milk is 60/40 and 50/50 in late lactation [4]. Likewise, the concentration of free amino acids ranges when compared to colostrum, transitional milk and mature milk. The amount of protein decreases rapidly during the first month and then stabilizes for a time, then again decreases slowly over the lactation. Colostrum has a high content in immunoglobulins

Table 2: Concentration of some of the proteins present in colostrum and breast milk.

	Concentration ($g.L^{-1}$)	
Proteins	**Colostrum**	**Milk**
Caseins (α, β, κ)	Traces	2,7
α-Lactoalbumin	4,9	3,4
Immunoglobulins (IgG, IgM, IgA)	Jun-48	0,3-1,8
Lactoferrin	10-May	0,1
Lactoperoxidase ($mg.L^{-1}$)	Traces	Traces
Lysozome	0,360	0,300

Data extracted from Jensen et al. [5], Korhonen et al. [3], Velona et al. [26], Marnila & Korhonen [22].

particularly IgA, lactoferrin, cells (lymphocytes and macrophages), oligosaccharides, cytokines and other defense factors that protect newborns from microorganisms of the environment and promote the maturation of the immune system [24]. This fluid is adapted to the specific needs of the neonates since their immature kidneys cannot filter large volumes of liquids, and additionally it also facilitates the elimination of meconium, preventing neonatal hyperbilirubinemia [22]. Colostrum contains intestinal enzymes that help in digestion (lactase and other intestinal enzymes are not produced in the newborn). The high concentration of immunoglobulins allows the endothelium of the digestive tract to be covered preventing the adhesion of pathogens. Furthermore, colostrum has antioxidants and quinone that protect the digestive tract from oxidative damage, it is also rich in growth factors that stimulate the maturation of both the digestive and immune systems [25].

Transitional / Mature Milk

The term transitional milk, or intermediary, refers to milk that is produced after the colostrum, on average between 7 and 21 days postpartum. In this period there is a sudden increase in milk production, which follows a sequential increasing until it reaches a volume of 600-700ml per day, between the 15^{th} to 30^{th} day of lactation [26]. This milk has an intermediate composition and varies from day to day, until it reaches the composition of mature milk. Changes in milk composition occur more slowly in this period than in the period immediately after birth [8]. After this period, what is called mature milk is produced and secreted, on an average, after 21 days postpartum. This composition also shows variability, but less than that observed during early lactation, the intermediate concentrations of fat and lactose increases, whereas the concentrations of proteins, growth factors, immune factors, particularly IgA, and minerals decrease [27]. Mature milk contains a variety of nutritive and non-nutritive components. The average volume of milk produced by a mature woman is 700-900 ml per day during the first 6 months postpartum, and if the mother has twins enough volume for each baby is produced. Moreover, lactation progresses to a phase of colostrum before merging into milk secretion [28].

The total protein content of human milk undergoes longitudinal variation however, unlike the fat little transverse variation. The total protein content is higher in colostrum (approximately 15.8 gL-1) and decreases during lactation (about 9.0 gL-1 in mature milk) [29]. The protein content ingested by

the infant, however, does not vary much during lactation, since the amount of ingested colostrum produced is smaller than the amount of mature milk, which compensates for variations in protein concentration during lactation [11]. The concentration of whey protein, which is also higher in colostrum is reduced with the passage of time in lactation. These changes result in a casein/whey protein ratio of approximately 10:90 in the first few days of lactation to 45:55 in mature milk [30]. In cow's milk the ratio of casein/whey protein is about 80:20, this ratio is quite different from that contained in human milk [1]. A change in milk composition during lactation is most pronounced during the first weeks of lactation.

BIOLOGICAL EFFECTS OF WHEY PROTEINS

The anticarcinogenic effect of whey proteins (or their peptides) was also observed in cultured cells. The development of breast and prostate cancer cell lines - and MCF-7 PC-3 - was inhibited when the whey proteins were added to the culture medium [31]. Increased levels of glutathione in the liver are also related to the anticarcinogenic effects of whey proteins which have been demonstrated in different studies with experimental animals. The effect of milk protein on reducing the size and frequency of intestinal tumors in rats induced by dimethylhydrazine was greater than that observed for meat and soybean proteins [32].

Few clinical studies correlate antineoplastic effect on milk protein, but the results are very promising. It is believed that, contrary to what occurs in normal cells, the glutathione concentration is high in tumor cells making them resistant to chemotherapeutic drugs [33]. In some cases, a 6 month administration of 30g/day dose of whey protein concentrate led to a decrease or stabilization of glutathione levels, resulting in stabilization or regression of the tumor.

The proteins present in the serum of colostrum and breast milk have several nutritional and physiological functions [3], some examples of the functions of these proteins are shown in Table 1. Through these examples, we can understand the large role played by these proteins, and their importance for the newborn. In addition to the proteins mentioned above, there exist other serum proteins, whose function has already been established, and others whose functions are not yet clear [17].

α-lactalbumin

The α-lactalbumin is a major protein found in human milk, making up 20-25% of the whey proteins. The primary structure of this protein consists of many different amino acids representing a readily available source of essential amino acids as well as branched amino acids , which is important from the nutritional point of view [34]. Some studies indicate that α-lactalbumin has a relevant role in the absorption of ions: it is known that the human α-lactalbumin is complexed with Ca^{2+} ion and can also have Zn^{2+} ion as a binder [35]. Although the maximum amount of calcium ions complexed with α-lactalbumin in breast milk is only 1% of the total calcium content of human milk [36], it is possible that α- lactalbumin may have a positive effect on the absorption of other minerals, possibly by the formation of peptides that facilitate the absorption of divalent cations.

Kelleher *et al*. [34] found that infant formula supplemented with bovine α- lactalbumin increases the absorption of zinc and iron in young rhesus monkeys, but there are still no concrete studies that relate the effect of human α-lactalbumin with mineral absorption in breastfed infants. Besides the nutritional function already discussed, new studies have been targeted at the analysis of the antimicrobial potential of α-lactalbumin. Polypeptides obtained after exposure of α-lactalbumin to proteases commonly found in the gastrointestinal tract have antimicrobial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus, Staphylococcus epidermidis, Streptococci,* and *Candida albicans* [37]. Since the primary structure of the human α-lactalbumin is similar to that of a monkey, it can be speculated that proteolysis of the human α-lactalbumin could produce the same antimicrobial peptides conferring protection to infants during the lactation period.

Immunoglobulins

It is believed that immunoglobulins represent about 10-15% of whey proteins. There are five classes of antibodies; IgA, IgD, IgE, IgG and IgM. These maternal antibodies are of particular importance because the secretory immune system of the newborn only becomes mature several months after birth [38-40]. However, the mother's immunity against some pathogens can be transferred to the infant in the form of IgA [41], allowing the immature immune system of the newborns to be driven by the mother's acquired immunity. IgA is the most abundant antibody in milk. The IgA concentrations are elevated in early lactation (1.2 g/L) and maintained between 0.5 and 1 g/L until the 2nd year of lactation [14]. IgA is produced by mammary gland cells; it is derived from the B cells of the small intestine and respiratory tract and then is transferred to the infant's digestive tract. IgA, due to its special molecular structure [14], is resistant to intestinal proteolysis Lindh [42], by being absorbed by the endothelial membrane, therefore it enters the systemic circulation and protects the neonate. The excretion of intact IgA was noted in breastfed neonates, and the amount of these proteins in the feces decreased according to their concentration in breast milk over the period of lactation [20]. IgA antibodies against pathogenic bacteria such as *Escherichia coli, Vibrio cholerae, Haemophilus influenzae, Streptococcus pneumoniae, Clostridium difficile* and *Salmonella*, and antibodies against viruses such as *rotavirus, cytomegalovirus, HIV* and *influenza virus*, and in addition to these antibodies against yeast such as *Candida albicans* were also found in breast milk [14], showing the amplitude of the defense system.

Lactoferrin

Lactoferrin (LF) belongs to the family of iron-binding proteins and has antimicrobial and immunotrophic function [43,44], being found in higher concentrations in the serum of human milk [45]. Due to this fact, a high proportion of the iron present in breast milk is bound to LF, which facilitates the mineral uptake by the intestinal cells. LF is relatively resistant to proteolytic degradation in the gastrointestinal tract when compared to other milk proteins, such as, casein etc., hence facilitating the absorption of LF from milk by the neonate. The peptides resulting from LF proteolysis also have antibacterial activities [46]. LF is absorbed in the intestine through specific membrane receptors, localized in intestinal cells [47]. When orally administered, LF

stimulates the immune response both locally and systemically, playing an important role in absorption of nutrients and also stimulating the proliferation of endothelial cells in the intestine and the growth of lymphoid follicles associated with the intestine [48]. This property suggests the possibility of using LF in premature infants and in patients with intestinal diseases [49]. LF controls the appropriate composition of intestinal microflora, by suppressing the growth of pathogenic bacteria and promoting the proliferation of Lactobacillus and Bifidobacterium [50]. The newborns fed with artificial diets develop a harmful intestinal microflora (*Enterococcus, Enterobacter, Bacteroides, Escherichia*).

The non-pathogenic microflora ensures a low pH, produces some vitamins, increases the activity of Natural Killer Cells (NK), T lymphocytes and macrophages, promotes the production of protective immunoglobulins and decreases the risk of allergies [51]. In studies in rats, LF showed a protective effect in cases of bacteremia and endotoxemia [52]. This protein stimulated the activity of cells of the reticulo-endothelial system and promoted myelopoiesis, thereby eliminating bacteria [53]. In a model of experimental endotoxemia, this protein inhibited the activity of pro-inflammatory cytokines, nitric oxide, and reactive forms of oxygen [54].

LF may also promote differentiation of T and B cells from immature precursors and increase the activity of *natural killer* cells (NK) and *lymphokine-activated killer* cells (LAK) [55]. It also protects against the toxicity of reactive oxygen radicals, and this property may be particularly relevant when the infant feeding is based on modified cow milk, containing iron mineral, since it is a source of free radicals [56]. Together, these experimental studies support the idea that natural human milk has the best nutritional value for the newborn. Supplementation of artificial food for newborns with LF seems to strongly enhance the protection and immunity in this category of food [57]. So much so that the commercially available infant feeding formulas for newborns in the United States and Japan are all supplemented with LF [58].

Enzymes

Whey also contains several classes of enzymes such as oxidoreductases, hydrolases, transferases, lyases, isomerases and ligases. The major whey enzyme is lactoperoxidase, which in the presence of hydrogen peroxide (formed in small amounts in different cell reactions) catalyzes the peroxidation of thiocyanate (which is present in biological fluids such as saliva and milk) forming hypothiocyanate, which is effective against both Gram-positive and Gram-negative bacteria [59]. Thus, lactoperoxidase in human milk may prevent infections in the mouth and upper gastrointestinal tract of the newborn [60]. This action is so promising that lactoperoxidase has been used in cow milk for decades by the dairy industry in developed countries to ensure microbiological quality of their products.

Lysozyme is a major component of whey protein fraction in human milk and is considered a bactericide by hydrolyzing the glycosidic bonds of type β (1→4) between the N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) , the peptidoglycan cell wall of Gram-positive bacteria. Recent studies show that the addition of recombinant human lysozyme to chicken feeding serves as a natural antibiotic [61].

Lysozyme has also shown to have bactericidal effect against Gram-negative bacteria *in vitro* for a synergistic action of lactoferrin [62]. Since lactoferrin is associated with the lipopolysaccharide removing them from the outer membrane of bacterial cells, it enables lysozyme to penetrate and break down membrane proteoglycan matrix, lysing the bacterial cell wall. The human lysozyme has *in vitro* anti-HIV activity [63]. Two of its peptides – HL8 and HL9– block infection and viral replication in MT-2 cell culture, and the second peptide also alters host cell gene expression involved in cell signaling [64]. The molecular mechanism of lysozyme action on HIV virus is not clear , however it is reasonable to assume that the antiviral effect is caused by the hydrolytic activity of lysozyme on viral polysaccharides.

Lipase present in human milk is Bile salt dependent (LDSB), it has a wide spectrum of functions allowing efficient use of cholesterol esters, mono-, di- and triglycerides, fat-soluble vitamins, long-chain fatty acids (> C18) lipoamides present in milk, either soluble and micellar [65]. In newborns, particularly preterms, the role of the bile-salt stimulated lipase is very important, accounting for approximately 30-40% in the digestion of lipids as they have low lipase enzyme activity and poor lipid utilization [66]. Human milk pasteurization destroys the bile-salt stimulated lipase, impairing lipid absorption in preterm infants fed with human milk that comes from milk banks [67].

CYTOKINES AND HORMONE

Cytokines are small and soluble glycoproteins, which act in an autocrine or paracrine manner by binding cellular receptors, on cascade operation, leading to the development and functioning of the immune system of the newborn. Human milk contains a number of pro-inflammatory cytokines, such as interleukin (IL) 1β, IL-6, IL- 8, tumor necrosis factor α, β factor and transforming growth factors (both TGF β1 and TGF β2) and TNF α- [68] and anti-inflammatory cytokines, such as IL-10 [69]. Although all of these cytokines are immunomodulatory, as already pointed out, it seems that the overall effect of these factors on milk is to lessen the anti-inflammatory response in neonates, despite beneficial, an exaggerated inflammatory response results in reducing absorption and damaging the infant's intestine [70]. These cytokines are present in low concentration (picograms), however their relative concentration is higher in colostrum, after being reduced on the 21st day [68]. These physiological modifications of the cytokine profile in different periods of lactation seem to be related to the required changes in the immune system of infants and neonates' needs for these cytokines.

Various hormones are also present in the human milk, such as cortisol, somatostatin [71], insulin, thyroid hormones, lactogenic hormones, oxytocin [72] prolactin [73], ghrelin adiponectin and leptin.

Human milk also contains substances that modulate growth, such as Epidermal Growth Factor (EGF), Nerve Growth Factor (NGF), Growth Factor Similar to Insulin (IGFs), and interleukins. The transforming growth factors (TGF-alpha and TGF-beta) and colony-stimulating factor granulocyte (G-CSF) were also detected in human milk [69]. These growth factors are secreted by the epithelial mammary gland cells, activated macrophages by lymphocytes (mainly T cells), or by neutrophils in milk [74].

Some peptides such as the growth factors; the Epidermal Growth Factor (EGF), Growth Factor Releasing Hormone (GHRF) and Insulin- Like Growth I (IGF-I) are present in milk, and when absorbed, can influence the metabolism and also promote the growth and differentiation of various organs and tissues of the neonate. It appears that these growth factors protect cells against toxic substances and toxins and reduce the risk of neonatal necrotizing enterocolitis [75].

Fibronectin is a protein that is involved in phagocytosis, and is present in human milk [76], and levels of this protein in serum are higher in breast-fed infants than those fed with commercial infant formulas. Comparison of fibronectin isolated from milk and the one present in the plasma showed that they were both very similar, and that fibronectin is ingested intact from colostrum.

New advances in the use of proteins in infant formula

The presence of protective factors against infections, the absence of allergenic factors and the narrow affective mother-child relationship are examples of the benefits feeding the newborn with human milk, which are absent when the milk is replaced by infant formulas [77]. The first important aspect observed when comparing human milk to infant formula is the qualitative and quantitative differences in certain nutritional components. Because of these differences, minimal recommendations are made for key compounds in human milk that are important for the development of the newborn [78]. To compensate for the lower digestibility of the treated proteins found in infant formulas in comparison to proteins naturally found in human milk, the formula pattern must have a minimum protein content of 1.8 g/100 kcal (12 g/L) on a formula of 670 kcal/L. Thus the proteins made available are 25% higher than the average provided in the first 6 months of lactation. Formulas with higher protein content do not represent an advantage, as they overwhelm the metabolic and excretory functions of the neonate [4].

Another important point is how the milk proteins are complexed with each other and divided into different fractions. These characteristics are essential for the absorption of these, and other nutrients in the gastrointestinal tract [5], such as the minerals, zinc, iron and copper [79]. Cases of deficiency of these minerals are reported more frequently in infants fed formulas prepared from cow milk than in those fed with human milk, although the amount of these minerals in the formulas is generally higher [80]. From these results, the authors concluded that these minerals in human milk showed a higher efficiency of absorption (bioavailability) [81], which seems to be due to the difference in distribution of these minerals in the fractions of milk (bovine and human) and infant formula. Moreover, differences in concentration of serum proteins should also be considered, such as lactoferrin and α-lactalbumin, which as already noted, also have an important role in the absorption and consequently the bioavailability of some of these minerals [6]. Conte-Junior [17] also described the presence of other proteins whose function appears to be related to absorption of minerals, but these proteins have not been fully identified and studied.

The interest in producing recombinant proteins of human milk in addition to infant formulas has been growing in recent years [82]. Microorganisms and transgenic animals can be used for the production of proteins with biological activity, however, benefits from the addition of each protein in cells should be evaluated in animal models and ultimately in neonates [83]. Another important point is to be careful with appropriate processing conditions so that the proteins added to formulas retain their biological acitivities. It is essential to use some processing conditions such as aseptic processing, sterile filtration etc. to maintain the unchanged tertiary structures of proteins, and consequently preserve the biological activities [84]. The importance of post-translational modifications should also be considered, because some proteins may require glycosylation and/or phosphorylation to present appropriate physiological activity [49].

In summary, knowledge of the composition of human milk and the factors that influence it has increased considerably over the past two decades. Human milk is the best nutrition for newborn because breastmilk is a complex fluid, rich in nutrients and in non-nutritional bioactive components.

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