Donkey and human milk: insights into their compositional similarities

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ABSTRACT

This paper reviews the main research results on donkey milk quality to highlight the nutritional similarities with human milk, the gold standard for infant feeding. The major differences between donkey and human milk are related to the fat yield, which thus means that quantitative and qualitative supplements are needed in pre-weaned children. The two milks are similar in terms of total protein content and amino acid profile. In addition, both donkey and human milk are poor in casein and rich in lactose and also share a similar unsaturated:saturated fatty acid ratio and cholesterol, sodium, potassium and vitamin C content. The triglyceride profile is also similar. Donkey milk seems to be a promising alternative food targeted at children's health. New, well-designed clinical studies in children and other sensitive groups are needed to examine in depth the clinical and metabolic outcomes and the potential positive health impacts.

Contents

1. Introduction

Donkeys play an important role as working animals in many rural economies, are used in donkey-assisted therapy, social recreational activities, for meat production, as well as to obtain skin gelatine used in Asian traditional medicines and for milk production intended for human nutrition and cosmetics. Donkey milk has gained interest in the scientific field for human nutrition, due to the applications in feeding the increasing number of children who suffer from cows' milk protein allergy (CMPA) (Vandenplas, De Greef, & Devreker, 2014). For allergic children who are not breastfed, there are commercially available formulas that include hydrolysates of cows' milk proteins, amino acid formulas and soy beverages. However, some products maintain the allergenic potential, need nutritional enhancement, or have a poor palatability.

Donkey milk nutritional quality monitoring is essential since infant nutrition has long-term effects on later development and health and infants with food allergy are also predisposed to poor growth (Isolauri, Sutas, Salo, Isosomppi, & Kaila, 1998). Interesting reviews focusing on protein bioactive components and on technological aspects of donkey milk have been recently published (Cunsolo et al., 2017; Souroullas, Aspri, & Papademas, 2018; Vincenzetti, Pucciarelli, Polzonetti, & Polidori, 2017); however, in the literature the papers that include a complete description of nutritional aspects and compositional variability are still few in number compared with other dairy species. Knowledge of donkey milk nutritional quality can contribute to a better estimation of the nutrient intake and of health effects of diets based on this milk**.** This paper reviews the main research results on donkey milk and highlights the nutritional similarities with human milk, the gold standard for infant feeding.

2. Physical characteristics and nitrogen fraction

The pH of donkey milk, as with human milk, is neutral or slightly alkaline (Table 1), likely due to the low content of casein and phosphate (Ozturkoglu-Budak, 2018). Compared with human milk, donkey milk contains less dry matter (Table 1), but shows similar average protein content: about 18 and 21 g L^{-1}, respectively. The similar protein content is important since higher protein intake in formula-fed infants is associated with an earlier adiposity rebound (Michaelsen & Greer, 2014). In human milk, protein content is naturally decreased during lactation to meet the change of full term infants' protein requirements and similarly also donkey has the same decreasing trends (Liao et al., 2017; Martini, Altomonte, Salari, & Caroli, 2014a).

Regarding proteins, the two milks share a low casein content (Table 1) that is, on average, 56% and 30% of the total protein in donkey and human, respectively (Liao et al., 2017; Martini et al., 2014a). Casein exists in milk as a colloid, known as the casein micelle, with a diameter of about 298 nm in donkey milk and 64 mm in human milk (Tidona et al., 2014). Due to the low content of casein, donkey and human milk may form a soft flocculation during digestion that contributes to a faster digestibility compared with the consistent clot of cow milk (Ragona et al., 2016).

Human milk and donkey milk also have a proportion of non-protein nitrogen (NPN), on average 4 and 5 g L^{-1}, respectively, consisting mainly of β-casein and α_{S2}-casein derived peptides in donkey (Chianese et al., 2010), and in free amino acids in human milk (Zhang, Adelman, Rai, Boettcher, & Lönnerdal, 2013). The nutritional significance of this fraction is not known, but bioactive actions have been reported for some molecules.

2.1. Casein

Regarding proteins, human and donkey milk share similar quantitative and qualitative characteristics in protein profiles (Table 2); for example, α-casein is a minor component of both milks. Only one α-casein has been identified in human milk while in donkey, two α-casein fractions (α_{S1}- and α_{S2}-casein) are present (Cunsolo et al., 2017; Liao et al., 2017).

Above all, a major similarity is linked to the primary structures of α_{S1}-, β- and κ-caseins that are closely related in the two milk types (Cunsolo et al., 2017); this is important from the point of view of the nutritional value of proteins for the infant's nutrition. In addition, a simulated in vitro digestion showed that donkey casein has rapid degradability and an almost complete digestibility (Tidona et al., 2014). This can also play a role in the reduced allergenicity of donkey milk since food protein allergenicity is also linked to the survival of the allergen in the gastrointestinal tract (Dupont & Mackie, 2015).

2.2. Whey protein

In human and donkey milk, a highly represented protein is α-lactalbumin (on average about 2 and 3 g L^{-1} in donkey and human, respectively; Table 2). α-Lactalbumin plays a key role in lactose synthesis in the mammary gland and provides a major source of essential amino acids in human milk. In addition, α-lactalbumin is resistant to digestion and reaches the gut tract almost intact, where it stimulates the mucosa and regulates the overall immune function of the infant (Lönnerdal, Forsum, & Hambraeus, 1976). α-Lactalbumin also inhibits the growth of several potential pathogens, both in vitro and in vivo, and since it is a calcium-binding protein that can bind iron and zinc, it has a stimulating effect on the absorption of minerals (Lönnerdal, Erdmann, Thakkar, Sauser, & Destaillats, 2017). The similar content of α-lactalbumin in the two milk types is interesting for infant welfare and nutrition, also considering that infant formulas based on bovine milk are poor in this protein and must often been enriched (Lönnerdal et al., 1976).

β-Lactoglobulin is absent in breast milk, but present in donkey milk in which there are two different molecular forms (β-lactoglobulin I and β-lactoglobulin II) and also genetic variants that differ in terms of the primary structure (Herrouin et al., 2000). β-Lactoglobulin was once thought to be responsible for the allergic reactions to cows' milk; however, the role of this protein in eliciting allergic reactions has been re-evaluated, and today it is generally accepted that cow milk caseins are the major protein allergens of milk (Docena, Fernandez, Chirdo, & Fossati, 1996).

Other main whey proteins are lysozyme and lactoferrin. Lysozyme is an enzyme that breaks the peptidoglycan layer of Gram-positive bacteria. The high concentrations of lysozyme in donkey milk probably contribute to its low bacterial count (Ragona et al., 2016). Data on human milk lysozyme show that the activity in raw breast milk is higher than donkey milk (about 22,000 U mL^{-1}) (Viazis, Farkas, & Allen, 2007). From investigations on donkey lysozyme seems that this enzyme quite resistant to human gastrointestinal enzymes (75% resistance) in vitro (Marletta, Tidona, & Bordonaro, 2016). Although studies on human babies are controversial (Lönnerdal et al., 2017), investigations on a mice model of Crohn's disease show that lysozyme can reach the intestinal tract intact, and also exert selective action on gut bacteria (Yvon et al., 2016).

Lactoferrin is one of the main proteins in breast milk, constituting about 20% of true protein (Lönnerdal et al., 1976), while in donkey milk, it is a minor component (0.097–0.133 g L^{-1}; Ozturkoglu-Budak, 2018). Lactoferrin is a protein with bacteriostatic effects that are derived, in part, from its ability to withhold iron from bacteria that require it for growth. It also exhibits antibacterial, antivirus, antifungal and antiprotozoan activities that are likely to be independent of iron chelation (Lönnerdal et al., 2017). In addition, lactoferrin of raw human milk has been found in stools of exclusively breastfed infants showing a certain resistance to digestion (Lönnerdal et al., 2017). However, during human lactation lactoferrin decreases and the newborns gradually acquire the capacity to digest it. This trend is linked with the development of the infant gut. During first infancy, when higher concentrations of lactoferrin in gut are found, this protein seems to promote cell proliferation; when lactoferrin concentrations decreases, gut cell differentiation is stimulated (Lönnerdal et al., 2017).

Although lactoferrin displays potential antibacterial activity, it can be estimated that pasteurisation of milk compromises realisation of this potential for infant feeding. Donkey lactoferrin has a poor thermal resistance and is 43% degraded on heating at 65 °C for 2 min (Ozturkoglu-Budak, 2018) and completely destroyed at 75 °C for 2 min, and human milk lactoferrin is significantly degraded when heated at 62.5 °C for 30 min (Holder pasteurisation treatment) (Guerra, Mellinger-Silva, Rosenthal, & Luchese, 2018).

2.3. Amino acid profile

In donkey milk the total amino acid and essential amino acid levels (Table 3) are quite similar to those in human milk (Guo et al., 2007; Zhang et al., 2013). A high degree of similarity between the two milks is interesting from a nutritional point of view since the composition of infant feed can be evaluated with an amino acid score that is based on the amino acid composition of human milk (Raiten et al., 1998 cited by Zhang et al., 2013).

In humans, the total and essential amino acid content decreases over time (Lönnerdal et al., 2017), with the largest changes in colostrum and transitional milk. According to Lönnerdal et al. (2017), these changes are related to the evolving needs of the growing infant, although the nutritional value of protein in breast milk, measured by the ratio of essential amino acids to total amino acids, appears to be consistent over time. In addition, no changes in the amino acid profile of donkeys have been observed during lactation, except for threonine and aspartic acid (Guo et al., 2007). Although this is an issue of interest, only this one study has focused on the amino acid composition of donkey milk.

3. Lactose and oligosaccharides

Both human and donkey milks have a high lactose content of about 60–70 g L^{-1} (Table 1) (Ballard & Morrow, 2013; Martini et al., 2014a); this is an important energy source for unweaned children. The high lactose content of donkey milk is also responsible for its good taste and contributes to a good palatability and approval rating among children with CMPA (Barni et al., 2018).

It seems that lactose may have stimulatory effect on intestinal calcium absorption, important in bone mineralisation; this lactose effect has been observed in animal models, while there are contradictory reports in humans (Kwak, Lee, & Lee, 2012). Lactose is also a significant source of galactose, which is important for early human development (Choelho, Berry, & Rubio-Gozalbo, 2015).

Human milk is particularly rich in oligosaccharides, with a total average content of 7–12 g L^{-1} (Coppa et al., 1999; Thurl, Munzert, Boehm, Matthews, & Stahl, 2017) and more than 200 different oligosaccharides (Sischo, Short, Geissler, Bunyatratchata, & Barile, 2017). Neutral oligosaccharides and acidic are the main human milk oligosaccharides (Barile & Rastall, 2013) with the most common being 2'fucosyllactose (2.6 g L^{-1}), difucosyl-lacto-N-hexose II (2 g L^{-1}) and trifucosyl-lacto-N-hexose (2.8 g L^{-1}) (Thurl et al., 2017).

Unlike human milk oligosaccharides, donkey milk oligosaccharides have been studied to a lesser extent and their total content is not known. To our knowledge only three acidic oligosaccharides have been quantified in donkeys: 3-sialyllactose (3-SL), 6-sialyllactose (6-SL) and disialyl-lacto-N-tetraose (DS-LNT). They are present in lower concentrations than in human milk: 3-SL is on average 0.014 g L^{-1} in donkey versus 0.20 g L^{-1} in humans, whereas 6-SL and DS-LNT are 0.019 and 0.008 g L^{-1}, respectively in donkeys versus 0.33 g L^{-1} and 0.50 g L^{-1}, respectively, in human milk (Monti, Cattaneo, Orlandi, & Curadi, 2015; Orlandi, Curadi, Monti, & Cattaneo, 2016; Thurl et al., 2017).

The nutritional importance of oligosaccharides is due to their probiotic role (reported for breast milk): oligosaccharides have a structural composition (sialylation and fucosylation in terminal positions) that makes them inaccessible to some bacteria and are antiadhesive and antimicrobials (Barile & Rastall, 2013). Finally, oligosaccharides also modulate lymphocyte cytokine production and provide essential nutrients for brain development and cognition (Bode, 2012).

4. Physical and chemical characteristics of lipids

From the point of view of the nutritional composition, one of the main differences between donkey and human milk is fat content; the fat percentage of donkey milk is usually three times lower than human milk (Table 1) and also energy is about twice in human compared with donkey milk (Ballard & Morrow, 2013).

According to nutritional recommendations of different international organisations, fat may provide about 40–60% of the daily energy intake in pre-weaned children (between 0 and 6 months) and should be gradually reduced to 35% in children through to 2 years old (Aranceta & Pérez-Rodrigo, 2012). From Table 1 it is possible to estimate that an exclusively donkey milk based diet can hardly cover the daily fat requirement in the children below six months and donkey milk fat levels would need to be supplemented, e.g., by adding vegetable oils. However, despite the low fat content of donkey milk, there is a fairly wide variability in fat content linked to endogenous and exogenous factors (e.g., breed, stage of lactation and milking strategy) (Alabiso, Giosuè, Alicata, Mazza, & Iannolino, 2009; Contarini, Pelizzola, Scurati, & Povolo, 2017; Martini et al., 2014a) that might also be used to increase the fat content.

Different from the case with donkey milk, human milk fat content seems less variable, even if some authors have found decreases (from 37.3 g L^{-1} in the first month to 33.7 g L^{-1} in the fourth month) followed by increases in late lactation (to 48.0 g L^{-1} in 17th month) (Larnkjaer, Christensen, Michaelsen, & Lauritzen, 2006). In humans, inter-individual, intra-individual, and daily variations of fat have also been reported as well as effects of the maternal diet (Demmelmair & Koletzko, 2017; Shi et al., 2011).

4.1. Fat globules

The differences between human and donkey milk fat are also present in the physical characteristics of lipids. Donkey milk fat globules are very small, with an average diameter of 2 microns, in contrast to human milk, in which the diameter is about 4 microns (Martini, Altomonte, & Salari, et al., 2014b; Michalski, Briard, Michel, Tasson, & Poulain, 2005). The size of the fat globules can affect the nutritional intake and influence the digestive kinetics (Martini, Salari, & Altomonte, 2016), even if the studies done so far concern other milks and are based on in vitro tests. The small size of the fat globules indicates a larger surface available for the lipase action, and could contribute to the faster digestibility of donkey milk fat (Martini et al., 2016).

In addition, since smaller fat globules have more membranes per unit volume of fat, the smaller fat globules in donkey milk may contribute to a higher uptake of the milk fat globule membrane (MFGM). MFGMs are also the principal source of polyunsaturated fatty acid (PUFAs), phospholipids and cholesterol in milk (Martini, Altomonte, & Salari, 2013).

4.2. Triglycerides and cholesterol

The position of fatty acids on the glycerol backbone, especially long chain saturated fatty acids, is very similar in donkey and human milk triglycerides. These similarities may influence fat absorption and fatty acids bioavailability.

Human milk contains triglycerides made up of chains from 36 to 54 carbon atoms; similarly, those of donkey milk are composed of chains from 30 to 54 carbon atoms. In both milks the majority of triglycerides (about 35% in humans and 22% in donkeys) are made up of 52 carbon atoms (Gastaldi et al., 2010).

Moreover, in donkey milk the position of fatty acids on the glycerol backbone is very similar to that in human milk: e.g., C16:0 (palmitic acid) is present mainly at sn-2. This type of esterification allows a more effective C16:0 absorption; 2-monoacylglycerols of the saturated fatty acids (SFAs) are much more easily absorbed than free fatty acids. From birth up to 5 months of life, human milk provides 10% of dietary energy as palmitic acid, and it has been hypothesised that in humans, the esterification of palmitic acid in position sn-2 constitutes an evolutionary advantage in terms of its absorption. Although often considered to have adverse effects on chronic disease in adults, C16:0 is an essential component of tissue lipids, acts in the secretion and transport of lipids, and in the formation of signal molecules (Innis, 2016).

In addition to the position of fatty acids on the glycerol backbone, the relatively high content of medium chain triglycerides (MCTs), makes the lipids in donkey milk, through quantitatively modest, highly bioavailable (Gastaldi et al., 2010). MCTs are also a source of short chain fatty acids (SCFAs) and medium chain fatty acids, which are a source of rapidly available energy, particularly important for patient with severe food allergies, as well as in prematurely born neonates (Łoś-Rycharska, Kieraszewicz, & Czerwionka-Szaflarska, 2016).

Donkey milk triglycerides have a higher degree of unsaturation than human milk triglycerides, due to the greater presence in the triglycerides of long chain PUFAs, while in human milk there are mainly monounsaturated fatty acids. The content of saturated triglycerides (about 12%) on the other hand, is similar in the two milks.

Cholesterol is the major milk sterol and a functional and structural component of all the animal cell membranes, necessary for hormone and vitamin synthesis and for the development of the nervous system. Variable cholesterol contents have been reported in mature human milk from a maximum value of 290 mg L^{-1} to minimum value of 34 mg L^{-1} (Table 4) (Álvarez-Sala, Garcia-Llatas, Barberá, & Lagarda, 2015; Kamelska, Pietrzark-Fiecko, & Bryl, 2012). In donkeys the few studies available report average values of 18.3 mg L^{-1} in bulk milk (with a range of 9 to 30 mg L^{-1}; Contarini et al., 2017).

4.3. Phospholipids

Donkey milk is rich in phospholipids per unit of fat (Contarini et al., 2017), (Table 4). However, given the low fat content, phospholipids per litre of milk are, on the whole, lower in donkey milk than in human milk (42.8–97.1 mg L^{-1} versus 98–474 mg L^{-1}, respectively).

Phosphatidylcholine it is the second more present phospholipid in both milks (19–38.4% and 10–18% of the total phospholipids in humans and donkeys, respectively). In contrast, the main milk phospholipids in humans is sphingomyelin (28.9–43.3% of the total phospholipids) (Cilla, Quintaes, Barberá, & Alegrìa, 2016), while in donkeys, the main phospholipids are phosphatidylethanolamine (17–23% of phospholipids) and phosphatidylcholine (10–18% of phospholipids) (Contarini et al., 2017). The content of sphingomyelin and phosphatidylcholine is considered of great importance for the development of infants, choline being required for organ growth and membrane biosynthesis. In the case of breast-feeding, it has been estimated that about 17% of choline received by the neonate comes from human milk polar lipids (Sala-Vila, Castellote, Rodriguez-Palmero, Campoy, & López-Sabater, 2005). The differences in the amount and type of phospholipids can imply different intakes between human milk and donkey milk. Probably some enrichment of donkey milk in children's diets should be considered; furthermore, there is a lack of nutritional recommendations with regard to phospholipids in the field of infant nutrition and the effective doses affording optimal growth and health benefits for infants still remain to be established.

4.4. Fatty acids

A correct diet in children below 2 years should include a content of both SFAs and PUFAs that does not exceed 8–15 % of the recommended energy intake (Aranceta & Pérez-Rodrigo, 2012).

SFAs and unsaturated fatty acids (UFAs) are contained in donkey milk fat at similar levels to those in human milk fat, although the lower fat level of donkey milk leads to a lower overall content of SFAs and UFAs than that for human milk (Table 4).

One similarity between human and donkey milk is the low amount of SCFAs, especially the chains shorter than C8, and the high quantities of long chain fatty acids (Martini et al., 2014b; Yuhas et al., 2006). The most common SCFAs are C8:0 and C10:0 ranging from 1.6 to 2.8 g kg^{-1} and from 14.6 to 25.3 g kg^{-1} of fat, respectively, in humans. In donkey milk C8:0 ranges from 40.3 to 24.5 g kg^{-1} of fat and C10:0 from 98 to 59.9 g kg^{-1} (Martemucci & D'Alessandro, 2012; Martini et al., 2015). This similarity is important for infants since the presence or deficiency of SCFAs affects the pathogenesis of some diseases (autoimmune, inflammatory diseases) and SCFAs have also antimicrobial activity and anti-inflammatory effects in the gut (Tan et al., 2014).

C16:0 is, in absolute terms, the most concentrated saturated fatty acid in the two milks (200–220 and 190–230 g kg^{-1} fat in donkey and human milk, respectively) (Martemucci &

D’Alessandro, 2012; Martini et al.; 2015; Yuhas et al., 2006). The importance of this fatty acid for health has been discussed previously (section 4.2).

Oleic acid (C18:1 OA) is the most represented UFA both in donkey and human milk (110–280 g kg^{-1} fat and 210–360 g kg^{-1} fat in donkey and human milk, respectively), while linoleic acid (C18:2n6 LA) is present in similar quantities in the two milks (90–170 g kg^{-1} fat versus 123–139 g kg^{-1} fat in donkey and human, respectively) (Martemucci & D’Alessandro, 2012; Martini et al.; 2015; Yuhas et al., 2006).

Linolenic acid is considered indispensable in children between 0 and 6 months and essential in children below 2 years so that some international documents have set recommended intakes for this fatty acid (Aranceta & Pérez-Rodrigo, 2012). Of the milk of all dairy animals, donkey milk is the richest source of α-linolenic acid (ALA; C18:3n3). In donkey milk there are between 20 and 140 g of ALA per kg fat (Martemucci & D’Alessandro, 2012; Martini et al., 2015; Massouras, Triantaphyllopoulos, & Theodossiou, 2017), thus higher amounts than human milk (8.5–11.5 g kg^{-1} fat). A litre of donkey milk provides, on average, 0.38 g of ALA, similarly to that contained in human milk (0.36 g L^{-1}); therefore, donkey milk can be considered a good source of this n3 precursor fatty acid, which is essential and indispensable for children for adequate growth, neurological development and cardiovascular health. International recommendations for this fatty acid from the different international organisations are 0.2–0.6% of daily energy intake or 0.5 g day^{-1} (Aranceta & Pérez-Rodrigo, 2012).

Donkey fat is also richer in eicosopentaenoic acid than human milk fat (0.1–7.1 versus 0–11 g kg^{-1} fat) (Koletzko et al., 2001; Martemucci & D’Alessandro, 2012; Martini et al.; 2015; Massouras et al., 2017). Docosahexaenoic acid (DHA) content in donkey milk is 0.3–0.4 (g kg^{-1} of fat), whereas in human milk its content varies considerably and is highly correlated to the mother’s intake of DHA-rich foods (<1 to >10 g kg^{-1} fat) (Jackson, Polreis, Sanborn, Chaima, & Harris, 2016; Yuhas et al., 2006). DHA is a constituent of the retina and of the nervous system and has an indispensable role in neuronal membranes. For DHA, there are recommended adequate intakes (0.10 g DHA in infants and young children between 6 and 24 months), given the low total fat intake of donkey milk, an enrichment of DHA, especially for pre-weaned children, is recommended.

The arachidonic acid (C20:4) content of donkey milk is comparable with that of human milk (about 3 g kg^{-1} of fat and 3 to 12 g kg^{-1}, respectively) (Koletzko et al., 2001; Martini et al.; 2015). Arachidonic acid is an important component of cellular membranes (Martini et al., 2013); however, whether or not the provision of preformed dietary arachidonic acid in infancy is of high importance remains controversial and no specific values have been set.

Regarding the n-3/n-6 ratio, the values in the literature for donkey milk range from 0.59 to 3.47 (Table 5) (Martemucci & D'Alessandro, 2012; Massouras et al., 2017), whereas in humans, a range from 0.10 to 0.20 has been reported (Luukkainen, Salo, & Nikkai, 1994; Makrides, Simmer, Neumann, & Gibson, 1995). The interest in the n-3/n-6 ratio derives from evidence that indicates that this ratio can give long-term consequences of early life intake on adult metabolic health. In pre-weaned mice low neonatal plasma n-6/n-3 PUFA ratios have been proved to regulate offspring adipogenic potential and future obesity resistance in adult life (Rudolph et al., 2018).

5. Minerals and vitamins

Minerals are essential in skeletal structure development and in many biological processes and are thus fundamental in the maintenance of a good health. In infant nutrition, a balanced amount of minerals, as well as of protein, is important for the renal load. A high renal solute load leads to a higher urine concentration, and when fluid intake is low and/or when external water losses are high, the renal concentrating ability of infants may be insufficient for maintaining the water balance and can lead to dehydration (Brown, 2007).

The average total mineral content is only slightly higher in donkey milk than in human milk (Table 1). In both milks, the main macro elements are potassium and calcium (Table 5). Calcium is present in human milk in lower quantities than in donkey milk (0.26 versus 0.77 g L^{-1} in human and donkey milk, respectively), as well as phosphorus and magnesium (phosphorus, 0.15 versus 0.58 g L^{-1} and magnesium, 0.03 versus 0.07 g L^{-1} in human and donkey milk, respectively), while sodium and potassium are present in similar quantities (Fantuz et al., 2012; Yamawaki et al., 2005). Regarding Zn, it is the main microelement in donkey and human milk (Fantuz et al., 2013). Infants, children and adolescents have increased requirements for zinc and thus, are at increased risk of zinc depletion. Zinc deficiency during growth periods results in growth failure (IZiNCG, 2004). Epidermal, gastrointestinal, central nervous, immune, skeletal, and reproductive systems are the organs most affected clinically by a zinc deficiency (Roohani, Hurrel, Kelishadi, & Schulin, 2013).

Adequate intake of calcium and the calcium/phosphorus ratio, as well as magnesium and vitamin D, are vital for bone health and development (Loughrill, Wray, Christides, & Zand, 2017). The appropriate uptake of calcium is important, especially in babies affected by CMPA and in allergic babies fed surrogate milk (soya beverages) who need calcium supplements (Seppo et al., 2005). Donkey milk and human milk fall within the recommended range of the calcium and phosphorus ratio (1–2:1), and are also under the limit recommended by World Health Organisation that suggests a Na/K ratio of ≤ 1 (WHO, 2009). A low Na/K ratio is recommended since in children it plays a crucial role in the future development and severity of hypertension.

The data available on vitamin content in donkey milk are reported in Table 5. Donkey milk has a total vitamin C content of 12–57 mg L^{-1}, similar to that of human milk (38–53 mg

L^{-1}) (Buss, McGill, Darlow, & Winterbourn, 2001; Lazarevic et al., 2017; Leaf, 2007; Vincenzetti et al., 2017). Vitamin C aids in the absorption of iron, has antioxidant effects and is essential for collagen formation. Moreover, vitamin C intake also seems to have a protective effect in the development of atopic dermatitis in high risk infants (Hoppu et al., 2005). Donkey milk can be considered a good source of this vitamin since about 500 mL of donkey milk covers the recommended daily intake (25 mg) for children aged 0–12 months (Leaf, 2007).

The B-vitamins comprise a group of eight water soluble vitamins that perform an essential role in cellular function, and act as co-enzymes in several catabolic and anabolic enzymatic reactions. As regards the content of B vitamins, the values in the literature vary considerably. In general, the amounts of B1 (thiamin) and vitamin B3 (Niacin) in donkey milk are on average higher than in human milk (Table 5) (Allen, 2012; Claeys et al., 2014; Vincenzetti et al., 2017).

Vitamin A consists of a group of active compounds known as retinoids and carotenoids (Gentili et al., 2013). Carotenoids are mostly found in vegetables and are converted by animals into retinol with an efficiency that varies in the different species. In general, the mammary gland derives free retinol from the liver and, after esterification, retinol is secreted in the core of the milk fat globules (Nozière et al., 2006). Vitamin A is especially important in growth, development, immunity, epithelial cells and eye health, and may give significant protection against gastrointestinal and respiratory infections (Pereira, 2014; Yao et al., 2016). Although retinol content is lower in donkey milk than in human milk (17–586 μg L^{-1} versus 300–2000 μg L^{-1}) (Claeys et al., 2014; Gentili et al., 2013) the recommended intake of this vitamin for 1–3 year-old children is 350–400 μg per day and, in general, there are no deficiencies of this vitamin in the Western Countries (Leaf, 2007). In human milk β-carotene is also present (63.2±23.3 ng mL^{-1} in mature milk) and provides potential sources of vitamin A for babies.

Vitamin D is a group of compounds with an antirachitic activity, playing a crucial role in calcium homeostasis and bone metabolism and also acting as a hormone. In infants and children, an intake of 400 and 600 IU per day, respectively, of vitamin D is recommended (Holick et al., 2011) to prevent deficiencies. Recent research shows that the total vitamin D content in donkey (23 μg L^{-1}; about 920 UI) milk is higher than the average values found in the literature for human milk (Martini, Altomonte, Licitra, & Salari, 2018; Við Streym et al., 2016).

Vitamin E consists of eight biologically active forms, represented by four tocopherols (saturated isoprenoid side chain) and four tocotrienols (unsaturated isoprenoid side chain) with an antioxidant activity, mainly present in the milk membranes. Donkey milk has a lower level of α-tocopherol compared with the average levels found in the literature for human milk (59–807 versus 1000–9840 μl L^{-1} respectively) (Gentili et al., 2013; Kamao et al., 2007; Lima, Dimenstein, & Ribeiro, 2014) and has also a quantity of γ-tocopherol (260 μl L^{-1}) (Gentili et al., 2013).

7. Possible uses of donkey milk for children's health

In preterm infants the actual protein intake from breast milk are suboptimal and fortification of human milk is needed. Fortifiers are generally designed with cows' milk proteins but recently, given similarities of donkey with human milk, a milk fortifier derived from donkey milk for preterm infants has been developed (Coscia et al., 2017). The feeding tolerance or the clinical, metabolic, neurological or auxological outcomes of preterm infants have not yet been published. Regarding clinical trials on humans, the majority of them have tested the efficacy of donkey milk on CMPA and atopic dermatitis in children over six months old. However, there have been fewer clinical studies on children under six months, carried out on a lower number of children (Tesse, Paglialunga, Braccio, & Armenio, 2009).

A recent study of Barni et al. (2018) reported that 93% of children with CMPA treated with donkey milk did not show any form of allergy and that donkey milk can be considered as a "hypoallergenic" milk according to the definition of the American Academy of Pediatrics (Baker et al., 2000). Trinchese et al. (2015**)** also suggest that donkey milk can modulate the intestinal microbiota, which according to Penders et al. (2013) is a key source of immune development and regulation in early life and could thus have a preventive role in the development of atopic dermatitis.

8. Conclusions

Donkey milk can be considered as the natural milk closest to human milk. Given its content of several functional components it could therefore be considered an alternative food targeted at children's health. Furthermore, the future research challenges should be concerned with some compositional improvements especially related to energy and fat content to make it more suitable for children below 6 months. In addition, new well-designed clinical studies in children and other sensitive groups are needed to analyse in depth the clinical and metabolic outcomes, and update the potential positive health impacts.

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Table 1

Maximum and minimum values of physico-chemical parameters reported in the literature for donkey and human milk. [a]

Parameter	Unit	Donkey milk		Human milk	
		min	max	min	max
pH		7.2	7.5	7.0	7.5
Total solids	g L^{-1}	85	118	108	152
Protein		15	22	7	35
Non-protein nitrogen		1.8	4.1	3.8	5.3
Casein		5.6	7.5	0.2	7
Whey/casein ratio		32:68	54:45	45:55	97:3
Fat	g L^{-1}	1	38	34	48
Lactose		61	73	67	78
Ash		3	4	1.7	2.1
Energy	kcal L^{-1}	313	414	602	884

[a] References: Addo & Ferragut, 2015; Ballard & Morrow, 2013; Carminati & Tidona, 2018; Guo et al., 2007; Liao et al., 2017; Lönnerdal et al., 1976; Martini et al., 2014a,b; Ozturkoglu-Budak, 2018; Salimei et al., 2004.

Table 2

Maximum and minimum values reported in the literature for the protein fractions of donkey and human milk (g L^{-1}). [a]

Protein fraction	Donkey milk		Human milk	
	min	max	min	max
Casein				
α_{S1}-Casein	0.730	1.116	0.20	0.70
α_{S2}-Casein	0.074	0.110	absent	
β-Casein (35–35 kDa fraction)	0.013	0.084	0.53	2.15
β-Casein (16–16.7 kDa fraction)	0.038	0.055		
κ-Casein	nq		0.45	1.05
Whey protein				
α-Lactalbumin	0.81	3.1	2.6	3.30
β-Lactoglobulin	2.4	5.4	absent	
Lysozyme	4	3	0.8	1.10
Lactoferrin	0.10	0.13	1.44	4.91

[a] nq, not quantitated. References: Caroli et al., 2015; Gubić et al., 2014; Guo et al., 2007; Liao et al., 2017; Lönnerdal et al., 2017; Ozturkoglu-Budak, 2018; Rai et al., 2014; Tsakali et al., 2016; Vincenzetti et al., 2011, 2017.

Table 3

Average amino acid composition of donkey and human milk (g kg^{-1}). [a]

Amino acid	Donkey milk	Human milk
Essential		
Histidine	0.36	0.27
Leucine	1.35	1.06
Lysine	1.15	0.73
Phenylalanine	0.68	0.43
Valine	1.02	0.60
Tryptophan	-	0.22
Threonine	0.56	0.50
Methionine	0.28	0.16
Isoleucine	0.87	0.57
Total essential amino acids	6.27	4.54
Non-essential		
Arginine	0.72	0.39
Aspartic acid	1.40	1.32
Alanine	0.55	0.42
Tyrosine	0.58	0.36
Proline	1.38	0.94
Glycine	0.19	0.26
Serine	0.98	0.48
Glutamic acid	3.58	1.88
Cysteine	0.07	0.23
Total amino acids	15.72	14.25

[a] References: Guo et al., 2007; Lönnerdal et al., 2017; Zhang et al., 2013.

Table 4

Maximum and minimum values reported in the literature for lipid fractions of donkey and human milk. [a]

Fraction	Units	Donkey milk		Human milk	
		min	max	min	max
Triglycerides	g kg^{-1} fat	800	850	970	980
Phospholipids	g kg^{-1} fat	11.4	21.4	2	20
Phospholipids	mg L^{-1}	42.8	97.1	98	474
Cholesterol	g kg^{-1} fat	3.30	5.60	2.50	5.00
Cholesterol	mg L^{-1}	9	30	34	290
SFA	g kg^{-1} fat	410	630	370	550
UFA		350	560	410	640
SFA	g L^{-1}	3.3	5.0	12.6	18.7
UFA		2.8	4.5	13.9	21.8
Fatty acids	g kg^{-1} fat				
C8:0		24.5	40.3	1.6	2.8
C10:0		59.9	98	14.6	25.3
C16:0		200	220	190	230
C18:1		110	280	210	360
C18:2n6		90	170	123	139
C18:3n3		20	140	8	11
C20:4n6		3	88	2	12
C20:5n3		0.1	7	0	11
C22:6n3		0.3	0.4	<1	>10
n3:n6 ratio		0.59	3.47	0.10	6

[a] Abbreviations are: SFA, saturated fatty acids; UFA, unsaturated fatty acids. SFA and UFA in g L^{-1} calculated considering 0.8% of fat for donkey milk and 3.4% for human milk.

References: Contarini et al., 2017; Martemucci & D'Alessandro, 2012; Martini et al., 2014b; Yuhas et al., 2006.

Table 5

Max and minimum values of minerals and vitamins in donkey and human milk. [a]

Component	Donkey milk		Human milk	
	min	max	min	max
Minerals				
Calcium	0.36	1.18	0.23	0.30
Phosphorus	0.32	0.84	0.13	0.17
Potassium	0.24	0.96	0.43	0.64
Sodium	0.11	0.26	0.11	0.24
Magnesium	0.02	0.11	0.02	0.03
Zinc	2.16	4.56	0.06	3.00
Ca/P	1.04	1.64	1.77	1.76
Na/P	0.14	0.26	0.26	0.34
Vitamins				
Vitamin B1	210	2550	20	350
Vitamin B2	40	970	10	550
Vitamin B3	73	-	571	687
Vitamin B12	1.1	-	21	-
Vitamin C	12000	57000	38000	53000
Vitamin A	17	586	300	2000
Vitamin D	0	2.3	0.07	-
Vitamin E	59.4	807	1000	9840

[a] Minerals in g L^{-1} except zinc in mg L^{-1}. Vitamins in μg L^{-1}. References: Allen, 2012; Buss et al., 2001; Fantuz et al., 2012, 2013; Gentili et al., 2013; Kamao et al., 2007; Lazarevic et al., 2017; Leaf, 2007; Lima et al., 2014; Martini et al., 2014a, 2018; Salimei et al., 2004; Vincenzetti et al., 2017; Yamawaki et al., 2005.