



Review

Equid milk for human consumption

Elisabetta Salimei^{a,*}, Francesco Fantuz^b^a *Dipartimento di Scienze e Tecnologie Agroalimentari, Ambientali e Microbiologiche, Università degli Studi del Molise, via De Sanctis, 86100 Campobasso, Italy*^b *Dipartimento di Scienze Ambientali, Università degli Studi di Camerino, via Gentile III da Varano, 62032 Camerino, Italy*

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ABSTRACT

Cows' milk allergy is an increasing problem in human infancy and clinical studies show interesting results on equid (horse and donkey) milk tolerability. Donkey milk is also considered useful in the prevention of atherosclerosis and has the ability to upregulate the immune response of healthy elderly humans. The mammary gland capacity in equids is low and milking technique and routine are of utmost importance. Details on milk proteins, fat fractions, minerals, and vitamins are discussed here with regard to milk nutritional value and tolerability; the hypolipid content must be balanced in the consumer's diet. The presence of bioactive and functional components in raw horse and donkey milk is also reviewed. Equid milk and milk derivatives could become valuable foods for elderly consumers; equid milk could be considered for probiotic beverage production, as health-promoting properties are claimed for koumiss or airag.

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Contents

1. Introduction	130
2. Equid milk in the human diet	131
3. Milking the dairy equid	131
4. Equid milk production	132
5. Equid milk hygiene and mammary gland health	133
6. Equid milk composition	134
6.1. Gross composition and energy content	134
6.2. Nitrogenous components	135
6.3. Lipid components	136
6.4. Minerals and vitamins	137
6.5. Functional and bioactive components	138
7. Fermented horse and donkey milk	138
8. Conclusions	139
Acknowledgements	139
References	139

1. Introduction

The domestic equid species (horse and donkey) belong to the taxonomic order Perissodactyla, family Equidae and genus *Equus*. The nutritional and therapeutic properties of horse (*Equus caballus*) milk have been known since ancient times according to the

historian Erodoto (V century BC), and horses are traditionally milked in central Asia and eastern Europe, where koumiss and other fermented horse milk products with claimed health benefits are produced (Uniacke-Lowe, Huppertz, & Fox, 2010). Donkeys (*Equus asinus*) evolved in different environmental conditions compared with horses (NRC, 2007; Rossel et al., 2008) and were mainly used as pack and riding animals. Donkeys remain essential for rural economies today in semiarid and mountainous areas of the world. The use of dairy products from donkeys was known in the Roman era and for a long time donkey milk was recognized as

* Corresponding author. Tel.: +39 0874 404850; fax: +39 0874 404652.
E-mail address: salimei@unimol.it (E. Salimei).

a common remedy. In the late nineteenth century, donkey milk was successfully used for feeding orphaned infants in France, as reported by D'Arval (1912). More recently, horse colostrum and milk production developed in France (Drogoul, Prevost, & Maubois, 1992) and then spread throughout Europe, also involving the donkey species, as part of projects on equid diversity preservation.

It must be noted that the economically relevant interest in the use of equid milks is also in cosmetology. Horse milk fat is considered an important ingredient in Mongolian cosmetics because of its high polyunsaturated fatty acid content (Temuujin et al., 2006). Milk proteins are also described as naturally active in skin hydration and skin ageing prevention (Cotte, 1991), but these issues are not within the scope of this review.

Although extensive reviews are available on horse and donkey milk composition (Doreau & Martin-Rosset, 2011; Doreau & Martuzzi, 2006a; Martuzzi & Doreau, 2006; Salimei, 2011; Salimei & Chiofalo, 2006; Uniacke-Lowe & Fox, 2011; Uniacke-Lowe et al., 2010), the aim of this paper is to provide information on raw equid milk production and its natural attributes that may be exploited in an innovative dairy-chain.

2. Equid milk in the human diet

Horse and donkey milk has been used successfully as an alternative food for infants with food allergies, e.g., cows' milk protein allergy (CMPA), a common food allergy in childhood with a prevalence of approximately 3% during the first 3 years of life. In particular, the first clinical evidence by Iacono et al. (1992) suggests that infants with food allergy could tolerate donkey milk (210–250 mL milk kg⁻¹ body weight d⁻¹). Clinical conditions and growth rate (average daily weight increase: 39.8 ± 12.0 g d⁻¹) of babies fed modified donkey milk for 15–35 days were considered satisfactory also in the follow up (Iacono et al., 1992). Subsequent clinical studies showed interesting results on equid milk tolerability (Table 1), most likely related to horse and donkey phylogenetic differences with ruminants (Order: Artiodactyla). When breastfeeding it is not possible or not advisable and hypoallergenic milk formulae are not tolerated (low palatability, cross-reactions, etc.), Restani, Ballabio, Di Lorenzo, Tripodi, and Fiocchi (2009)

Table 1
Clinical studies on tolerability of horse and donkey milk of children with food allergies.

Species	Experimental conditions	Tolerability (%)	References
Donkey	9 unweaned children (age 26–79 d) with multiple food allergies	100	Iacono et al. (1992)
Horse	25 children (age 19–72 months) with severe IgE-mediated CMPA	96	Businco et al. (2000)
Donkey	21 children (age 10 d–9 months) with food allergies and hydrolysed proteins intolerant	86	Carroccio et al. (2000)
Donkey	46 CMPA children (age 12–149 months) intolerant to common CMP substitute	82.6	Monti et al. (2007)
Donkey	26 CMPA and atopic dermatitis children (age 6 mo–3 yr), crossover design	88	Vita et al. (2007)
Donkey	30 children (age 6 mo–11 yr) with mild to moderate CMA	96	Tesse et al. (2009)

support the taxonomic approach in the search for alternative milk from unconventional dairy species, as the cross-reactivity among proteins from different species generally depends on phylogenetic relationships.

The flavour and appearance of equid milk have been found to be attractive to children, which is of particular importance to young consumers. However, results on equid milk tolerability cannot be considered conclusive (Iacono et al., 1992; Vita et al., 2007): the data in Table 1 suggest that both horse and donkey milk can be regarded as dietary alternatives for children with cows' milk allergy IgE- and non IgE-mediated but tolerability must be tested first (Businco et al., 2000).

Children with CMPA fed supplemented donkey milk showed significant increases in weight and other growth related parameters (Monti et al., 2007; Tesse, Paglialonga, Braccio, & Armenio, 2009), but a case of growth impairment and nutritional deficiencies has been described in a five month old baby with CMPA underfed unmodified donkey milk (D'Auria, Mandelli, Ballista, DiDio, & Giovannini, 2011). Although results from clinical studies on the nutritional adequacy of equid milk need to be confirmed, its use must be balanced in a varied diet, according to child growth and age (Tesse et al., 2009).

Nutrition is also considered crucial in the immune recovery of elderly consumers and equid milk may be an interesting food also for this consumer group. Due to its immunological activities, raw horse and donkey milk, and fermented derivatives are, in fact, considered useful in the prevention of atherosclerosis (Chiofalo, Drogoul, & Salimei, 2006a) and have the ability to upregulate the immune response of healthy elderly consumers (Jirillo et al., 2010). Moreover, donkey milk whey proteins have been observed to exert in vitro anti-proliferative and anti-tumour activity (Mao et al., 2009).

According to clinical evidence it must be noted that relationships between respiratory sensitisation to equid dander allergens and allergic reactions to horse and donkey milk protein have been reported in adults (Fanta & Ebner, 1998; Robles et al., 2007; Salimei & Chiofalo, 2006). Diets of allergic infants and adults should always be supervised by nutritionists.

Besides specific knowledge on milk nutrients and potential allergens, the consumption of equid milk by sensitive consumers, such as allergic patients and elderly people, also requires the adoption of specific milking equipment and routine to achieve a high quality product and economically feasible production.

3. Milking the dairy equid

Horse and donkey milk production differs greatly from that of conventional dairy species, especially in terms of milk supply. The equid mammary gland has a low average capacity (max. 2.5 L in heavy-horse breeds) so that milking may be carried out 2 or 3 h after separation from the foal (Doreau, 1991; Drogoul et al., 1992). According to Le Du (1986), who first introduced machine milking for horses in Europe, kinetics of milk ejection shows 2 peaks: the first represents the emission of the cisternal milk, while the second represents the emission of alveolar milk (75–85%) as natural response to oxytocin release during milking, which is often insufficient for complete milk removal from the udder of dairy equids (Dzidic, Knopf, & Bruckmaier, 2002; Salimei, Fantuz, Simoni, Varisco, & Chiari, 2004b). As a consequence, low fat contents are reported for both horse and donkey milk (Smiddy, Huppertz, & VanRuth, 2011), whereas residual milk, accounting on average for 30% of total milk extracted, is the richest fraction in fat (Cherepanova & Belokobylenko, 1974; Salimei et al., 2004b).

For a maximum response of milk ejection in heavy-horse breeds, e.g., Breton and Comtois breeds, the presence of the foal

during milking is recommended (Doreau, 1991). However, when foals are not physically present, the milking routine is more manageable in terms of both human and animal safety, and for optimal milk extraction, according to previous experience with donkeys (Simoni, Salimei, & Varisco, 2004). Due to the relatively small amount of milk per milking and depending on the wavering consumer demand, mostly limited by local availability and lack of communication, dairy equids may need to be milked many times a day (Doreau, 1991; Drogoul et al., 1992).

Caroprese et al. (2007) found that mechanical milking positively affects milk yield and composition of Murghese horses, an Italian saddle breed, not milked previously. In donkeys, although manual milking could be as efficient as machine milking in terms of quantity of milk extracted per milking, production varies less when donkeys are machine milked (Salimei, 2011) and the risk of contamination, a crucial issue, can be significantly reduced compared with manual milking (Sorrentino et al., 2010).

In a productive system of “on demand” raw horse and donkey milk, as is the case at present, mechanical milking is preferred. The milking plant for dairy equids consists of milk and pulse tubes, a portable or wheeled bucket or graduated jar, connected through a vacuum tube to the rotary vacuum pump (140 L min^{-1} at 50 kPa), a 15 L vacuum tank, a 150 mL claw, a stainless steel mastitis detector for visual evaluation of milk and a sheep cluster. Because of the rapid milk ejection, ranging from 40 to 90 s per milking (Caroprese et al., 2007; Dzidic et al., 2002; Simoni et al., 2004), the adoption of a rapid pulsation rate ($120 \text{ cycles min}^{-1}$) is recommended to avoid both the liner back flow and milk stress (Salimei, Cattaneo, Chiofalo, & Dell’Orto, 1996b). In addition, other optimised parameters of the mechanical equipment for horse milking also apply to donkey (42–45 kPa of vacuum level, 50:50 pulsation ratio) (Caroprese et al., 2007; Doreau, 1991; Salimei et al., 2004a,b).

Milking routine is crucial in equids with regard to the specific management of the dams that live with their suckling foals until natural weaning (>7 month of age). Dairy horses and donkeys could be milked up to 8 times per day but, among the management conditions essential to achieving 1000 L per heavy-horse breed per season (6–7 months of lactation), dams should be milked from 20 to 90 d after foaling, three times a day, following a strict milking preparation and routine consisting of 3 h physical separation from foal, udder massage and teat cleaning (Bayle-Labouré, 2007; Doreau & Martin-Rosset, 2011).

Such a milking procedure requires appropriate facilities that take into account both safety conditions and animal welfare. A suitable milking stable for horses (Bayle-Labouré, 2007) consists of a series of individual boxes with waterers and feeders and a covered area for the foals adjacent to the dams but separated from them by a manger. Robust fences delimit the milking areas between two animals allowing space for both operator and portable milk bucket. The stable also include a milk room, containing equipment for cooling and bottling as well as for sanitising the milking plant and tank.

In general, the presence of the foal during milking does not affect milk ejection in donkeys adapted to the milking procedure (Salimei et al., 2000), which is a significant advantage compared with dairy horses and allows the modification of the milking parlour, routine and facility. In fact, a specific facility has been developed for a small dairy donkey farm (60 lactating donkeys) that consists of a milking parlour and a milk room. In addition to the milking plant, the parlour is equipped with an electric pump (for delicate liquid foods) to feed milk into a refrigerated tank, and all the accessories for cleaning. The adjacent milk room, connected only by the milk pipeline, is equipped with the refrigerated tank and all the accessories for bottling (Simoni et al., 2004). The unit is divided into these two areas to minimize environmental contamination.

After 3 h of physical separation from the foal at a visible distance, dams move to the waiting area to be milked in the parlour. Donkeys adapt quickly to the routine (including udder massage, teat cleaning, approaching and leaving the parlour) that requires about 3 min per animal (Simoni et al., 2004). During lactation, housing elements in a dairy donkey farm must provide a healthy environment for dams stabled with their foals, usually in barns with a large external paddock and a covered feeding alley. The unit also includes additional boxes where dams are gathered before each milking (Salimei, 2011).

4. Equid milk production

At present, horse and donkey milk yield studies are focussing on defining the most appropriate management of dairy equids in an intensive production system. However, variability of milk production reported in the literature is high due to many other factors, such as individual milkability, nutrition, genetics, management of reproduction, etc., in addition to milking management.

Data on donkey milk production are more limited than those related to horses. Moreover, different approaches have been applied in the studies on nursing and dairy horse production, related mainly to foal nutrition and growth, but increasingly to human consumption, while the recent literature on donkey milk production is mainly concerned with its use as food for sensitive consumers.

Variability due to inter- and intrabreed differences is reported but data available in the literature are inconclusive with regard to the dairy aptitude of different equid breeds, and milk production is assumed to be proportional to body weight ($2.5\text{--}3.0 \text{ kg } 100 \text{ kg}^{-1}$ body weight) (Doreau & Martin-Rosset, 2011; Salimei, 2011).

According to Doreau and Martin-Rosset (2011) the lactation curve in heavy-horse breeds gradually declines from approximately 13 kg d^{-1} to 5 kg d^{-1} , and the peak is reported to be within the 3rd month of lactation (Doreau & Martuzzi, 2006a) but more frequently it is considered to occur at the 2nd month of lactation (Martin Rosset, Austbo, & Coenen, 2006). Moreover, the Wood’s lactation model has been applied to the lactation curve of Lusitano nursing horses showing the peak of milk yield (14 kg d^{-1} , 2.6 kg milk 100 kg^{-1} body weight) on the 31st day of lactation with a persistency between 90 and 95% per month (Santos & Silvestre, 2008). It is also estimated that light horses, such as the Murghese breed (average 480 kg body weight), produce approximately 14.0 kg milk per day, while for heavy horses, such as the Tiro Pesante Rapido (average 880 kg body weight), the daily yield is 22 kg milk at the peak of lactation (Pinto, Faccia, Di Summa, & Mastrangelo, 2001). However, in light-horse breeds, such as Haflinger, dams in good body condition and machine milked twice a day produced $0.9 \pm 0.25 \text{ L}$ milk per milking during mid-late lactation (Salimei et al., 1996b).

Adaptation to milking routine is crucial for optimal milk extraction: according to Caroprese et al. (2007), untrained horses and stockmen result in lower milk yield. In trained donkeys, milk yield does not differ between morning and afternoon milking (Giosuè, Alabiso, Russo, Alicata, & Torrisi, 2008).

Data on donkey milk yield are usually expressed in mL per milking “session”, i.e., from 3 h before mechanical milking until the exit of the milking parlour. As also observed in horses, studies on dairy donkeys are often carried out in different experimental conditions and highly variable productions are obtained with manual ($466 \pm 260 \text{ mL}$ milk per milking; mean value and standard deviation calculated from Alabiso, Giosuè, Alicata, Mazza, & Iannolino, 2009; Chiofalo, Azzara, Piccolo, Liotta, & Chiofalo, 2004; Guo et al., 2007; Ivankovic et al., 2009) or mechanical milking ($772 \pm 148 \text{ mL}$ milk per milking; mean value and standard

deviation calculated from D'Alessandro, De Petro, Claps, Pizzillo, & Martemucci, 2009; Fantuz et al., 2007, 2010; Giosuè et al., 2008; Salimei et al., 2000; Salimei, Fantuz, Varisco, Maglieri, & Polidori, 2005a; Simoni et al., 2004).

As depicted in Fig. 1A, the average milk yield per mechanical milking in non-pregnant Martina Franca donkeys (average body weight 280 kg) shows an initial decline from d30 to the 4th month of lactation with an estimated persistency of 85–90% per month. Subsequently, milk production stabilizes at 600–800 mL until the 9th month of lactation (Salimei & Chiofalo, 2006). This trend is confirmed by data on Ragusana population that show seasonal variation of milk yield, presumably due to foaling period (Giosuè et al., 2008).

Individual variation is high in dairy donkeys (Fig. 1B), suggesting that the adoption of adequate selection programmes and reproductive management would lead to a substantial improvement in milk production, as already experienced with dairy horses (Doreau & Martuzzi, 2006a).

The interval between two consecutive milking sessions did not statistically affect the daily milk production of donkeys even if longer intervals (20 h vs. 4 h) can increase the milk fat content (Salimei et al., 2006). However, data on the effects of milking management and routine in dairy equids require further investigation.

5. Equid milk hygiene and mammary gland health

The growing interest in horse and donkey milk as a food for sensitive consumers, such as infants with food allergy or elderly people, implies strict regulations for food safety. In this regard, the

European Union laid down specific rules for the production of raw milk from any mammal (Regulations EC 852/2004, 853/2004 and 1662/2006) and restrictions are applied by Member States for safety reasons (Colavita, Amadoro, & Salimei, 2011).

Only limited data are available on horse milk hygiene, but recent studies confirm that the total bacterial count is generally lower (below 40×10^3 cfu mL⁻¹) than that reported for conventional dairy species (Dankov, Wojtowski, Pikul, Znikowski, & Cais-Sokolinska, 2006; Doreau & Martin-Rosset, 2011). Furthermore, current data on donkey milk show its suitability for human consumption when hygiene standards are observed during milking, even though the total bacteria count was found to vary from 2.5×10^2 to 7.4×10^5 cfu mL⁻¹ (minimum and maximum from Alabiso et al., 2009; Colavita et al., 2011; Conte, Calabrò, & Monsù, 2003; Conte et al., 2004; Coppola et al., 2002; Pilla, Daprà, Zecconi, & Piccinini, 2010; Salimei et al., 2000, 2004b, 2005a, 2006; Simoni et al., 2004; Sorrentino et al., 2005, 2010).

A rare presence of undesirable microorganisms, such as *Streptococcus equi*, *Streptococcus equisimilis*, *Staphylococcus* spp., and the absence of *Listeria monocytogenes* and *Salmonella* spp. are reported in the literature on equid milk (Colavita et al., 2011; Pilla et al., 2010). However, results from a biennial study on raw bulk donkey milk (from 10 dairy donkeys) showed occasional isolation of *Escherichia coli* and an ample variability of coliform contamination (<10 cfu mL⁻¹ up to 3.7×10^6 cfu mL⁻¹). These data highlight the importance of raw milk management since its hygiene can be compromised as soon as correct procedures are disregarded during milking and collection. Moreover, the presence of *Listeria* spp. in donkey milk must be ascribed to ineffective sanitisation of equipment and/or milking facilities (Colavita et al., 2011).

Lactic acid bacteria are the most widely represented microbiota in donkey milk (Coppola et al., 2002; Sorrentino et al., 2010). However, the presence of undesirable microorganisms found after storage at 4 °C for 3 days in manually milked bulk donkey milk must be attributed not only to the milking technique, but also to the hygiene management of the animals.

The heat treatment necessary for milk sanitisation involves irreversible alterations of some endogenous compounds in milk. Data reported in Table 2 confirm that both whey protein and lipid components of donkey milk decreased with increasing temperature and time of heat processing (Sorrentino et al., 2005).

Thermal damage is therefore determined by the hygienic characteristics of raw milk. In this regard, it is also important to consider the rapid inactivation of alkaline phosphatase (ALP) in horse milk compared with cow milk (Marchand, Merchiers, Messens, Coudijzer, & De Block, 2009). Therefore this endogenous enzyme, present in smaller quantities than in cow milk and universally

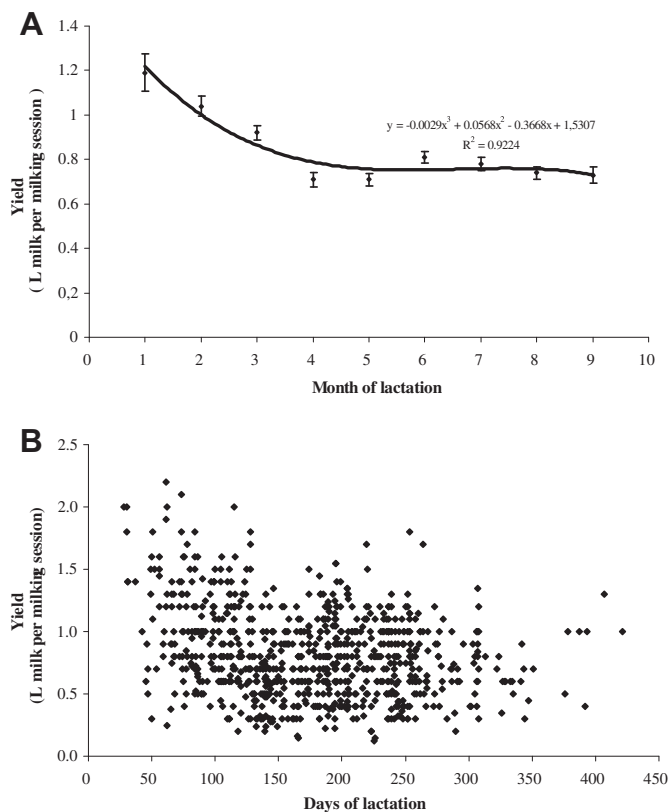


Fig. 1. Donkey milk yield during lactation (mean values \pm s.e.): A, non-pregnant donkeys (adapted from Salimei et al., 2005a); B, milk yield per milking in donkeys ($n = 815$ observations on 23 dairy jennies, unpublished data).

Table 2

Changes in nitrogenous and lipid components in raw donkey milk and after thermal treatments.^a

Component	Raw	66 °C 10 min	63 °C 30 min	70 °C 1 min	90 °C 1 min
Nitrogenous components					
N (g kg ⁻¹ milk)	2.1	2.2	2.2	2.2	2.2
NCN (g kg ⁻¹ milk)	1.3	1.3	1.2	1.2	0.6
Casein (g kg ⁻¹ milk)	0.8	0.9	0.9	1.0	1.6
NPN (g kg ⁻¹ milk)	0.3	0.3	0.3	0.3	0.3
WP (g kg ⁻¹ milk)	0.9	0.9	0.9	0.8	0.3
WP/N (%)	44.8	42.3	42.3	38.5	14.5
Furosine (mg 100 g ⁻¹ protein)	15.43	20.84	18.53	19.71	112.89
Lipid components					
Trans retinol (μg kg ⁻¹ milk)	16.7	16.4	13.9	14.2	14.1
α tocopherol (μg kg ⁻¹ milk)	51.4	47.3	43.5	46.7	30.3

^a Adapted from Sorrentino et al. (2005); N, total nitrogen; NCN, non-casein nitrogen; NPN, non-protein nitrogen; WP, whey protein.

accepted as reference method for the determination of ALP in cow milk derivatives, is not suitable as an indicator for the correct pasteurisation of horse milk (Marchand et al., 2009). A high furfural content, as molecular index of thermal and storage processes, was found in powdered horse milk products, with and without the addition of vitamins (1230 and 950 mg 100 g⁻¹ protein, respectively; Marconi & Panfili, 1998).

Due to the sensitivity of equid milk consumers, it is also important to consider the increasing concern with regard to the observed persistence of allergenicity in heat-treated cow milk, and the effect of heat treatments in promoting milk protein sensitisation. Therefore, development of new food technologies or the combination of chemical and physical processes should be studied for hypoallergenic products free of residual antigenic activity (Restani et al., 2009; Roth-Walter et al., 2008).

As already noted with regard to microbial quality, the mammary gland status of dairy horses is generally considered good, with a low average somatic cell count (4.61 log SCC mL⁻¹; Dankov et al., 2006). Furthermore, mastitis does not seem to represent a limiting factor in horse milk production (Doreau & Martin-Rosset, 2011); it is rare in dairy donkeys and mainly of traumatic origin (Salimei, 2011). More extensive studies on mammary gland status carried out on dairy donkeys confirm the low somatic cell count of equid milk, ranging from 3.5 to 4.5 log point mL⁻¹ (Salimei, 2011). Among the cell populations studied in donkey milk, Beghelli et al. (2009) identified lymphocytes, 13% of total somatic cells, monocytes/macrophages, 18% of total somatic cells, polymorphonuclear neutrophils, 40% of total somatic cells, and epithelial cells, 28% of total somatic cells. The neutrophil predominant cells in milk secretion during mid-late lactation are reported to be characteristic of equid physiology (Beghelli et al., 2009).

6. Equid milk composition

After foaling, the colostral phase is shorter in equids than in bovines: as reported for total solids, fat and protein content (Fig. 2), horse and donkey mammary secretion is close to the mature milk composition 24–36 h after foaling. Like in many other mammals, equid colostrum contains more proteins than mature milk (Fig. 2), immunoglobulins and enzymes in particular (Uniacke-Lowe et al., 2010). There is a limited interest in producing horse colostrum for pharmaceutical purposes or for orphan high-value foal feed (Doreau & Martin-Rosset, 2011; Drogoul, Clement, Ventorp, & Orlandi, 2006), and normally dairy equid species are milked starting 30 d after foaling. Besides its role in newborn immunity, mammary secretion provides nutrients, cells, enzymes, hormones and trophic factors so that in the first month of life they are responsible for the rapid growth of the foal.

6.1. Gross composition and energy content

Published data on horse and donkey milk gross composition (Doreau & Martin-Rosset, 2011; Doreau & Martuzzi, 2006a,b; Salimei, 2011; Summer, Sabbioni, Formaggioni, & Mariani, 2004) confirm the closer resemblance to human milk (Hosoi et al., 2005; Yamawaki et al., 2005) for lactose, protein and ash levels (Table 3) when compared with cow, sheep and goat milk (Park, Juarez, Ramos, & Haenlein, 2007; Pulina & Nudda, 2002). Methodological differences among studies contribute to the observed variability in horse and donkey milk components.

Despite the high lactose content of equid milk, the average energy content is lower compared with human milk (Table 3). According to Oftedal and Jenness (1988) the low energy content of equid milk is related to the large amounts of milk secreted to meet the nutritional requirements of the foal for its rapid growth.

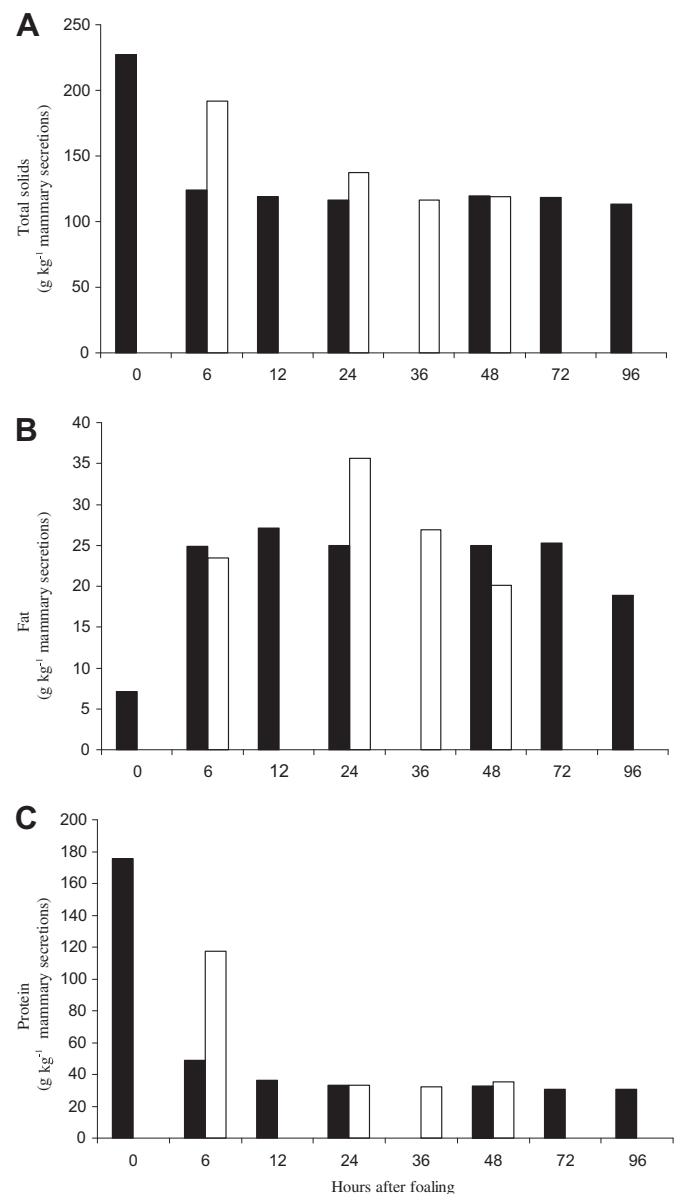


Fig. 2. Evolution of horse (■) and donkey (□) mammary secretion composition up to 96 h after foaling: A, total solids; B, fat; C, protein. Adapted from: Maglieri & Salimei (unpublished data); Salimei et al. (2002); Ullrey et al. (1966).

However, the fat fraction contributes only 10–20% of horse milk gross energy (Mariani et al., 2001). Therefore, the hypolipid content of horse and donkey milk (Table 3) must be taken into account from a nutritive point of view: for this purpose, when used in infant

Table 3

Horse and donkey milk gross composition and energy content compared with human milk.^a

Parameter	Horse	Donkey	Human
Total solids (g kg ⁻¹)	110.0	95.3	125.0
Lactose (g kg ⁻¹)	61.0	65.8	64.4
Total N × 6.38 (g kg ⁻¹)	21.4	16.5	12.5
Fat (g kg ⁻¹)	14.0	7.6	34.6
Ash (g kg ⁻¹)	4.5	4.1	1.9
Gross energy (MJ kg ⁻¹)	2.10	1.76	2.60

^a Horse values averaged from: Doreau and Martin-Rosset (2011), Doreau and Martuzzi (2006b), Mariani et al. (2001), Martuzzi et al. (2004); donkey values averaged from: Salimei (2011); human values averaged from: Hosoi et al. (2005), Yamawaki et al. (2005).

nutrition, donkey milk is usually supplemented with vegetal oil (4 mL 100 mL⁻¹ milk) to conform to human milk energy (Iacono et al., 1992).

In donkey milk significant positive correlations between milk yield and total solids or fat content were observed. These results confirm that higher milk fat is associated with a more complete udder evacuation in dairy donkey. Moreover, total solids were positively correlated to protein, fat and ash content, whereas the protein content was directly related with fat and ash contents but negatively correlated to lactose (Ivankovic et al., 2009; Salimei, Maglieri, Varisco, La Manna, & Fantuz, 2009).

According to circadian studies, milk lipid and lactose contents have been observed to peak in donkey during the night, whereas the protein content peaked during the day (Piccione, Fazio, Caola, & Refinetti, 2008). However, internal rhythms are known to be affected by environmental factors, such as management of feeding and the light–dark cycles.

Among the variables associated with changes in the equid milk gross components, the effect of stage of lactation has been studied on both horse and donkey milk composition.

The protein content decreases by 20–25% from d 28 to d 150 in horse (Doreau & Martin-Rosset, 2011; Mariani et al., 2001; Martuzzi, Summer, Formaggioni, & Mariani, 2004; Summer, Tirelli, Formaggioni, Malacarne, & Mariani, 2005; Ullrey, Struthers, Hendricks, & Brent, 1966) and donkey milk (Giosuè et al., 2008; Guo et al., 2007; Salimei et al., 2004a). In equids, the milk nitrogen fractions are also reported to be influenced by the length of the lactation period. The casein content declines by approximately 20–30% in horse (Mariani et al., 2001; Martuzzi et al., 2004; Summer et al., 2005) and donkey milk (Fantuz, Maglieri, Varisco, La Manna, & Salimei, 2009b; Giosuè et al., 2008) while results on the non-protein nitrogen (NPN) milk content during lactation are not conclusive. No significant trends are reported for horse milk NPN by Mariani et al. (2001) and Summer et al. (2005) but not by Martuzzi et al. (2004), who observed a significant decline during lactation, consistent with reports by Guo et al. (2007) for donkey milk. However, the latter authors did not observe significant changes of the casein content during lactation. Finally, the increasing trend reported for milk NPN content in dairy donkeys could reflect a nutritional imbalance of the diet in late lactation (Giosuè et al., 2008).

No significant differences were observed in the variable fat contents of horse and donkey milk produced from 20 to 150 days after parturition (Giosuè et al., 2008; Mariani et al., 2001; Salimei et al., 2004a) but a negative trend during lactation was observed in horse milk (Doreau & Martin-Rosset, 2011; Martuzzi et al., 2004; Ullrey et al., 1966).

As expected due to its osmotic role in milk secretion, the lactose content of mature milk was found unaffected by stage of lactation in dairy horse and donkey (Martuzzi et al., 2004; Salimei et al., 2004a, 2009); however, a tendential increase throughout lactation is also reported in the literature (Doreau & Martin-Rosset, 2011; Guo et al., 2007; Mariani et al., 2001; Ullrey et al., 1966). There is a lack of data on oligosaccharides in the literature for horse colostrum and milk that show the presence of molecular similarity with some human milk oligosaccharides (Boehm & Stahl, 2007; Urashima, Saito, Nakamura, & Messer, 2001).

The intense neonatal foal growth also requires a suitable mineral content in milk: in this regard, the ashable content of horse and donkey milk declines from 28 to 150 d of lactation (Guo et al., 2007; Mariani et al., 2001; Martuzzi et al., 2004; Salimei et al., 2009; Schryver, Oftedal, Williams, Soderholm, & Hintz, 1986).

The gross energy content of horse milk is also reported to decrease during lactation by approximately 15% (Mariani et al., 2001; Martuzzi et al., 2004; Ullrey et al., 1966). However, due to

the high variability observed in milk fat content, Salimei et al. (2004a) did not note any effect of lactation phase on the energy content of donkey milk.

6.2. Nitrogenous components

The non-protein nitrogen (NPN) accounts for an average of 10–16% of total nitrogen in horse and donkey milk, which is lower than values reported for human milk but higher than those of domestic ruminants (Salimei, 2011; Uniacke-Lowe et al., 2010). Among the NPN components of equid milk, the average urea content (20–35 mg 100 g⁻¹) is consistent with data from cow milk, but slightly lower than those from human milk (Darragh & Lonnerdal, 2011; Dell'Orto et al., 1994; Fantuz et al., 2009b; Martuzzi et al., 2004; O'Connell & Fox, 2011) and it represents approximately 40% of the NPN fraction in horse and donkey milk (Fantuz et al., 2009b; Salimei, Varisco, & Rosi, 2002). This could be related to the different metabolic abilities among mammals in recycling urea.

The amino acid profile of the donkey milk proteins shows a slightly lower percentage of essential amino acids (36.7–38.2 g amino acid 100 g⁻¹ protein) than in human milk proteins (40.7 g amino acid 100 g⁻¹ protein), according to Guo et al. (2007). Moreover, tryptophan was recovered only in horse milk protein (1.2 g amino acid 100 g⁻¹ protein; Csapò-Kiss, Stefler, Martin, Makray, & Csapò, 1995) and both cysteine and leucine were found at lower levels in donkey milk proteins than human milk proteins (Guo et al., 2007).

However, the free amino acid content of horse milk, more rapidly available to gut absorption, is intermediate between the lower values in cow milk and the higher values of human milk (Uniacke-Lowe et al., 2010). Moreover, in donkey milk the bioactive amines spermine (5.79–32.78 µg L⁻¹), spermidine (5.20–51.06 µg L⁻¹) and putrescine (2.94–374.79 µg L⁻¹) were identified and their content was found to be lower than the values reported for mature human milk (La Torre, Saitta, Potorti, Di Bella, & Dugo, 2010).

It must also be noted that in horse and donkey milk the protein content is characterised by an average casein to whey protein ratio of 1.0–1.5:1 (Csapò-Kiss et al., 1995; Fantuz et al., 2009b; Guo et al., 2007; Malacarne, Martuzzi, Summer, & Mariani, 2002; Martuzzi & Doreau, 2006; Martuzzi et al., 2004; Salimei et al., 2004a; Summer et al., 2004), being higher than the average value reported for human milk, which shows significant variation during lactation (Lönnerdal, 2003).

In this regard, many patients who tolerated equid milk (Table 1) experienced intolerance to goat or sheep milk, characterised on average by a 3–3.5:1 casein to whey protein ratio (Uniacke-Lowe et al., 2010): this effect may be due to specific levels of major allergenic milk components.

Among the most possible allergens of milk, α_{s1} -, α_{s2} -, β - and κ -casein have been identified in horse and donkey milk, thanks to the recent advances in proteomic technology (Chianese et al., 2010; Uniacke-Lowe et al., 2010). However, the casein fractions are not yet quantified in donkey milk (Table 4). The percentage of homology between each cow milk casein and whey protein and corresponding equid and human milk protein is also reported in Table 4, as reviewed by Restani et al. (2009).

A variable amount of γ -like caseins is reported in the literature for cow, horse, and donkey milk casein fractions, probably due to lysis of β -casein by the action of indigenous milk protease such as plasmin (Egito et al., 2002; Fantuz et al., 2001; Humbert, Chang, & Gaillard, 2005).

The polymorphism of α_{s1} - and β -casein has only been extensively studied in horse milk (Uniacke-Lowe et al., 2010); in donkey milk from the Ragusana breed, Criscione et al. (2009) found, in one

Table 4
Casein and whey nitrogen, distribution in milk.^a

	Horse	Donkey	Human	Cow
Casein N (mg 100 g ⁻¹ milk)	172	120	75	407
Fractions (%)				
α _{s1} -casein	17.9 (43.3)	Identified (60.0)	32 (31.9)	41 (100)
α _{s2} -casein	1.4 (n.a.)	Identified (n.a.)	Not identified	10.8 (100)
β-casein	78.5 (60.5)	Identified (n.a.)	Max 85 (56.5)	33 (100)
κ-casein	1.8 (57.4)	Identified (n.a.)	<15 (53.2)	12 (100)
Whey N (mg 100 g ⁻¹ milk)	130	107	97	99
Components (%)				
β-lactoglobulin	30.7 (59.4)	29.8 (56.9–51.6)		50.8 (100)
α-lactalbumin	28.5 (72.4 A; 69.1 B/C)	22.6 (71.5)	40.3 (73.9)	19.0 (100)
Serum albumin	4.4 (74.5)	6.2 (74.1)	7.7 (76.6)	6.3 (100)
Immunoglobulins	19.6	11.5	15.5	12.7
Lactoferrin	7.0	4.48	26.6	1.6
Lysozyme	10.5	21.0	5.5	Traces

^a Data are from (horse and cow) Uniacke-Lowe et al. (2010), (donkey) Chianese et al. (2010), Fantuz et al. (2009b), Salimei et al. (2004a) and (human) Lönnnerdal (2003); percentage homology with cow milk caseins and whey proteins (Restani et al., 2009) are given in parenthesis (n.a., not available).

case, a milk protein profile lacking two components of α_{s1}-casein. In this regard, the variability associated with genetic polymorphism of caseins may drive breeding strategies in the dairy horse and donkey enterprises for sensitive consumers (Chianese et al., 2010; Mateos et al., 2009) even though the complexity of casein component expression should also be taken into account.

The main components of whey proteins in equid milk are β-lactoglobulin, α-lactalbumin, immunoglobulin, serum albumin, lactoferrin and lysozyme (Table 4). The β-lactoglobulin content of donkey milk, which is most likely related to its weak allergenicity, shows two molecular forms (I and II): two variants (A, B) and 3 variants (A, B and C) have been identified for β-lactoglobulin I and II, respectively (Godovac-Zimmermann, Conti, James, & Napolitano, 1988; Godovac-Zimmermann, Conti, Sheil, & Napolitano, 1990; Herrouin et al., 2000). Criscione et al. (2009) found a significant percentage of Ragusana donkeys (>23%) that produced milk lacking in β-lactoglobulin II, while others identified in donkey milk the fourth variant of β-lactoglobulin II, named D (Cunsolo, Saletti, Muccilli, & Foti, 2007). In horse milk, β-lactoglobulin is present as a monomer, while this whey protein is dimeric in ruminant milk (Fessas, Iametti, Schiraldi, & Bonomi, 2001).

Horse milk α-lactalbumin (Table 4) exists in three genetic variants (A, B and C) (Godovac-Zimmermann, Shaw, Conti, & McKenzie, 1987) and lysozyme in donkey milk occurs in at least two forms (A and B) (Godovac-Zimmermann et al., 1988; Herrouin et al., 2000). With regard to the potential allergenic role of equid milk proteins, Gall, Kalveram, Sick, and Sterry (1996) reported on the IgE-mediated horse milk allergy in one adult, most likely caused by heat-labile proteins α-lactalbumin and β-lactoglobulin in milk. For details on chemistry, structure and nutritional significance of horse milk proteins, the reader is referred to the review by Uniacke-Lowe et al. (2010).

Digestion of milk proteins from different species has been studied in vitro through a two-step simulation using human gastric and duodenal enzymes (Inglingstad et al., 2010; Tidona et al., 2011). These studies showed that the overall digestibility of caseins is high and comparable for horse, donkey, cow, goat, and human milk, the percentage of undigested caseins after gastric and duodenal steps being 4%, 7%, 4%, 6% and 5%, respectively. However, after gastric digestion the percentage of undigested caseins was almost double for cow and goat caseins compared with horse and human milk (Inglingstad et al., 2010).

Lactoferrin in milk from the same species is also highly digested; however, similarly to cow and goat β-lactoglobulin, horse and donkey β-lactoglobulin is resistant to gastric enzymes although after digestion with human duodenal enzymes only 25% and 30% of

β-lactoglobulin remain intact respectively in horse and donkey milk, compared with the higher concentration observed for cow (64%) and goat (62%) milk (Inglingstad et al., 2010; Tidona et al., 2011). Equid, as well as cow and human, α-lactalbumin were very resistant to gastric and duodenal digestion, with a percentage of intact protein at the end of the process ranging from 91% to 95% (Inglingstad et al., 2010; Tidona et al., 2011). Lysozyme from equid milk is also relatively resistant to digestion with 64% and 75% of undigested protein in horse and donkey milk, respectively (Inglingstad et al., 2010).

Differences in digestibility of potential milk allergens (Inglingstad et al., 2010; Tidona et al., 2011), in amount and distribution of nitrogen components (Salimei, 2011; Uniacke-Lowe et al., 2010), and in primary structure of milk proteins (Cunsolo et al., 2011) may help explain the hypoallergenic properties of equid milk compared with milk from conventional dairy species.

6.3. Lipid components

As mentioned above, the fat content of horse and donkey milk is low, and is characterised by a high multifactorial variability, consistent with findings reported for conventional dairy species (Fox, 2003). Notwithstanding the low energy content of equid milk, dietary immunonutrients must also be considered as vitamins and long-chain polyunsaturated fatty acids regulate the immune function and may be involved in the development and severity of symptoms of inflammation (Hoppu, Kalliomaki, & Isolauri, 2002).

Contrary to human and cow milk, horse milk triglycerides account for approximately 80% of total lipids, phospholipids and sterols for approximately 5% each, and free fatty acids represent approximately 10% of total lipids (Malacarne et al., 2002). The cholesterol content in equid milk ranges from 50 to 88 mg L⁻¹ (Barello et al., 2008; Malacarne et al., 2002; Marconi & Panfilii, 1998). The diameter of horse milk fat globules is 2–3 μm (Doreau & Martin-Rosset, 2011) while in donkey milk it varies from 1 to 10 μm, showing a dimensional distribution consistent with that reported for cow milk (Salimei, 2011).

With regard to milk fatty acid composition (Table 5), saturated fatty acids are the most represented class in horse and donkey milk compared with monounsaturated or polyunsaturated fatty acids; although a wide variability can be observed among experimental data. Such variability is most likely related to dietary and/or body condition differences, as reviewed for monogastric herbivores (Salimei & Chiofalo, 2006). Moreover, the high levels of total n3 polyunsaturated fatty acids (PUFAs) are well balanced by the total n6 PUFA content of horse and donkey milk (Table 5). The equid milk

Table 5
Fatty acids composition of horse, donkey and human milk.^a

Fatty acids	Horse	Donkey	Human
C4:0	0.3–0.9	0.32–0.6	
C6:0	0.3–1.4	0.28–1.22	
C8:0	0.8–6.1	8.52–12.8	
C10:0	2.3–16.7	18.65–20.42	
C12:0	3.8–14.6	10.67–15.9	
C14:0	4.7–19.2	5.77–10.59	5.48
C14:1 n5	0.1–2.6	0.22–0.88	
C15:0	0.2–0.9	0.32–0.57	
C16:0	12.4–28.5	11.47–29.17	23.98
C16:1	2.2–9.7	2.37–3.93	2.32
C17:0	0.0–1.2	0.22–0.52	
C17:1	0.0–1.1	0.27–0.73	
C18:0	0.3–3.0	1.12–3.91	9.52
C18:1 n9	9.4–31.6	9.7–22.15	38.85
C18:2 n6	3.6–20.3	8.15–15.17	12.71
C18:3 n3	2.2–26.2	6.32–16.33	0.71
SFA, % total fatty acids	43–49	46.7–67.7	39.41–42.24
MUFA, % total fatty acids	26.8–36.2	15.3–35.0	44.30–45.11
PUFA, % total fatty acids	19–20	15.2–30.5	15.48
PUFA n3, % total FA	8.66–11.97	9.45–9.64	1.27–2.19
PUFA n6, % total FA	7.06–11.77	11.57–13.09	11.17–14.21

^a Values are presented as g 100 g⁻¹ fatty acids: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Data are adapted from: (horse) Doreau and Martuzzi (2006b), Marconi and Panfili (1998), Salimei et al. (1996a); (donkey) Chiofalo et al. (2005), Chiofalo et al. (2006b), Salimei and Chiofalo (2006), Salimei et al. (2004a); (human) Kaila, Salo, and Isolauri (1999), Marangoni et al. (2002).

is in fact characterised by a PUFA n6:n3 ratio averaging from 0.57 to 1.40 while this ratio between pro-inflammatory (derived from n6 long chain PUFA) and anti-inflammatory (derived from n3 long chain PUFA) immunonutrients is reported to vary from 5.1 to 11.2 in human milk (Kaila Salo, & Isolauri, 1999; Marangoni et al., 2002). It is interesting to note that in lactating donkeys the transfer from blood to milk of n3 PUFAs was found to be more efficient than that of n6 PUFAs (Chiofalo, Piccolo, Maglieri, Riolo, & Salimei, 2007).

As hind gut fermenter, the horse large intestine mucosa shows significant differences in the relative rate of transport of volatile fatty acids generated by the microbiota activity compared with rumen mucosa (Van Soest, 1994). Digested dietary fats, soluble carbohydrates, proteins and minerals (except for escaped nutrients and part of phosphorus) are mainly absorbed by the small intestine of the horse and donkey; the scarce or null biohydrogenation before absorption suggests the direct influence of diet on the fatty acid composition of milk, as previously suggested by Marangoni et al. (2002) for human milk.

Besides the dietary factors, the low stearic acid (C18:0) content of equid milk (Table 5) could also be explained by the Δ^9 desaturase activity in the mammary gland (Doreau & Martuzzi, 2006b). A high content of both linoleic (C 18:2 n6) and linolenic (C 18:3 n3) acids is reported for horse and donkey milk. Conjugated isomers of linoleic acid (CLA) have not been detected in donkey milk (Salimei & Chiofalo, 2006) but small amounts are reported in the literature for horse milk (Doreau & Martuzzi, 2006b), suggesting a very limited absorption of both CLA and trans-vaccenic acid from the large intestine of equid species (Hoffman et al., 1998). According to Jahreis, Fritsche, Mockel, Schone, and Moller (1999), the higher CLA content of ruminant milk compared with non-ruminant species is negatively correlated with the PUFA and, to a certain extent, MUFA content.

Assuming that no odd chain length fatty acids synthesised in the large intestine are absorbed, due to the relatively high pH (higher or equal 4.0) in the non-glandular stomach of the horse, some bacterial activity exists (Andrews, Buchanan, Elliot, Clariday, & Edwards, 2005), and synthesis of microbial fatty acids results in odd chain and trans-fatty acids in milk (Hoffman et al., 1998).

Among the “minor” PUFA (<0.5 g 100 g⁻¹ fatty acids), horse and donkey milk contains eicosapentaenoic (EPA, C20:5 n3), and docosaesanoic (DHA, C22:6 n3) acids (Doreau & Martuzzi, 2006b; Salimei & Chiofalo, 2006), whose roles in both the development of the neonatal brain and retina (Al et al., 1995) and the inflammatory response pathways (Laiho, Ouweland, Salminen, & Isolauri, 2002; Liu, Li, & Neu, 2005) are reported.

It must be noted that dietary supplementation with vegetable or marine oils modified the fatty acid profile of horse and donkey milk, even though it had a negative or null effect on the milk fat percentage (Alabiso et al., 2009; Hoffman et al., 1998; Salimei, Bontempo, & Dell’Orto, 1996a; Salimei et al., 1996b). Increased intake of soluble fibre also affects the donkey milk fatty acid composition by increasing the total saturated fatty acid percentage and reducing the total n6 PUFA content (Chiofalo, Piccolo, Riolo, Maglieri, & Salimei, 2006b). Moreover, the effect of dietary factors on the palatability of donkey milk is important: when dams were fed fresh forage vs. hay, C6 and C9 alcohols and aldehydes with a distinctive taste have been identified in donkey milk, along with beta-pinene, beta myrcene, limonene, *p*-cymene, and gamma-terpinene, accounting for the green-grassy flavour (Chiofalo, Polidori, Costa, & Salimei, 2005).

The lipid fraction of donkey milk shows a triglyceride (TAG) pattern with 30–54 acyl carbon atoms (Chiofalo et al., 2006a; Pagliarini, Solaroli, & Peri, 1993) and the most frequent TAG components found in donkey milk were palmitic–oleic–linolenic (6.69%), palmitic–oleic–oleic (6.22%), palmitic–palmitic–oleic (5.25%), capric–palmitic–oleic (4.63%), palmitic–oleic–linoleic (4.48%) and palmitic–palmitoleic–oleic (4.18%) (Chiofalo et al., 2006a).

Donkey and human milk show a certain degree of qualitative similarity in the TAG fractions, human milk being characterised by prevalence of palmitic–oleic–oleic (approximately 24%) and palmitic–oleic–linoleic (approximately 19%), followed by palmitic–palmitic–oleic, oleic–oleic–oleic, palmitic–palmitoleic–oleic and linoleic–oleic–oleic (3–8%) (Morera Pons, Bargallo, Folgoso, & Lopez Sabater, 2000; Morera Pons, Castellote, Jauregui, Casals, & Lopez Sabater, 2003). In comparison, in the TAG composition of cow milk butyric–oleic–palmitic, butyric–palmitic–palmitic and butyric–myristic–palmitic are prevalent and no TAGs containing polyunsaturated fatty acids are reported (Chiofalo et al., 2006a).

As already suggested with respect to the native nitrogenous components of milk, technologies and processes for extending horse and donkey milk shelf-life should also consider the potential side effects on the lipid bioactive substances due to both the high lipase activity, most likely as a result of a thermolabile lipoprotein lipase (Chilliard & Doreau, 1985), and the possible lipid peroxidation of unsaturated fatty acids.

6.4. Minerals and vitamins

Considering essential macro minerals in equid milk (Table 6), although published data on donkey milk are scarce, the Ca and P concentrations are similar in horse and donkey milk and are approximately 3 times higher than in human milk. In contrast, Ca and P concentrations are approximately 1.5–2 times lower than in cow milk (Gaucheron, 2005). However, the average Ca:P ratio (1.5) observed in donkey milk is slightly lower than that in human and horse milk. The concentrations of K, Na, and Mg in horse and donkey milk appear to be similar to those in human milk. All macro minerals in horse and donkey milk vary significantly during lactation (Fantuz et al., 2011; Summer et al., 2004). Here it is important to highlight the contribution of the milk mineral content to the particular physicochemical and rheological properties of horse milk and fermented derivatives (Bornaz et al., 2010).

Table 6
Essential milk macro minerals, trace elements and vitamins in horse, donkey, and human.^a

Component	Horse	Donkey	Human
Ca	500–1300	330–1140	278
P	200–1200	320–650	140
K	300–800	240–747	530
Na	167–200	100–268	180
Mg	40–110	40–83	35
Ca/P	1.72	0.93–2.37	1.7
Fe	0.22–1.46	0.43–2.64	0.72
Zn	0.9–6.4	1.23–3.19	1–3
Cu	0.2–1.0	0.08–0.30	0.2–0.4
Mn	0.01–0.05	trace	0.003–0.006
Vitamin A	0.093–0.34	0.017	0.3–0.7
Vitamin D	0.003		0.0004
Vitamin E	0.26–1.13	0.051	3–8
Vitamin K	0.029		0.003–0.015
Vitamin C	17.2–147		50–100
Vitamin B2	0.37		0.4–0.6

^a Values are given in mg L⁻¹ and are referenced from: (horse) Csapò, Stefler, Martin, Makray, and Csapò-Kiss (1995), Doreau and Martin-Rosset (2011), Marconi and Panfili (1998); (donkey) Fantuz, Maglieri, Lebboroni, and Salimei (2009a), Salimei and Fantuz (2010), Sorrentino et al. (2005); (human) Darragh and Lonnerdal (2011).

From a nutritional point of view, it must be noted that the renal load of solutes, mainly determined by the amount of protein and inorganic substances in the diet, is very similar in both breast-fed infants and those fed donkey milk (Iacono et al., 1992).

As far as the trace element profile of equid milk is concerned, data reported in the literature show high variability (Table 6) and need to be confirmed. However, Fe and Zn are reported to be similar to or higher than the levels in human milk. Mn is present at µg levels in horse and human milk. Furthermore, dietary trace element supplementation did not affect the donkey milk macro-mineral profile (Fantuz et al., 2011) but did significantly increase the human-like triiodothyronine content of donkey milk according to Todini, Salimei, Malfatti, Ferraro and Fantuz (2011). In addition, supplementing late gestating and lactating horses with higher dietary trace element levels than estimated requirements had no influence on the concentration of Cu, Zn and Fe in milk (Kavazis, Kivipelto, & Ott, 2002).

The equid milk vitamin contents (Table 6) show a high level of vitamin C in horse milk and ample variability of the fat-soluble components, whose concentration in milk fat is similar in cow milk, consistent with data from Pagliarini et al. (1993).

6.5. Functional and bioactive components

Among the functional proteins detected in horse and donkey milk, there are molecules active in antimicrobial protection such as lysozyme (17 kDa) and, at a lower level, lactoferrin (75 kDa) (Table 4). The lactoferrin content of equid milk is intermediate between the lower values of cow milk and the higher values of human milk, which on the contrary has a lower content of lysozyme than equid milk. Lysozyme in donkey milk ranges from 100 mg 100 mL⁻¹ (Vincenzetti et al., 2008), which is consistent with data on horse milk (Doreau & Martin-Rosset, 2011), to 400 mg 100 mL⁻¹ (Coppola et al., 2002), depending on the analytical method used (chemical or microbiological). Lysozyme in equid milk is highly thermo-stable (Coppola et al., 2002; Di Cagno et al., 2004). Lysozyme activity is intense against Gram-positive bacteria and much weaker on Gram-negative bacteria, as described by Kato (2003). Results from a pilot technological application of donkey milk as alternative source of lysozyme in Italian hard cheese making, show that cheese yield at 24 h was lower when donkey

milk was added instead of hen egg lysozyme; however, no differences were found in clotting time and curd-firming time. Similarly, sensory profiles showed no perceptible differences between cheeses made with hen egg lysozyme or donkey milk (Galassi, Salimei, & Zanazzi, in press).

Donkey milk lysozyme is very resistant to acid and protease and may play a significant role in the intestinal immune response (Tidona et al., 2011). Mammary secretions contain a multitude of compounds with very diverse functions: in human milk, many of these are proteins involved in the synthesis and expression of milk, but the majority appears to have evolved to provide local and systemic physiological activities in breast-fed infants (Lonnerdal, 2010). In this regard, functional peptides are mainly considered as fragments encrypted in caseins and whey proteins and released by the digestion process or fermentation with specific biological activities. The presence of biomolecules released during the digestive proteolysis may further contribute to the antimicrobial activity of donkey milk (Nazzaro, Orlando, Fratianni, & Coppola, 2010; Tidona et al., 2011). Evidence of their presence is also limited in horse milk and derivatives (Uniacke-Lowe et al., 2010).

In an in vitro study simulating gastrointestinal digestion of donkey milk, Bidasolo, Ramos, and Gomez-Ruiz (2011) identified one β-casein derived peptide with potent angiotensin converting enzyme (ACE)-inhibitory activity. Furthermore, in horse and donkey mammary secretion, defatted or not, growth factors and hormones have also been determined through radioimmunoassay or ELISA methods that use human peptides as standard and specific antibodies, properly validated for the equid species. In detail, horse and donkey mammary secretions contain human-like leptin at levels (3.35–5.32 ng mL⁻¹ milk; Salimei et al., 2002, 2005b; Salimei, Rosi, Maglieri, Magistrelli, & Fantuz, 2007) close to human milk (Bonnet et al., 2002). The bioactive peptides insulin-like growth factor 1 (IGF-1, 9.81–13.50 ng mL⁻¹ milk), ghrelin (4.26–4.63 pg mL⁻¹ milk; Magistrelli, Rosi, Amicone, Maglieri, & Salimei, 2008) and triiodothyronine (T3, 4.0 ng mL⁻¹ milk; Todini, Malfatti, Salimei, & Fantuz, 2010) were also found in frozen donkey milk. These molecules, and many others present in human milk, are increasingly receiving attention from a nutraceutical point of view because of their potential direct role in regulating food intake, metabolism, and infant body condition (Agostoni, 2005; Dvorak, 2010; Lonnerdal, 2010).

7. Fermented horse and donkey milk

Equid milk can be considered a suitable substrate for probiotic beverage production. The use of fermented horse milk is an ancient tradition in central Asia, where koumiss or airag are considered beverages with health-promoting properties (Uniacke-Lowe, 2011). Koumiss, a lactic-alcoholic beverage derived from horse milk, is an effective combination of raw milk and indigenous microbial populations, mainly lactic acid bacteria and yeast whose diversity is of increasing interest (Batdorj et al., 2006; Di Cagno et al., 2004; Watanabe et al., 2008). A novel *Bifidobacterium* species, *Bifidobacterium mongoliense* sp. nov. has been isolated from airag (Watanabe et al., 2009) while a novel *Lactobacillus casei* strain, *Lb. casei* Zhang, is under investigation for its probiotic potential (Guo, Wang, Yan, Liu, & Zhang, 2009). Bacteriocines produced by lactic acid bacteria in airag have recently been isolated and characterised by Batdorj et al. (2006). Koumiss is also rich in ACE-inhibitory peptides, supporting the claim of its beneficial effects on cardiovascular health (Chen et al., 2010).

Donkey milk could also be valorised as a good base ingredient for probiotic and therapeutic food preparations. Pasteurised donkey milk has been inoculated with some strains of *Lactobacillus*

rhamnosus, with probiotic properties (Jacobsen et al., 1999; Shu et al., 1999): *Lb. rhamnosus* strains remained highly viable after 15 days of storage at 4 °C and at low pH (3.7–3.8). The high lysozyme content only partially influenced the growth of the strains tested without any significant effect on their acidifying activity (Chiavari, Coloretti, Nanni, Sorrentino, & Grazia, 2005; Coppola et al., 2002).

Texture and flavour of fermented horse and donkey milk, on the other hand, may be a constraint to the acceptability of the products, so that fortification with Na-caseinate, pectin, and threonine or the addition of flavours can enhance the rheological and sensory quality (Chiavari et al., 2005; Di Cagno et al., 2004). However, the production of fermented milks by means of a standardised manufacturing protocol should be considered crucial for consumers and markets, according to Di Cagno et al. (2004).

8. Conclusions

Recent clinical evidence has renewed the interest in horse and donkey milk because of high tolerability in infants with cows' milk protein allergy. Although the use of extensively hydrolysed proteins or soy-bean derived formulae is preferred in the treatment of this disease, alternative foods, such as equid milk, are required for highly problematic patients. Due to its compositional resemblance to human milk and its palatability, when equid milk is produced hygienically it is considered a valid substitute of hypoallergenic formulae but the low fat content must be appropriately balanced in the infant's diet. However, for their low fat content and the unique fatty acid composition, horse and donkey milk and their derivatives could become valuable foods for elderly consumers.

Moreover, the presence of endogenous bioactive compounds may help explain the health-promoting properties of raw horse and donkey milk. If confirmed by more in depth studies, the dairy equid species enterprise could be exploited in an agro-medical chain, where animal nutrition and management of animals and milk should be carefully evaluated and regulated for safety reasons. In this regard, specific milk processing technologies are needed to improve equid milk shelf life, preserving both natural attributes of equid milk and health of sensitive consumers.

The scenario of the innovative "dairy" chain for human consumption could revitalize many marginal areas of the world, where soil stability and animal diversity are becoming serious concerns.

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