Review Article

Nutritional and nutraceutical quality of donkey milk

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ABSTRACT

In the last few decades, there has been a renewed interest in donkeys by the scientific community involved in the recovery of biodiversity, in the rescue of some donkey breeds that have become almost extinct, and in the rediscovery of donkey milk. In addition, due to the increase in food allergies, attention has been focused on the need for a "natural" milk with a good taste, which could be used in some childhood illnesses such as allergy to cow's milk proteins allergy (CMPA).

Donkey milk is very similar to human milk, especially in terms of its protein profile and lactose content, which increases palatability, stimulates intestinal absorption of calcium, and thus provides an excellent substrate for the development of enteric flora. The high content of lysozyme in this milk favours selective action against pathogenic microorganisms. In addition, the mineral content (such as calcium) and

liposoluble vitamins make it an excellent nutraceutical product. A project aimed to create a donkey milk supply chain from the Amiata native breed has recently been developed in Central Italy (Tuscany). In this paper, the nutritional and nutraceutical characteristics of donkey milk are reviewed in detail. In addition, some of the potential uses of donkey milk have been briefly described: in the diet of children with CMPA, in the diets of the elderly and of people who need to lose weight by virtue of the low fat content and the good contribution of omega 3.

Keywords:
Donkey milk
Milk quality
Nutraceutical and nutritional quality

1. Introduction

For centuries, donkeys were used as a working animal. However, as a consequence of the industrialization of agriculture, in Europe the number of local donkey breeds has decreased significantly. Safeguarding the native donkey breeds will help in protecting biodiversity and is also an opportunity to develop marginal agricultural areas. The properties of donkey milk have been known since ancient times. Donkey milk was considered not only as an elixir of youth, but also used as a therapeutic product for sick elderly people and children. However, since then, donkey milk use has been almost forgotten in Europe.

In the last few decades, there has been renewed interest in donkeys by the scientific community involved in the recovery of biodiversity, in the rescue of some donkey breeds that have become almost extinct and in the rediscovery of the milk. Donkey milk is also becoming increasingly common as an alternative productive option for farms. The majority of investigations into donkey milk quality have been carried out in Italy; however, data are also available on milk from Chinese and Balkan donkey breeds [1,2]. In addition, due to the increasing spread of food allergies worldwide [3], attention has been focused on the need for “natural” milk, which tastes good and is suitable in some childhood diseases such as cow's milk protein allergy (CMPA). Cow’s milk protein is the main cause of food allergies in children younger

than three years. In their first year of life, between two and three per cent of children are allergic to cow's milk proteins. Recent epidemiological data show that CMPA is increasing in early childhood, especially in those countries with the highest socioeconomic levels [4]. The symptoms appear within one hour of milk ingestion: gastrointestinal (regurgitation, vomiting, diarrhoea), dermatological, respiratory, and general symptoms, including anaphylactic shock [5]. The only effective therapy is to exclude cow's milk and the formulas based on cow's milk from the diet. Suitable alternative milks include hydrolysates of cow's milk proteins, amino acid formulas, soy and rice milk. Some of these products still have an allergenic potential and are not a complete food, and therefore need to be integrated. In addition, some alternative types of milk do not have a pleasant taste and are therefore often rejected by children, thus compromising their growth. Therefore, the search for a natural, tasty and hypoallergenic milk is an issue of interest.

Recently in central Italy (Tuscany) an Amiata donkey milk chain has been set up and an experimental farm was created. The farm currently rears 160 Amiata native donkeys. The donkeys are reared outdoors and are fed concentrates and hay. The milk is pasteurized, bottled and labelled, and the average farm milk yield is about nine thousand litres per year. The majority of Amiata donkey milk is produced for babies with CMPA. It should be noted that, using a single donkey breed for milk production limits the variability of the physical and chemical milk composition and facilitates genetic selection on animals aimed at improving the milk yield and quality. The main results of research on nutritional and nutraceutical quality of donkey milk and on the Amiata donkey breed are reviewed.

2. Nutritional quality of donkey milk

2.1 Dry Matter

Donkey milk is, as a whole, richer in water and has a lower dry matter content than ruminant milk. Human milk has an average dry matter content (11.7 g/100ml), which is intermediate between donkey and cow's milk (about 9.5 and 12.5 g/100ml, respectively) [6,7].

2.2 Protein

The average total protein content of human and donkey milk is very similar (1.42 and 1.63 g/100ml, respectively) and it is about a half that of cow's milk (3.25 g/100ml) [7,8]. The low total protein content in human and donkey's milk does not produce an excessive renal load of solute in children [9]. In addition, not only the total amount of proteins but also the profile of the protein fractions is very similar in human and donkey milk. In fact, human and donkey milk contain a low casein content (about 0.4 and 0.7 g/100ml of milk, respectively) [8,10], whereas total protein, and also casein fraction is highly represented in ruminants. In cow's milk, the total casein content is on average 2.6 g/100ml [8], and is more than three times higher than donkey milk.

Milk caseins are present in different fractions: alpha S1, alpha S2, beta casein and K casein. Donkey caseins have not been extensively studied. However, all the four fractions have been identified [11], though there are only a few quantitative data which report low four casein fraction contents. The most represented caseins in cow's milk are alpha S1 and beta. In contrast, caseins constitute a minor component of human and donkey milk. Beta casein is the main fraction in both milks (about 0.27 and 0.37 g/100 ml, respectively) [12,13], whereas alpha S1 and k casein are only found at very low levels, and alpha S2 is absent in human milk [11].

On the contrary, whey proteins are more present in human and donkey milk, while lower quantities are detectable in cow's milk (about 0.76, 0.64 and 0.57 g/100 ml, respectively) [1,8]. Beta-lactoglobulin is absent in breast milk [11] but present in cow's and donkey milk (about 0.32 and 0.27 g/100ml, respectively) [12,14,15]. Recent *in vitro* studies with human gastrointestinal enzymes have shown that donkey milk beta-lactoglobulin is more digestible than cow beta-lactoglobulin [16]. Alpha-lactalbumin is mainly represented in human milk (about 0.34 g/100ml) followed by donkey milk (about 0.21 g/100ml) [12-14]. Instead alpha-lactalbumin is lower in cow's milk (about 0.125 g/100ml), which is less than half that of human milk [15].

Lysozyme (LZ) is a powerful natural antimicrobial present in body fluids and also in milk. *In vitro* recent studies have highlighted the poor digestibility of donkey LZ by human gastrointestinal enzymes [16]. It therefore seems that this protein can reach the intestinal tract and exert selective action on gut bacteria [17]. This protein has

different concentrations in milk of different species. It is higher in donkey milk and in particular in the Amiata breed, it is about 1.5 g/L [12], and a lower content is found in human (about 0.420 g/L) and cow milk (about 0.0013 g/L) [18]. Donkey LZ activity varies throughout lactation on average from 4000-5000 U/ml [10,19] with maximum values at the beginning of lactation which probably promotes the low bacterial count of donkey milk. LZ activity in bovine milk is just detectable, it is on average 0.0292 U/ml [20]. LZ activity in raw human milk from breast banks is higher than donkey and cow's milk (about 39000 U/mL).

In vitro studies have shown that casein precipitation leads to its longer stay time in the infant stomach as compared to whey protein [21]. Thus, the difference between the casein:whey protein ratio in human, donkey and cow's milk results in a softer clot in humans and donkeys and contributes to a better digestibility. The casein:whey protein ratio is more weighted towards casein in cow's milk (4.6), whereas it is about four times lower in donkey milk (1.1) and about nine times lower in human milk (0.5) [1,8,10]. Studies suggest that the i*n vitro* degradability and digestibility of donkey casein and whey proteins are higher than cow [22]. Protein digestibility is also important since food protein allergenicity is linked to the survival of the allergen in the gastrointestinal tract [23].

2.3 Lactose

Lactose is a disaccharide formed by glucose and galactose, it is highly present in human and donkey milk (about 7 g/100g), while in ruminants its contribution is lower (about 4.6% in cow milk) [7,24]. Lactose is an important source of galactose, a key source of energy and a crucial structural element in complex molecules, which is particularly important for early human development. In addition, galactose may be beneficial in several diseases, particularly in those affecting the brain [25]. The high lactose content of donkey milk also increases its palatability. In fact, our preliminary data on an evaluation of the approval rating shows that 75% of children appreciate it (Personal communication).

Lactose is also one of the dietary factors that stimulates intestinal calcium and phosphorus absorption, which is important for bone mineralization and in the prevention of osteoporosis [26]. Therefore, it is important both for children and the

elderly. Lactose also provides a substrate for the growth of enteric flora, and has a probiotic role [17]. Recent studies suggest that donkey milk also contains sialylated oligosaccharides in amounts comparable to ruminant milk but lower than human and mare milk. Sialylated oligosaccharides have been well studied for their antimicrobial and prebiotic effect and their capacity in stimulating the immune system [27].

2.4 Minerals

The average ash content in donkey milk is 0.36% [7], which is more similar to human (about 0.22%) than cow milk (about 0.76%) [8]. Ash in cow's milk is double that of donkey milk. Also the lower amount of minerals in donkey milk reduces the load of minerals for the kidneys.

The content of calcium, phosphorus and magnesium in donkey milk is intermediate between human and cow milk. Also the sodium and potassium content in donkey milk are intermediate between human and cow milk but more similar to those of human milk [28,29].

2.5 Lipids

From the point of view of the macro components, donkey milk differs from human milk mostly in terms of lipid content. In fact, the average fat percentage in donkey milk ranges from 0.50-1.7%, whereas the milk fat percentage of human and bovine milk are on average 3.1% and 3.7%, respectively [1,7]. The low lipid content gives donkey milk a lower energy value compared to human and bovine milk: 40 vs 62 and 65 kcal per 100 g, respectively (approximate values indicating the calories) for protein 4 kcal/g, for lactose 4 kcal/g and for fat 9 kcal/g.

To date, studies on the use of donkey milk in subjects with CMPA have been carried out mainly on children over six months old. There are few data available on children under six months, and include few subjects [30,31]. In order to fill this gap, clinical studies are currently underway at the Meyer Children Hospital in Florence (Italy). The donkey milk used in this study was supplemented with amount of lipids depending on the age of the children. Supplementation was made up of medium-chain triglycerides and olive oil in children; in addition a DHA integration has been

made. Donkey milk is currently the only milk alternative to the specific formulas in the case of CMPA considered by the Italian Society of Pediatric Nutrition in post weaned children [32].

With regard to the physical characteristics of lipids, donkey milk fat globules are very small, with an average of 1.92 microns, in contrast to ruminant milk, which has a more than double average globule size. This low size of the fat globules indicates a larger surface available for the lipase action, which could contribute to the higher digestibility of donkey milk [33].

From the point of view of the lipid fractions, saturated fatty acids (SFA) are contained in donkey milk fat in similar amounts to human milk (about 57 and 45 g/100g of fat, respectively) and lower than cow milk (about 71 g/100g of fat) [10,34,35]. The amount of unsaturated fatty acids (UFA) is also similar in donkey and human milk (about 43 and 55 g/100g of fat, respectively) and higher compared to cow milk (about 29 g/100g of fat), while the UFA:SFA ratio in donkey milk is intermediate (0.75) between human (1.22) and cow's milk (0.41). From a nutritional point of view, donkey milk leads to a lower SFA intake, about 3.02 g/L in Amiata donkey milk, while SFA are on average 13.95 and 26.27 g/L in human and cow milk respectively. Furthermore, despite being rich in UFA, donkey milk provides a limited amount of fat, thus the total intake of UFA per 1 L of milk is lower than human and cow milk (about 2.28 vs 17.05 vs 10.73 g/l, respectively) [10,34,35].

The average content of oleic acid (C18:1 OA) in donkey milk is comparable to cow milk (17 and 18.35 g/100g of fat, respectively) and is lower than human milk which has about double oleic acid content (31 g/100g of fat). Conversely, linoleic acid (C18:2n6 LA) is similar in donkey and human milk (about 9.5 and 12.29 g/100g of fat, respectively) and higher than cow milk (about 2.05 g/100g of fat) [35-37]. Of the milk of all farm animals, donkey milk has the richest sources of α-linolenic acid (C18:3n3 ALA) (about 7.25 g/100g of fat), in higher amounts than both human milk and cow milk (about 1.15 and 0.56 g/100g of fat, respectively). The fat of donkey milk is rich in EPA (about 0.26 g/100g of fat) compared to human milk (about 0.11 g/100g of fat) and cow milk (about 0.05g/100g of fat). However, the DHA content in donkey milk is about a half that the average values reported in human milk (0.28 and 0.51 g/100g of fat, respectively) [36], while in cow milk, it is undetectable. In

addition, donkey milk is not an important source of arachidonic acid (C20:4 AA). Therefore, because of the low fat, in particular in the babies fed an exclusive-milk diet, the lipid intake must be adjusted, both in terms of total and qualitative intake [31,38].

2.6 Vitamins

The literature on donkey milk reports that this milk is rich in vitamin C, while there are lower amounts of A and E and other water-soluble vitamins compared to cow milk [15]. Vitamin C aids in the absorption of iron, has antioxidant effects and is essential for the collagen formation. The reported total vitamin C average content is 5700 μg/100 ml in donkey milk, similar to human milk (about 5600 μg/100 ml), whereas it is lower in cow milk (about 1500 μg/100 ml) [39,40]. Recent research carried out by our research group shows a higher vitamin D content (2.3 μg/100 ml ± 0.86) (about 92 UI) in Amiata donkey milk than the average values found in the literature for bovine (0.03 μg/100 ml) and human milk (0.03 μg/100 ml) [34,41]. The amount of Vitamin D in donkey has shown seasonal variations, as reported in cows. In particular, a higher concentration of vitamin D was found in summer than in winter, probably due to the different sun exposure of the animals [42].

3. Possible uses of donkey milk for human health

The composition of donkey milk, as reported by many researchers, gives this food many potential uses, and thus it can be considered as a functional food.

The majority of clinical trials on humans have tested the efficacy of donkey milk on CMPA and atopic dermatitis [43,44]. The studies show that in children this milk leads to significant improvements in atopic dermatitis and it is well tolerated in cases of CMPA. In addition, children's growth parameters are good. A current on-going research project (Personal communication) on Amiata donkey has found that donkey milk can be considered as a "hypoallergenic" milk according to the definition of the American Academy of Pediatrics [45]. In fact, our findings show that the 93% of treated children with CMPA did not show any form of allergy and tolerated the milk. Among other the benefits of donkey milk, the most commonly reported is the ability to upregulate immune responses, which has been observed in vitro [46], in

animal models [47-49] and in a clinical study on elderly humans [50]. Other studies on rats have shown that donkey milk increases antioxidant and detoxifying enzyme activities [47], while LZ from donkey milk has been shown to directly suppress tumour proliferation on cell lines [46]. In addition, hypolipidemic effects, increase in body protein content vs body fat, improvement in energy metabolism, and weight control have been observed in animal models [47-49]. Therefore, these studies support the possibility of using donkey milk also in low-calorie diets, for the elderly and dyslipidemias.

4. Conclusions

Donkey milk has a closer composition to human milk than ruminant milk. It has similar protein quantities and profile to breast milk, as well as similar lactose, linolenic acid, vitamin C and mineral contents. The digestibility of the protein fraction is better than cow's milk proteins, and the smaller size of the fat globules also presupposes a faster and better lipid digestibility, while the total lipid content is lower than human and cow milk. Recent studies have shown it is a good supply of vitamin D. The most demonstrated beneficial effect of donkey milk is the good tolerability of children who suffer from CMPA. Thus, its use in pediatrics is recommended, and this milk could be a better substrate than cow milk to produce formulas for newborns and infants. In this case, a formulation require adequate increases in fat and adjustment in some fatty acid content (e.g. DHA).

Given its anti-inflammatory, antitumor, hypolipidemic actions and the effects on body composition proven in experimental models, as well as the vitamin D content, it could be used by the elderly and in obesity therapy. Donkey milk would therefore also be an alternative food targeted at consumer health. New well-designed clinical studies in these sensitive population groups are needed in order to confirm and update the potential positive health impacts.

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