



Donkey milk composition and its therapeutic applications

ANURADHA BHARDWAJ, YASH PAL*, RAM AVTAR LEGHA, PARVATI SHARMA, VARIJ NAYAN, SANJAY KUMAR, HEMA TRIPATHI and B N TRIPATHI

ICAR-National Research Centre on Equines, Hisar, Haryana 125 001 India

Received 29 May 2019; Accepted 24 October 2019

ABSTRACT

Milk contains plentiful nutrients. In Western countries, cow's milk fat is supposed as a risk factor for health because it is a source of a high fraction of saturated fatty acids. There has been increasing attention in donkey's milk. Donkeys' milk is preferential as a potential new dietetic food and a good alternative for infant nutrition in the case of bovine milk protein allergy. The microbiota present in this food may be responsible for its beneficial effects. Considering the potential health benefits, an increase in utilization of donkeys' milk is expected. The present review addresses various aspects of donkey milk with special emphasis on milk yield, composition, shelf life, antimicrobial, therapeutic and cosmetic properties.

Keywords: Antimicrobial, Composition, Cosmetic, Donkey, Milk, Therapeutic

Donkey (*Equus asinus*) is a member of the equine family, which has been used as a working animal mainly as pack since ancient times. It still remains an important work animal in difficult hilly terrains. Based on the livestock census 2012, total donkeys' population in India was 3,17,878 and decrease of 27% was observed over the last 5 years. The adult donkey females were recorded as 89,603 only and decrease of 37% was observed over the last 5 years. The decrease in donkey population is mainly due to mechanization of agriculture and transport.

Milk has all nutritional requirements for every mammalian newborn of different species. Specificity depends by the needs of the neonate and must ensure survival and growth during the early months of life. There is enhanced interest from consumers for donkey milk in southern states, while there are no official reports or published data with reference to its production, quality and hygiene. Also, donkey milk is not gaining wider consumer acceptance, because of a lack of the related food product legislation. In western countries, donkey dairies are emerging to produce an alternative milk source for human infants.

Milk yield

Donkey milk production differs greatly from that of conventional dairy species, especially in terms of milk supply. Recent studies on donkey lactation curves showed that individual milk yield ranged between 1.54 and 1.73 kg/day on specialized farms (Bordonaro *et al.* 2013), which generally raise animals in semi extensive conditions and care about their wellness. Equid mammary gland has a low average capacity (maximum 2.5 L); therefore, to increase

milk supply, dairy equids may need to be milked many times a day (Salimei and Fantuz 2012). The studies showed that the highest milk yield corresponded to three milkings per day every 3 hours, while daily milking regimen of six milkings per day did not increase milk production and had a negative influence on the health of the mammary gland. Alabiso *et al.* (2009) also showed that the highest milk yield can be obtained with three milkings per day compared to two per day, with an increase in milk fat content, too. The donkeys that gave birth in an autumn–winter period yielded more milk than donkeys foaling in spring–summer period since seasonal thermal stress can have detrimental effect on the quantity and quality of milk.

Milk composition

The composition of donkey's milk differs considerably from the milk of the important dairying species (cow, buffalo, goat, camel and sheep). In comparison with bovine milk, donkey's milk contains less fat, protein and inorganic salts but more lactose, with a concentration close to that human milk. Lactose is a source of fast energy. It makes this milk sweet, palatable and well accepted by children. On the other hand, the majority of adults and certain ethnic groups exhibit intolerance to milk sugar (Guo *et al.* 2007). The high lactose content also stimulates the intestinal absorption of calcium, which is essential for bone mineralization and for nervous system development in infants (Schaafsma 2003). Moreover, the high lactose content suggests use of donkey milk for probiotic purpose (Coppola *et al.* 2002) because it is an ideal substrate for a correct development of intestinal lactobacilli and makes donkey's milk an ideal matrix for the preparation of probiotic drinks following the incubation with *Lactobacillus*

*Corresponding author e-mail: yashpal1888@gmail.com

rhamnosus strains (Coppola *et al.* 2001). However, lactose content in donkey milk was observed as 4.70% in our studies (Pal *et al.* 2018).

The gross composition of milk is affected by genetic and environmental factors, including the breed, individuality of animals, stage of lactation, frequency and completeness of milking, maternal age, health and type of feed. The lower fat content in donkey milk compared to human and cow's milk (3.1% and 3.7%, respectively) (Saarela *et al.* 2005, Guo *et al.* 2007) could be a limiting factor in its use in infant nutrition in a diet exclusively based on milk. Many times fat content in donkey milk was estimated negligible in our studies (Pal *et al.* 2016) and hence, donkey milk is termed as natural defatted milk. Henceforth, it is encouraging for studies on the possible use of donkey milk in dietotherapy.

The pH of donkey milk, as well as human milk, is neutral or slightly alkaline, likely due to low content of caseins and phosphates, in comparison to cow milk (Pal *et al.* 2018). The average size of donkey milk fat globules may be important for milk digestibility. Although there are no explanatory results in the literature, it seems that the diameter of the milk fat globules has a different effect on the way in which fat is digested and metabolized (Michalski *et al.* 2005a). In fact, some authors have speculated that smaller native milk fat globules may have the best digestive parameters due to the larger surface available for the lipase action (Raynal-Ljutovac *et al.* 2008). Differences between donkey and other dairy species have also been found regarding the number of milk fat globules/mL of milk. In fact, the number of donkey milk fat globules has been found to be lower than that found in cows, goats and sheep (Martini *et al.* 2013a). Donkey milk fat globules showed an average diameter of 2 μm for 70% of total globules, resulting similar to the horse ones but smaller than both human (4 μm) and cow (2.8–4.6 μm) fat globules (Martini *et al.* 2013, Claeys *et al.* 2014). Moreover, equine milk does not cream due to the lack of cryoglobulin, a protein that adsorbs onto the fat globules as the temperature is reduced, and thus the agglutination of fat globules occurs very slowly (O'Mahony and Fox 2014).

Milk contains numerous nutrients. The content of most minerals is higher in donkey milk than in human milk, but significantly lower than in ruminant milk (Fantuz *et al.* 2012). The vitamin content of donkey milk is generally comparable or slightly lower than human milk, and, on average, lower than the vitamin content of ruminant milk, except for the vitamin C level (Claeys *et al.* 2014).

The principal salts in donkey milk are calcium, phosphorus, potassium, sodium, and magnesium. The concentrations of these elements, except potassium, are higher in donkey milk than in human milk but all are considerably lower compared with species such as cows, buffaloes, goats or sheep. The calcium, phosphorus concentrations were observed to be about 2–3 times higher than in human milk (Salimei and Fantuz 2012). Casein micelles are primarily a source of amino acids, calcium,

phosphate and bioactive peptides for neonates (Shekar *et al.* 2006). Although the mechanism of tolerance of donkey's milk has not yet been fully clarified, it is reasonable to hypothesize that the reduced allergenic properties of this milk can be related to structural differences of its protein component with respect to cow's milk. The lower casein concentration in donkey's milk (about 50% of total protein) compared with bovine milk (80% of total protein) (Zicker and Lonnerdal 1994) and the relevant percentage of essential amino-acids make this milk a new dietetic food and a promising breast milk substitute (Guo *et al.* 2007). A more recent study on donkey milk has revealed the percentage of four caseins ($\alpha\text{s}1$, $\alpha\text{s}2$, β and $\kappa\text{-CN}$) in decreasing order: β (54.28) > $\alpha\text{s}1$ (35.59) > $\alpha\text{s}2$ (7.19) > $\kappa\text{-CN}$ (2.79) as evaluated by Urea-PAGE analysis at pH 8.6, followed by immuno-detection with polyclonal antibodies, coupled to densitometric analysis (Cosenza *et al.* 2019).

Shelf life of donkey milk

Shelf life of milk is a very important aspect as it helps in its storage, processing, packaging and supply. It is the actual time period in which it deteriorates to an unacceptable level. Higher is the shelf-life; better would be its acceptability. Mare and donkey milk samples collected aseptically were incubated at 37°C for 24 h and periodical change in acidity and pH in equine milk was studied at 2 h interval regularly. Shelf life results indicated that mare and donkey milk was stable at 37°C up to 8 h and 10 h, respectively (Pal *et al.* 2016). Lysozyme acts as a natural preservative, conferring a lengthy shelf life to raw donkey's milk (Zhang *et al.* 2008, Šariæ *et al.* 2012).

Antimicrobial properties

The low microbial content and the rare presence of pathogenic bacteria were ascribed to the natural antimicrobial substances, mainly lysozyme and lactoferrin, present in donkey milk. Furthermore, the mammary gland status of dairy donkey is generally healthy due to presence of lactoferrin, lactoperoxidase and lysozyme. Mastitis was not seen at our farm since inception and it does not represent a limiting factor in milk production. The implementation of hygienic practices and regulations, such as pasteurization and Hazard Analysis and Critical Control Point (HACCP) plans, should further improve the production of donkey milk of high quality and safety (Zhang *et al.* 2008).

In the microbiota of donkey milk, the lactic acid bacteria (LAB) represent the major portion. The LAB usually reaches counts of 3–4 log CFUs/mL, representing nearly 80% of the total microbial content (Zhang *et al.* 2008, Carminati *et al.* 2014, Cavallarin *et al.* 2015). LAB counts decreased during cold storage of donkey milk, and this was attributed to a possible antimicrobial effect of lysozymes that inhibit the LAB development (Salerno *et al.* 2011, Cavallarin *et al.* 2015).

Therapeutic properties of donkey milk

Cows are the primary dairy animal species throughout

the world because of the abundance of their lacteal secretion, needed to satisfy the demand of milk and dairy products for human nutrition. However, cow milk is not suitable for the infants affected by cow milk protein allergies (CMPA), the prevalence for which was estimated between 5% and 15%, including infants who show symptoms related to adverse reactions to cow milk protein (Vandenplas *et al.* 2007). The hypoallergenic properties, together with other peculiar and health promoting aspects reported by many authors, initiated great interest in donkey milk as confirmed by the increased number of articles published in the last few years.

Donkey milk has been successfully used in clinical studies, in children with cow's milk protein allergy (CMPA), and has good palatability (Monti *et al.* 2007, Dello Iacono and Limongelli 2010). Its composition is more similar to human milk than ruminant milk, however it is poor in lipids, and adequate lipid integration is needed for a toddler's diet (D'Auria *et al.* 2011). Recently, the potential role of donkey milk has also been of increasing interest in the prevention of atherosclerosis and cardiovascular diseases (Tafaro *et al.* 2007).

The good tolerance of donkey milk in children suffering from CMPA (Monti *et al.* 2007) could thus be due to the levels of its major allergenic milk components, in fact its low casein content and casein:whey protein ratio play an important role in the sensitization capacity of the milk (Lara-Villoslada *et al.* 2005). Other factors may also help to explain the good tolerability of donkey milk, for example the number of casein fractions, the primary structure of the milk proteins, and the differences in digestibility of potential milk allergens, factors which have not yet been analyzed in depth (Salimei and Fantuz 2012). In addition, the lactose content represents a substrate for the development of intestinal microbiota with health-promoting properties (Coppola *et al.* 2002).

In fact, milk contains a variety of bioactive compounds with special properties associated with the development, growth and survival of infants beyond those provided by nutrition alone (Schanbacher *et al.* 1998). The major antimicrobial proteins in milk are immunoglobulins, lactoferrin, lactoperoxidase and lysozyme (Tanaka 2007). The protein, α -lactalbumin has a property of antiviral, antitumor and anti-stress. Cow's milk represents the most common feeding during the infant weaning and widely used as a substitute for human milk, but the cow's milk is one of the most common food allergies in children, it can lead to an abnormal immunological response (Criscione *et al.* 2009). Cow milk protein allergy (CMPA) is the most common food allergy in early childhood; affecting 2 to 5% of the child population with less than three years of age (Huang and Kim 2012).

Cosmetic properties of donkey milk

The use of donkey milk as a moisturizer in cosmetics is more fabulous. In cosmetics, donkey's milk is used for its cleaning and hydrating action combined with an antioxidant action that prevents the aging. In fact, the fat content in

donkey's milk nourishes the skin and gives it softness. Cleopatra and other privileged ladies of ancient times were taking their bath in donkey milk in order to maintain their skin fresh, lenitive and shiny. Nowadays, so-called therapeutic and cosmetic properties of donkey milk seem to be validated by many trials (Brumini *et al.* 2016). Donkey milk is rich in vitamins and polyunsaturated fatty acids (Aspri *et al.* 2016) and contains anti-ageing, anti-oxidant and regenerating compounds, which are described as naturally active in skin hydration and skin ageing prevention. The lipid fraction is characterized by high levels of essential fatty acids and low saturated fatty acids (Gastaldi *et al.* 2010). Compared with ruminants' milk, the considerable presence of unsaturated fatty acids found in donkey milk makes it very useful in the prevention of the cardiovascular, auto-immune and inflammatory diseases (Martemucci and D'Alessandro 2012, Martini *et al.* 2014). In addition, donkey milk could act therapeutically in numerous cases, such as liver problems, infectious diseases, fevers, asthma, etc. (Hippocrates 1843, Pliny the Elder 1893).

Agri-entrepreneurship through donkey dairy farming

It has been observed that donkey milk has tremendous medicinal value as curative agent for metabolic and allergic diseases along with its use in cosmetics. In some part of the country especially the southern regions, donkey milk is sold for medicinal uses. The donkey milk fetch a good amount of money for donkey milk providers and is in fair demand. Donkey milk based cosmetics and health mixtures can be an attractive agribusiness for equine farmers in India. This new possibility of increasing the income of the equine owners is very widespread among general public.

Since time immemorial the donkey milk was known for its unique therapeutic values. However, as cow's milk became a more popular choice for the masses due to high production and breeding policies, the values of donkey's milk was forgotten with time. But, economic gains from donkey milk are still on air, it is the costliest milk around the world fetching around 2,000 to 5,000 per litre according to the demand. Not only this now-a-days the cosmetic properties of donkey milk are gaining momentum. One agri-entrepreneur from Kerala, Mr Aby Baby decided to embark on a journey to bring back the lost glory of donkey's milk. Mr Aby Baby is now a man with a donkey farm. Aby Baby's one of its kind donkey farm at Ramamangalam (a mid-sized kraal with 19 donkeys) is the source of donkey milk used in a range of natural wellness and skincare products manufactured by him and getting favourable economic gain. Mr Aby Baby is finally finding joy in his venture after receiving positive feedback from consumers. A client in Hollywood, another, a sheikh from Qatar and people locally have responded to his products convincingly.

Constraints

- In India, donkey farms are small and are not as yet well-organized.

- Donkey milk is not gaining wider consumer acceptance, because of a lack of the related food product legislation.
- Social taboo.
- Low milk yield.

Future perspectives

While the donkey was shunned aside for its 'asinine' values, its milk was venerated as the elixir of life. With increasing numbers of research reports worldwide, donkey milk is now being taken as "gold mine" for future. In contrast to the affluent Western societies in India donkey serves for the livelihood of the landless, small and marginal farmers. Maximum donkeys are found with nomads and labour at brick kilns who don't know how to gain economic benefit from the animal except as pack and load carrying animal. If these donkey owners and marginal donkey farmers are made aware of the benefits and the products of donkey milk, this can be a great financial boon to them. The future prospects are really mountainous by proper breeding, milking and product marketing.

REFERENCES

- Alabiso A, Giosuè C, Alicata M L, Mazza F and Iannolino G. 2009. The effects of different milking intervals and milking times per day in jennet milk production. *Animal* **3**: 543–47.
- Aspri M, Economou N and Papademas P. 2016. Donkey milk: An overview on functionality, technology, and future prospects. *Food Reviews International* **33**(3): 316–33.
- Bordonaro S, Dimauro C, Criscione A, Marletta D and Macciotta N P P. 2013. The mathematical modeling of the lactation curve for dairy traits of the donkey (*Equus asinus*). *Journal of Dairy Science* **96**: 4005–14.
- Brumini D, Criscione A, Bordonaro S, Vegarud G E and Marletta D. 2016. Whey proteins and their antimicrobial properties in donkey milk: a brief review. *Dairy Science and Technology* **96**: 1–14.
- Carminati D, Tidona F, Fornasari M E, Rossetti L, Meucci A and Giraffa G. 2014. Biotyping of cultivable lactic acid bacteria isolated from donkey milk. *Letters in Applied Microbiology* **59**: 299–305.
- Cavallarin L, Giribaldi M, Soto-Del Rio M D, Valle E, Barbarino G, Gennero M S and Civera T. 2015. A survey on the milk chemical and microbiological quality in dairy donkey farms located in NorthWestern Italy. *Food Control* **50**: 230–35.
- Claeys W L, Verraes C, Cardoen S, De Block J, Huyghebaert A, Raes K, Dewettinck K and Herman L. 2014. Consumption of raw or heated milk from different species: an evaluation of the nutritional and potential health benefits. *Food Control* **42**: 188–201.
- Coppola R, Salimei E, Sorrentino E, Nanni M, Succi M, Belli Blanes R and Grazia L. 2001. Latte d'asina: un substrato ideale per la preparazione di bevande probiotiche. In Proc. 36° Simp. Internazionale Zootecnico Ancona, Italy, 57–61.
- Coppola R, Salimei E, Succi M, Sorrentino E, Nanni M, Ranieri P, Belliblanes R and Grazia L. 2002. Behaviour of *Lactobacillus rhamnosus* strains in ass's milk. *Annals of Microbiology* **52**: 55–60.
- Cosenza G, Mauriello R, Garro G, Auzino B, Iannaccone M, Costanzo A, Chianese L and Pauciullo A. 2019. Casein composition and differential translational efficiency of casein transcripts in donkey's milk. *Journal of Dairy Research* **86**(2): 201–07.
- Criscione A, Cunsolo V, Bordonaro S, Guastella A M, Saletti R, Zuccaro A, D'Urso G and Marletta D. 2009. Donkey milk protein fraction investigated by electrophoretic methods and mass spectrometry analysis. *International Dairy Journal* **19**: 190–97.
- D'Auria E, Mandelli M, Ballista P, Di Dio F and Giovannini M. 2011. Growth impairment and nutritional deficiencies in a cow's milk-allergic infant fed by unmodified donkey's milk. *Case Reports in Pediatrics* **2011**: 1–4.
- Dello Iacono I and Limongelli M G. 2010. Impiego del latte di asina nel bambino con allergia alle proteine del latte vaccino: nuovi contributi. *Rivista di Immunologia e Allergologia Pediatrica* **4**: 10–15.
- Fantuz F, Ferraro S, Todini L, Piloni R, Mariani P and Salimei E. 2012. Donkey milk concentration of calcium, phosphorus, potassium, sodium and magnesium. *International Dairy Journal* **24**(2): 143–45.
- Gastaldi D, Bertino E, Monti G, Baro C, Fabris C, Lezo A and Conti A. 2010. Donkey's milk detailed lipid composition. *Frontiers in Bioscience* **E2**: 537–46.
- Guo H Y, Pang K, Zhang X Y, Zhao L, Chen S W, Dong M L and Ren F Z. 2007. Composition, physiochemical properties, nitrogen fraction distribution and amino acid profile of donkey milk. *Journal of Dairy Science* **90**: 1635–43.
- Hippocrates, Francis Adams (trans.). *The Genuine Work of Hippocrates*, Vol. 1. Sydenham Society; 1843.
- Huang F and Kim J S. 2012. IgE-mediated cow's milk allergy in children. *Pediatric Allergy and Immunology* **12**(6): 630–40.
- Lara-Villoslada F, Olivares M and Xaus J. 2005. The balance between caseins and whey proteins in cow's milk determines its allergenicity. *Journal of Dairy Science* **88**: 1654–60.
- Martemucci G and D'Alessandro A G. 2012. Fat content, energy value and fatty acid profile of donkey milk during lactation and implications for human nutrition. *Lipids in Health and Disease* **11**: 113–27.
- Martini M, Altomonte I and Salari F. 2014. Amiata Donkeys: Fat globule characteristics, milk gross composition and fatty acids. *Italian Journal of Animal Science* **13**(1): 3118.
- Martini M, Altomonte I and Salari F. 2013a. Evaluation of the fatty acid profile from the core and membrane of fat globules in ewe's milk during lactation. *Lebensmittel-Wissenschaft Technologie* **50**: 253–58.
- Michalski M C, Briard V, Desage M and Geloën A. 2005a. The dispersion state of milk fat influences triglyceride metabolism in the rat—a ¹³CO₂ breath test study. *European Journal of Nutrition* **44**: 436–44.
- Monti G, Bertino E, Muratore M C, Coscia A, Cresi F, Silvestro L, Fabris C, Fortunato D, Giuffrida M G and Conti A. 2007. Efficacy of donkey's milk in treating highly problematic cow's milk allergic children: an *in vivo* and *in vitro* study. *Pediatric Allergy and Immunology* **18**: 258–64.
- O'Mahony J A and Fox P F. 2014. Milk: an overview (Chapter 2). (Eds.) Boland M, Sing H and Thompson A. *Milk Proteins: From Expression to Food*, second ed. Elsevier, Oxford, UK, pp. 19–73.
- Pal Y, Kumar S, Mohanty A K and Bhardwaj A. 2016. *Annual Report 2015–2016*, ICAR-National Research Centre on Equines, Hisar, pp 31–32.
- Pal Y, Legha R A, Kumar S, Bhardwaj A and Tripathi B N. 2018. Composition of equine milk in comparison to different milk

- species. XV Annual Convention of Society for Conservation of Domestic Animal Biodiversity (SOC DAB) and National Symposium on ‘Sustainable Management of Livestock and Poultry Diversity for enhancing the Farmers’ Income’ February 8–10, 2018, RAJUVAS, Bikaner, Rajasthan, pp 234.
- Pliny the Elder, *Naturalis Historia*. Second volume, book XXVIII. Bostock J and Riley H T (trans.). (Ed) H.G. Bohn; 1893.
- Raynal-Ljutovac K, Lagriffoul G, Paccard P, Guillet I and Chilliard Y. 2008. Composition of goat and sheep milk products: an update. *Small Ruminant Research* **79**: 57–72.
- Saarela T, Kokkonen J and Koivisto M. 2005. Macronutrient and energy contents of human milk fractions during the first six months of lactation. *Acta Paediatrica* **94**: 1176–81.
- Salerno M, Paterlini F and Martino P A. 2011. *Microbiologia e attività battericida del latte di asina*. (Eds.) Milonis E and Polidori P. Latte di asina: produzione, caratteristiche e gestione dell’azienda asinina. *Fondazione Iniziative Zooprofilattiche e Zootecniche*, Brescia, Italy. ISBN: 978-88-904416-6-0: 193–205.
- Salimei E and Fantuz F. 2012. Equid milk for human consumption. *International Dairy Journal* **24**: 130–42.
- Šariæ L, Šariæ B M, Mandiæ A I, Torbica A M, Tomiæ J M, Cvetkoviæ D D and Okanoviæ D G. 2012. Antibacterial properties of domestic Balkan donkeys’ milk. *International Dairy Journal* **25**: 142–46.
- Schaafsma G. 2003. Nutritional significance of lactose and lactose derivatives. *Encyclopaedia of Dairy Science* (Roginski Fuquay & Fox. London) **3**: 1529–33.
- Schanbacher F L, Talhouk R S, Murray F A, Gherman L I and Willett L B. 1998. Milk-borne bioactive peptides. *International Dairy Journal* **5**(6): 341–598.
- Shekar C P, Goel S, Rani S D S, Sarathi D P, Alex J L and Singh S. 2006. k-Casein-deficient mice fail to lactate. *Proceedings of the National Academy of Sciences, USA* **103**: 8000–8005.
- Tafaro A, Magrone T, Jirillo F, Martemucci G, D’Alessandro A G, Amati L and Jirillo E. 2007. Immunological properties of donkey’s milk: its potential use in the prevention of atherosclerosis. *Current Pharmaceutical Design* **13**: 3711–17.
- Tanaka T. 2007. Antimicrobial activity of lactoferrin and lactoperoxidase in milk, pp. 101–115. *Dietary proteins research trends*. Nova Science Publishers, New York.
- Vandenplas Y, Koletzko S, Isolauri E, Hill D, Oranje A P, Brueton M, Staiano A and Dupont C. 2007. Guidelines for the diagnosis and management of cow’s milk protein allergy in infants. *Archives of Disease in Childhood* **92**: 902–08.
- Zhang X, Zhao L, Jiang L, Dong M and Ren F. 2008. The antimicrobial activity of donkey milk and its microflora changes during storage. *Food Control* **19**: 1191–95.
- Zicker S C and Lönnerdal B. 1994. Protein and nitrogen composition of equine (*Equus caballus*) milk during early lactation. *Comparative Biochemistry and Physiology* **108A**: 411–21.