Composition, Characteristics, Nutritional value and Health Benefits of Donkey Milk-A Review

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Abstract

Donkey milk differs from other ruminant milk as it contains lower fat, higher minerals and lactose. The major antimicrobial proteins in milk are immunoglobulins, lactoferrin, lactoperoxidase and lysozyme. Donkey milk has higher antibacterial activity against *Listeria monocytogenes* and *Staphylococcus aureus* bacteria and it could be effective in the treatment of listeriosis and staphylococcosis. Lysozyme, which is dominant in donkey milk, possesses effective in inactivation of certain viruses, anti-microbial activity, angiogenic inhibition and antitumour. Donkey milk whey protein possesses potent antiproliferative activity which has potential in the treatment of lung cancer. The protein, α-lactalbumin has a property of antiviral, antitumor and anti-stress. Donkey milk has low amount of casein very close to human milk and contains lower amount of β-lactoglobulin compared to cow milk. Donkey milk has higher amount of lactose, helps in good palatability, avoids allergy in infants and is an option for infants suffering from cow's milk protein allergy (CMPA) and lactose intolerance. Donkey milk has multiple effects on the osteogenesis process,

arteriosclerosis therapy rehabilitation of patient coronary heart disease, premature senescence and in hypocholesterolemic diets. Donkey milk is used in the production of fermented beverages, it has probiotic *lactobacilli* strains. Donkey milk is enriched with good amount of vitamins, minerals and poly unsaturated fatty acids (PUFA), thus offering the skin care. Due to less awareness and availability, the uses of donkey milk are limited. Further studies should be conducted on the chemical composition and medicinal value of donkey milk.

Keywords: Donkey milk, Chemical composition, Medicinal value, Protein, Probiotic

Introduction

Donkey is a complex creature, capable of many moods. It can be friendly, affectionate, patient, independent and especially intelligent. It has a keen sense of curiosity and an incredible memory. But it is stubborn in an original way. It is a hard animal and resistant to disease, normally it loves the hot and dry climates. Donkey has a slow gait and it is most active in the evening.

The donkey (*Equus asinus*) is a member of the horse family, domestication began about 6000 BC in North Africa, over the centuries donkeys have spread in Asia, India, South-America and South Europe (Bordonaro et al. 2012). According to Food and Agricultural Organization Statistics (FAOSTAT 2013), among the Asian countries, India reported to be 8th position in donkey's/asses (300000) population, after China, Pakistan, Iran, Afghanistan, Yemen, Iraq and Uzbekistan. According to Dept. of Animal Husbandry, Dairying and Fisheries (DOAHDF 2012) and 19th livestock census report, the per cent share of donkey population during 2012 was reported to be 5.12% in Karnataka and highest of 25.56% was reported in Rajasthan state.

The size of donkey varies considerably from 0.9 m to 1.6 m. The gestation is approximately of 12 months (Mariani 2008). The lactation period of Domestic Balkan donkey breed varies from 45 to 200 days (Gubic et al. 2014). Life of donkey is 25-30 years.

Few species exist in the wild: some of them are the African, Asiatic and Himalayan (Mariani 2008). A male donkey is referred to as a jack and a female donkey is called a jenny or jennet (Hagstrom 2004).

The classifications of African and Asiatic wild donkeys were based on their height and colour. Food and Agriculture Organization (FAO) has reported three distinct types of Indian asses *viz.,* Indian, Indian wild and Kiang. Indian wild asses are available in Rann of Kutch (Gujarat) while Kiang is available in Sikkim and Laddakh. They are dark red brown with white under parts and patch behind the shoulder. Among Indian, two major types of donkeys *i.e.,* those of larger size and smaller size are common. The larger size donkeys are light grey to almost white in colour. The smaller size ones are dark grey in colour (www.nrce.nic.in). Donkeys are majorly reared for four reasons: work, breed, milk and meat. Among these work is more important. Donkeys are used mainly as pack animals, either for carrying loads or for riding.

Donkeys remain essential for rural economies 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 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). Compared with ruminant milk, donkey milk has been studied less in the past, but in the last years, research interest and capital investment in donkey milk have increased because its composition is similar to that of human milk. The physico-chemical composition is represented in Table 1 (Polidori and Vincenzetti 2013).

Importance of milk

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. Milk proteins appear to be an exciting link between nutrition,

dietetics and therapy; today, consumers can expect more than just nutrition from intake of milk proteins. 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). 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 child hood; affecting 2 to 5% of the child population with less than three years of age (Huang and Kim 2012).

Symptoms of CMPA can appear immediately or start several hours or even days after the intake of moderate to large amounts of cow milk or its infant formula. Clinical symptoms arise from skin (e.g. pruritus and urticaria), the gastrointestinal (e.g. abdominal pain and diarrhoea) and respiratory (e.g. asthma) tracts (Carroccio et al. 2000; Sicherer 2000). Bovine milk proteins (β-LG and β-casein) is regarded as one of the a major food allergies, which affects primarily infants who's enzyme system isn't developed yet, but may also persist throughout adulthood (Villoslada et al. 2005). Casein acts as a potent allergen in CMPA where each different fraction (S1, S2, β and κ-casein) can induce specific Ig-E responses. The largest phosphorylation sites appear to be a major allergen epitope in caseins and changes in these regions could affect the allergenicity of these (Naspitz et al. 2004). According to a study by Reis and Vaz (2004), caseins were predominantly allergenic and immunogenic in patients with IgE-mediated CMPA compared to whey proteins (Rangel et al. 2016). Several studies revealed that caseins and β-lactobglobulin are the main allergens in cow milk (Monti et al. 2007; Tafaro et al. 2007; Tesse et al. 2009). In the last few years, milk from non-bovine mammals has been studied to

identify the best natural substitute for human milk (Businco et al. 2000; Muraro et al. 2002; Restani et al. 2009). Donkey milk has been successfully used in clinical studies on children with Cow Milk Allergy and found to provide nutritional adequacy and good palatability.

Physico-chemical composition of donkey milk

The donkey milk is as white, thin, with a slightly sweet pleasant taste, pleasant milky aroma, sweet flavour and no persistent after taste. Milk has very low somatic cell counts and total microbial counts (Malissiova et al., 2016). According to most authors, the composition of protein content varies considerably among species and is influenced by breed, stage of lactation, feeding, climate, parity, season and udder health status (Gubic et al. 2016).

Milk proteins appear to be an exciting link between nutrition, dietetics and therapy. The major antimicrobial proteins in milk are immunoglobulins, lactoferrin, lactoperoxidase and lysozyme (Tanaka, 2007). Immunoglobulins (Ig-G, Ig-M and secretory Ig-A) act by a specific mode of action involving antigen-antibody reactions (Malacarne et al. 2002). The detailed chemical composition of donkey milk is reviewed hereunder.

Proteins: Milk protein is a very heterogeneous group of molecules, classified into five main categories: caseins, whey proteins, milk fat globule proteins, enzymes and other miscellaneous minor proteins (Hang 2003). The protein composition of donkey milk is significantly different from cow milk: the total content is lower (1.5-1.8 g/100g) and quite similar to that of human and mare milk: this condition avoids an excessive renal load of solute (Malacarne et al. 2002).

Lactose: Donkey milk has high amount of lactose content (5.8-7.4) which is higher than cow milk and much similar to human milk. The high content is responsible for the good palatability of milk and facilitates the intestinal absorption of calcium that is essential for infant's bone mineralization. Lactose gives good taste to donkey milk (Iacono et al. 1992;

Monti et al. 2007) and is also a precious source of galactose, essential for the development of the nervous system.

Compared with ruminant milk, donkey milk presents high lactose content, therefore resulting as inadequate for people suffering from lactose intolerance (10 to 60% of the population). (Heyman 2006; Lomer et al. 2008) reported that the fermented milk products such as yogurt can be tolerated by lactose-intolerant people because they contain live bacteria, which help to convert the lactose into lactic acid. Nevertheless, the main differences are given by the presence in donkey milk of minor components at low partition number (PN) values and Triacylglycerols (TAGs) with high unsaturation degree, due to the larger amount of PUFA in this milk.

Donkey milk is a valuable and safe source for the nutrition of cow milk intolerant infants as well as an interesting nutraceutical food. Moreover, milk confirms a natural source of lipids with high similarity to human milk and therefore high nutritional value in the regulation of the immune inflammatory system (Chiofalo et al. 2011). The three major whey proteins in donkey milk are α-lactalbumin, β-lactoglobulin and lysozyme. Donkey milk α-lactalbumin has two isoforms with different isoeletric point (Giuffrida et al. 1992). Recently, it has been shown that α-lactalbumin presents antiviral, antitumor and antistress properties.

In particular, human breast milk shows that the α-lactalbumin forms a complex with oleic acid called HAMLET (Human Alpha-lactalbumin Made Lethal to Tumor cells) that proved to be able to induce tumour-selective apoptosis. This complex may be considered as a potential therapeutic agent against various tumour cells (Zhang et al. 2009). Furthermore it has shown that the α-lactalbumin possesses antiinflammatory activity exerted by the inhibition of cyclooxygenase-2 (COX-2) and phospholipase A2 (Yamaguchi et al. 2009). One of the main protein allergens in children is β-lactoglobulin that is the major whey protein in

cow milk (Carroccio et al. 1999), which is absent in human milk. The content of β- lactoglobulin in donkey milk is approximately 40% of the whey proteins equal to the level in mare milk and lower than that in cow milk (Miranda et al. 2004). This condition may be related to the hypoallergenic characteristic of donkey milk (Carroccio et al. 2000; Businco et al. 2000; Iacono et al. 1992).

With references to the many authors the donkey milk shown well tolerated by children with cow milk protein allergy (CMPA) in terms of clinical tolerability (Carroccio et al. 2000; Muraro et al. 2002; Restani, et al. 2002; Monti et al. 2007; Monti et al. 2012). Donkey milk shows the best aspirant as a substitution of human milk for clinical tolerability, palatability and nutritional adequacy for children affected by CMPA, furnishing additional physiological functions as well, such as providing antibacterial substances, digestive activity molecules, growth factors and hormones (Polidori et al. 2015).

The results revealed the donkey milk has low allergenicity is mainly due to the low casein content (Vincenzetti et al. 2007) and is very close to the casein content determined in human milk. The caseins composition in donkey milk, (Vincenzetti et al. 2008; Criscione et al. 2009) showed the presence of αS-1 and β-caseins that are present in different phosphorylated and glycosylated forms, while the presence of κ-casein and αS2-casein is in very small amounts, which is differently to cow milk (Bertino et al. 2010; Creamer 2002).

The mechanism for tolerance may be related to the specific levels of the major allergenic components in the milk. Donkey milk has three genetic variants for β-lactoglobulin: one presents three amino acid substitutions while the others have two amino acid exchanges (Herrouin et al. 2000). Donkey milk β-lactoglobulin is a monomer whereas this protein is a dimer in ruminant's milk. The protein β-lactoglobulin is belongs to the lipocalin family and has high affinity for a wide range of compounds opening the way to various suppositions about its function. In fact, it has been shown that this protein is involved

in hydrophobic ligand transport and uptake, enzyme regulation and the neonatal acquisition of passive immunity, other authors demonstrated that β-lactoglobulin forms complexes with folic acid suggesting that these complexes could be used as an effective carrier of folic acid in functional foods (Liang et al. 2008).

Different amino acids like Ser, Glu, Arg, Val were higher in donkey milk and have lower level of Cys, which exhibited unique nutritional characteristics and has optimal potential to be used as a new dietetic food and breast milk substitute because of similar results found in physic-chemical composition of human milk (Rathore et al. 2011). Donkey milk is a good source of essential fatty acids; these subjects are in fact at risk of developing a deficiency in essential fatty acids and particularly in PUFA n–3, which are absolutely necessary for adequate growth, neurological development and cardiovascular health (Echevarria et al. 2008). Donkey milk shows an high content of both linoleic (C18:2) and linolenic (C18:3) acids, respectively 9.0 g/100 g and 5.1 g/100 g of total fatty acids, when compared with ruminant species milk, in which the contents of the above mentioned polyunsaturated fatty acids are always lower (Carroccio et al. 1999; Iacono et al. 1992).

Fat: Donkey milk shows a lower fat content compared to human milk (Dugo et al. 2005) ranges between 0.03 and 1.18 kg/kg (Tidona et al. 2011a) with an increasing, nonlinear, trend from partum to the end of lactation (Salimei et al. 2004; Guo et al. 2007), it is beneficial in the diet therapy to prevent cardiovascular, autoimmune and inflammatory diseases. In particular, the high value of polyunsaturated fatty acids (PUFA) (ω6 and ω3) (52.2%), the low ω6 to ω3 ratio and the advantageous values of atherogenic and thrombogenic indices (Martemucci and D'Alessandro 2012) tend in human diet. To lower the level of cholesterol in blood, to prevent the formation of atherosclerotic plaques, removing the risk of coronary heart disease, hypertension and thrombosis (D'amico et al. 2007; Agostino et al. 2007),

suggested the donkey milk as a functional food for infant nutrition, but also for adults that have to follow particular diets.

The pH value is around 7.2, which is slightly higher than cow milk (6.7), but closer to human (7.3) and mare milk (7.18). It varies with respect lactation, even if the difference is not statistically significant (Guo et al. 2007).

Minerals: Minerals are very important to human body growth and development of their skeletal structure. Milk is the main source of minerals. Due to differences in breed and stage of lactation an average of 0.3 to 0.9 g/100 g is present in donkey milk which is similar to human milk and higher than cow milk (Fantuz et al. 2012), even with respect to lactation the composition will varies. Donkey milk is also a rich source of various minerals like Ca, P, Na, K, Mg, Fe, Zn, Cu (Salimei et al. 2004; Salimei et al. 2012).

Vitamins: The amount of several vitamins has not yet been detected in donkey milk, it is known that mare milk contains a significantly higher level of vitamin C than cow milk. In donkey milk, with exception for niacin (vitamin B_3), the amount of thiamine (vitamin B_1), riboflavin (vitamin B_2), cobalamin (B_3) and vitamin C is higher than in human milk (Salimei et al. 2012).

Health benefits of donkey milk

Donkey milk could be effective in the treatment of listeriosis and staphylococcosis, (Tidona et al. 2011b) reported, high resistance of donkey milk lysozyme to the degradation by gastro-intestinal enzymes, while (Nazzaro et al. 2010a) detected the presence of compounds in hydrolyzed donkey milk, different from cow milk, capable of inhibiting the growth of *S. aureus*. Donkey milk has higher antibacterial activity against *L. monocytogenes* and *S. aureus bacteria*, which was tested at 38 °C after 8 h of incubation of raw milk. The lysozyme had stronger activity against *S. aureus.* This probably indicates that lysozyme is not the only antimicrobial agent in donkey milk which can also acts against tested Gram positive

bacteria, since it is a complex medium with numerous different compounds which could contribute to the overall antimicrobial potential. The experimental results revealed that lysozyme could be marked as the major antibacterial agent in donkey milk, because it is present in high concentration (Saric et al. 2014).

The concentrations of three proteins with antimicrobial effect are compared from donkey, human and cow milk. From these data it is evinced that human and donkey milk contain considerable amounts of lysozyme and lactoferrin but lactoperoxidase is in small amounts. The lysozyme content in donkey milk is 1.0 g/L, human milk is 0.12 g/L and only trace amounts presents in bovine milk which is less as compared to human and donkey milk. Lactoperoxidase is lower in donkey milk (0.11 mg/L) compare to human milk (0.77 mg/L) and bovine milk (30-100 mg/L). Lactoferrin is also lower in donkey milk (0.080 g/L) compared to human (0.3-4.2 g/L) and bovine (0.10 g/L) milk (Polidori and Vincenzetti 2012).

Lysozyme, lactoperoxidase and lactoferrin have been recognized as antimicrobial and bacteriostatic agents and could be useful to prevent intestine infections in infants. Their action may extend the conservation of fresh donkey milk and the relative potential commercial supply (Polidori and Vincenzetti 2013). Lactoferrin is a protein of the transferring family and is particularly resistant to the proteolytic degradation in contrast to other milk protein (Iver and Lonnerdal 1993) and it is an iron-binding protein that displays many biological functions: regulation of iron homeostasis, cellular growth, antimicrobial and antiviral functions and protection against cancer development and metastasis (Ward et al. 2005).

Also, it controls the proper composition of the intestinal microflora suppressing the growth of pathogenic bacteria and promoting the multiplication of non-pathogenic *Lactobacillus* and *Bifidobacterium* (Mariani 2008). The amount of lysozyme, β-lactoglubulin and α-lactalbumin concentrations (mg/ml) varied at different stages of donkey lactation (60,

90, 120, 160 and 190 days after parturition), which was determined by RP-HPLC analysis (Polidori and Vincenzetti 2012).

The amount of lysozyme in donkey's milk varied considerably during the different stages of lactation, with a mean value of 1.0 mg/ml and proved to be higher with respect to that in bovine (traces) and human (0.12 mg/ml) (Stelwagen 2003). The mean β-lactoglobulin content in donkey milk (3.75 mg/ml) was very close to bovine milk (3.3 mg/ml). The α-lactalbumin content increased in the three months after parturition till the value of 1.8 mg/ml, close to the α-lactalbumin content in human milk (1.6 mg/ml). Lysozyme in donkey milk could contribute to its anti-inflammatory and antimicrobial activity (Salimei et al. 2004; Zhang et al. 2008). In addition, lysozyme has other physiological functions, including inactivation of certain viruses, immunoregulatory activity, antiinflammatory and antitumour activity (Ibrahim and Aoki 2003).

Several studies have indicated that lysozyme possesses effective angiogenic inhibition and anti-tumour activities (Jiao et al. 2004; Sava 1989; Ye et al. 2008). The antitumour effect of donkey milk is naturally rich in lysozyme. The basic whey proteins in donkey milk are β-lactoglobulin, α-lactalbumin, immunoglobulins, blood serum albumins, lactoferrin and lysozyme (Vincenzetti et al. 2008). The β-lactoglobulin is present in donkey milk as a monomer (Polidori and Vincenzetti 2012) and has better digestibility in newborns due to higher digestibility and absorption of soluble monomer proteins (Lonnerdal, 2003; Barłowska, 2011). Donkey milk whey protein possesses potent antiproliferative activity, may have potential in the treatment of lung cancer, although invivo evidence would be required to determine such possible actions (Mao et al. 2009), whey protein content also varies with respect to lactation period. The deep knowledge of the protein composition and variability could be beneficial for a more appropriate use in infant feeding. The high content of protective antimicrobial compounds in donkey milk taken from the early and middle lactation

period suggested its beneficial impact on gut health and immune defense system (Gubic et al. 2014; Gubic et al. 2016).

The fatty acids composition of the milk sample was determined using capillary gas chromatography. The role of PUFA, important in the development of the neonatal brain as well as in the development of the retina and cognitive functions (Fleith et al. 2005). The high content of n3 fatty acids in donkey milk could have a significant effect on the development of the neural system, vision and infant growth. The beneficial health effects of n3 fatty acids include inflammatory disease, such as rheumatoid arthritis (Simopoulos 2002), dermatitis (Horrobin 2000), cancer (Dupertuis et al. 2007), depression and dementia and it can be potentially used to treat lateonset Alzheimer's disease (Ruxton et al. 2004). The high PUFA, n3 content in donkey milk could have a functional effect on the immunological system. Donkey milk exhibited the ability to induce Ig-G secretion and the release of interleukins (IL12, IL1β and IL10) and TNFα is important to the immune treatment of immune related disease and a high release of nitric oxide (NO), a potent promoter in the prevention of atherosclerosis.

Indeed, NO is a strong vasodilator of terminal vessels, improves blood flow, and is an effective antimicrobial agent in the development of atherosclerosis, at moderate intake level (200 mL/day) (Jirillo et al. 2010), can upregulate the immune response in elderly hosts. The overall unsaturated fatty acid (UFA) content of the milk is 48.02 ± 2.97% and is increased during the lactation period. The mean UFA/SFA ratio was 0.92, which underwent a significant increase over time, reaching a value greater than 1% at day 120 and a peak of 1.50% at day 180. This result reflects the higher PUFA n3/n6 ratio at the 7 month of lactation (day 210) (Martemucci and D'Alessandro 2012).

Donkey milk consists of lower percentages of 5,8,11,14,17-eicosapentaenoic acid (EPA), 4,7,10,13,16,19-docosahexaenoic acid (DHA) with low LC-PUFA n-6 content (with

particular reference to amino acids and the better quality of the lipid pattern in donkey milk versus cow milk observed, which is recognized to play an important role in cognitive development and therapy of atopy) (Echevarria et al. 2008), make donkey milk inadequate as an exclusive food in infants for the first year of life. In donkey milk the position of fatty acids on glycerol backbone, above all of long chain fatty acids (LC-SFA), is very similar to that of human milk: this fact, in conjunction with the relatively high contents of medium chain triacylglycerol (MCT), makes the lipids in donkey milk, through quantitatively modest, highly bioavailable. Within a well balanced and integrated diet, donkey milk is a precious source of essential fatty acids. These fatty acids are of particular importance in the diet of subjects with CMPA, especially if affected by multiple food allergies (Gastaldi et al. 2010).

The use of donkey milk by humans for alimentary and cosmetic purposes has been popular since ancient times, but it was not until the renaissance that the first real scientific consideration was given to this exceptional milk, more recently it has been used successfully as a substitute for human milk in Western Europe (Vincenzetti et al. 2008), as an alternative food for infants with food allergies and to upregulate the immune response of healthy elderly humans (Salimei and Fantuz 2012) and used in the production of fermented beverages, it has probiotic *lactobacilli* strains also (Nazzaro 2010b). Moreover, donkey milk whey proteins showed *in vitro* antiproliferative and antitumor activity (Mao et al. 2009). These properties have allowed its diffusion as fresh milk, milk powder and even in cosmetic products (Iannella 2015). Basically, donkey milk has nutritional properties that make it more similar to human milk, potential to be used as a new dietetic food and breast milk substitute. Hence, it is suggested that milk powder based on donkey milk could be a good alternative for all those infants or new born remain deprived from mother's milk (Rathore et al. 2011).

Conclusions

Donkeys can play a vital role in the economy. Donkeys are majorly reared for four reasons: work, breed, milk and meat. Among these work is more important and recently the concentration on donkey milk has increased because of its multidisciplinary usages such as health issues, medicinal, cosmetics and substitute to infants because of the most similar physicochemical composition compared to human milk. Donkey milk is unique containing various protective proteins like α-lactalbumin, lysozyme, lactoferrin, lactoperoxidase, Immunoglobulins which exert antioxidatives, antibacterial, antiviral, antifungal, hypoglycaemic, antiparasitic, growth promotion, aging prevention, autoimmune diseases and anti-tumour activity.

Donkey milk cures several food allergies, skin diseases and especially it can be used for infants who are suffering from CMPA and lactose intolerance. Donkey milk can be considered as a substitute of human milk, when infant is weaned or mother is ill. Although donkey milk has such values, it is less appreciated thus its consumption is restricted because of lack of awareness and myths.

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