Nutritional qualities of donkey milk

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Introduction

Milk is a biological fluid designed to contain all nutritional requirements of a specific mammalian newborn; therefore, the composition of milk differs by the needs of the neonate of different species. Although much research has been devoted to milk composition in the domestic horse, donkey's milk has recently aroused scientific interest, above all among paediatric allergologists and nutritionists. Clinical studies have demonstrated that donkey milk may be considered a good replacer for dairy cow's milk in feeding children with severe Ig-E mediated cow's milk protein allergy, when human milk can not be given (*Carroccio, A. et al., 2000*). For these patients, donkey milk is not only useful (*Monti, G. et al., 2007*), but also safer compared with milk obtained by other mammalian species (*Polidori, P. et al., 2009*), due to the high similarity with human milk, especially considering protein fractions content (*Salimei, E. et al., 2004; Vincenzetti, S. et al., 2008*).

The donkey (*Equus asinus*) is a member of the horse family and its progenitor was the small gray donkey of northern Africa (*Equus africanus*) domesticated around 4000 BC on the shores of the Mediterranean Sea. It worked together with humans for centuries; the most common role was for transport. It still remains an important work animal in the poorer Regions. Compared with ruminant's 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 (see Table 1).

Table 1

Comparison of chemical composition and physical properties of donkey and human milk (*Guo, H.Y et al., 2007*).

	Donkey	**Human**
pH	7.0-7.2	7.0-7.5
Protein (g/100g)	1.5-1.8	0.9-1.7
Fat (g/100 g)	0.3-1.8	3.5-4.0
Lactose (g/100 g)	5.8-7.4	6.3-7.0
Ash (g/100 g)	0.3-0.5	0.2-0.3
Total Solids (g/100 g)	8.8-11.7	11.7-12.9
Caseins (g/100 g)	0.64-1.03	0.32-0.42
Whey Proteins (g/100g)	0.49-0.80	0.68-0.83

Cow's milk protein allergy

Adverse reactions to food are currently classified into toxic and non-toxic reactions. Non-toxic adverse reactions to milk are primarily caused by either lactose intolerance or milk allergy. Milk intolerance is due to the inherited lack of the specific enzyme, β-galactosidase that is required to hydrolyze lactose. For lactose intolerance, the most common therapeutic approach excludes lactose-containing milk from the diet.
Cow's milk protein allergy (CMPA) is defined as an immunological reaction to one or more milk proteins (*Hill, D.J et al., 1986*). A variety of symptoms can be suggestive for CMPA. CMPA is suspected clinically in 5-15% of infants (*Høst, A., 2002*), while most estimates of prevalence of CMPA vary from only 2 to 5 %. Confusion regarding CMPA prevalence is often due to differences in study population, and a lack of defined diagnostic criteria for CMPA. The importance of

defined diagnostic criteria needs to be emphasised. It precludes infants from an unnecessary diet (*Vandenplas, Y. et al., 2007*) and avoids delay in diagnosis, which can lead to malnutrition (*Viera, M. et al., 2010*).

Cow's milk is a member of the "Big-8" food allergens that include egg, soy, wheat, peanuts, tree nuts, fish and shellfish in terms of prevalence (*Crittenden, R.G. and Bennett, L.E., 2005*). The incidence of CMPA varies with age. CMPA is prevalent in early childhood with reported incidences between 2 and 6% (*Garcia-Ara, M.C. et al., 2004*) and decreases into adulthood to an incidence of 0.1–0.5% (*Woods, R.K et al., 2002*). It has been suggested that infants have milk allergies because milk is usually the first source of foreign antigens that they ingest in large quantities, and the infant intestinal system is insufficiently developed to digest and immunologically react to milk proteins. When milk is eliminated, the inflammation response is controlled. After several years, oral tolerance is developed, and milk can again be tolerated (*Høst, A., 1999*).

For human beings cow's milk represents the most common feeding during the infant weaning, but also the first allergen in life. In many countries cow's milk is the most important food allergen in babies and children (*Hill, D.J. and Hosking, C.S, 1996*). Adverse reactions to cow's milk were found in 2% of babies during the first year of life: 30% of cases at the first month, 60% before the third and 96% within the twelfth (*Stintzing, G. and Zetterstrom, R., 1979*). Symptoms can even appear during the breast-feeding because newborn reacts against a small amount of cow milk proteins present in maternal milk (*Høst, A. et al., 1988*). Children followed for the first 3 years of life, 56% of cases had recovered from cow's milk allergy at 1-year age, 77% at 2 years and 87% at 3 years age (*Høst, A. and Halken, S. 1990*). However allergy can persist for all life. Considering the possible use of alternative milk sources for human in cases of cow's milk allergy, the use of goat's milk should be avoided because of the high risk of cross-reactivity , while mare's and donkey's milks, used in popular practice for allergic children, are valid alternative protein sources when appropriately evaluated from the hygienic point of view (*Restani, P. et al., 2002*). The discussion on the use of soy-based infant formula is difficult, since scientific societies have different recommendations. There is a broad consensus on the following statements: the incidence of soy allergy in soy formula-fed infants is comparable to that of CMPA in cows' milk formula-fed babies (*De Greef, E. et al, 2012*). Cross reactivity to soy has been reported in 10 to 35% of infants with CMPA, regardless whether they were positive or negative for specific IgE for CMPA. In particular, infants with multiple food allergies and eosinophilic enterocolitis also react to soy protein; therefore, different specialist groups have different standpoints on the use of soy formula for CMPA, but is generally not recommended before the age of 6 months (*De Greef, E. et al, 2012*).

Donkey milk protein fractions

In a study performed in order to determine the different protein fractions in donkey milk (*Vincenzetti, S. et al, 2008*) it was possible to separate 9 peaks that were identified as β-caseins (sequence: REKEELNVSS) and α_{S1}-caseins (sequence: RPKLPHRQPE), having different molecular weights. Reversed-phase chromatography on HPLC (RP-HPLC) followed by 15% SDS-PAGE and N-terminal analysis was performed on the skimmed donkey's milk giving as a result three main peaks identified as lysozyme (sequence, KVFSKXELA), α-lactalbumin, (sequence, KQFTKXELSQVLXSM), and β-lactoglobulin (sequence TNIPQTMQ), respectively (Table 2).

This study revealed the presence of β-caseins (sequence: REKEELNVSS) and αs_1-caseins (sequence: RPKLPHRQPE), which presented marked homology with αs_1- and β-caseins from mare's milk (*Egito, A.S. et al. 2002*), while the presence of other types of caseins, such as α s_2-, γ- and k- were not determined in donkey milk. This result show another high similarity between donkey and human milk: in fact, the presence of αs_2-caseins in human milk has not been demonstrated (*Egito, A.S. et al. 2002*).

Table 2

Donkey's milk protein fractions (*Vincenzetti, S. et al, 2008).*

Protein	kDa	N-terminal sequence
Lysozyme	14.60	KVFSKXELA
α-lactalbumin	14.12	KQFTKXELSQVLXSM
β-lactoglobulin	22.40	TNIPQTMQ
αs_1-casein	33.30	RPKLPHQPE
β-casein	37.50	REKEELNVS

Thanks to RP-HPLC analysis, it was possible also to calculate the lysozyme, β-lactoglubulin and α-lactalbumin concentrations (in mg/ml) at different stages of lactation (60, 90, 120, 160 and 190 days after parturition), the results are shown in Table 3.

Table 3

Quantitative determination of lysozyme, β-lactoglobulin, α-lactalbumin in different stages of lactation (*Vincenzetti, S. et al, 2008).*

Days after parturition	**Lysozyme (mg/ml)**	β-lactoglobulin **(mg/ml)**	α-lactalbumin **(mg/ml)**
60	1.34	Not determined	0.81
90	0.94	4.13	1.97
120	1.03	3.60	1.87
160	0.82	3.69	1.74
190	0.76	3.60	1.63

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 tothat in bovine (traces), human (0.12 mg/ml) and goat'smilk (traces), whereas, it was very close to mare's milk (0.79 mg/ml), as demonstrated by Miranda, G. et al. (2004). The mean β-lactoglobulin content in donkey's milk (3.75 mg/ml) was very close to that of bovine milk (3.3 mg/ml) and pony mare's milk (3.0 mg/ml), whereas in human milk the β-lactoglobulin is absent (*Egito, A.S. et al. 2002*). 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) but lowest compared to the pony mare's α-lactalbumin content (3.3 mg/ml) (*Uniacke-Lowe, T. et al., 2010*).

Conclusions

In conclusion, donkey milk has been indicated as a valuable and safe source for the nutrition of cow-milk intolerant infants as well as an interesting nutraceutical food for older people, therefore as a brand new functional food. Moreover, the results here discussed confirm that it represents a natural source of proteins with high similarity to human milk. Nevertheless, the use of donkey milk for therapeutic purposes is hampered by several factors: difficulties in finding food, ensuring continuity of supply, medical legal risk associated with the administration of non-conventional diet therapy. In this context the valorisation of "donkey milk" as food, considering that the Regulation (EC) No 853/2004 lays down specific hygienic rules for food on animal origin including donkey raw milk, could represent an incentive for the breeding of donkeys and, therefore, an indispensable factor to preserve the animal biodiversity. Therefore, the implementation of the good practices throughout the global "dairy donkey" food chain is a crucial topic to guarantee the safety and quality of donkey milk for Public health.

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