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MAIN LECTURE

Donkey milk production: state of the art

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ABSTRACT - Milk is one of the most common causes of food allergies among children under one year of age. No specific therapy exists for this allergy, and thus the only feasible response is to avoid assumption of milk and derived products. Studies conducted on the serum of children with hypersensitivity to milk have shown that caseins are the proteins with the greater allergenic potential. However, in some cases, children have also shown hypersensitivity to the β -lactoglobulines and to the α -lactalbumins. When food intolerance is diagnosed in an infant, it is often necessary to impose a period of total parenteral feeding, followed by breast feeding, considered the most correct method of re-feeding. When human milk can not be given, alternative food sources must be sought. Clinical studies have demonstrated that donkey milk could substitute breast feeding in infants affected by severe Ig-E mediated milk allergies. In these subjects, donkey milk is not only useful, but also safer than other types of milk. In fact donkey milk composition in lipids (high levels of linoleic and linolenic acid) and proteins (low caseins content) is very close to human milk. Lysozyme content in donkey milk resulted to be very high (mean value 1.0 mg/ml) if compared to bovine (traces), caprine (traces) and human milk. The high lysozyme content of donkey milk may be responsible of the low bacterial count reported in literature and also makes this milk suitable to prevent intestine infections to infants. Among seroproteins, β -lactoglobulin and α -lactalbumin content in donkey milk was respectively 3.75 and 1.80 mg/ml and remained substantially the same during the different stages of lactation.

Key words: Donkey milk, Milk quality, Milk chemical composition.

Introduction - Milk is a fluid secreted by female mammals for the nutrition of its offspring. In addition to meeting the complete nutritional requirements of the neonate, milk serves several physiological functions, including protective (immunoglobulins and other antibacterial agents), digestive aids (enzymes and enzyme inhibitors, binding or carrier proteins) and growth factors/hormones (Fox, 2003). Milk has a fundamental nutritional importance in infancy. Breast milk of a healthy, well-nourished woman is the best reference for nutritional requirements during the early neonatal period (Donnet-Hughes *et al.*, 2000). Commercial infant formulas have been developed as normal nutritional substitutes for breast milk and mimic, where possible, the levels and types of vitamins, minerals and other nutrients present in human milk. Despite this, differences in response to infection, development of allergies and atopic diseases have been reported in formula-fed compared with breast-fed infants (Restani *et al.*, 2002).

When a mother cannot breastfeed, or chooses not to breastfeed, the use of a milk substitute must provide the best option to meet the nutritional and health needs of the infant.

Cows' milk is widely used as a substitute for human milk but, in an increasing number of cases, it can lead to an abnormal immunological response (Criscione *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.*, 2002). Donkey's milk has successfully been used in clinical studies on children with Cow Milk Allergy, and found to provide nutritional adequacy and good palatability (Monti *et al.*, 2007).

Definition of cow milk allergy (CMA) - The term allergy or hypersensitivity is used for immune-mediated reactions, and the term intolerance for nonimmune-mediated reactions (Pelto *et al.*, 2003). Immune-mediated reactions may be immunoglobulin (IgE)-mediated or non-IgE-mediated, whereas intolerance may be enzymatic, pharmacologic or undefined food intolerance. The terms allergy and hypersensitivity are used variably in literature: normally the term allergy is used in infants and children, while hypersensitivity is used in adults for adverse clinical reactions based on any type of abnormal immune response to milk as an allergen. Cows' milk proteins are the first source of foreign proteins given in large quantities to an infant, and in the immature intestine it may provoke allergic reactions (Sampson, 1999). Cows' milk contains several proteins known to be potential antigens: they can be generally classified in two major groups: caseins and whey proteins (Restani *et al.*, 2002). Because β -lactoglobulin is absent from human milk, it has long been considered the most important of cows' milk allergens (Savilahti and Kuitunen, 1992), but several studies have demonstrated that the casein fractions, too, have an important allergic potential (Muraro *et al.*, 2002). Cows' milk is one of the most common food allergies in children; the incidence of CMA ranges from 0.3 to 7.5% in population based studies in different countries; early childhood allergies may predispose the development of atopic allergies in later life (Iacono *et al.*, 1992). The wide range in these estimates may be due mainly to different diagnostic criteria in addition to other factors such as race, age of tested patients, type of infant feeding, as well as the duration of observations (Taylor, 1986). Symptoms of CMA 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/or respiratory (e.g. asthma) tracts (Carroccio *et al.*, 2000; Sicherer, 2000). Delayed reactions happen after T-cell dependent mechanisms have been elicited and can be operative both at the skin and the intestinal level (Taylor, 1986). Cows' milk contains more than 20 proteins (allergens) that can cause allergic reactions (El-Agamy, 2007). Whey proteins include: α -lactalbumin, β -lactoglobulin, bovin serum albumin and immunoglobulin. In addition to those, other minor proteins are also present in cow milk (Vincenzetti *et al.*, 2008). Several studies revealed that caseins and β -lactoglobulin are the main allergens in cow milk, and the interest on the use of donkey milk for the treatment of infant population suffering for cow milk allergy is greatly increasing in the recent years (Monti *et al.*, 2007; Tafaro *et al.*, 2007; Tesse *et al.*, 2008).

Donkey milk composition - Dairy products have been a part of human diet for more than 7000 years (Piccione *et al.*, 2008). The properties of equine milk differ from that one of other mammals in many ways that include important differences in nutritional value; moreover, its composition does not permit the production of cheese due to the high content of whey proteins that represent 35-50% of the nitrogen fractions (Chiavari *et al.*, 2005).

Table 1. Range of variation of chemical composition and pH values of donkey, human and cow milk.

	Donkey	Human	Cow
Total Solids (g/100g)	8.8-11.7	11.7-12.9	12.5-13.0
Protein (g/100g)	1.5-1.8	0.9-1.7	3.1-3.8
Fat (g/100g)	0.3-1.8	3.5-4.0	3.5-3.9
Ash (g/100g)	0.3-0.5	0.2-0.3	0.7-0.8
pH	7.0-7.2	7.0-7.5	6.6-6.8

Source: adapted from Guo *et al.*, 2007.

Furthermore donkey milk also contains an high amount of lysozyme, which is practically absent in the milk of cows, ewes and goats (Vincenzetti *et al.*, 2007); this enzyme possesses bactericidal properties as it hydrolyzes the polysaccharides of bacterial cell walls and inhibits bacteria development (Vincenzetti *et al.*, 2005). Recently, research interest in donkey milk has increased in Europe, especially in Italy, because its composition is similar to that of human milk (Carroccio *et al.*, 2000; Salimei *et al.*, 2004). Data available about chemical composition of donkey milk have been reviewed by Guo *et al.* (2007); the level of fat in donkey milk ranges from 0.28% to 1.82% (Table 1). Because of the low level of fat, Carroccio *et al.* (1999) suggest the use of donkey milk enriched with medium-chain tryglicerides in a cows' milk free diet in infancy. Dugo *et al.* (2005) determined in total 55 triacylglycerols in donkey milk fat, while fatty acids composition had been evaluated during lactation by Chiofalo *et al.* (2003; 2005). The knowledge of lipid fraction is obviously very important in order to better characterize donkey milk from a bionutritional point of view.

Considering the possible use of donkey milk as a safe and valid treatment for infants affected by cow milk allergy or to other food used as possible human milk replacers, many studies had been recently conducted with the aim of better characterizing donkey's milk protein profile (Cunsolo *et al.*, 2007a; 2007b; Guo *et al.*, 2007; Marletta *et al.*, 2007; Vincenzetti *et al.*, 2005; 2007; 2008). An accurate determination of total caseins, whey proteins, lysozyme, α -lactalbumin and β -lactoglobulin in donkey, sheep and human milk had been performed by Vincenzetti *et al.* (2007), the results are shown in Table 2.

Table 2. Determination of total whey proteins, total caseins, lysozyme, α -lactalbumin and β -lactoglobulin in donkey, sheep and human milk.

	Donkey (g/L)	Sheep (g/L)	Human (g/L)
Total whey proteins	7.50	17.0	8.00
Total caseins	6.60	39.0	5.60
Lysozyme	1.00	trace	not detectable
β -lactoglobulin	3.75	A variant 16.7 B variant 11.0	----
α -lactalbumin	1.80	1.63	2.2

Source: adapted from Vincenzetti *et al.*, 2007.

Donkey milk production - The asinine species is considered a seasonal polyestrous one, but the latitude in which the farm is located can greatly influence the reproduction cycle; considering a recent investigation runned in the south of Italy, specifically in Sicily, the local latitude determined small photoperiod oscillations between the different seasons, and under these conditions the donkeys have a continuous reproductive cycle, the same was observed also in sheep (Giosuè et al., 2008). In Sicily it is in fact possible to obtain donkey milk throughout the year by adequately planning the breeding seasons; results showed that donkeys foaling in winter and in summer produced more milk than those foaling in the other seasons (Table 3). Considering the different milk yield obtained when donkeys were milked twice a day, in the morning and in the afternoon (Salimei et al., 2004), the average milk yield of the morning milking was found to be statistically lower than that observed for the afternoon milking (549.2 mL vs. 949.3 mL). D'Alessandro and Martemucci (2007) have investigated on the effects of daily milking number and frequency on donkey milk production: the results obtained in that study demonstrated that the highest milk yield corresponded to three milkings per day every three hours, while daily milking regimen of six milking per day did not increase milk production and had a negative influence on the health of the mammary gland (Table 4). Also Alabiso et al. (2006) have demonstrated that the highest milk yield can be obtained with three milking per day compared to two milking per day, with an increase in milk fat content, too (Table 5).

Donkey milk hygiene should also be evaluated, as recently stated by Conte (2008). The EC Regulation n. 853/2004 allows the sale of ass' milk being these animals included in the "other milk species". According to this EC Regulation, the donkey milk should have a to-

Table 3. Milk yield and chemical composition of donkey milk across the different foaling seasons (mean \pm s.d.).

	Autumn	Winter	Spring	Summer
Milk (kg)	447 \pm 58	600 \pm 89	392 \pm 69	517 \pm 69
Lactation (day)	301 \pm 18	296 \pm 32	277 \pm 22	290 \pm 25
Milk/day (kg)	1.4 \pm 0.1	2.0 \pm 0.2	1.5 \pm 0.1	1.7 \pm 0.1
Fat (%)	0.52 \pm 0.1	0.38 \pm 0.2	0.28 \pm 0.2	0.58 \pm 0.2
Protein (%)	1.8 \pm 0.03	2.0 \pm 0.05	1.8 \pm 0.04	1.9 \pm 0.04
Lactose (%)	6.3 \pm 0.1	6.6 \pm 0.2	6.2 \pm 0.2	6.6 \pm 0.2

Source: adapted from Giosuè et al., 2008.

Table 4. Effect of milking number per day on milk production (mean \pm s.e.).

	Milking parameters		Milk production (mL)
	Number	Frequency	Mean/milking
Group A	1		346.0 \pm 7.2
Group B	3	3 h	512.2 \pm 7.6

Source: adapted from D'Alessandro and Martemucci, 2007.

Table 5. Effect of milking number per day on milk production and on milk chemical composition.

	Milking Number	
	3	2
Milk yield (kg/d)	1.62	1.23
Fat (g/100g)	0.59	0.42
Protein (g/100g)	2.08	2.05
Lactose (g/100g)	6.35	6.35

Source: adapted from Alabiso *et al.*, 2006.

Table 6. Mineral content of donkey milk (mg/kg).

Mineral	Mean	Min.	Max
Ca	676.7	360	1140
P	487.0	320	650
K	497.2	244	640
Chloride	336.7	140	500
Na	218.3	100	68
Mg	37.3	7	48

Source: adapted from Salimei *et al.*, 2004.

mammary barrier or de novo intramammary synthesis). Many of these proteins are the so called «acute phase proteins» (APP). In bibliography no data exist on the differential leukocyte cell count (DCC) for donkey milk, neither on the acute phase proteins (APP) fluctuations during an entire lactation period and/or during a mastitis. The peculiarity of donkey milk together with the potential consumers require indeed an accurate evaluation of milk quality and udder health that should pass through the evaluation of DCC, together with the acute phase proteins (APP) assessments.

Mineral content in donkey milk has been determined consistent with data reported in the literature for mare's milk (Salimei *et al.*, 2004); values determined in donkey milk were closer to human milk except for the higher levels of calcium and phosphorus (Table 6). However, the ratio Ca/P in donkey milk ranged between 0.93 and 2.37, with an average value of 1.48, which is in between the lower values of cow's milk and the higher values of human milk (Pagliarini *et al.*, 1993).

Conclusions - Human milk is obviously the best food for children under one year of age. The chemical composition of human milk has been used as a guide for the preparation of infant formulas and/or human milk replacers. It is very important to remember that, unlike infant formulas, the composition of human milk is not uniform. Significant changes in the composition of breast milk occur not only among individual women, but also throughout

tal bacterial plate count of less than <1,500,000/ml at 30°C. But, if this milk is assigned to infants or elderly human beings, it's absolutely necessary that, besides the nutritional requirements, the sanitary aspects will be evaluated too. Conte (2008) has already discussed the European indications as inadequate for the control of milk quality in donkeys, demonstrating that in presence of some milk bacteria (*S. aureus*, *S. intermedius*, *S. dysgalactiae*) there are no evidences, in donkeys, of clinical signs and/or

increased somatic cell count (SCC). The dairy industry is currently focusing on SCC as marker of both milk quality and hudder health, and the important role of SCC in the pathogenesis of mastitis is well described (Morgante *et al.*, 1996). Peeler *et al.* (2000) have demonstrated that sometimes, in an «infected» milk (in cows, goats and ewes), the changes does not regard (or not only) the number of cells but the type of them. Furthermore, as the severity of inflammation associated with the mammary gland disease increases, the protein composition of milk changes approaching that of blood (as a consequence of increased permeability of the blood

the day, throughout the lactation period, with changes in maternal diet and as a result of other external factors such as exercise or metabolic illnesses. Today there is still much to learn about the biology of human milk, and consequently there is much to study about the best composition of infant formulae. Donkey milk can be considered the closest natural milk to human milk, and the results obtained by pediatric scientists seem to confirm the nutritional value of this milk, known from ancient times in many Countries of the world. Considering its unique nutrient profile and economic potential, donkey milk could surely be exploited to fulfill the nutritional requirements of particular consumers and to increase the income of donkey farmers as well.

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