INVESTIGATION ON THE PROTEIN FRACTION OF DONKEY'S MILK PRODUCED IN SICILY BY ELECTROPHORETIC METHODS

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SUMMARY

In the last few years in Sicily the consumption of donkey's milk arised in importance, particularly for babies whose mothers cannot nurse them. Equidae milk appears to be the most similar to human milk. Donkey's milk is often well tolerate by infants and adults affected by cow's milk protein allergy; this is probably due to its protein fraction and its low casein-to-whey protein ratio. This empiric evidence, confirmed by few clinical trials, needs to be better investigated. A preliminary survey on the protein fraction of donkey's milk produced in Sicily was carried out by means of electrophoretic techniques. Fifty-eight individual milk samples have been collected in three farms. Isoelectric focusing (IEF) in ultrathin polyacrylamide gels with carrier ampholytes as well as sodium dodecil sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) methods were perfomed. Electrophoretic pattern of the individual samples presented a remarkable variability, IEF showing the presence/absence of protein bands belonging to both fractions, caseins and whey proteins. An apparent reduced amount of casein fraction was also highlighted by SDS.

INTRODUCTION

There is universal consensus that breast milk is the perfect food for newborns with healthy mothers who have appropriate nutritional stores. Notwithstanding this in some cases babies cannot be breast-fed so it is necessary to find out what kind of milk is the most suitable from case to case. Cow's milk allergy is a common disease of infancy and early childhood affecting about 2,5% of infants (Bock 1987; Saarinen et al., 1999). Infant formula, an industrial milk product usually based on either cow or soy milk, strives to be an adequate artificial substitute for natural breast milk. However, breast milk cannot be fully reproduced and babies can suffer from allergy when fed infant formula (Iacono et al., 1992; Carroccio et al., 2000). Although it is not still clear which are the major allergens, β-lg and α_s-caseins, absent in human milk, seem to be the most relevant. In the last few years many milks from different species (goat, donkey, horse, camel) have been studied to identify the best substitute for human milk (Hachelaf et al., 1993; Iacono et al., 1992; Businco et al., 2000; El-Agamy et al., 1997). Equidae milk has an excellent nutritional value and is easily digested (Miranda et al., 2004), moreover its unique nitrogen composition, with a low casein-to-whey protein ratio (Zicker and Lonnerdal, 1994; Csapo-Kiss et al., 1995), makes it a good alternative to human milk in infant nutrition. A comparative study on milk from different species showed that the antigenic similarities between donkey and human milk proteins are stronger than to that of milk of the other milking species (cow, goat, horse, buffalo) (El-Agamy et al., 1997). Sicily is the first region in donkey breeding and in the last few years the consumption of donkey's milk increased in importance. Only limited data are available for genetic polymorphism of donkey's milk proteins. The aim of this work was to provide preliminary informations about milk protein variability.

MATERIAL AND METHODS

Individual milk samples were collected from fifty-eight lactating donkeys in three sicilian farms and immediately cooled to 4°C. Milk samples were defatted by centrifugation at 3500 rpm, 15°C for 30 min. The fat layer was solidified at –20°C for 15 min and drawn up. According to Ochirkhuyag et al. (2000) whole casein was obtained from skim milk by isoelectric precipitation (pH 4.6) at 22 °C, using 1 mol·L^{-1} HCl. The precipitate was washed twice with distilled water at pH 4.6, solubilized at pH 7 by addition of 1 mol·L^{-1} NaOH, precipitated again at pH 4.6 with 1 mol·L^{-1} HCl and washed 3 times with distilled water. Finally, the whole casein was solubilized at pH 7, freezedried and stored at –20 °C. The supernatant, containing the whey proteins, was recovered by centrifugation at 4500 rpm at room temperature for 30 min and subsequently stored at -20°C. Isoelectric focusing (IEF) in ultrathin polyacrylamide gels with carrier ampholytes was performed in Multiphor II Flatbed Electrophoresis System (Amersham Biosciences). Gels were stained with Coomassie blue R350. SDS-PAGE was performed in a standard vertical gel electrophoretic apparatus (Protean© II xi Cell –BioRad) using 14% polyacrylamide gels which were stained with Coomassie blue R250 (Laemmli, 1970).

RESULTS AND DISCUSSION

IEF was used to obtain a first screening of donkey's milk protein pattern as well as of the precipitated casein and whey protein profile. Donkey's milk samples exhibited marked heterogeneity and showed five different IEF patterns. Most of the milk samples (64,3%) showed a common pattern, named A (fig.1) and used as a reference, whereas some others

were characterized by the presence/absence of some protein bands. IEF of milk, caseins and whey proteins of selected samples indicated which protein fraction the bands belonged to. Pattern B (1,78%) and C (21,42%) appeared to be defective, lacking some bands in the casein and in the whey protein fraction, respectively (fig. 1a). Pattern D (8,92%) and E (3,58%), on the contrary, showed the presence of split bands apparently belonging to ca2sein and whey protein fraction respectively (fig. 1b). Moreover two milk samples, collected in two farm from donkeys at the end of lactation, showed an unexpected IEF pattern corresponding only to the whey protein profile; animals producing this kind of milk were not apparently afflicted with mastitis. SDS-PAGE confirmed what previously obtained by Salimei et al. (2004) and also revealed a marked reduced amount of the casein fraction of the defective milk sample (IEF pattern B) compared to the reference (fig. 2).
In order to better describe the observed polymorphism three milk samples (A, B and C type) were chosen to be fully characterized by Mass Spectrometry analysis. Preliminary results indicate that defective patterns B and C present polymorphism at α_{s1}-casein and β-lactoglobulin II respectively (Cunsolo et al., 2006).

CONCLUSIONS

Individual donkey's milk samples, collected in Sicily, showed a great variability when analysed by isoelectric focusing. Each of the five observed protein pattern needs to be further investigated at molecular level and used in immonological test (*in vivo* and *in vitro*) in order to assess their allergenic capacity. The observed polymorphism, occurring at casein and whey protein fraction, could be useful to better exploit donkey breeding and particularly to promote donkey's milk utilization.

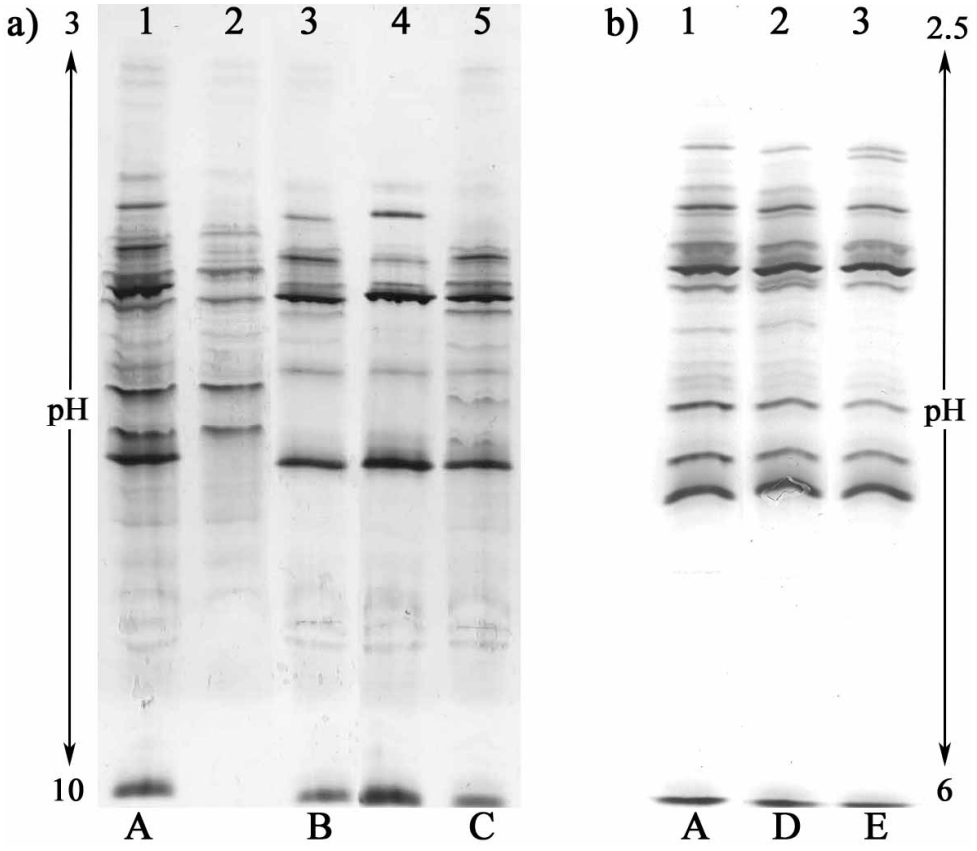

Figure 1. Isoelectric focusing of donkey's skim milk and protein fractions. a) 1: Reference milk sample, pattern A; 2: Casein fraction of reference sample; 3: Defective milk sample, pattern B; 4: Whey protein fraction of reference sample; 5: Defective milk sample, pattern C. b) 1: Reference milk sample, pattern A; 2: Milk sample with split casein band, pattern D; 3: Milk sample with split whey protein band, pattern E.

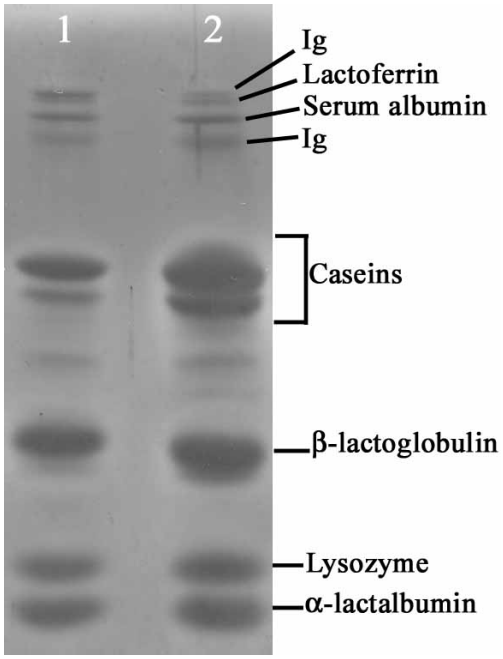

Figure 2. SDS-PAGE of donkey's skim milk. 1: Defective milk sample, pattern B; 2: Reference milk sample, pattern A.

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