α_{s1}-casein in goat milk: identification of genetic variants by Capillary Zone Electrophoresis compared to Isoelectric Focusing

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RIASSUNTO – Caratterizzazione dei latti caprini per la frazione α_{S1}-caseina mediante focusing isoelettrico ed elettroforesi capillare zonale – *Il polimorfismo al locus α_{S1}-caseina è stato studiato in campioni individuali di latte caprino proveniente da razze italiane, mediante isoelettrofocalizzazione (IEF) ed elettroforesi capillare zonale (CZE). L'elettroforesi capillare ha permesso di identificare le principali varianti genetiche della frazione α_{S1}-caseina. I risultati ottenuti sono stati confrontati con quelli derivanti dall'analisi IEF mettendo in evidenza come l'elettroforesi capillare, grazie al grado di risoluzione e al livello di automatizzazione raggiunti, sia in grado di fornire, in alternativa all'IEF, risultati altrettanto precisi con una maggiore ripetibilità.*

KEY WORDS: *Capra hircus*, α_{S1}-casein, capillary zone electrophoresis, isoelectric focusing.

Abbreviation key: CE = capillary electrophoresis, **CZE** = capillary zone electrophoresis, **IEF** = isoelectric focusing.

INTRODUCTION – Alpha$_{S1}$ casein fraction in caprine milk is characterized by an important polymorphism due to substitution, deletion of amino acids and post trascriptional modifications (Grosclaude *et al.*, 1994; Ferranti *et al.*, 1997). This structural polymorphism is associated to a quantitative variability in protein expression related to different milk quality and dairy properties (Pierre *et al.*, 1998; Remeuf, 1993; Vassal *et al.*, 1994). Classical electrophoretic methods were applied to characterize the phenotypic variants at α_{S1}-casein fraction (Addeo *et al.*, 1988; Russo *et al.*, 1986). During the last ten years capillary electrophoresis became an analytical technique for rapid and automated analysis requiring small sample volume and small solvent waste. These characteristics, together with the high resolution and the chance to give quantitative results, made this technique a useful tool in studying milk protein characterization and in detecting adulteration (Cattaneo *et al.*, 1996a; 1996b) in different application fields. CZE was applied to the study of caprine milk proteins to quantify high, medium and low α_{S1}-casein content and to identify genetic variants α_{S1} A, B and C on the basis of their different migration time (Recio *et al.*, 1997). The aim of this work was to test a CZE procedure able to identify and discriminate the main α_{S1} caprine variants A, B, E and F through specific and repeatable electromigration patterns. Comparison between CZE and IEF assays is discussed.

MATERIAL AND METHODS – *Samples:* Forty individual raw milk samples were collected from Sarda, Nera di Verzasca, Frontalasca and Alpine goat breeds (1:1:1:1) and stored at -20°C. Before analysis milk samples were defatted by centrifugation at 1000g for 10 min at 4°C. Caseins were separated from defatted milk by precipitation at the isoelectric point.

CE *Sample treatment:* Sample buffer (pH 8.6±0.1) was prepared by mixing 10 M urea, 167 mM TRIS,

42 mM MOPS, 67 mM EDTA and and 17 mM dithiotreitol. The solution was filtered over a 0.45 mm filter (Sartorius, Göttingen, Germany). Milk samples were diluted 1:1.5 (w/w) in sample buffer. Isoelectric casein was dissolved at 4% (w/v) in a sample buffer diluted (60% v/v) solution. Both milk and casein samples were incubated 5 minutes at room temperature, centrifuged at 10000 g for 10 minutes then analysed by CZE. (Recio *et al.*, 1996) *Capillary zone electrophoresis:* Electromigrations were carried out using a Biofocus 2000 capillary system (Bio-Rad Laboratories, Hercules, Ca, USA). Separations were performed at 38°C using a 550 mm x 50 μm i.d. Bio-Rad Biocap hydrophilically coated capillary with a running electrolyte (pH 3 ±0.1) made up of 20 mM sodium citrate buffer, 0.05% MHEC and 6 M urea. Voltage was set up at 20.00 kV with polarity from positive to negative, pressure injection 10 psi*sec and UV detector at 214 nm. (Cattaneo *et al.*, 2002).

IEF The experimental procedure is described in a previous work (Feligini *et al.*,2002).

Data repeatability was tested using at least five samples characterised by the same α_{S1}-CN genotype, AA genotype at k-CN locus and AA, AC, AB genotype at α_{S2}-CN locus. Alpha $_{s1}$-CN reference samples were genotyped by DNA analysis (Spallanzani laboratories).

RESULTS AND CONCLUSIONS – A good correlation between α_{S1} genotype, CZE peak areas and the intensity of IEF corresponding bands was found, although not quantitative results were achieved. CZE, by the procedure here described, was found to be a suitable technique to identify α_{S1} A, B, E and F caprine milk genetic variants (Fig. 1), showing a good comparability with IEF results (Fig. 2). Partial discrimination was obtained between variants such as B and E, which show similar electrophoretic properties. However, the same problem occured also when IEF was applied.

IEF confirmed to be a suitable electrophoretic technique able to give accurate results although particularly influenced by the operator effect (Feligini and Nudda, 1999), due to the manual steps. On the basis of the reduced amount of toxic solvents, the automated technology used and the simple procedure, CZE can be proposed for genetic screenings as feasible substitute of IEF method in routine labs with this specific aim.

Figure 1. Electropherograms obtained by CZE-urea of individual caprine milk samples.

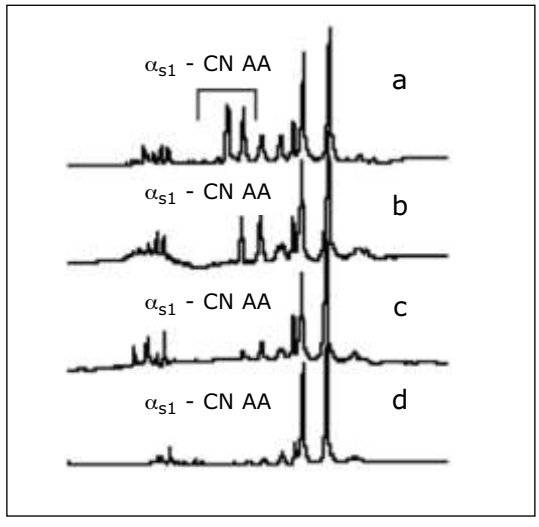
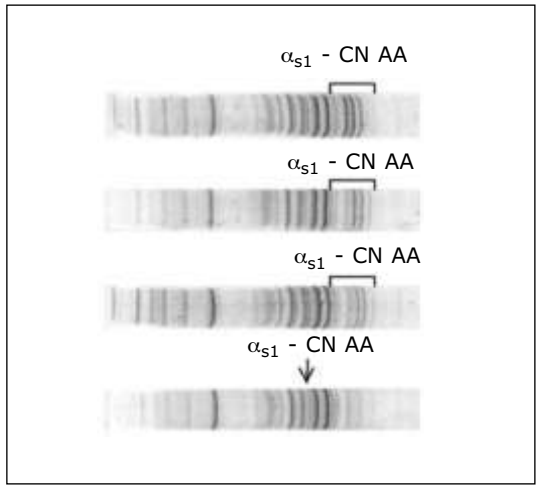


Figure 2. IEF in pH gradient 2.5-6.5 of individual caprine milk samples.



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