

Short Communication

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Identification of caseins in goat milk

The importance of goat milk in infant diet is growing, because it is reported that goat's milk in some cases is less allergenic than cow's milk. This is due probably to the lower presence of caseins associated with a specific type of α_{s1} -casein. In caprine breeds, four types of α_{s1} -casein alleles are identified and associated with various amounts of this protein in milk. The contribution of strong alleles to the goat milk is approximately 3.6 g/L of α_{s1} -casein, while for middle alleles is only 1.6 g/L, weak alleles 0.6 g/L. The contribution of null allele is very low (or non-existent). The quantity of total caseins in caprine milk is positively correlated with the amount of α_{s1} -casein. Milk from animals possessing strong alleles contain significantly more total caseins than milk from animals without those alleles. This is important because animals with mild alleles can be employed to produce milk for allergic subjects while the other animals can be used to produce milk for the dairy industry. This work shows casein profiles of two types of classified goat milk (B, strong α_{s1} allele, 0, null α_{s1} allele) with two-dimensional electrophoresis coupled with matrix-assisted laser desorption/ionization-time of flight mass spectrometry, and it confirms the different polymorphisms at locus α_{s1} casein.

Keywords: Caseins / Milk / Two-dimensional gel electrophoresis

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Investigations on the polymorphism of milk proteins are less extensive in the goat than in the cow. Studies on the goat α_{s1} casein polymorphism [1, 2] revealed the existence of 14 alleles producing seven electrophoretic variants. Different rates of α_{s1} casein synthesis are associated with different variants: variants A, B and C called "strong" alleles, E "intermediate", D and F "weak" and 0 "null". While alleles α_{s1} -Cn^A, α_{s1} -Cn^B and α_{s1} -Cn^C each contributed approximately 3.6 g casein *per* litre of milk, α_{s1} -Cn^E contributed only 1.6 g, α_{s1} -Cn^F and α_{s1} -Cn^D 0.6 g, and α_{s1} -Cn⁰ appeared to be a null allele [3].

The genetic mechanism explaining this quantitative effect was elucidated by Leroux *et al.* [2]. The importance of a null allele is studied for its implication in milk intolerance, possibly developed in infancy. Moreover, β -casein has a role in the stimulation of autoimmune response, while κ -casein may have a role of protection against allergy, because it suppresses the release of histamine from mast cells [4]. Food allergy is principally a problem in infancy and early childhood. Infants will develop food allergy most often in the same order in which the foods are introduced into their diets. Adverse reactions to foods, mainly cow's milk proteins, are most common in the first years of life. The total cumulative incidence of food allergy

and food intolerance during the first 3 years of life has been estimated to be approximately 7–8% [5]. In other prospective studies, the incidence of cow's milk protein allergy/intolerance in infancy was estimated to be around 2–3% [6, 7]. In type I diabetic patients, much attention has been given to infant's early diet [8, 9] and in particular to a possible role of cow's milk [10]. Saruger *et al.* [11] suggests that humoral responses to α - and β -casein are elevated in Italian patients with type I diabetes.

There are few studies of caseins in milk using 2-DE [12–14, 24] and only for human and bovine milk; thus, the aim of this work was to detect, by 2-DE coupled with MALDI-TOF mass spectrometry, two types of genotyped goat milk. These milk samples have defined characteristics. They came from animals classified on the basis of the presence of certain α_{s1} casein alleles. The importance of this preliminary work is for the eventual use of goat's milk in infant food (α_{s1} -Cn⁰) as well as indicating milk from animals with strong α_{s1} alleles for use in cheese transformation (strong alleles produce more cheese because the quantity of total caseins in caprine milk is positively correlated with the amount of α_{s1} -casein).

Goat milk samples, (Frisa Valtellinese breed; genotyped by mean of PCR-RFLP method) of 2 mL were collected in polypropylene tubes and kept refrigerated. The samples were ultracentrifuged according to Iametti *et al.* [15] to precipitate casein. Dilutions were made in 7 M urea, 2 M thiourea and 4% CHAPS (as solubilizing agents) [16]. IEF was performed using immobilized pH gradient strips (home-made), which

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had a linear pH range of 3–7 (11 cm long). The samples were applied to the strips using the in-gel rehydration method, as described by Rabilloud *et al.* [17] and Sanchez *et al.* [18]. The samples were diluted to a total volume of 200 μ L in rehydration solution, which contained 8 M urea, 0.5% CHAPS, 15 mM DTT, 0.2% Ampholine (50% 4–6.5 and 50% 3–6.5; Amersham Biosciences, Uppsala, Sweden). The strips were rehydrated and focused on an IPGphor (Amersham Biosciences) at 20°C. Rehydration was allowed for 10 h at low voltage (5 h at 30 V and 5 h at 50 V). Focusing was obtained by application of 500 V for 1 h, 1000 for 1 h, 8000 for 10 h. The current was limited to 50 μ A per strip. The equilibration step was performed for 22 min. The equilibration solution was 6 M urea, 2% SDS, 30% glycerol in Tris-HCl buffer, 0.05 M, pH 6.8, plus DTT (0.1%) for 15 min that was replaced by iodoacetamide (130 mM). Second dimension was carried out on ExcelGel SDS, homogenous, 15% (Amersham Biosciences). The run was performed for about 2 h, at 600 V, 30 mA, 50 W.

Protein identification by MALDI-TOF and ESI-ion trap MS was carried out as previously described [20]. After visualization with Coomassie blue staining, electrophoretic spots were excised and protein digestion was carried out as described [19]. Spectra were acquired using a Ettan MALDI-TOF mass spectrometer (Amersham Biosciences). Mass fingerprinting database searching was carried out using ProFound (www.proteometrics.com) and PeptIdent (<http://www.expasy.ch>), on-line available softwares. Peptide sequencing by MS/MS analysis was carried out using a LCQ DECA ion trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA). Database searching was carried out using TurboSEQUEST software (Thermo Finnigan) and MASCOT MS/MS ion search software (Matrix Science, London, UK).

Matching was performed using Image Master 2D Elite software, V.3.1, between samples from goats with strong alleles and null alleles.

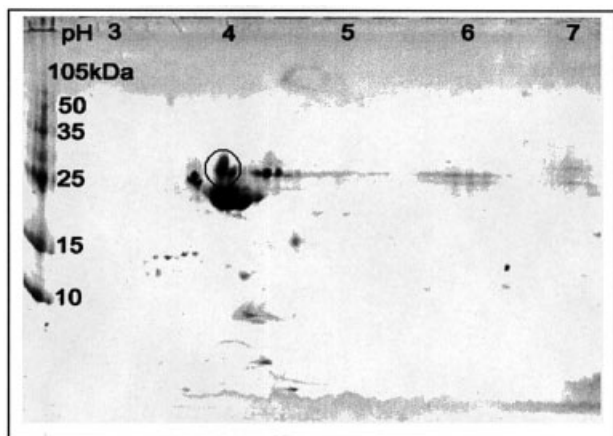


Figure 1. 2-D PAGE stained with Coomassie blue of caprine caseins, strong alleles.

Isoelectric focusing in IPGs was performed according to Righetti [21]. The pH range used for analysing the casein fractions of null allele was 3–6.5. Chromatographic separation of the caprine caseins fractions was performed by RP-HPLC on a Vydac C4 column (0.46 $\times$ 25 cm) (Grace-Vydac, CA, USA) according to Jaubert and Martin [22] with some modifications, as described by Iametti *et al.* [23].

The protein profile resulting from 2-D PAGE separation of casein fractions using a linear 3–7 gel is shown in Figs. 1 and 2. The proteins are visualized by Coomassie staining. The first evident result is that a zone in null allele milk was absent with respect to the area on the gel from the sample with strong allele, as shown by the circle.

This is the α_{s1} -casein zone, as confirmed by an IPG 3–6.5 (urea + additives, same conditions as the first dimension of 2-DE) of the same sample but purified in each fraction casein by RP-HPLC (Fig. 3). In fact in null allele α_{s1} -casein is absent and there is also a minor quantity of other casein. This is because the quantity of total caseins in caprine milk is positively correlated with the amount of α_{s1} -casein. The diminished level of expression of α_{s1} -casein was not counterbalanced by an increase in the levels of the other caseins.

The matching of casein performed by Image Master Software is shown in Fig. 4, where numbers indicate spots excised for subsequent mass analysis.

Figure 5a shows the mass spectrum of α_{s2} casein (excised spot no. 1 in Fig. 4). In Fig. 5b there is the mass spectrum of β - and κ -casein. This is confirmed by peptide sequencing by MS/MS analysis of 2183 and 1251 peptide (Fig. 5c and 5d). The sequence of 2183 peptide is YPVEPFTESQSLTLTDVEK and it corresponds with a

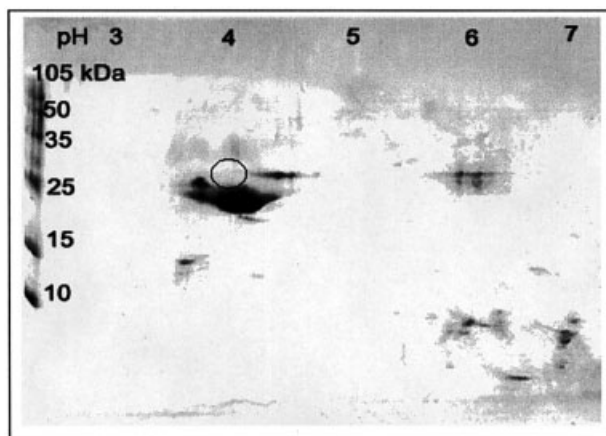


Figure 2. 2-D PAGE stained with Coomassie blue of caprine caseins, null allele.

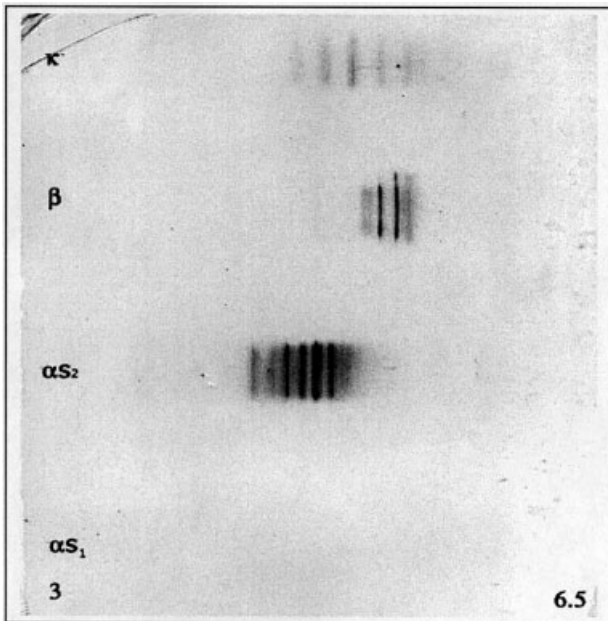


Figure 3. IEF on IPG of purified casein fractions by RP-HPLC, null allele.

region of β -casein precursor amino acid sequence. The sequence of 1251 peptide is YIPIQYVLSR and it corresponds with a region of κ -casein precursor amino acid sequence.

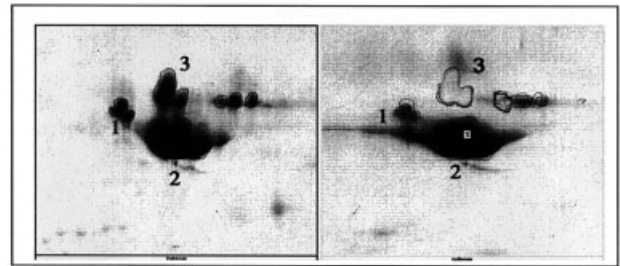


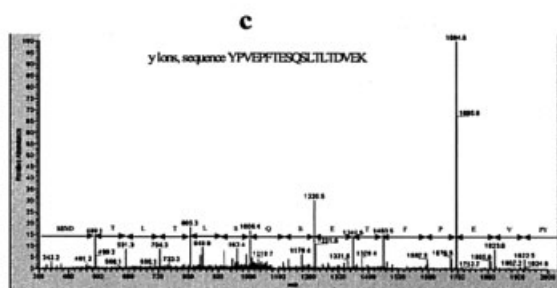
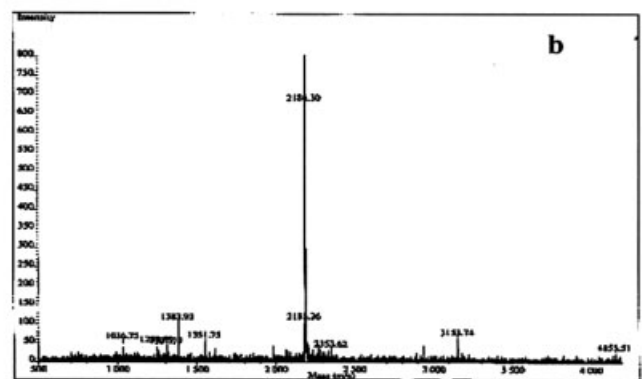
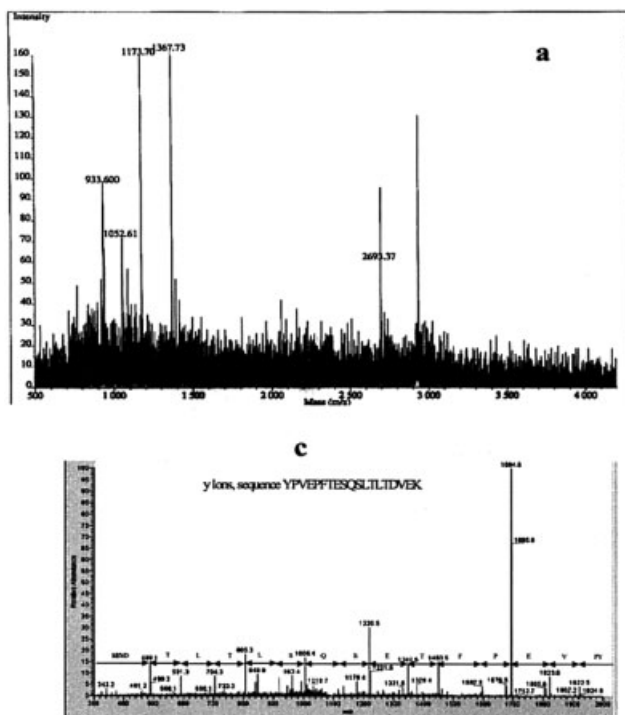
Figure 4. 2-D matching between strong alleles (left) and null allele (right). Spot 1: α_{S2} casein; spot 2: $\beta + \kappa$ -casein; spot 3: α_{S1} casein. Note that after matching with Image Master software common spots are circled and specific spots are represented as a synthetic grey circle, as in the right image for α_{S1} casein, that is not present.

Two-dimensional electrophoresis coupled with MALDI-TOF represents a key method to investigate and classify milk caseins with a relative short-time response compared with RP-HPLC.

These data confirm that goat milk from animals with null allele has less total casein than milk from goats with strong alleles and thus it should be taken into consideration for potential use in infant feeding.

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