ELECTROPHORETIC STUDY OF DROMEDARY WHEY PROTEINS

[1]ALIM N., [2]BRAMBILLA A., [2]FONDRINI F., [1]TENIOU R., [2]FELIGINI M., [2]ENNE G.
[1]Institut National de la Médecine Vétérinaire d'Alger, Bp 125 Hassen Badi El-Harrach Alger, Algérie.
[2]Istituto Sperimentale Italiano "Lazzaro Spallanzani", Laboratorio di Epigenomica Applicata, Viale Giovanni XXIII 7, I-26900 Lodi, Italy. E-mail: maria.feligini@isils.it

Résumé:

La composition des protéines sériques du lait de dromadaire Algérien a été analysée par isoélectrofocalisation, éléctrophorése sur gel de polyacrylamide en présence du sodium dodécasyllabe, électrophorèse sur gel polyacrylamide en milieu alcalin et électrophorèse capillaire. Les principaux composants des protéines sériques ont été caractérisés par l'utilisation des standards et marqueurs.
Aucune analogie directe n'a été observée entre le sérum du lait de dromadaire et celui du bovin sur la mobilité électrophorétique, par contre une hétérogénéité dans la même race a été observée.
Trois différentes formes de l'alpha-lactalbumine ont été observée à ~23, ~32 and ~43 kDa, en autre la béta-lactoglobuline n'a pas été observée. Au environ de 19 kDa une bande protéique a été observée seulement dans un échantillon, dans le même échantillon a été constatée l'absence de l'alpha-lactalbumine au environ de 32 kDa. Le lysozyme et la lactoferrine sont présents avec une concentration élevée dans tous les échantillons en comparant avec le sérum bovin. Le lait de dromadaire peut être une solution alternative pour la formule du lait pour les enfants et les personnes allergiques au lait et produits laitiers bovins vue sa composition particulière (taux élevée en albumine sériques et l'absence de la béta- lactoglobuline) qui est similaire au lait maternelle.

Mots clés: Camelus dromedarius, protéines sériques, électrophorèse

Abstract:

Protein composition of whey from Algerian dromedary (Camelus dromedarius) was analysed by isoelectric focusing, sodium dodecilsulfate-polyacrylamide gel electrophoresis, alkaline polyacrylamide gel electrophoresis and capillary electrophoresis. Bovine whey proteins standards and markers were used to better characterize the main protein components.
No direct analogy between bovine and dromedary whey proteins electrophoretic mobility was found and heterogeneity between samples was observed. Three different bands characterising the alpha-lactalbumin were observed at ~23, ~32 and ~43 kDa. Beta-lactoglobulin was not found. A protein band at ~19 kDa was found by SDS-PAGE only in one whey sample, while in the same sample □-La band at ~32 kDa was absent. Lysozyme and lactoferrin were present at high level in all the samples if compared to bovine whey. Due to the particular composition (high amount of serum albumin and absence of beta-lactoglobulin), dromedary milk could be an alternative in infant milk formula and to people with allergy in cow milk dairy products.

Key words: Camelus dromedarius, whey proteins, electrophoresis

Abbreviation words: IEF = isoelectric focusing, CZE = capillary zone electrophoresis, SDS = sodium dodecylsulfate, PAGE = polyacrilamide gel electrophoresis, CN = casein.

INTRODUCTION

Algerian dromedary (*Camelus dromedarius*) belongs to the genus Camelus and is characterized by one hump compared to the two–humped *Camelus bactrianus* (Farah, 1993). Up to now the majority of studies on dromedary were carried out to investigate physiological adaptation to desert conditions, anatomical features and traditional management, while few studies exist on milk yield and composition, probably due to the nomadic life of animals.

Dromedary milk is characterized by a high content of vitamin C, the highest among dietary milks (three times more than cow milk), which gives it antimicrobial and protective activities. Besides, κ–casein content is three times less than in cow milk and also the content in lactose and cholesterol is low (Faye,1997; Ramet, 1991/2). These peculiar features make this precious milk particularly suitable for allergic subjects (FAO, 2001). On the other hand, dromedary milk has peculiar physical and chemical characteristics which seem to negatively influence its technological properties (Attia et al. 2000). In fact the relatively large size of dromedary milk micelle and κ-casein low content determine difficulties in the coagulation process. Research on camels, especially on protein composition of dromedary milk, is indeed scarce until today if compared to research on other species.

In a study on milk whey from Somali dromedary (Conti et al. 1985) the amino acid composition and N- terminal sequence of the protein was elucidated and the existence of two α-lactalbumin (A and B) characterized by a different pI (5.5 and 5.2 respectively) was reported. Recent studies on the whey fraction from dromedary milk show the absence of β-lactoglobulin (Ochirkhuyag et al. 1998; Kappeler, 1998). Dromedary casein has been studied focusing on the protein fractions αs1, αs2, κ and β-CN and the presence of a small amount of κ-casein compared to the whole casein fractions was reported (Ochirkhuyag et al. 1997). The apparent isoelectric points were determined for αs1-CN (4.41 and 4.40), αs2-CN (4.58), β-CN (4.66) and κ–CN (4.10) (Kappeler et al. 1998).
In the present work, individual Algerian dromedary milk samples from Larbaa breed were analyzed by electrophoretic methods to study whey protein fractions. The major whey proteins were characterized by IEF, CZE, PAGE and SDS-PAGE using known standards and markers and a comparison with protein electrophoretic patterns from cow milk was done.

MATERIALS AND METHODS

1. Milk samples collection. Three individual Algerian dromedary milk samples were collected from Larbaa breed in Laghaouat region. Sample collection was carried out in the morning, manually, from one primipara female 2 years old (D1) and two multiparous females 15 (D2) and 5 (D3) years old. After collection milk samples were immediately frozen at -20°C without preservatives. Whey proteins were separated by ultracentrifugation at 27 000 g for 1 hour and 30 minutes at 4°C.

2. Isoelectric focusing. Whey proteins were diluted (1:1 v/v) with a denaturing solution prepared according to Krause and Belitz (1985). The gel matrix was prepared with 30% (w/v) acrylamide/bis (37.5:1), 8M urea and 12.2% (w/v) glycerol (87%). pH gradient was performed by 2.5% of 2.5-5 and 4-6.5 Pharmalyte (2:1 v/v) and 15 µl/sample were loaded. Electrophoresis run was carried out at 10°C by a Multiphor II apparatus (Amersham Biosciences) for 2 hours at a constant current of 4 mA, max 250 V/cm and 20 W. Gels were stained overnight with a staining-destaining solution containing Coomassie G-250 according to Blankesley and Boezi (1977). Individual samples of bovine whey were analysed as reference samples.

3. SDS-PAGE 14%. Dromedary whey samples were diluted (1:5 v/v) in a reducing buffer containing SDS according to Laemmli (1970) and 5 µl/lane were loaded. Electrophoresis was carried out by a vertical apparatus Mini Protean II (Bio-Rad, Richmond, USA) and run was conducted at 150 V constant for 1 hour. Gel was stained with Coomassie R-250 for 1 h. Destaining was performed with a solution containing acetic acid, methanol and double distilled water (1:4:5 v/v/v). The identification of main dromedary whey proteins was done on the basis of the relative migration time of known standards (α-lactalbumin, β-lactoglobulin, lactoferrin and serum albumin, SIGMA) and markers (RNP 800, Amersham Biosciences).

4. PAGE 14%. Dromedary whey was diluted (1:3 v/v) with 9.6M urea (w/v) according to Medrano and Sharrow (1998). Gel was prepared with a solution of acrylamide/bis (30% T; 2.5% C) and 0.375M Tris-HCl pH 8.9. Running buffer contained 0.025M Tris-base and 0.2M Glycine, pH 8.3. Electrophoresis was performed in a vertical apparatus Mini Protean II (Bio-Rad) and 4 µl/lane of each sample were loaded. Run was conducted at 150 V constant for 2 hour. Gel was stained for 1 hour with Coomassie R-250; and the destaining phase was performed with acetic acid, methanol and double distilled water (1:4:5 v/v/v).

5. Densitotometric analysis. Computerized densitometry (densitometer: GS 800; software: Quantity One 1-D Analysis version 4.4, BioRad) was used to quantify whey protein bands from SDS-PAGE of individual samples.

6. Capillary electrophoresis. Sample buffer (pH 8.6±0.1) was prepared by mixing 10 M urea, 167 mM TRIS, 42 mM MOPS, 67 mM EDTA and 17 mM dithiotreitol. The solution was filtered over a 0.45 µm filter (Sartorius, Göttingen, Germany). Milk and whey samples were first diluted in water (1:1 v/v), then in sample buffer (1:1.5 v/v) and incubated 5 minutes at room temperature, centrifuged at 10000 g for 10 minutes then analysed by CZE.

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 (Recio et al. 1996; Cattaneo et al. 2002). Individual samples of bovine milk, bovine whey and protein standards (α-Lactalbumin, β-Lactoglobulin and serum albumin, SIGMA) were analysed as reference samples.

RESULTS AND DISCUSSION

Isoelectric focusing of individual whey samples from Algerian dromedary (Fig. 1) shows two main bands corresponding to α-La A and B respectively, according to Conti et al. (1985) and Ochirkhuyag et al. (1998). A slight difference in the α-La apparent isoelectric point was found between these results (5.5) and previous studies (5.2), probably due to the specific pH range applied (2.5-5 and 4-6.5). In individual whey sample D3 a smaller amount of α-La was observed if compared to samples D1 and D2, this suggesting a possible heterogeneity among samples

Figure 2 shows SDS-PAGE profiles of dromedary whey (lanes 2 to 4) compared to bovine whey proteins (lane 5, 6 and 7). In dromedary whey using molecular weight size standards (lane 1), we can observe bands corresponding to BSA (66 kDa) and lactoferrin (76 kDa) according to Elagamy et al. (1996), while a band corresponding to β-Lg (~18 kDa) is not evident, as already observed by Ochirkhuyag et al. (1998) and Merin et al. (2001). Three different bands characterising α-La can be observed at ~23, ~32 and ~43 kDa (Farah, 1986; Merin et al. 2001) and the dromedary whey basic protein (WBP) is shown at ~20 kDa as observed by Ochirkhuyag et al. (1998).
If we consider that the molecular weight of lysozyme from dromedary milk is similar to the one of lysozyme from cow milk (14.4 kDa) (Duhaiman, 1988) we can argue that the band (fig. 3, lane 6) with MW in the region 14.4 kDa represents exactly this enzyme.
Polyacrylamide gel electrophoresis profiles (Fig. 4) of individual whey samples (lanes 3, 4 and 5) show three main bands each sample characterized by a lower mobility than bovine milk whey protein mobility, according to Farah (1986). A band corresponding to β-Lg is not evident, according to Beg et al. (1987) and Ochirkhuyag et al. (1998). No differences among individual whey profiles were observed by this technique.

Figure 5 shows capillary electropherograms of individual dromedary whey obtained by CZE-urea. It is not possible to define a simple and direct analogy among the electrophoretic profiles from individual bovine and dromedary milk samples (Fig 5 A). Comparing milk and whey from an individual dromedary milk sample (Fig. 5 B) we can observe the presence of one main whey protein peak, partially co-migrating with a small casein fraction in whole milk. The main dromedary whey peak has electrophoretic characteristics similar to bovine serum albumin while peaks with the same electrophoretic characteristics of bovine α-La and β-Lg are not observed (Fig. 5 C).The irregular shape of the top of this main peak suggests the presence of more than one protein fraction underlying, probably characterized by similar charge/mass features, whose separation could be improved testing different buffer conditions.

Results of densitometric analysis of SDS-PAGE from one individual whey sample are shown in figure 6. The identification of the major whey proteins was performed using markers and standards on the basis of bibliographical information. The protein band (NI) at ~19 kDa was found only in one whey sample by densitometric analysis, while in the same sample α-La2 band was absent (Fig. 2). Table 1 shows percent quantitative analysis of densitometric peaks from three individual whey samples (Fig. 6) performed by Quantity One. We can observe that both lysozyme and lactoferrin are present at high level in all the samples if compared to bovine whey while the basic protein is characterized by a great variability its content ranging from 3.2 to 12.2.

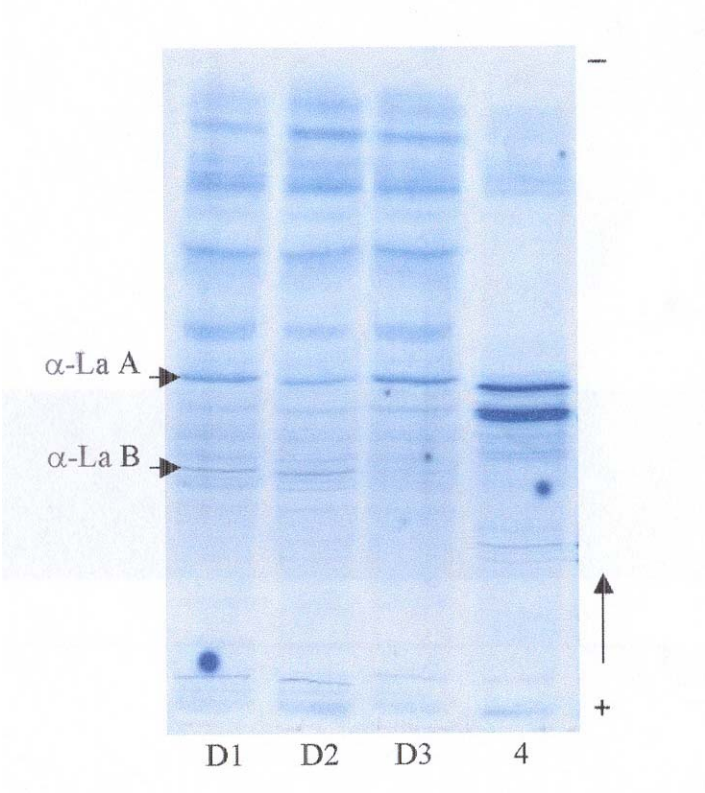

Fig 1. Isoelectric focusing pH gradient 2.5-6.5 of individual whey samples (lane 1 to 3) from Algerian dromedary, lane 4: bovine whey.

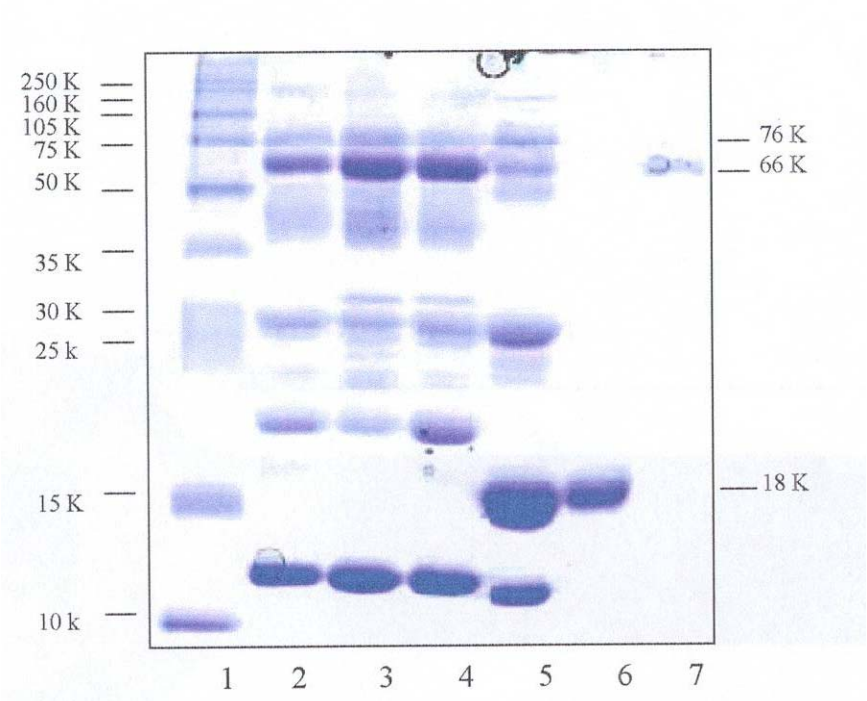

Fig 2. 14%T SDS-PAGE gel separation of individual dromedary whey samples (lane 2: D3, lane 3: D2, lane 4: D1), lane 1: marker (Amersham Biosciences), lane 5: bovine whey proteins, lane 6: bovine β-Lg (Sigma), lane 7: BSA (Sigma).

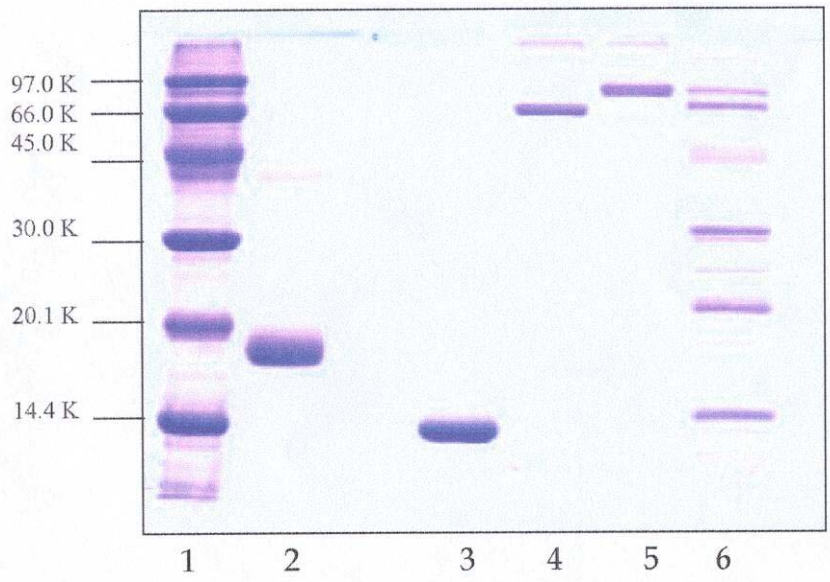

Fig 3. 14%T SDS-PAGE of individual dromedary whey sample. Lane 1: marker (Amersham Biosciences); lane 2: bovine β-Lg (Sigma); lane 3: bovine α-La (Sigma); lane 4: BSA (Sigma); lane 5: bovine lactoferrin (Sigma); lane 6: dromedary whey.

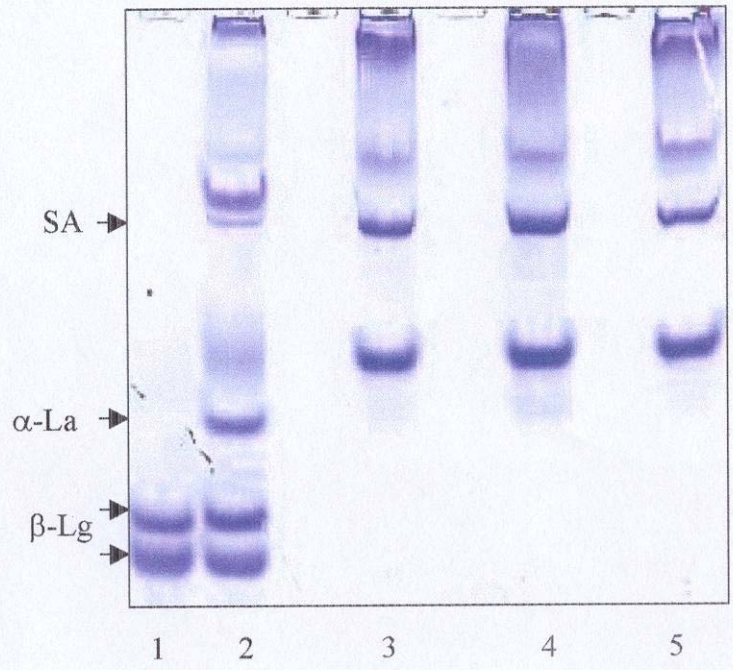

Fig. 4. Separation of individual whey proteins (lane 3: D1, lane 4: D2, lane 5: D3) from Algerian dromedary by 14%T PAGE technique, lane 1: bovine β-Lg (Sigma), lane 2: bovine whey proteins.

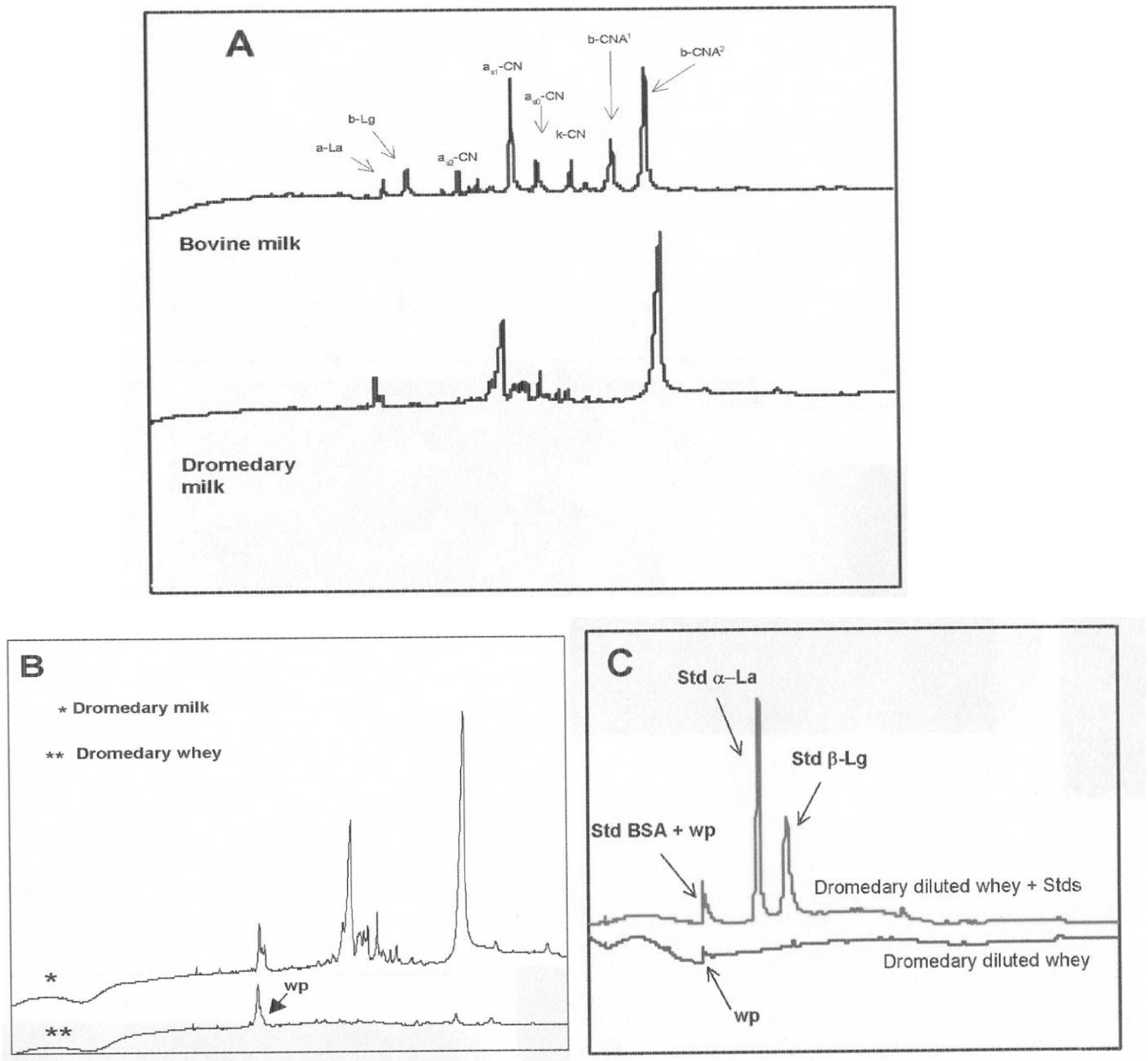

Fig 5. Capillary electropherograms obtained by CZE-urea analysis of dromedary whey. (A) individual bovine and dromedary milk samples (bovine peaks identification from Recio et al. 1997); (B) individual dromedary milk and whey; (C) dromedary whey diluted with water (1:3) or with the addition of bovine α-La, β-Lg and BSA. Wp = dromedary whey protein.

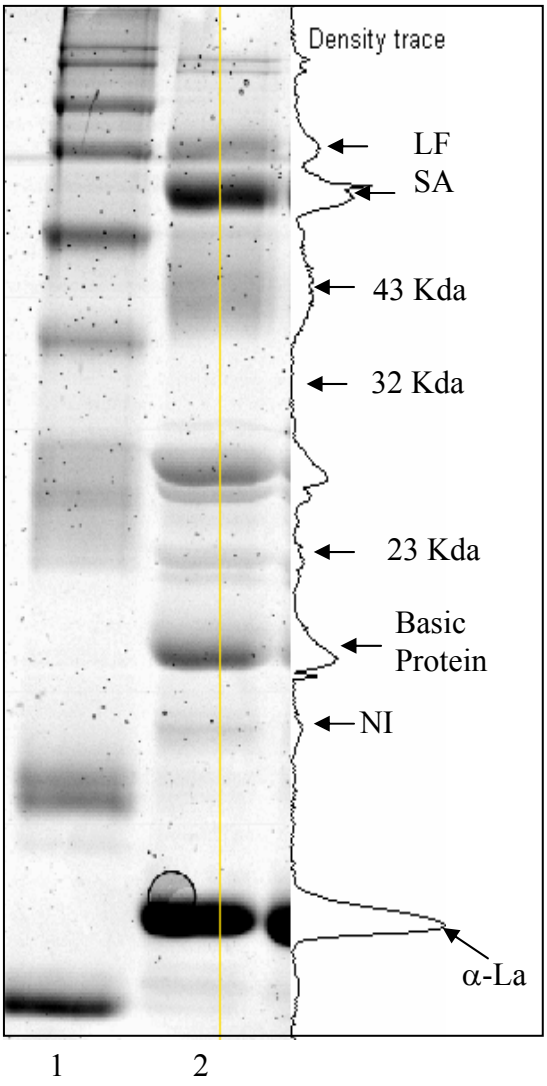

Fig. 6 Densitometric analysis of individual whey protein. Lane 1: marker; lane 2: individual whey protein LF: lactoferrin; SA: serum albumin; α-La: alpha-lactalbumin; NI: not identified.

Tableau 1 : Densitometer values (%) of the protein band intensity from the SDS-PAGE shown in Fig 2.

	LF	SA	43 kda	32 kda	23 kda	Basic Protein	a-La	NI	other
Whey 1	5.6	12.9	7.7	-	2.7	8.4	32.6	2.1	28.0
Whey 2	5.1	17.7	5.7	2.9	3.2	3.2	33.4	-	28.8
Whey 3	2.4	17.2	5.4	1.7	2.1	12.2	33.0	-	26

CONCLUSIONS

Results from this study confirm the absence of β-Lg in dromedary whey, mainly constituted of α–lactalbumin in three different forms, serum albumin, lactoferrin, whey basic protein and lysozyme.
Due to the high amount of serum albumin and the absence of β-Lg dromedary milk could be an interesting alternative in infant milk formula and to people with allergy in cow milk dairy products. In the course of this study on Larbaa breed, interesting differences in the protein composition of whey samples emerged if compared to the results of previous studies (Conti et al 1985., Farah 1986., Ochirkhuyag et al 1998., Merin et al 2001) on dromedaries located in Somalia, Kenya, Mongolia and Israel. Nevertheless further investigations are necessary to better characterize whey proteins and to identify the protein polymorphism.

Acknowledgements
The authors thank Dr. Maktouf Lakhdar for milk sample collection.

BIBLIOGRAPHY

[1]Attia H., Kherouatou N., Nasri M., Khorchani T. Characterization of the dromedary milk casein micelle and study of its changes during acidification. Lait 2000; 80: 503-515.
[2]Beg O U., Van Bahr-Lindstrom H., Ziadi H.Z., Jornavall H. Charcterization of a heterogeneous camel milk whey non-casein protein. Federation of European Biochemical Societies 1987; 216(2): 270-274.
[3]Blankesley R.W., Boezi J.A. A new staining technique for proteins in proteins in polyacrylamide gel using Coomassie Brilliant Blue G-250. Anal. Biochem 1977; 82: 580-582.
[4]Cattaneo T.M.P., Civardi G., Maraboli A. Ricerche ed innovazione nell'industria alimentaire V. Chiriotti Ed., Pinerolo (TO) 2002; 1100-1109.
[5]Conti A., Godovac-Zimmermann J., Napoletano L., Liberatori J. Identification and characterization of two α-lactalbumins from Somali Camel milk (Camelus dromedarius). Milchwissenschaft 1985; 40 (11): 673-675.
[6]Duhaiman A S. Purification of camel milk lysozyme and its lyctic effecton Escherishia coli and Micrococcus lysodeikticus. Comp.Biochem. Physiol. 1988; 91 B: 793-796.
[7]Elagamy E I., Ruppanner R., Ismail., Champagne C P., Assaf R. Purification and Characterisation of lactoferrin, lactoperoxidase, lysozyme and immunoglobulins from camel's milk. International Dairy Journal 1996; 6:129-145.
[8]FAO. Du fromage de dromadaire sur votre table? FAO Archives de l'actualité 2001.
[9]Faye B. Le guide de l'élevage. Ed. Sanofi, Libourne1997.
[10]Farah Z. Effect of heat treatment on whey proteins of camel milk. Milchwissenschaft 1986; 41: 763-765.
[11]Farah Z. Composition and characteristics of camel milk. J. Dairy Res 1993; 60: 603-626.
[12]Kappeler S. Compositional and structural analysis of camel milk proteins with emphasis on protective proteins. Dissertation ETH (Swiss Federal Institute of Technology) N° 12947, Zurich 1998.
[13]Kappeler S., Farah Z and Puhan Z. Sequence analysis of Camelus dromedarius milk caseins. J. Dairy Res 1998; 65: 209-222

[14]Krause I., Belitz H.D. Differenzieung von milchproteinen verschiedener tierarten nachweis von kulmich in schafzieghen und buffelmilch bzw-kase. Lebensmittelchem .Gerichtl. Chem 1985; 39: 29-46.

[15]Laemmli U.K. Clevage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227: 680-685.

[16]Medrano J and Sharrow L. Milk protein typing of bovine mammary gland tissue used to generate a complementary deoxyribonucleic acid library. J. Dairy Sci.1988; 72:3190-3196.

[17]Merin U., Bernstein S., Bloch-Damti A., Yaghil R., Van Creveld C., Lindner P., Gollop. A comparative study of milk serum proteins in camel (Camelus dromedarius) and bovine colostrums. Livestock Production Science 2001; 67: 297-301.

[18]Ochirkhuyag B., Chobert J M., Dalgalarrondo M., Choiset Y., Haertlé T. Characterisation of caseins from Mongolian yak, khainak, and bacterian camel. Lait 1997; 77: 601-613.

[19]Ochirkhuyag B., Chobert J M., Dalgalarrondo M., Choiset Y., Haertlé T. Characterisation of whey proteins from Mongolian yak, khainak, and bacterian camel. Journal of food biochemistry 1998; 22: 105-124.

[20]Ramet J.P. La transformation du fromage de dromadaire. Rev. Mond. Zoot. FAO 1991/2 67: 20-28.

[21]Recio I., Olieman C. Determination of denatured serum proteins in the casein fraction of heat-treated milk by capillary zone electrophoresis. Electophoresis 1996; 17: 1228-1233.

[22]Recio I., Pérez-Rodríguez M L., Ramos M., Amigo L. Characterization of ewe's milk by capillary zone electrophoresis. J. Chromatography A 1997; 768: 47-56.