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Short communication

A comparative study of milk serum proteins in camel (*Camelus dromedarius*) and bovine colostrum

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Abstract

Camel (*Camelus dromedarius*) whey proteins were detected and compared to bovine whey proteins using size exclusion chromatography columns on HPLC. Camel whey proteins such as serum albumin and α-lactalbumin appear to possess molecular weights similar to the respective bovine whey proteins. Camel whey lacks β-lactoglobulin and consists of large amount of serum albumin, compared to bovine whey. Camel colostrum is rich in IgG and serum albumin, which reduce in amount already after 3 days postpartum. The main protein in camel colostrum whey is serum albumin while that of bovine colostrum whey is β-lactoglobulin. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Camel milk; Colostrum; Whey proteins

1. Introduction

In bovine whey, there are five major protein fractions with defined molecular weights, i.e., immunoglobulins (Igs), mainly immunoglobulin G (IgG), bovine serum albumin (BSA), α-lactalbumin (α-la), β-lactoglobulin (β-lg) and proteose peptones (Whitney, 1977; Eigel et al., 1984). Serum proteins that were identified in camel milk include α-la, serum albumin, lactophorin A, lactoferrin, lactoperoxidase and Igs. Three other fractions include whey acidic protein (WAP), peptidoglycan recognition protein (PGRP) and blood serum albumin (Beg et al., 1985, 1986, 1987; Conti et al., 1985; Farah, 1993; Kappeler, 1998). It appears that most of the whey proteins in camel milk resemble those of bovine whey proteins, except for the lack of β-lg (Ochirkhuyag et al., 1998). Nevertheless, proteins of molecular weight around 23 and 43 kDa were reported to be bands of bovine β-lg (Farah, 1986).

Colostrum differs in composition from regular milk, especially in its high content of fat, protein, ash and whey proteins. The latter contain the immunoglobulins (Ig), which are needed to provide the newborn with immunity and are passed to the calf in the colostrum (Schmidt, 1971; Jenness, 1985).

In the present work, camel colostrum was studied in comparison to bovine colostrum, to determine the composition of the serum protein fractions and their changes immediately postpartum, using size exclu-

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sion chromatography. The protein's fractions were compared and identified according to known markers using SDS–PAGE and gel filtration columns on HPLC.

2. Materials and methods

2.1. Colostrum samples

Camel colostrum was collected immediately postpartum from three she camels of the experimental farm at The International Camel Center, Sapir Center, Central Arava. Each female was milked 1 h postpartum, after the newborn started to suckle, and every 4 h thereafter and the colostrum was pooled for a whole day. Cow colostrum was obtained in the same manner from three cows from the experimental farm at the Volcani Center, Bet Dagan.

2.2. Preparation of serum proteins

Serum proteins were prepared after the removal of fat by centrifugation at $3000 \times g$ at 4°C, and the precipitation of the casein according to British Standard (1963). According to the method used, the whey proteins were diluted by the preparation procedure by 1:50. The clear filtrate without further dilution was used for injection to the HPLC column and for other uses.

2.3. HPLC determination

For the determination of the different proteins served an L-6200 Merck–Hitachi HPLC, equipped with an L-4200/L-4300 UV/UV-Vis detector. Separation was obtained on a pre-column, followed by GF-250 (4 μm, 150A) and GF-450 (6 μm, 300A) size exclusion columns in series (Zorbax, Rockland Technologies, USA) and a 100-μl injection loop. Mobile phase consisted of sodium phosphate at pH 7.0 with 200 mM NaCl, at 1 ml/min in isocratic conditions, detected at 220 nm.

2.4. Calibration proteins

A series of proteins with varying molecular weights (MW) were purchased from Sigma (Sigma, St. Louis, MO) and served as markers. These included: thyroglobulin (669 kDa), apoferritin (443 kDa), β-amylase (200 kDa), alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa), carbonic anhydrase (29 kDa), bovine β-lactoglobulin (18.2 kDa) and bovine α-lactalbumin (14.4 kDa).

2.5. SDS–PAGE electrophoresis

Camel and bovine serum proteins for gel electrophoresis were prepared as described in Section 2.2. The proteins were loaded on a 12.5% T acrylamide gel, with 5% acrylamide staking gel, using Mini-Protean II apparatus with a power supply (BioRad, Hercules, CA) operated at constant current of 100 V, for about 1 h. Markers (purchased from Sigma), included ovalbumin (45.5 kDa), carbonic anhydrase (29 kDa) and myoglobin (18 kDa).

All tests were performed in triplicates from the daily-pooled colostrum samples that were collected as explained in Section 2.1.

3. Results and discussion

A molecular weight calibration curve for the Zorbax columns was prepared as shown in Fig. 1. It was intended for the determination of the peak for IgG and other Igs that have a high MW of over 150 kDa. The correlation performed revealed that the two Zorbax columns in series could serve as a good tool to separate whey proteins in the range of 10 to over 700 kDa. The MW of the eluted proteins were calculated according to the fit equation of Fig. 1 ($r = 0.987$):

$$y = 9.09 - 0.227x \quad (1)$$

where $y = \log \text{MW}$; $x =$ retention volume.

In order to identify the different whey proteins in camel and bovine whey, SDS–PAGE electrophoresis and gel filtration of whey samples of the two species were compared to one another and to whey proteins, which were purchased commercially. In Fig. 2 lane 4 (bovine whey) it is possible to observe α-la, β-lg and BSA. Several faint bands probably correspond to α-la dimers (28 kDa) and β-lg dimers and octamers. Lanes 3 and 4 are camel whey. While lane 3 is a

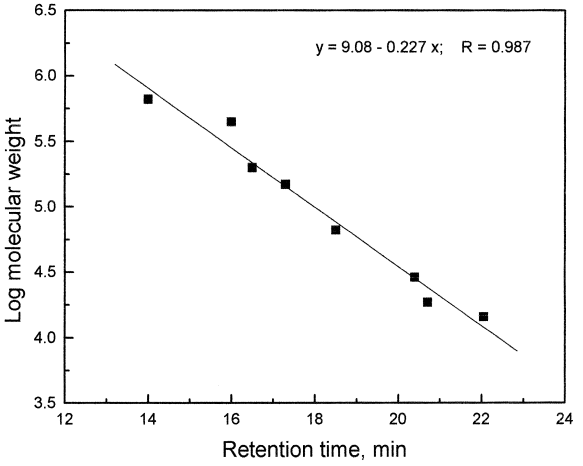

Fig. 1. Calibration curve of the Zorbax GF-250 and GF-450 size exclusion HPLC columns. Markers in increasing order of retention: thyroglobulin (669 kDa), apoferritin (443 kDa), β-amylase (200 kDa), alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa), carbonic anhydrase (29 kDa), bovine β-lactoglobulin (18.2 kDa), bovine α-lactalbumin (14.4 kDa). Regression: $y=9.08–0.227x$; $r=0.987$ (see text for details).

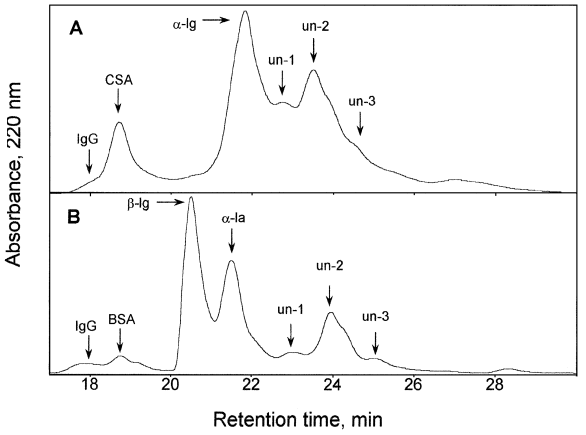

Fig. 3. Exclusion chromatograms of camel (A) and bovine (B) whey. (A, camel) IgG, immunoglobulin G; CSA, camel serum albumin; α-la, α-lactalbumin; un-1,2,3, unknown proteins of 8000, 5400 and 800 MW. (B, bovine) IgG, immunoglobulin G; BSA, bovine serum albumin; β-lg, β-lactoglobulin; α-la, α-lactalbumin; un-1,2,3, unknown proteins of 8000, 5400 and 800 MW.

little overloaded, in lane 4 it is possible to observe similar band to α-la, bands of ~23, ~32, 43 kDa, and similar band to BSA at 66 kDa. Farah (1986) also observed similar bands of 23 and 43 kDa. The band of 32 kDa could result from an α-la dimer.

A chromatogram of camel whey (A) is presented in Fig. 3 in comparison to bovine whey (B) of pooled herd milk collected in mid lactation. It can be assumed that the first relevant peak (Fig. 3A,B) at elution time of about 18 min is IgG with molecular weight of about 160 kDa (Eigel et al., 1984; Conti et al., 1985; Jenness, 1985). IgG is presumed to be eluted similarly to alcohol dehydrogenase (150 kDa) due to its close MW. The other known proteins are

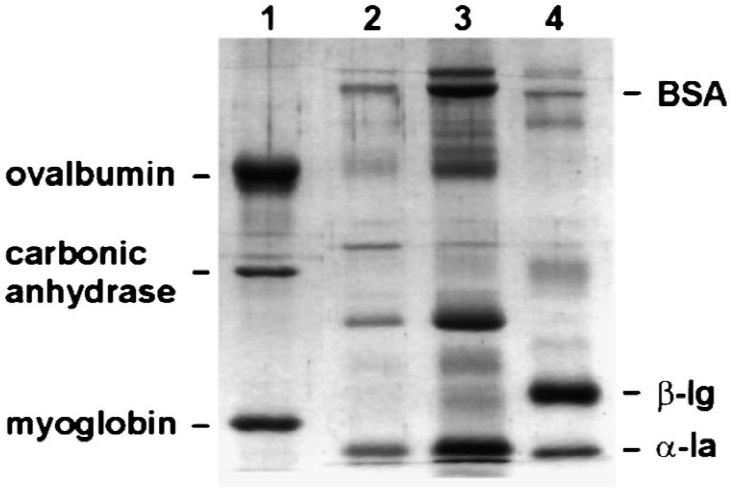

Fig. 2. SDS–PAGE of camel and bovine whey proteins. (1) molecular weight markers; (2) camel whey (diluted ×2 of No. 3); (3) camel whey; (4) bovine whey.

BSA (Fig. 3, BSA) and its corresponding camel serum albumin, CSA (Fig. 3A, CSA). The identification of BSA was done by injection of whey with external commercial BSA. It was eluted in a single enhanced peak, which is a common accepted practice for protein identification in gel filtration. Therefore, it could be assumed that the protein eluted at a similar time in camel whey by the size exclusion and as seen by its positioning on the SDS–PAGE is a BSA analog. β-lg (Fig. 3B) appears only in bovine milk, which is in agreement with published data (Farah, 1986; Ochirkhuyag et al., 1998). Lack of β-lg is also reported for milk of other species including human milk (Jenness, 1985) and is not due to preparation artifact, since in the SDS–PAGE electrophoresis no band in the vicinity of 18 kDa was detected in camel whey (Fig. 2). α-la was detected in both whey (Fig. 3A,B). The identifications of α-la and β-lg were performed similarly to BSA as noted above. The lower MW proteins, referred to as proteose peptones in bovine whey, appear in both samples at equal or different elution times (Fig. 3A,B, un-1,2,3). It should be noted that the proportions between the whey proteins of the two species are different, especially that of CSA and BSA (Fig. 3A,B). Nevertheless, the total amount of whey proteins is equal in the two milks (Merin et al., 1998) and the proportions of bovine whey proteins assume the relative concentrations that appear in the literature (Whitney, 1977; Jenness, 1985). It is assumed that other whey components such as lactoferrin (75–76 kDa), lactoperoxidase (69 kDa) and the 43-kDa fraction (Kappeler, 1998), are contained within the serum albumin peaks, since they have molecular weights that will not be separated on the columns used in this work. It would be reasonable to assume that the whey proteins of camel and bovine that appear at similar elution times are actually identical as was presented by sequencing of the different camel whey proteins by Ochirkhuyag et al. (1998).

Camel and bovine whey of colostrum of the first day reveal an interesting pattern (Fig. 4). Relative higher contents of Igs are found in camel colostrum (Fig. 4A,B). Similar fraction of Igs, were reported for camel milk by Conti et al. (1985). The major components of camel colostrum are IgG and CSA (Fig. 4A) compared with bovine colostrum IgG and

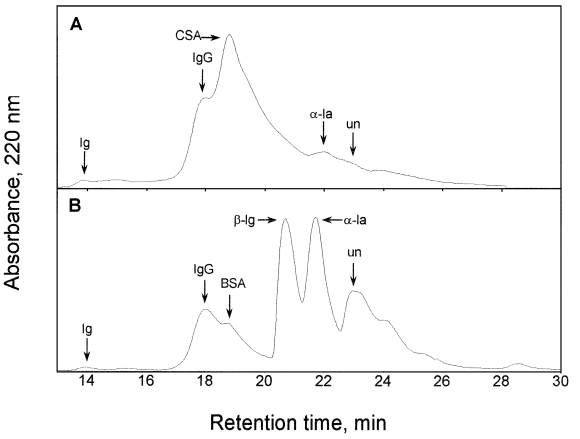

Fig. 4. Exclusion chromatograms of whey of first day camel colostrum (A) and first day bovine colostrum (B). (A, camel) Ig, immunoglobulins; IgG, immunoglobulin G; CSA, camel serum albumin; α-la, α-lactalbumin; un, unknown (8000 MW). (B, bovine) Ig, immunoglobulins; IgG, immunoglobulin G; BSA, bovine serum albumin; β-lg, β-lactoglobulin; α-la, α-lactalbumin; un, unknown (8000 MW).

BSA (Fig. 4B). Interesting is the different proportions between the various colostrum components, and especially the relative small amount of α-la in camel colostrum compared to CSA. Fraction un of 8000 MW (Fig. 4A,B) is found in both preparations and it probably belongs to the proteose peptone fraction that is present in both preparations. Fig. 5 presents the change in the different whey proteins fractions present in camel colostrum during 3 days

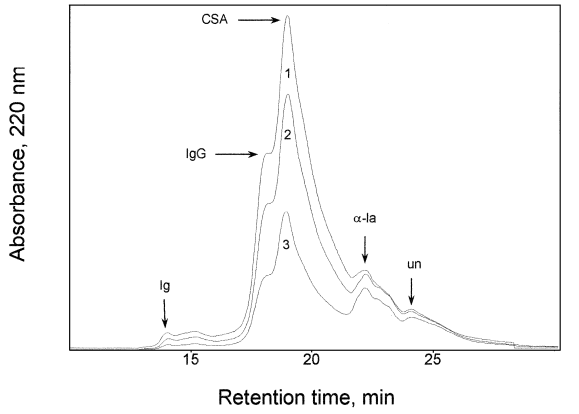

Fig. 5. Exclusion chromatograms of whey from camel colostrum. (1) Day one; (2) day two; (3) day three postpartum, respectively. Ig, immunoglobulins; IgG, immunoglobulin G; CSA, camel serum albumin; α-la, α-lactalbumin; un, unknown (8000 MW).

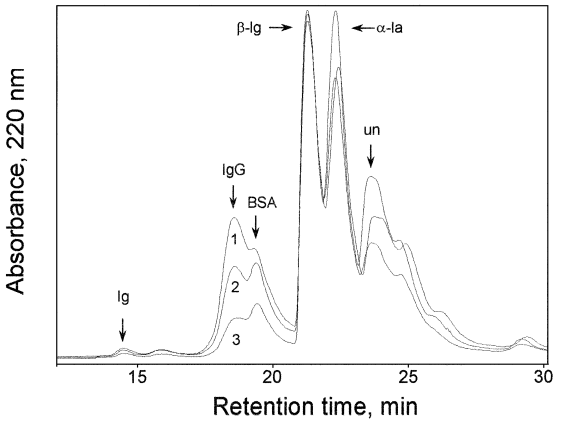

Fig. 6. Exclusion chromatograms of whey from bovine colostrum. (1) Day one; (2) day two; (3) day three postpartum, respectively. Ig, immunoglobulins; IgG, immunoglobulin G; BSA, bovine serum albumin; β-lg, β-lactoglobulin; α-la, α-lactalbumin; un, unknown (8000 MW).

postpartum (Fig. 5, 1–3). It is clear that there is a major decrease in Igs and IgG in the first 3 days (Fig. 5, 1–3). Due to the large amounts of whey proteins in the colostrum during the first days the major peaks present in Fig. 3A for camel whey are hardly visible in Fig. 5, 1–3 (absorption is one order of magnitude larger than that of Fig. 3). It should be noted that the proportions of the serum proteins that appear in Fig. 5 in camel colostrum are different from those, which appear in bovine colostrum (Fig. 6). While in camel colostrum the major fraction is CSA (Fig. 5, 1–3) in bovine colostrum the main protein is β-lg (Fig. 6, 1–3). In addition, the amount of serum albumin is different in the milk of the two species as can be seen in Fig. 3A,B. The unknown fractions in Figs. 5 and 6 (un-1,2,3) are probably fractions of the proteose peptones.

4. Conclusions

It was shown in colostrum and milk that most camel serum proteins are similar in molecular weights to bovine whey proteins. The main differences between the serum protein samples are the lack of β-lg and the high amount of camel serum albumin and the different proportion of the various proteins. Camel colostrum is rich in IgG and CSA, which are reduced in amount already after 3 days postpartum. The main protein in whey of camel colostrum is CSA while that of whey of bovine colostrum is β-lg.

References

Beg, O.U., von Bahr-Lindstrom, H., Zaidi, Z.H., Jornvall, H., 1985. The primary structure of α-lactalbumin from camel milk. Eur. J. Biochem. 147, 233–239.

Beg, O.U., von Bahr-Lindstrom, H., Zaidi, Z.H., Jornvall, H., 1986. A camel milk whey protein rich in half-cystine. Primary structure, assessment of variations, internal repeat patterns, and relationship with neurophysin and other active polypeptides. Eur. J. Biochem. 159, 195–201.

Beg, O.U., von Bahr-Lindstrom, H., Zaidi, Z.H., Jornvall, H., 1987. Characterization of a heterogeneous camel milk whey non-casein protein. FEBS Lett. 216, 270–274.

British Standard 1741, 1963. Methods for the Chemical Analysis of Liquid milk and Cream. British Standards Institution, UK.

Conti, A., Godovac-Zimmermann, J., Napolitano, A., Liberatori, J., 1985. Identification and characterization of two α-lactalbumins from Somali camel milk (*Camelus dromedarius*). Milchwissenschaft 40, 673–675.

Farah, Z., 1986. Effect of heat treatment on whey proteins of camel milk. Milchwissenschaft 41, 763–765.

Farah, Z., 1993. Composition and characteristics of camel milk. J. Dairy Res. 60, 603–626.

Eigel, W.N., Butler, J.E., Ernstrom, C.A., Farrel, H.M., Harlwalker, V.R., Jenness, R., Whitney, R.McL., 1984. Nomenclature of the proteins of cow's milk: fifth revision. J. Dairy Sci. 67, 1599–1631.

Jenness, R., 1985. Biochemical and nutritional aspects of milk and colostrum. In: Larson, B.L. (Ed.), Lactation. The Iowa State University Press, Ames, IA, pp. 164–197, Ch. 5.

Kappeler, S., 1998. Compositional and structural analysis of camel milk proteins with emphasis on protective proteins. Dissertation ETH (Swiss Federal Institute of Technology) No. 12947, Zurich.

Merin, U., Rosen, B., Bernstein, S., Yagil, R., Gollop, N., 1998. Effects of husbandry practices on the composition of Israeli camel milk. Milchwissenschaft 53, 680–682.

Ochirkhuyag, B., Chobert, J.M., Dalgalarrondo, M., Choiset, Y., Haertle, T., 1998. Characterization of whey proteins from Mongolian yak, khainak, and bacterian camel. J. Food Biochem. 22, 105–124.

Schmidt, G.H., 1971. In: Biology of Lactation. W.H. Freeman, San Francisco, CA.

Whitney, R.M.c.L., 1977. Milk proteins. In: Graham, H.D. (Ed.), Food Colloids. Avi Publ. Co, Westport, CT, pp. 66–151, Ch. 2.