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Short communication: Caseins and α-lactalbumin content of camel milk (*Camelus dromedarius*) determined by capillary electrophoresis

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ABSTRACT

Camel milk has unique physical, nutritional, and technological properties when compared with other milks, especially bovine. Because proteins confer many of the properties of milk and its products, this study aimed to determine the proteins of camel milk, their correlations, and relative distribution. Raw milk samples were collected from 103 dromedary camels in the morning and evening. Capillary electrophoresis results showed wide variation in the concentrations (g/L) of proteins between samples as follows: α-lactalbumin, 0.3 to 2.9; α_{S1}-casein, 2.4 to 10.3; α_{S2}-casein, 0.3 to 3.9; β-casein, 5.5 to 29.0; κ-casein, 0.1 to 2.4; unknown casein protein 1, 0.0 to 3.4; and unknown casein protein 2, 0.0 to 4.6. The range in percent composition of the 4 caseins were as follows: α_{S1}, 12.7 to 35.3; α_{S2}, 1.8 to 20.8; β, 42.3 to 77.4; and κ, 0.6 to 17.4. The relative proportion of α_{S1}-, α_{S2}-, β-, and κ-caseins in camel milk (26:4:67:3, wt/wt) differed from that of bovine milk (38:10:36:12, wt/wt). This difference might explain the dissimilarity between the 2 milks with respect to technical and nutritional properties.

Key words: camel milk, protein, α-lactalbumin, casein, capillary electrophoresis

Short Communication

Dromedary one-humped camels (*Camelus dromedarius*) are the only dairy animals in the world that can survive the harsh desert conditions of high temperature and drought (Wernery, 2006). Camel milk (**CM**) is an important source of nutrients and has several health benefits, including antidiabetic and antiallergic effects (Izadi et al., 2019). However, difficulties are encountered in the processing of CM into fermented products and UHT treatment (Berhe et al., 2017). The CM proteins are mainly composed of caseins (50–88%) and whey proteins (20–25%; Shuiep et al., 2013; Mati et al., 2017). Camel milk is rich in α-LA, but is devoid of the whey protein β-LG, the main whey protein in bovine milk (**BM**; El-Hatmi et al., 2015). The relative distribution of caseins differs between CM and BM, especially for β- and κ-caseins (Kappeler et al., 1998). Several reports have investigated the concentrations of major proteins in CM, but only in a limited number of samples (Kappeler et al., 1998; Omar et al., 2016; Ryskaliyeva et al., 2018). In this study, we have used capillary electrophoresis to investigate a large number of CM samples for the variability in the concentrations of casein proteins (α_{S1}, α_{S2}, β, κ) and α-LA. In addition, we investigated the variability in the relative proportions of the different caseins, which might affect the properties of CM with respect to commercial processing and health benefits (Ghnimi and Kamal-Eldin, 2015).

Fresh CM samples were collected from 103 dromedary camels in the evening and morning of consecutive days (206 milk samples total). The animals were reared in the farm of the company Emirates Industry for Camel Milk and Products (EICMP, Umm Nahad 3, Dubai, United Arab Emirates). The total milk from an individual animal was collected from an automated milking system through tubes into a stainless-steel container as described in Nagy et al. (2013) and mixed manually before aliquots were collected in sterile bottles (250 mL). The samples were transported to the laboratory in a thermo cool box, aliquoted, and then frozen at −20°C. The total protein concentrations in the CM samples (g/L) were determined using a midinfrared spectroscopy instrument (Foss Milkoscan FT-120, Foss A/S, DK-3400 Hillerød, Denmark). Somatic cell count per milliliter was determined by a Fossomatic Minor instrument (Foss A/S, DK- 3400).

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Milk proteins were separated by capillary electrophoresis (7100 A, Agilent Technologies, Palo Alto, CA) system equipped with a UV light–diode array detector, and Open Lab Chemstation software was used to control the instrument as described by Johansson et al. (2013). Preparation of sample buffer, running buffer, and milk samples was done as described by Åkerstedt et al. (2012). The fused silica packed capillary column (length = 80.5 cm; outside diameter = 360 μm; inside diameter = 50 μm) was preconditioned for 3 min with water and 5 min with running buffer. Conditions included a voltage of 25 kV and injection pressure of 5 kPa. The column was washed with NaOH (0.1 *M*) after running 4 samples to remove any adsorbed contaminants from the capillary walls. Separated peaks were detected via UV light absorbance at 214 nm. Sigma (St. Louis, MO) bovine protein standards [α-casein (>85%), β-casein (>80%), κ-casein (>80%), α-LA (>85%)] were prepared at several concentrations (1–9 mg/mL) using deionized water and analyzed by capillary electrophoresis to determine their corresponding peak area. Standard calibration curves were prepared for each bovine protein by plotting peak areas versus concentration. The slope of the plot for β-casein was used to calculate the concentration of unknown casein proteins (1 and 2). Protein concentrations were determined using the following equation:

$$\text{Concentration (mg/mL)} = \text{peak area (mAU)}/\text{slope of standard curve of bovine protein} \times \text{dilution factor.}$$

Figure 1 presents representative electropherograms of BM and CM samples. The assignment of peaks to the different proteins was based on the electrophoretic mobilities of standard BM proteins. The identified proteins included α_{S1}-, α_{S2}-, β-, and κ-caseins and the whey protein α-LA. Capillary electrophoresis is reported to provide good separation of caseins and some whey proteins and to identify genetic variants, phosphorylations, and glycosylations (de Jong et al., 1993; Heck et al., 2008; Johansson et al., 2013). Milk proteins move through the coated fused silica capillary column according to their electrophoretic mobility, which is determined by their charge-to-mass ratio. We used buffer additives to optimize the selectivity and fine-tune protein separation by stabilizing the proteins and preventing their adsorption onto the capillary wall (Schwartz and Pritchett, 1994). In this study, the separation of milk proteins, especially the caseins, was improved over that obtained by Omar et al. (2016). Our results have shown that the CM samples were devoid of the whey protein β-LG, in agreement with others (Hinz et al., 2012; El-Hatmi et al., 2015). Lactoferrin was not detected in our electropherograms, but was detected by Omar et al. (2016) when the whey proteins were separated from caseins. It is possible that in our study the detection of lactoferrin was hampered by presence of the other milk proteins. Lactoferrin can induce interactions with whey and casein proteins due to the basic isoelectric point (8.0–9.5) and the almost positive charge (Riechel et al., 1998). Determination of lactoferrin in bovine whey reported as impossible was enhanced by different approaches (Riechel et al., 1998; Li et. al., 2012) and lead to im-

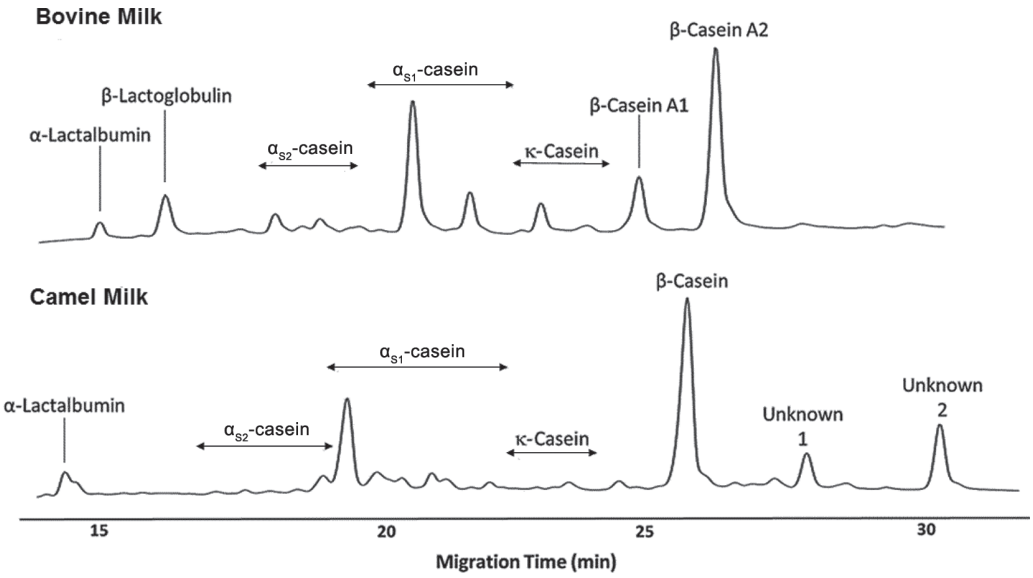

Figure 1. Representative electropherogram of bovine and dromedary camel milk samples determined by capillary electrophoresis.

proved resolution from interfering proteins. The last 2 peaks (unknown proteins 1 and 2) were present in the electropherogram of the casein fraction separated from a CM sample, suggesting that these 2 peaks belong to casein proteins. In CM from a Kazakhstan hybrid breed (*Camelus dromedarius* × *Camelus bactrianus*), Ryskaliyeva et al. (2018) reported the presence of 2 unknown proteins with molecular weights (22,939 Da and 23,046 Da), in addition to a short isoform of β-casein that was 946 Da lighter than the full length β-casein.

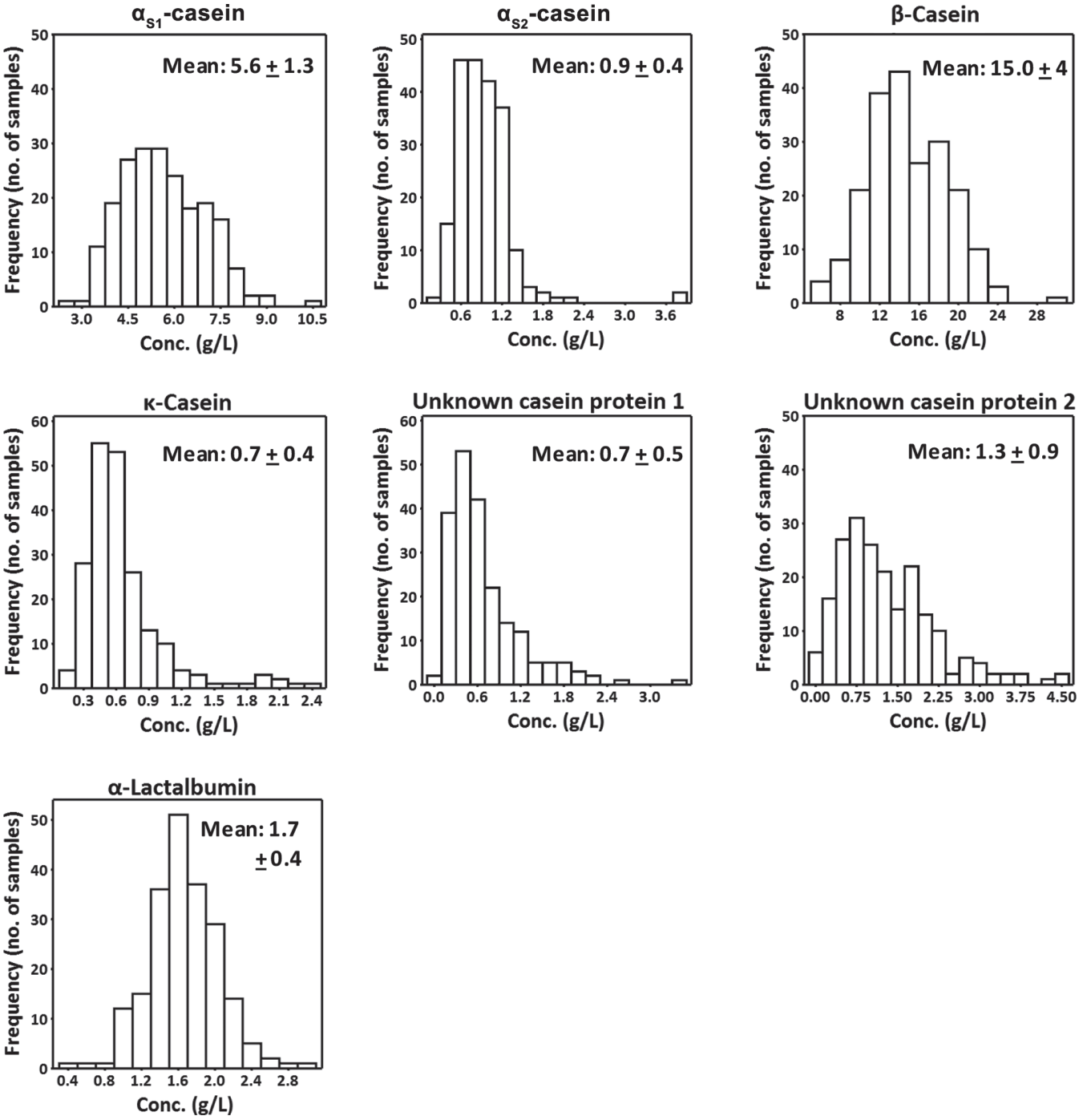

Figure 2. Histograms of concentration (conc.; g/L) of α_{S1}-casein, α_{S2}-casein, β-casein, κ-casein, unknown casein protein 1, unknown casein protein 2, and α-LA in morning and evening dromedary camel milk samples (n = 206).

The concentrations of α_{S1}-, α_{S2}-, β-, and κ-caseins and α-LA in CM samples (n = 206) are shown in Figure 2. The ranges of protein concentrations (g/L) were as follows: α-LA (0.3–2.9), α_{S1}-casein (2.4–10.3), α_{S2}-casein (0.3–3.9), β-casein (5.5–29.0), and κ-casein (0.1–2.4), which agree with values previously reported for pooled and individual CM samples (Kappeler et al., 1998; Hamed et al., 2012; Ryskaliyeva et al., 2018). Because no significant differences in protein concentrations were observed between the morning and evening milk samples (results not shown), all values were combined and are presented as histograms in Figure 2. The mean concentrations of α- and β- caseins (6.5 and 15 g/L, respectively) observed in this study are higher than the corresponding values of 3.6 and 12.8 g/L, while those of κ-casein and α-LA (0.7 and 1.7 g/L, respectively) are lower than the values of 1.7 and 2.0 g/L, respectively, reported by Omar et al. (2016). The lack of CM protein standards and rough purity of the bovine protein standards used for calibration may have led to some uncertainty in the quantifications (Kappeler et al., 1998; Omar et al., 2016; Ryskaliyeva et al., 2018). However, because all researchers used the same standards and had reached agreement between results for CM samples (using HPLC or capillary electrophoresis), there is validity to the results.

Pearson correlation tests were applied by using Minitab statistics package (version. 18, Minitab Inc., State College, PA). Table 1 presents Pearson correlation coefficients (r) between the different CM proteins, percentage of caseins in total proteins, and SCC. The results showed a weak positive correlation (r = 0.266, $P < 0.01$) between SCC and total protein, in agreement with previous results (Hamed et al., 2012). Somatic cell count, a quantitative index of mastitis condition of ruminants, has been linked with a decrease in casein content, proteolysis, and changes in the protein fraction distribution in BM (Le Roux et al., 1995; Musayeva et al., 2016; Johansson et al., 2017). Here, SCC correlated negatively with β-casein (r = −0.325, $P < 0.01$) and the percentage of caseins in total proteins (r = −0.39, $P < 0.01$), but it had a highly positive correlation with κ-casein (r = 0.76, $P < 0.01$). This agrees with Musayeva et al. (2016), who found that the percentage of caseins in total proteins decreased when the SCC increased in BM. Subclinical and clinical mastitis are known to be associated with increased activity of plasmin, the major milk proteolytic enzyme (Le Roux et al., 1995; Stelwagen, 2011). β-Casein is the most susceptible casein to plasmin hydrolysis, and κ-casein is very resistant (Fox and Kelly, 2004). No correlation was found between the concentration of β-casein and the unknown casein proteins, which can be attributed to the large variations in the β-casein levels. However,

Table 1. Pearson correlation coefficients (r) for dromedary camel milk proteins and SCC

Item[1]	Total protein (g/L)	SCC (cell number/mL)	Casein/total protein (%)	α-LA (g/L)	α_{S1}-Casein (g/L)	α_{S2}-Casein (g/L)	β-Casein (g/L)	κ-Casein (g/L)	UCP 1 (g/L)
SCC (cell number/mL)	0.266**								
Casein/total protein (%)	−0.158*	−0.393**							
α-LA (g/L)	0.488**	0.252**	−0.166*						
α_{S1}-Casein (g/L)	NS	NS	0.182**	0.514**					
α_{S2}-Casein (g/L)	0.348**	0.145*	−0.200**	0.474**	0.365**				
β-Casein (g/L)	NS	−0.325**	NS	0.379**	0.791**	0.365**			
κ-Casein (g/L)	0.566**	0.761**	−0.372**	0.445**	0.156*	0.405**	NS		
UCP 1 (g/L)	0.402**	NS	NS	0.407**	0.149*	0.170*	−0.214**	0.292**	
UCP 2 (g/L)	0.363**	NS	NS	0.415**	0.307**	0.193**	NS	0.157*	0.814**

[1]UCP 1 = unknown casein protein 1; UCP 2 = unknown casein protein 2.
**P-value <0.01, *P-value <0.05.

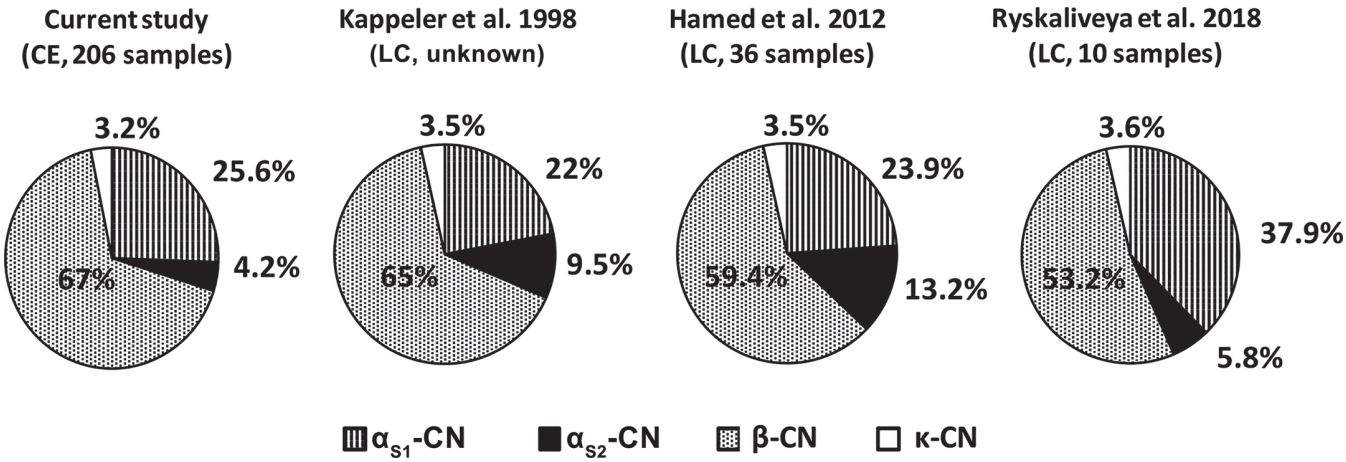

Figure 3. Relative proportion (%) of α_{S1}-, α_{S2}-, β-, and κ-caseins in dromedary camel milk as determined in the current and previous studies. CE = capillary electrophoresis; LC = liquid chromatography. The number of samples in the Kappeler et al. (1998) study was unknown.

the correlations between the relative proportions (%) of β-casein and the unknown casein proteins 1 and 2 were high and significant (−0.844 and −0.778, $P < 0.01$, respectively). Significant correlations were obtained between the concentrations (g/L) of β-casein and α_{S1}-casein (r = 0.79, $P < 0.01$) and between the unknown casein proteins 1 and 2 (r = 0.81, $P < 0.01$). α-Lactalbumin (g/L) correlated positively ($P < 0.01$) with all of the casein proteins (g/L), a correlation that we cannot explain.

The relative percentage of the 4 caseins in the CM samples (n = 206) is shown in Figure 3. In agreement with previous studies, β-casein was the major casein in CM (67%; Kappeler et al., 1998; Hamed et al., 2012; Ryskaliyeva et al., 2018). We observed that the range of the relative percentage was very wide for all of the caseins (α_{S1} = 12.7–35.3%; α_{S2} = 1.8–20.8%; β = 42.3–77.4%; κ = 0.6–17.4%), with α_{S2}- and κ-casein having the widest ranges. The average relative percentages of α_{S1}-, α_{S2}-, β-, and κ-caseins in CM were 25.6%, 4.2%, 67%, and 3.2%, respectively. Our results are in close agreement with those of Kappeler et al. (1998) and Hamed et al. (2012), whereas Ryskaliyeva et al. (2018) reported a higher average value for α_{S1}-casein (37.9%), a value close to the maximum of the range observed in our study (35.3%).

Our results suggest that the relative ratio of α_{S1}-,α_{S2}-, β-, and κ-caseins in CM is approximately 26:4:67:3 (wt/wt), in contrast to approximately 38:10:36:12 (wt/wt) in BM (Fox and Kelly, 2004). This difference and the dominance of β-casein in CM may be important in explaining some of the special properties of this milk. When processing CM to cheese, a weak coagulum is formed over a long coagulation time and the yield is low because a significant amount of the DM is lost with the whey (Ramet, 2001; Berhe et al., 2017). The yogurt curd from CM is fragile and heterogeneous, and consists of dispersed flakes (Attia et al., 2001; Berhe et al., 2017). The κ-casein concentration and its proportion in relation to α_{S1}- and β-caseins were reported to be low in poorly coagulating and noncoagulating BM (Wedholm et al., 2006). It was recently reported that the noncoagulating property of milk from red cattle significantly correlated with higher relative concentrations of α-LA and β-casein and lower relative concentrations of β-LG and κ-casein (Nilsson et al., 2020). The anticoagulation properties of β-casein can be explained by its chaperone-like activity (Zhang et al., 2005).

We observed a wide variation in the concentrations of the 4 caseins (α_{S1}-, α_{S2}-, β-, and κ-) and α–LA in the 206 CM samples, which may be attributed to the individual variability of animals from different breeds and physiological conditions. Moreover, the relative proportions of the 4 casein proteins in CM are different than in BM, a disparity likely responsible for several peculiarities of CM including poor gelation properties.

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