Vol 18 No 1, p 1-6

COMPOSITION, CONSTITUENTS AND PROPERTIES OF DUTCH CAMEL MILK

M.G. Smits[1], T. Huppertz[2], A.C. Alting[2] and J. Kiers[2]
[1]European Camel Research Society, Johanniterlaan 7, 6721 XX Bennekom, The Netherlands.
[2]NIZO Food Research, PO Box 20, Ede, The Netherlands

ABSTRACT

Samples of Dutch fresh raw, tunnel-frozen and home-frozen whole camel milk and raw whole bovine milk were studied with respect to composition and drying properties. Furthermore, the protein fraction of the milk was characterised in more detail by liquid chromatography and gel electrophoresis. Digestibility of the proteins of camel milk and bovine milks was studied under stimulated physiological conditions.

Camel milk contained less protein, fat and lactose than bovine milk, which is in agreement with literature data. Spray-drying of raw whole camel milk did not cause noticeable damage to its protein fraction and camel milk powder had a higher solubility than powder prepared from bovine milk. Characteristics of caseins and whey proteins in camel milk differed significantly from those in bovine milk, both in terms of molecular mass and hydrophobicity. The whey protein β-lactoglobulin, which is the main allergen in bovine milk, could not be detected in camel milk. Several unidentified proteins were characterised in camel milk.

Digestibility of camel milk proteins was comparable to that of bovine milk proteins, with the caseins being almost fully digested after simulated gastric digestion; whey proteins were resistant to gastric digestion, but were rapidly digested during subsequent simulated duodenal digestion. The results obtained with raw camel milk did not differ from those obtained with tunnel-frozen, home frozen and camel milk powder.

The characteristics of tunnel-frozen, home frozen camel milk and camel milk powder do not differ from those of fresh raw camel milk. The protein fraction of camel milk differs considerably from that of bovine milk. It contains several unidentified proteins. As in camel milk from other countries also Dutch camel milk does not contain the protein β-lactoglobulin, which is considered the main allergen in bovine milk.

Key words: Camel milk powder, camel, dromedary, health, home-frozen, tunnel-frozen, β-lactoglobulin

Camel milk contains several protective proteins including immunoglobulins, complements, lysozyme, lactoferrin etc (El-Agamy, 2006). In contrast with bovine milk, camel milk does not form a coagulum in acid environment (Abu-Lehia, 1989; Wangoh, 1993). This lack of coagulum formation allows camel milk to rapidly pass through the stomach, together with undamaged protective proteins. This has been raised to explain the superiority of several health and medicinal properties of camel milk above those of bovine milk. The hypo-allergenicity is ascribed to the lack of β-lactoglobulin, the main cause of cow's milk protein allergy (Nodake *et al,* 2010). Currently, it is not known which constituents, or combinations thereof, in camel milk contribute to the acclaimed health-benefits and medicinal properties.

In the thus far only Dutch camel dairy farm 15 dromedaries are milked, using a milking machine (Smits and Monteny, 2009). To optimise the market potential of camel milk detailed knowledge and understanding of its constituents and properties as to the health-benefit aspects is required.

Therefore we studied the gross composition of Dutch camel milk, the characterisation of its protein fraction and the digestibility there of to guide further studies on potential nutritional and health aspects. Furthermore we established the influence of freezing and spray-drying on camel milk constituents and properties. Samples were bench-marked against bovine milk in all cases.

Materials and Methods

Milk samples

Aliquots (3 L each) of raw camel milk, tunnel frozen camel milk and camel milk frozen in the home-freezer were provided to NIZO food research by the Dutch camel dairy farm. Part of the raw camel milk was spray-dried on the day of receipt; the remainder of the milk samples was frozen at -40°C prior to further analysis. Raw whole bovine milk (5 L) was

SEND REPRINT REQUEST TO M.G. SMITS email: mgsmitsm@online.nl

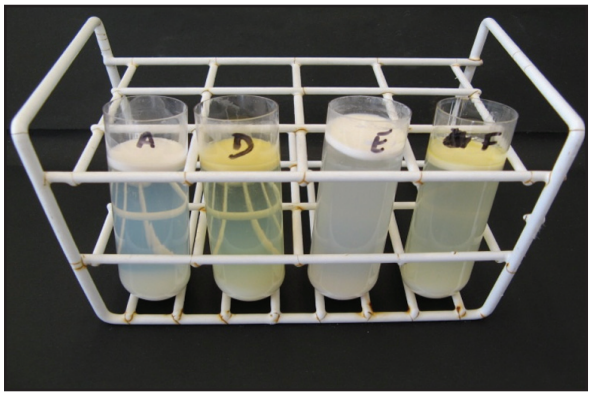

Fig 1. Milk samples following ultracentrifugation; (A): raw whole camel milk, (D) raw whole bovine milk; (E) reconstituted whole camel milk powder and (F) reconstituted whole bovine milk powder.

obtained from a local dairy farm. Part of this milk was spray-dried and the remainder was frozen just like the camel milk.

Spray-drying of milk

Aliquots (2 L each) of raw camel milk and bovine milk were spray-dried. The inlet temperature of the air was 180°C and the outlet temperature 90°C.

Reconstruction of milk powder

Spray-dried whole camel or bovine milk was reconstituted in demineralised water at a level of 10% (m/m) by dissolving 25 g of milk powder in 225 g of water, prior to characterisation of the protein fraction therein.

Ultracentrifugation

To separate the fat, serum and micellar phases, samples of fresh, frozen and reconstructed camel and bovine milk were subjected to ultracentrifugation at 60,000 x g for 75 min at 20°C. Frozen samples were first thawed overnight at 5°C and subsequently equilibrated at room temperature for 4 hours.

Analytical methods

Compositional analysis

The nitrogen content of milk samples was determined using the Kjedahl method. Nitrogen content was converted to protein content using a multiplication factor of 6.38. The fat content of milk samples was determined butyrometrically using the Gerber method. The lactose content of milk samples was determined by reversed-phase high performance liquid chromatography (RP-HPLC).

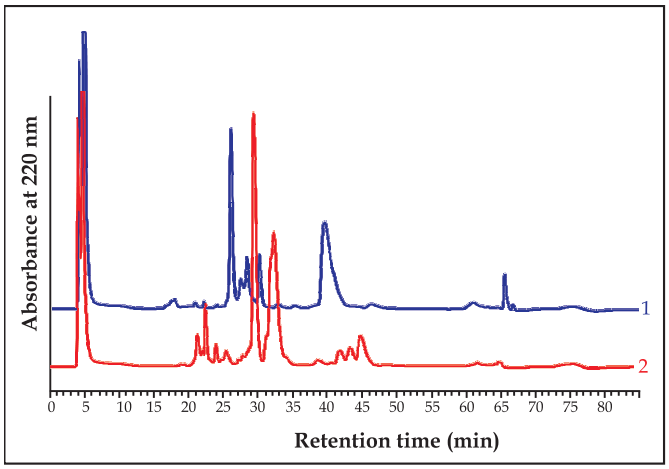

Fig 2. Reversed-phase chromatograms of raw camel milk (1) and raw bovine milk (2).

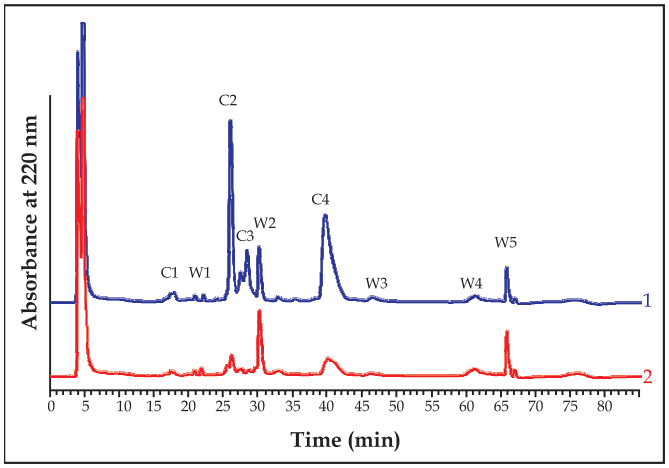

Fig 3. Reversed-phase chromatograms (RP-HPLC) of (1) raw whole camel milk and (2) its ultracentrifugal serum. Peaks tentatively identified as caseins and marked with C whereas those tentatively identified as whey proteins are marked with W in the chromatogram for raw whole camel milk.

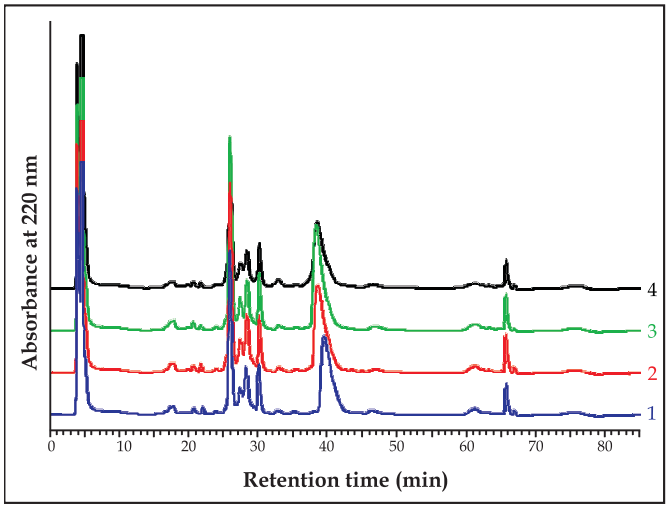

Fig 4. RP-HPLC chromatograms of (1) raw whole camel milk, (2) tunnel-frozen whole camel milk, (3) home-frozen whole camel milk, and (4) reconstituted whole camel milk powder.

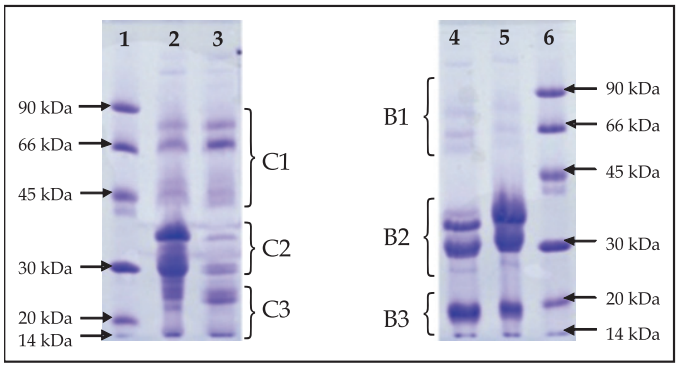

Fig 5. SDS-Page electrophoretograms of (1) molecular mass marker; (2) raw whole camel milk; (3) ultracentrifugal serum of raw whole camel milk; (4) ultracentrifugal serum of raw whole bovine milk; (5) raw whole bovine milk; (6) molecular mass marker. The molecular masses of the proteins included in the molecular mass marker are indicated.

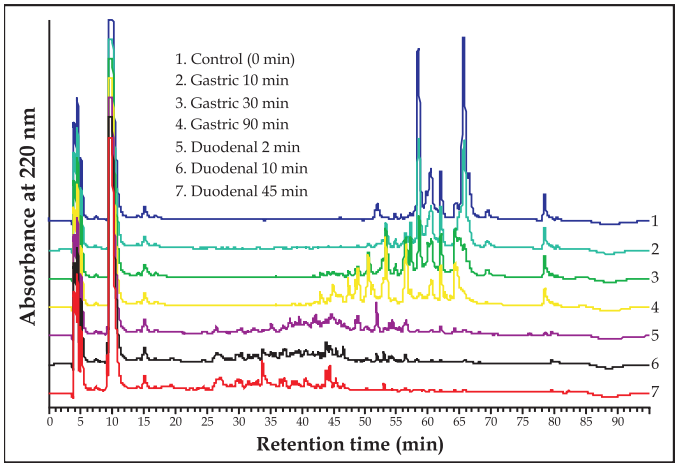

Fig 6. RP-HPLC chromatograms of raw whole camel milk subjected to simulated gastric digestion and subsequent simulated duodenal digestion.

The protein fraction of camel milk was characterised in more details using analytical RP-HPLC and sodium dodecyl sulphate poly acrylamide gel electrophoresis (SDS-PAGE). RP-HPLC was carried out according to a method adapted from Visser *et al* (1991) using a 250 x 4.6 mm Widepore C18 column (Bio-Rad Laboratories, Richmond, CA, USA) with a C18 cartridge (Bio-Rad) as a guard column. The solvents used were mixtures of acetonitrile, water and trifluoroacetic acid (TFA). Detection was carried out by UV absorption at 220 nm. Separation is largely based on differences in hydrophobicity of the proteins and peptides. SDS-PAGE was performed under reducing conditions on 12.5% homogenous gels using a PhastSystem (Amersham Biosciences) and subsequently stained using Coomassie Brilliant Blue. SDS-PAGE is an electrophoretic technique wherein the proteins are separated dependent of the size of the protein molecules. The smaller molecules migrate faster than the larger ones (O'mahony *et al,* 2003).

Digestion of proteins of camel and bovine milk under simulated gastric and duodenal conditions was performed using the SIMPHYD protocol, which is a protocol of NIZO food research that is based on earlier published work (Agudelo *et al,* 2004; Jakobsson and Beneditsson 1982; Kiers *et al,* 2000 and Sakai *et al,* 2000). Samples were taken at 10, 30 and 90 minutes of simulated gastric digestion and after 2, 10 and 45 minutes of subsequent duodenal digestion, and were analysed by RP-HPLC.

Solubility of milk powder was estimated by determination of nitrogen solubility index (NSI) according to the AOCS method. NSI is a measure for the amount of nitrogenous material (e.g. proteins, peptides, amino-acids and urea) which becomes non-sedimentable in a certain time under standardised conditions.

Results

Protein, fat and lactose content are outlined in Table 1. Protein content was slightly lower than that of bovine milk, the fat content of camel milk was comparable to that of bovine milk and the lactose content of camel milk (4.2%) was considerably lower than that of bovine milk (4.9%).

Camel milk and bovine milk powder looked nearly identical. As shown in Table 2 the composition of camel and bovine milk powder were near identical. Following reconstruction of a 10% (m/m) basis, near identical protein contents were observed for reconstituted camel and bovine milk powders. The NSI value of camel milk powder (74%) was higher than that of bovine milk powder (63%).

Table 1. Protein, fat, lactose content (%) of camel and bovine milk samples.

Sample	Protein	Fat	Lactose
Raw whole camel milk	3.34	3.84	4.21
Tunnel-frozen whole camel milk	3.41	4.41	4.49
Home-frozen whole camel milk	3.34	5.78	3.78
Raw whole bovine milk	3.57	4.70	3.93

Ultracentrifugation separated the milk into a cream layer on top, a proteinaceous pellet and a slightly turbid intermediate layer of serum. As shown in fig 1, the colours of the fat layer and the serum of camel milk (samples A and E) differed considerably of that of bovine milk (samples D and F).

Table 2. Protein, fat and lactose content and nitrogen solubility index (NSI) of milk powder prepared from raw whole camel or bovine milk and milk powder reconstituted from powder at 10% m/m.

	Camel milk		Bovine milk	
	Powder	Reconstituted powder	Powder	Reconstituted powder
Protein (%,m/m)	26.3	2.6	26.1	2.5
Fat (%,m/m)	30.9		32.3	
Lactose (%,m/m)	35.1		34.2	
NSI (%)	74.0		62.9	

The protein contents of the ultracentrifugal sera of milk samples are summarised in Table 3. For the 3 camel milk samples, about 45% of protein remained in the serum after ultracentrifugation.

Table 3. protein content (%, m/m) of milk samples and their ultracentrifugal serum.

	Milk	Ultracentrifugal supernatant
Raw whole camel milk	3.34	1.54
Tunnel-frozen camel milk	3.41	1.58
Home-frozen camel milk	3.34	1.62
Raw whole bovine milk	3.57	1.89
Reconstituted camel milk powder	2.60	1.21
Reconstituted bovine milk powder	2.53	1.06

For reconstituted camel milk, about 45% of total protein remained in serum, whereas for bovine milk only about 40% remained in the serum. For camel milk spray-drying did not result in increased formation of sedimentable material as the proportion of sedimentable material was comparable for milk samples and reconstituted milk powder. For raw bovine milk, however about 50% of protein was non-sedimentable, but only about 40% of protein was non-sedimentable in reconstituted bovine milk powder (table 3). Consequently bovine milk powder contains more poorly soluble material, which may have formed during spray-drying, and is in agreement with the higher NSI value of camel milk powder than of bovine milk powder.

The RP-HPLC chromatograms of raw whole camel milk and bovine milk are displayed in Fig 2. The chromatogram of raw bovine milk is representative to that commonly observed for bovine milk and comparable to those reported by Visser *et al* (1991). The peaks in the chromatograms with a retention time of < 10 min represent low-molecular weight components (urea, citrate and dithiothreitol) which are used in the sample preparation, whereas the peak at 77 min is inherent to the acronitrile gradient used in this experiment.

Table 4. Tentative identification of peaks in the RP-HPLC chromatogram of camel milk shown in fig 3.

Peak	Retention time	Protein
C1	17	κ-casein
C2	25	α_{s1}-casein
C3	27-28	α_{s2}-casein
C4	40	β-casein
W1	21-22	?
W2	30	α-lactalbumin
W2	47	serum albumin
W3	62	Immunoglobulin
W4	67	?

The RP-HPLC chromatograms of raw whole camel milk and its ultracentrifugal serum are shown in Fig 3. Although a definite identification of the nature of the various peaks in the camel milk chromatograms cannot be made with the existing knowledge, literature data can be used for the purpose of tentative identification. Results hereof are presented in Table 4. Caseins could be identified based on the studies of Kappeler (1998) and El-Agamy (2006) using comparable RP-HPLC conditions. Identification of the whey proteins is more difficult because comparable RP-HPLC data or isolated standards are not available to date. Assuming broad similarities with their bovine counterparts, peaks W3 and W4 in Fig 3 could potentially represent serum albumin and immunoglobulin. Furthermore, based on reports that α-lactalbumin is the major whey protein in camel milk (El-Agamy, 2006; Farah 1996; Kapeller, 1998) . It is conceivable that W2, the major whey protein, represents α-lactalbumin. Other whey proteins believed to be present in camel milk are lactoferrin and lactophorin (El-Agamy, 2006; Farah, 1996; Kapeller, 1998). However, with existing knowledge, these proteins cannot be assigned to peaks W1 and W5 with any certainty.

Chromatograms for tunnel-frozen camel milk, home frozen camel milk or reconstituted camel milk powder were comparable to that of raw whole camel milk, and are shown in Fig 4.

The SDS-PAGE electrophoretograms of raw whole camel milk and raw whole bovine milk are presented in Fig 5, showing considerable differences between the protein fractions of camel and bovine

milk. In bovine milk fractions B1 and B3 contain the non-sedimentable whey proteins, whereas fraction B2 contains the sedimentable caseins. The upper band in fraction B3 represents β-lactoglobulin, the lower α-lactalbumin. In camel milk the bands in section C1 and C3 were whey proteins, whereas section C2 contains the caseins; the 2 major bands herein were attributed to α_{s1}-casein (top) and β-casein (bottom). Of the whey proteins the bottom band in section C3, with a molecular mass of about 66 kDa was considered the blood serum albumin (El-Agamy, 2006). The identity of the camel proteins with a molecular mass of 20-25 kDa (section C3) and 40-50 and about 80 kDa (section C1) is at present unknown. The latter is potentially lactoferrin, which is isolated from camel milk and shown to have a mass of about 80 kDa (Kapeller *et al,* 1999). For the former three proteins counterparts were not detected in bovine milk.

RP-HPLC chromatograms of raw whole camel milk subjected to simulated physiological digestion using the SIMPHYD protocol are shown in Figure 6. Chromatograms for tunnel-frozen and home-frozen raw camel milk and reconstituted camel and bovine milk powder were comparable to those of raw whole camel milk. The gradient applied for RP-HPLC analysis of the milk digests differed from that of those applied for the identification of proteins to enable better visualisation of the peptides produced during digestion. As a result, retention times of proteins differ from those in Figure 2 and figure 3 but the order of elution is retained.

For camel milk, the peaks previously described as caseins decreased progressively with increasing time during gastric digestion and were almost completely absent from the chromatograms after 90 minutes. Concomitantly, a number of new peaks appeared with increasing degree of digestion, with retention times lower than those of the original proteins; these peaks most likely present proteolysis products (Fig 6). In contrast the peaks of previously identified as whey proteins were largely resistant to gastric digestion, but were fully degraded during 2 min. of subsequent duodenal digestion (Fig 6). These data indicate that the caseins in camel milk are largely digested in the stomach, but most of the whey proteins survive the passage almost intact, and is digested only in the duodenum. The digestive pattern of whole bovine milk was comparable to that of whole camel milk.

Discussion

This explanatory study showed that the gross composition of camel milk does not change by home and tunnel freezing or by making camel milk powder and that camel milk contains several thus far unidentified proteins. Furthermore it confirmed earlier findings showing that camel milk, just as human milk, is devoid of β-lactoglobulin (El-Agamy, 2006).

The composition of raw whole camel milk is changed by heat treatment (Farah, 1986). This suggests that camel milk loses the health benefits and medical properties when it is pasteurised. Therefore it is recommended to use only fresh raw camel milk when its supposed beneficial effects are desired. The present study shows that then also frozen camel milk and camel milk powder can be used.

During spray-drying the temperature is increased. However this concerns mainly the environmental temperature. The temperature in the milk droplets themselves is hardly increased. This explains why spray-drying does not negatively influence gross composition of camel milk.

The unidentified proteins might be involved in the immunological (El-Agamy, 2006) and antidiabetic properties (Agrawal *et al,* 2005 and Mohamad *et al,* 2009) of camel milk.

More than 80% of cow's milk protein allergy is caused by allergy to β-lactoglobulin (Nodake *et al,* 2010). As camel milk does not contain β-lactoglobulin it can be assumed that camel milk is suitable for patients with cow's milk allergy due to allergy for β-lactoglobulin.

In industrial practice, bovine milk commonly undergoes various pre-treatments prior to powder production, e.g. homogenisation to decrease the fat globules, and evaporation to concentrate the sample prior to drying (Kelly *et al,* 2003; Walstra 2006). Particularly homogenisation and evaporisation are known to increase solubility of whole milk powders (Kelly *et al,* 2003). As the solubility of camel milk is higher than that of camel milk further improvements on the solubility of camel milk powder appear readily achievable, if required. These pre-treatments seem to be less necessary for camel milk powder, as we found that camel milk powder is better soluble than bovine milk powder.

Protein, fat and lactose content of the Dutch camel milk was representative for those reported in camels from different African and Asian countries (El-Agamy 2006). Also protein fractions analysed using RP-HPLC and SDS-PAGE were similar to those reported earlier (El-Agamy 2006; Farah 1996; kappeler 1998; O'mahony *et al,* 2003). Consequently findings in

milk from camels in other countries generally apply to Dutch camel milk and vice versa.

Our study does not support the hypothesis that camel milk proteins are absorbed better in the gastro-intestinal tract than bovine milk proteins.

Strength of the present exploratory study was the careful analysis using modern techniques, including the SYMPHYD protocol. Nevertheless several limitations need consideration.

Vitamin A content is reported to be about 3 fold lower in camel milk than in bovine milk (Farah *et al*, 1992) thus explaining the whiter colour of camel milk fat.

Following centrifugation less (about 45%) of proteins remained in serum in camel milk than in bovine milk (about 50%). This higher degree of sedimentation of proteinaceous material from camel than bovine milk is in agreement with the larger size of camel casein micelles than of bovine casein micelles (Farah and Ruegg, 1989) as sedimentation speed increases with increasing particle size.

A remarkable finding in our study was the lack of detailed knowledge of the composition and characterisation of many camel milk proteins.

References

Abu-Lehia JH (1989). Physical and chemical characteristics of camel milk fat and its fractins. Food Chemisty 34:262-271.

Agrawal RP, Beniwal R, Kochar DK, Tuteja FC, Ghorui SK, Sahani MS and Sharma S (2005). Camel milk as an adjunct to insulin therapy improves long-term glycemic control and reduction in doses of insulin in patients with type-1 diabetes A 1 year randomized controlled trial. Diabetes Research in Clinical Practice 68:176-177.

Agudelo RA, Gauthier SF, Pouliot Y, Marin J and Savoie L (2004). Kinetics of peptide fraction release during in vitro deigestion of casein. Journal of the Science of Food and Agriculture 84:325-332.

El-Agamy ESI (2006). Camel milk. In: Y.P. Park and G.F.W. Haenlein (Ed) Handbook of Milk of Non-Bovine Mammals. Blackwell Publishing, Oxford pp 297-344.

Farah (1986). Effect of heat treatment on whey proteins of camel milk. Milchwissenschaft 41:763-765.

Farah Z (1996). Camel Milk; Properties and Products. St Gallen.

Farah Z, Rettenmaier R and Atkins, D (1992). Vitamin content of camel milk. International Journal of Vitamin Nutrition Research 62:30-33.

Farah Z and Ruegg MW (1989). The size distribution of casein micelles in camel milk. Food Microstructure 8:211-216.

Jakobsson ILT and Beneditsson B (1982). In vitro digestion of cow's milk proteins by duodenal juice from infants with various gastrointestinal disorders. Journal of Pediatrics, Gastroenterology and Nutrition 1:183-191.

Kapeller SR (1998). Compositional and Structural Analysis of the Camel Milk Proteins with Emphasis on Protective Proteins. ETH, Zurich, Switzerland.

Ref Type: Thesis/Dissertation

Kapeller SR, Ackermann M, Farah Z and Puhan Z (1999). Sequence analysis of camel (*Camelus dromedarius*) lactoferrin. International Dairy Journal 9:481-486.

Kelly A, O'Connell J and Fox PF (2003). Manufacture and properties of milk powders. In: P.F. Fox and P.L.H. McSweeny (Ed) Advanced Dairy Chemistry 1: Proteins. Kluwer Academic / Plenium Publishers, New York 1027-1061.

Kiers JL, Van Laeken AE, Rombouts FM and Nout MJ (2000). In vitro digestibility of bacillus fermented soya bean. International Journal of Food Microbiology 60:163-169.

Mohamad RH, Zekry ZK, Al Mehdar HA, Salama O, El Shaieb SE, El Basmy AA, Al said MG and Sharawy SM (2009). Camel milk as an adjuvant therapy for the treatment of type 1 diabetes: verification of a traditional ethnomedical practice. Journal of Medicine Food 12:461-465.

Nodake Y, Fukumoto S, Fukasawa M, Sakakibara R and Yamasaki N (2010). Reduction of the immunogenicity of beta-lactoglobulin from cow's milk by conjugation with a dextran derivative. Bioscience, Biotechnology and Biochemistry 74:721-726.

O'mahony JA, Ardo Y and Mc Sweeney PL (2003). H. Electrophoresis. Encyclopedia of Dairy Sciences. academic Press, Amsterdam. pp 67-74.

Sakai K, Yoshino K, Satter MA, Ota F, Nii Y, Fukuta K, Ueda N, Shimizu Y and Yamamoto S (2000). Effects of pH variation and NaCl on in vitro digestibility of cow's milk proteins in commercially available infant formulas. Journal of Nutrition Science Vitaminology (Tokyo) 46:325-328.

Smits MG and Monteny GJ (2009). Ammonia emission from camel dairy in The Netherlands. Journal of Camel Practice and Research 16:139-142.

Visser S, Slangen CJ and Rollema HS (1991). Phenotyping of bovine milk proteins by reversed-phase high-performance liquid chromatography. Journal of Chromatography 548:361-370.

Walstra PWJTM (2006). Dairy Science and Technology, 2nd edition. Boca, Raton.

Wangoh J (1993). What steps towards camel Milk technology? International Journal of Animal Science 8:9-11.