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# VARIABILITY OF THE PROXIMATE AND PROTEIN COMPOSITION OF CAMEL MILK (Camelus dromedarius)

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This dissertation is submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

Under the Supervision of Professor Afaf Kamal-Eldin

April 2021

## Declaration of Original Work

I, Huda Musa Elsayed Mohamed, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this dissertation entitled "*Variability of the Proximate and Protein Composition of Camel Milk* (*Camelus dromedarius*)", hereby, solemnly declare that this dissertation is my own original research work that has been done and prepared by me under the supervision of Professor Afaf Kamal-Eldin, in the College of Food and Agriculture at UAEU. This work has not previously been presented or published or formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my dissertation have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this dissertation.

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## Abstract

Dromedary camel milk (*Camelus dromedarius*) has unique physical, nutritional, and technological properties when compared with other milks. Unlike bovine milk, the processing of camel milk into fermented products and its treatment using ultra-high temperatures is technically challenging. Therefore, this research aimed to determine the variability in the proximate composition and the protein composition of camel milk collected from individual animals that are reared under intensive management in the UAE. To determine the proximate composition of samples (n = 217) were analysed by NIR and MIR spectroscopy methods. The results obtained by the two methods were also compared. The results of proximate composition showed a wide variation in the concentration of proteins (2.4 - 4.0%), fats (1.2 - 7.3%), lactose (3.0 - 5.7%) and total solids (9.1 - 15.2%). Excellent positive correlations between the two methods were obtained ($p < 0.001$); for protein ($r \geq 0.96$), fat ($r \geq 0.99$), lactose ($r = 0.82$) and total solids ($r = 0.90$). The mean of the relative difference ((MIR values - NIR values)/0.5 (MIR values + NIR values) × 100%) were: for protein (+13.4%), fat (+0.9%), lactose (-0.7%) and total solids (-3.4%). The difference between the two methods may be due to the effects of differences in milk homogeneity, especially with respect to casein micelles and fat globules.

Because proteins confer many of the properties of milk and its products, this research aimed to determine the concentrations of camel milk proteins, their correlations, and relative concentration of the caseins. Raw milk samples were collected from individual dromedary camels (n = 206) 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; αS1-casein, 2.4 to 10.3; αS2-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 of percent composition of the 4 caseins were as follows: αS1-, 12.7 to 35.3; αS2-, 1.8 to 20.8; β-, 42.3 to 77.4; and κ-, 0.6 to 17.4. The relative proportion of αS1-, αS2-, β-, and κ-caseins in camel milk averaged (26:4:67:3, wt/wt) which is different from that of bovine milk (38:10:36:12, wt/wt). This difference might explain the dissimilarity between the two milks with respect to technical and nutritional properties.

Dromedary camel milk includes several bioactive whey proteins with potential health effects. This research also aimed to study the variability in the concentrations of several bioactive whey proteins in milk collected from individual Dromedary camels. Milk samples (n =140) were collected from individual camels reared under intensive management. The concentrations of Insulin (IN), Insulin-Like Growth Factor-I (IGF1), Insulin-Like Growth Factor-II (IGF2), Lactoferrin (LF), Immunoglobulin G (IgG), Peptidoglycan Recognition Protein-1 (PGRP1), Lysozyme (LZ), and Lactoperoxidase (LPO), were determined using camel-specific quantitative sandwich enzyme linked immuno-sorbent assay (ELISA) kits. The range of concentration of the studied proteins were: IN (17.8 - 51.1 mIU/L), IGF1 (1.4 - 736.1 ng/ml), IGF2 (13.7 - 82.6 ng/ml), LF (639.4 - 2,094.9 ug/ml), IgG (7.3 - 17.9 mg/ml), PGRP1 (1.6 - 22.3 ng/ml), LZ (23.3 - 71.4 ug/ml), and LPO (7.1 - 15.5 ng/ml). Significant Pearson correlations ($p \leq 0.05$) were observed between IN & LZ ($r = 0.759$), IN & IgG ($r = 0.502$), IN & PGRP1 ($r = 0.6702$), LZ & PGRP1 ($r = 0.641$), IgG & LPO ($r = 0.698$) and IgG & PGRP1 ($r = 0.398$). There is a wide variability in the concentrations of the studied bioactive whey proteins in Dromedary camel milk. IGF1 and IGF2 are present in concentrations much higher than reported values in bovine and human milk shedding a light on possible importance in human nutrition.

**Keywords:** Camel milk, protein, fat, lactose, total solids, Near Infrared, Mid Infrared spectroscopy, α-lactalbumin, casein proteins, capillary electrophoresis, insulin, insulin-like growth factors, lactoferrin, immunoglobulin, peptidoglycan recognition protein-1, lysozyme, lactoperoxidase.

**Title and Abstract (in Arabic)**

## التباين في التكوين التقريبي ومكونات بروتين حليب النوق (*Camelus dromedarius*)


***الملخص***

يتميز حليب النوق (*Camelus dromedarius*) بخصائص فيزيائية وتغذوية وتكنولوجية فريدة بالمقارنة مع أنواع الحليب الأخرى. على عكس حليب الأبقار، فإن معالجة حليب النوق إلى منتجات مخمرة ومعالجته باستخدام درجات الحرارة عالية جدًا يمثل تحديًا تقنيًا. لذلك، يهدف هذا البحث الى تحديد المكونات التقريبية لحليب النوق (عدد العينات = 217) الذي تم جمعه من حيوانات منفردة تحت التربية المكثفة ومن ثم تحليل مكونات البروتين لهذه العينات. تم تحليل العينات بطرق التحليل الطيفي باستخدام الأشعة تحت الحمراء القصيرة (NIR) والمتوسطة (MIR) ومن ثم تمت مقارنة النتائج المتحصل عليها من الطريقتين. أبدت النتائج تباين واسع في محتوى (%) البروتين (2.5-4.0)، الدهون (1.2-7.3)، اللاكتوز (3.0-5.7) والمواد الصلبة الكلية (9.1 – 15.2). تم الحصول على ارتباطات (pearson correlation) ممتازة وموجبة بين الطريقتين (p-value<0.05): البروتين (r = 0.96)، الدهون (r = 0.99)، اللاكتوز (r = 0.82) والمواد الصلبة الكلية (r = 0.90). بينما كان متوسط الإختلاف النسبي ( relative % difference): للبروتين (+ 13.4 %)، الدهون (+ 0.9 % )، اللاكتوز ( −0.7 % ) والمواد الصلبة الكلية ( −3.4 % ). قد يرجع الإختلاف بين الطريقتين إلى تأثير الإختلاف في تجانس الحليب، خاصة فيما يتعلق بمذيلات الكازين وكريات الدهون.

نظرًا لأن البروتينات تمنح العديد من خصائص الحليب ومنتجاته، فقد هدف هذا البحث أيضاً إلى تحديد تراكيز بروتينات حليب النوق، وترابطها، والتركيز النسبي للكازين. تم جمع عينات الحليب الخام من حيوانات منفردة في الصباح والمساء (عدد العينات = 206). أظهرت نتائج الإرتحال الكهربائي الشعري تباينًا كبيرًا في تراكيز البروتينات (جم / لتر) بين العينات و كانت النتائج على النحو التالي: ألفا لاكتالبومين: 0.3 إلى 2.9؛ αS1- كازين ، 2.4 إلى 10.3 ؛ αS2- كازين ، 0.3 إلى 3.9 ؛ β- كازين ، 5.5 إلى 29.0 ؛ κ- كازين ، 0.1 إلى 2.4 ؛ بروتين الكازين غير المعروف 1 ، 0.0 إلى 3.4 ؛ و بروتين الكازين غير المعروف 2 ، 0.0 إلى 4.6. مدى النسبة المئوية للكازين كان على النحو التالي:αS1 : 12.7 إلى 35.3؛ αS2 ، 1.8 إلى 20.8 ؛ β ، من 42.3 إلى 77.4 ؛ و κ ، 0.6 إلى 17.4. اختلف المحتوى النسبي للكازين αS1- ، αS2- ، β- ، κ- في حليب النوق (26: 4: 67: 3 ، بالوزن / الوزن) عن تلك الموجودة في حليب الأبقار

(38:10:36: 12 ، بالوزن / بالوزن). قد يفسر هذا الإختلاف عدم التشابه الموجود بين حليب النوق والأبقار فيما يتعلق بالخصائص الفنية والغذائية.

يحتوي حليب النوق على العديد من البروتينات النشطة بيولوجيًا التي لها آثار صحية محتملة. تعتبر بروتينات مصل اللبن مصدرًا مقترحًا للخصائص الطبية لهذا الحليب. كانت من أهداف البحث أيضا دراسة التباين في تراكيز العديد من بروتينات مصل اللبن النشطة بيولوجيًا في عينات حليب النوق التي تم جمعها من حيوانات منفردة تم تربيتها في الإمارات العربية المتحدة. تم تحديد تركيز هذه البروتينات في عدد كبير من عينات حليب النوق. جمعت عينات الحليب (عدد العينات = 140) من نوق تحت التربية المكثفة. تراكيز كل من الأنسولين (IN)، عامل النمو الشبيه بالأنسولين I (IGF1)، عامل النمو الشبيه بالأنسولين II (IGF2)، اللاكتوفيرين (LF)، الغلوبولين المناعيG (IgG)، بروتين التعرف على الببتيدوغليكان (PGRP1)، الليزوزيم (LZ)، ولاكتوبيروكسيديز (LPO) تم تحديديها باستخدام طرق ال ELISA للتقدير الكمي المخصصة للإبل. كان نطاق تركيز البروتينات المدروسة: IN (17.8-51.1 mIU/لتر)، IGF1 (1.4-736.1 نانوغرام / مل)، IGF2 (13.7-82.6 نانوغرام / مل)، LF (639.4 - 2.094.9 ميكروغرام / مل)، IgG (7.3-17.9 مجم / مل)، PGRP1 (1.6-22.3 نانوغرام / مل)، LZ (23.3-71.4 ميكروغرام / مل)، وLPO (7.1-15.5 نانوغرام / مل). لوحظ وجود إرتباطات كبيرة ($p \leq 0.05$) بين IN & LZ (r = 0.759)، IN & IgG (r = 0.502)، IN & PGRP1 (r = 0.6702)، LZ & PGRP1 (r = 0.641)، IgG & LPO (r = 0.698) و IgG & PGRP1 (r = 0.398). توجد البروتينات النشطة بيولوجيًا في حليب النوق بتراكيز واسعة النطاق. يوجد IGF1 وIGF2 بتركيزات أعلى من حليب الأبقار والحليب البشري مما يلقي الضوء على الأهمية المحتملة في تغذية الإنسان.

**مفاهيم البحث الرئيسية**: حليب النوق، البروتين، الدهون، اللاكتوز، المواد الصلبة الكلية، الأشعة القصيرة والمتوسطة تحت الحمراء، التحليل الطيفي، ألفا لاكتالبومين، الكازينات، الهجرة الكهربية الشعرية، الأنسولين، عامل النمو الشبيه بالأنسولينI، عامل النمو الشبيه بالأنسولينII، لاكتوفيرين، الغلوبولين المناعيG، بروتين التعرف على الببتيدوغليكان، ليزوزيم، لاكتوبيروكسيدز.

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Emirates University I enjoyed this journey with you, thank you for your kindness and support.

# Dedication

*To my beloved parents, brothers, sisters, and friends*

*To all my teachers*

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# List of Abbreviations

| | |
|---|---|
| AA | Amino-Acid |
| BM | Bovine Milk |
| CM | Camel Milk |
| CE | Capillary Electrophoresis |
| ELISA | Enzyme-Linked Immunosorbent Assay |
| IGF1 | Insulin-Like Growth factor-I |
| IGF2 | Insulin-Like Growth Factor-II |
| IgG | Immunoglobulin G |
| IN | Insulin |
| LF | Lactoferrin |
| LLA | Lower Limit of Agreement |
| LPO | Lactoperoxidase |
| LZ | Lysozyme |
| MIR | Mid InfraRed |
| NIR | Near InfraRed |
| PGRP1 | Peptidoglycan Recognition Protein-1 |
| PTM | Post translational Modification |
| SDS | Sodium Dodoecyl Sulphate Polyacrylamide Gel Electrophoresis |
| UAE | United Arab Emirates |
| ULA | Upper Limit of Agreement |

# Chapter 1: Introduction

## 1.1 Overview

Milk produced by different animals is a good source of macro- and micro-nutrients and contributes to the nourishment of people of all ages around the world. Dromedary camel milk continues to be an optimum and stable source of nourishment in the arid areas of the world including the United Arab Emirates. Earlier Dromedary camel milk was valued for its medicinal properties and nowadays around the world and it is renowned for these properties. According to the most recent Food and Agriculture Organization statistics (FAOSTAT, 2019), 87.1% of the camel's population lies in Africa and 12.9% in Asia (FAOSTAT, 2019). In Asia, the United Arab Emirates (UAE) comes in the 4$^{th}$ rank with a population of 457,000 animals after Pakistan (1,090,000), Saudi Arabia (492,853) and Yemen (461,788). Figure 1 displays the population of Dromedary camels and the milk production in the UAE for the years (1974 – 2018) and (1968 – 2018), respectively (FAOSTAT, 2019). On the onset of the 20$^{th}$ century, a great transformation for camel milk production took place in the UAE; camel rearing and milk production was shifted from rural production only to world class animal intensive management, husbandry and commercial milk production and processing. Currently the UAE has two camel milk processing plants, one of them is the largest in the world.

Dromedary one humped or Arabian camels (*Camelus dromedarius*) distinctively can survive and adapt to the harsh arid conditions due to their physiological peculiarities (Wernery, 2006; Faye, 2014). They are the most efficient domestic animal for converting vegetative matter into work, milk, and meat (Wilson et al., 1990; Farah,

1993). Especially with the current climatic changes, Dromedary camels are a remarkable enhancer of arid lands because of their productive potential and their role in the agro-ecosystem balance (Faye, 2014).

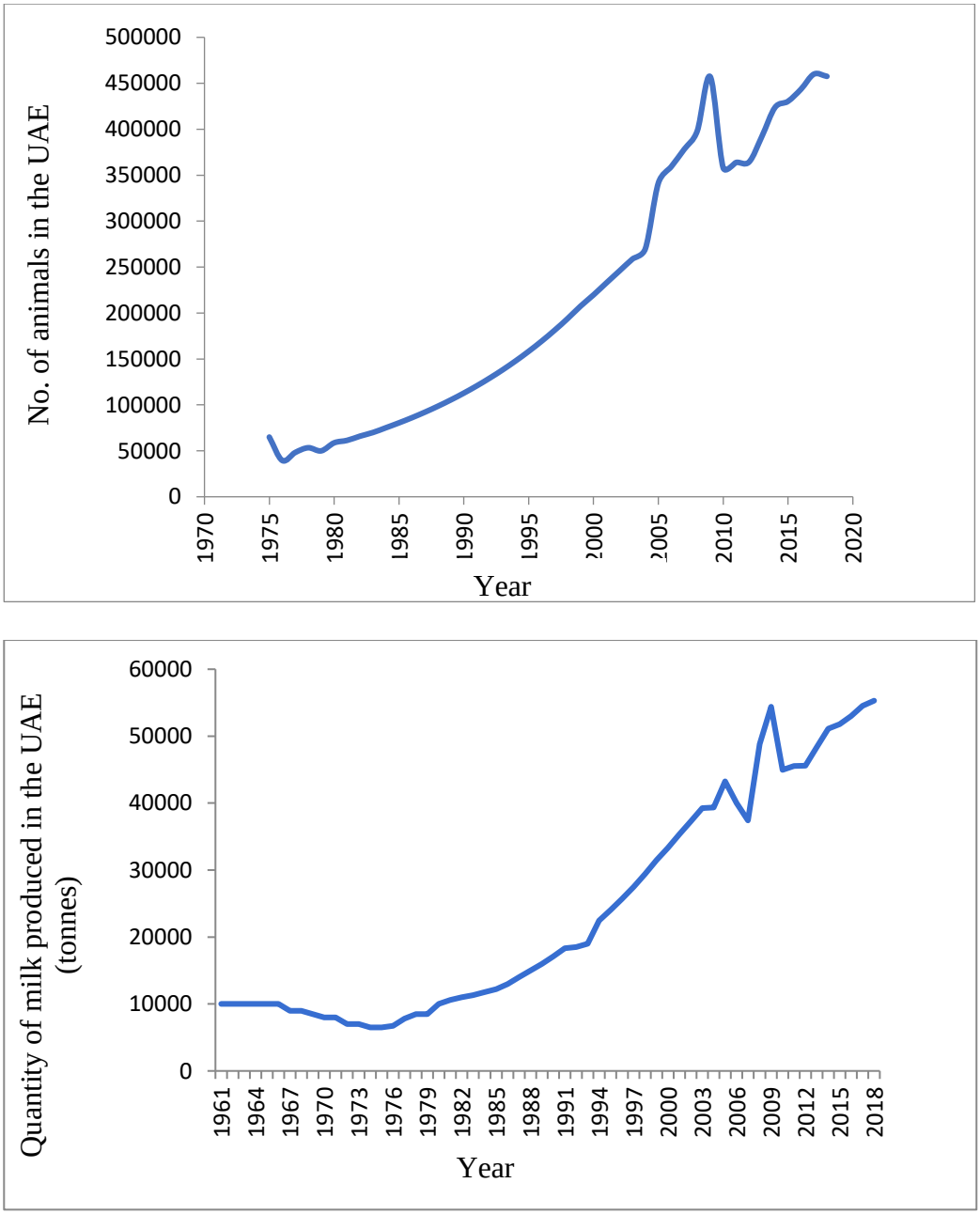


Figure 1**:** Population of Dromedary camels and quantity of milk (tonnes) produced in the UAE (FAOSTAT, 2019)

The proximate composition (protein, fat, lactose, ash, and total solids) of camel milk is roughly the same as cow milk but the structure of their molecules is different (Berhe et al., 2017). The concentrations of the specific proteins (caseins and whey proteins) that form the overall protein also differs between milk producing species. El-Hatmi et al. (2015) compared the milks of humans, camels, cows, goats, and donkeys, they reported that camel milk like human milk lacks β-lactoglobulin and is rich in β-casein and α-lactalbumin. β-casein has better digestibility and being devoid of β -lactoglobulin (major allergen) makes camel milk a substitute to children with cow milk protein allergy (Brezovečki et al., 2015; Izadi et al., 2019).

The results of different studies showed that Dromedary camel milk has medicinal properties and contributes significantly to health and wellness. Whey proteins are a major source for these biological activities of camel milk.

### 1.2 Statement of the Problem

Despite its unique potential and increased contribution to food security through its milk and meat production, less attention has been paid to camels compared to other livestock species (Faye, 2015). The UAE is rewarded by a large Dromedary camel population and its arid lands are the natural habitat of this species, adding value to local products can substantially contribute to maintaining food security and achieving the UAE sustainability goals. Moreover, camels are physiologically adapted to the arid land climate and have low demand for water. The information available from previous research on the protein composition of Dromedary camel milk and the variability in the concentrations of casein and whey proteins is very scarce. Up till now there are no studies done on a large number of samples to give information on the concentration range of these proteins in camel milk. In depth knowledge about the variability in the

concentration of proteins (α-s1, α-s2, β-, κ- caseins and α-lactalbumin) is necessary for explaining the properties of camel milk and interpreting the challenges encountered in camel milk processing therefore expanding the use of camel milk. Intensive qualitative and quantitative camel milk proteins research is a prerequisite to develop food products from camel milk, including yoghurts, cheeses, and long shelf-life milk (Ghnimi & Kamal-Eldin, 2015). Currently there are challenges encountered in processing camel milk, it is expected that the protein composition, i.e., concentrations of casein proteins of camel milk that are dissimilar to cow milk underlie these challenges (Kappeler, 1998; Berhe et al., 2017). Camel milk is reported to have medicinal and health benefits, with whey proteins being the major source of these biological activities (Mati et al., 2017). Very few reports exist on the concentration of camel proteins and in the published studies only a few or pooled samples were analyzed (Elagamy et al., 1996; Kappeler, 1998; Ereifej et al., 2011; Hamed et al., 2012; Omar et al., 2016; Ryskaliyeva et al., 2018).

Dromedary camel milk is valued for its proven health effects and whey proteins are a suggested source for the medicinal properties of this milk. Several properties have been reported for camel milk including antidiabetic, anti-anti-bacterial, anti-allergic, and anti-autistic effects, but the exact components of the milk that might be responsible for these effects and their mechanisms of action are still unknown. Data regarding the concentrations of the bioactive whey proteins (Insulin (IN), Insulin-Like Growth Factor-I (IGF1), Insulin-like Growth Factor-II (IGF2), Lactoferrin (LF), Immunoglobulin G (IgG), Peptidoglycan Recognition Protein-1 (PGRP1), Lysozyme (LZ), and Lactoperoxidase (LPO)) in camel milk is extremely scarce and sometimes not available.

These limitations in previous research does not allow generalized inferences with reference to these values. The lack of comprehensive data on camel milk proteins encouraged us to perform these studies in a large number of samples from individual animals using approved and optimal procedures for representative milk sampling.

The aims of the research were:

1. To study the variability in the proximate composition of Dromedary camel milk collected from individual animals by using Near InfraRed (NIR) and Mid Infrared (MIR) spectroscopy methods.
2. To study and compare the results of the NIR and MIR spectroscopy methods.
3. To study the variability in the protein composition of camel milk collected from individual animals using Capillary Electrophoresis.
4. To study the variability in the concentration of bioactive whey proteins in camel milk collected from individual animals using quantitative sandwich ELISA methods.

## 1.3 Relevant Literature

### 1.3.1 Camels and their Domestication Around the World

Camels belong to the family Camelidae that belong to the order of Artiodactyla (even toed ungulates), and the suborder Tylopoda (pad footed animals). The large camelids (old world camels) are represented by two domesticated species: the one-humped camel (Dromedary, *Camelus dromedarius*) and the two-humped camel (Bactrian, *Camelus bactrianus*), the first living in the hot arid lands of western part of Asia and Africa, the second in the cold steppes and deserts in Central Asia. Worldwide the one-humped camels are dominant. The small camelids (New world camels) originate from South America and include two domestic species (lama and alpaca) and two wild species (guanaco in the genus Lama and vicuna in genus Vicugna). Scientists believe

that ancestors of the modern camel lived in North America at least 40 million years ago and migrated to Asia. Figure 2 demonstrates the magnificent migration of the Camelids and the areas where Dromedary camels are currently domesticated. The distribution areas of Dromedary and Bactrian camels overlap in Western and Central Asia, especially in Turkey, Iran, India, Afghanistan, and Kazakhstan. Hybridization of the two species is most common in Kazakhstan (Soliman, 2015; Brezovečki et al., 2015; Burger et al., 2019).

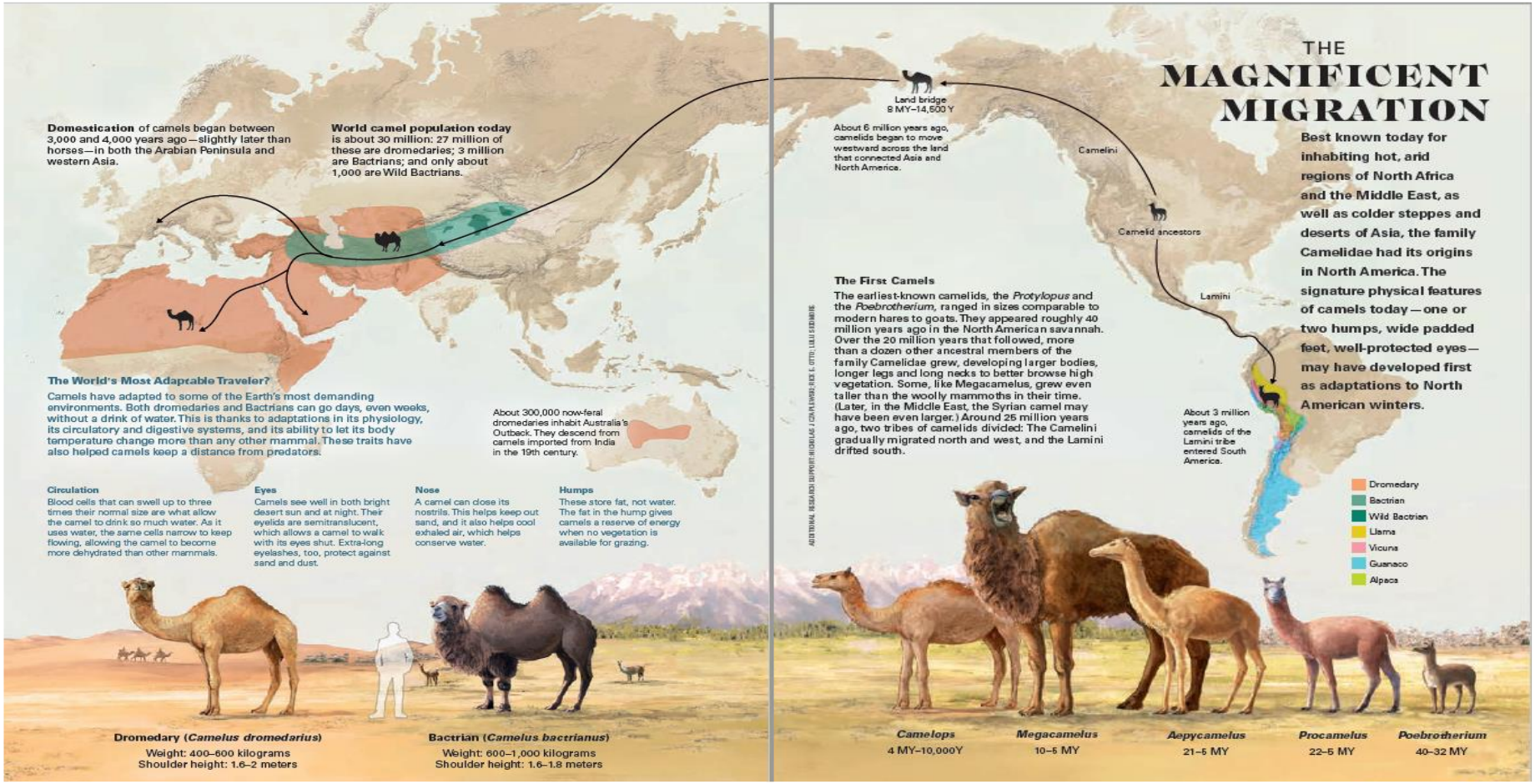


Figure 2: The origins of the Camelidae and the areas where Dromedary camels are currently domesticated. Photo from AramcoWorld (2018) reprinted after permission from AramcoWorld.

### 1.3.2 Body Features of Dromedary Camels

All camels have 74 chromosomes with a very similar morphology. The Arabian camel genome is the first mammalian genome to be sequenced in the Middle East. The findings suggested the possibility of camel-specific evolution to adapt to desert environments. Dromedary camels survive in hot dry desert due to their anatomical structure and its natural adaptations. Figure 3 summarizes the relevant features of Dromedary camels. Other important features include that the body temperature keeps fluctuating from 34°C to 41.7°C (93°F - 107°F) to reduce the sweating. The red blood cells of camels are small and oval to let the flow of blood continue even in a dehydrated state and to prevent them from rupturing due to osmosis. Camels' kidneys are capable of concentrating urine noticeably to reduce water loss. Blood glucose after ten days of water deprivation increases from 20 to 80% without glucosuria. An extremely long large intestine absorbs every drop of water from the digested foods (Soliman, 2015).

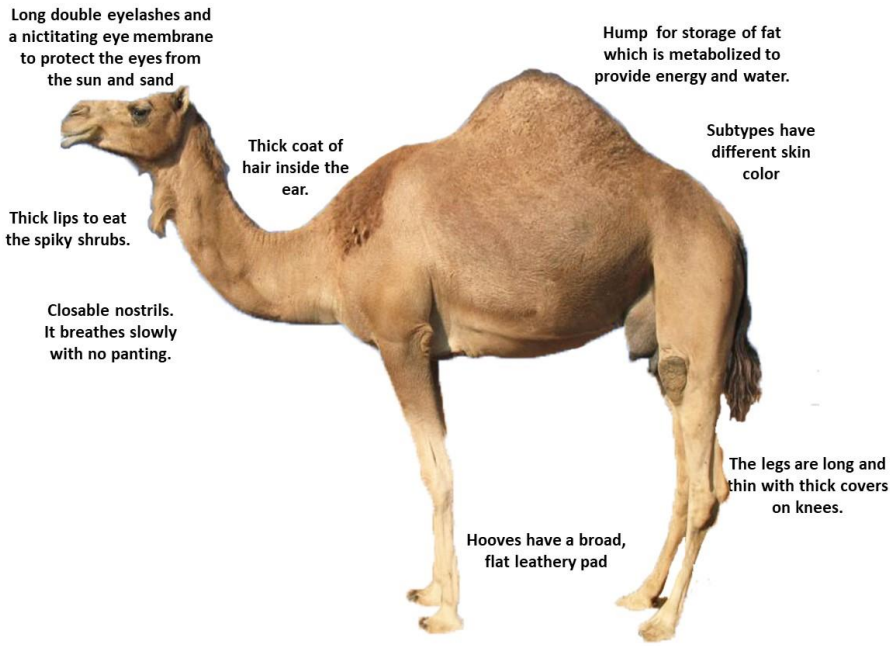


Figure 3**:** Features of Dromedary camels (*Camelus dromedarius*)

### 1.3.3 Lactation Period and Milk Yield

Dromedary camels weigh 400 - 600 kg and daily can produce daily an average of (3 to 10 L) of milk and can reach to more than 10 litres/day (Farah et al., 2007). The average daily milk production, the mean length of lactation and the mean total milk production per lactation of 174 Dromedary camels were studied by Nagy et al. (2013). The results were 6 ± 0.1 kg, 586 ± 11 days, and 3314 ± 98 kg, respectively. The lactation curve reached its peak during the $4^{th}$ month postpartum (8.9 ± 0.04 kg), then it declined slowly and by the $16^{th}$ month, it reached to (4.3 ± 0.06 kg).

### 1.3.4 General Characteristics and Particularities of Dromedary Camel Milk

Camel milk is opaque white with normal odor, has a sharp, sweet taste and sometimes very salty, the changes in taste are mainly caused by the type of fodder and availability of drinking water. Saltiness is attributed to feeding on halophilic plants. The opaque white color is attributed to the fats that are finely homogenized throughout the milk. The average density of camel milk is 1.029 g/$cm^3$ (Farah, 1993). Camel milk is less viscous than bovine milk; the viscosity of camel milk is 1.72 mPa-s measured at 20℃, while the viscosity of bovine milk at the same dry matter content and under the same conditions is 2.04 mPa-s (Kherouatou et al., 2003). Camel milk is frothy when shaken slightly. The pH of fresh camel milk ranges from 6.5 to 6.7 compared to 6.7 in cow milk (Farah, 1993; Walstra et al., 2006). Camel milk contains very high concentrations of vitamin C (169.7 mg/L), 6.7 times higher than cow milk (Sboui et al., 2016), making it a good source of Vitamin C in arid and semi-arid areas were vitamin sources like fruits and vegetables are scarce (Wernery et al., 2005). When water is restricted the water content of milk increases as a natural adaptation to provide fluids for dehydrated calfs (Yagil & Etzion, 1980). This was confirmed by Haddadin et al. (2008) who

observed that that the water content in milk was 861 g/l during the winter (December) and increased to 898 g/l in the summer when the temperature was (40-45℃). Simultaneously the total solids dropped from 139 g/l in January to 102 g/l/in August.

### 1.3.5 Health Benefits and Nutraceutical Properties of Dromedary Camel Milk

Camel milk like human milk contains a high percentage of β-casein, which is more sensitive to peptic hydrolysis than $\alpha_s$-caseins, this reflects its higher digestibility rate and lower incidence of allergy in the gastro-intestinal-tract of infants (El-Agamy et al., 2009; Kaskous & Pfaffl, 2017). Camel milk is also devoid of the allergic bovine whey protein β-lactoglobulin. People who are lactase deficient can consume camel milk without allergic response (Sakandar et al., 2018).

Camel milk is a rich source of bioactive proteins with biological and protective activity; insulin, lactoferrins, lysozyme, lactoperoxidase, serum albumin, whey acidic protein, peptidoglycan recognition protein, small peptides and various classes of immunoglobulins are responsible about these effects (El Agamy et al., 1992; El-Agamy, 2006; Mati et al., 2017).

Human intervention studies have proven that camel milk has benefits in patients with diabetes (Agrawal et al., 2011; Shori, 2015; Mihic et al., 2016, Izadi et al., 2019), autism (Al-Ayadhi & Elamin, 2013; Bashir & Al-Ayadhi, 2014) and allergy (Navarrete-Rodríguez et al., 2018; Talarico et al., 2019). Camel milk adjuvant effect to insulin therapy of diabetic patients have been reported. Shori (2015) reported that camel milk has an influential effect in reducing blood glucose levels and therefore insulin requirements and limits diabetic complications such as elevated cholesterol levels and delayed healing of wounds. A study on alloxan induced diabetic rats have

shown that camel milk has possible benefits in the treatment of diabetes and plays a role in reducing its complications (Shehata & Moussa, 2014).

Malik et al. (2012) reported that camel milk insulin is encapsulated in nanoparticles (lipid micro-vesicles), that allows its passage through the stomach and entry into the circulation. Ayoub et al. (2018) speculated that there are mechanisms other than insulin also responsible about the anti-diabetic properties of camel milk and reported another camel milk health benefit that is diabetic wound healing. Ashraf et al. (2021) investigated the molecular basis for the anti-diabetic properties of camel milk. Investigation carried out in cell lines, camel milk whey proteins and their hydrolysates showed inhibition of dipeptidyl peptidase IV (related to the progression of diabetes) and positively activated the human insulin receptor and glucose uptake.

Camel Immunoglobulins (Igs) are called nano-antibodies because they are significantly smaller than human and bovine antibodies. While human IgG failed, camel milk IgG showed capability to recognize and inactivate Hepatitis C virus peptides with a significant titer (Mullaicharam, 2014; El-Fakharany et al., 2012). Camel milk has also demonstrated efficacy in Hepatitis C patients, viral load in majority of patient sera was reduced after consumption of camel milk (El-Fakharany et al., 2017). By improving the cellular immune response and inhibiting the replication of the virus DNA, camel milk promoted the recovery from chronic hepatitis B patients (Saltanat et al., 2009). A study on experimental animals have proofed that mature and colostral camel milk have anti-schistosomal properties (Sakandar et al., 2018). Administration of camel milk to experimental animals caused immune potentiating effects and reversed the leukopenia and weight loss which are caused by the cytotoxic anticancer drug Cyclophosphamide (CYP) (Khan, 2017).

### 1.3.6 Milk Biosynthesis

Milk is an excellent functional biological fluid. It is a sterile lacteal secretion from mammary glands that provides the offspring with the macro components (protein, lipids, lactose) and micro-components (minerals and vitamins) essential for their growth and wellbeing. The young of the species depends on the mother's milk not only for growth and development, but also for vital immune support during early stage of life. The nutritional and physiological requirements of different species are different; therefore, milk composition is species-specific. The mammary gland, where intense bioprocessing of milk occurs, is situated in the udder (Chandan et al., 2015). The camel udder consists of four glandular quarters, the anterior and posterior quarters are independent and totally separated (Alluwaimi et al., 2017). Mammary secretory cells are epithelial in nature and are arranged in alveoli which are connected to ductal tissue. The secretory epithelial cells are surrounded by a layer of myoepithelial cells, which can contract and expel milk into the ducts in response to the hormone oxytocin (Farrell et al., 2006). For biosynthesis of milk constituents, the precursors extracted by mammary epithelial cells from blood include glucose, amino acids, fatty acids, β-hydroxy butyrate, and salts (Chandan et al., 2015). The synthesis of milk components occurs for the greater part in the secretory epithelial cells of the mammary gland. Figure 4 shows a diagram of a secretory epithelial cell. At the basal end precursors of milk components are taken up from the blood, and at the apical end milk components are secreted into the lumen. Proteins are formed in the endoplasmic reticulum and transported to the Golgi apparatus. A signal peptide (made up of nearly 20 hydrophobic amino acids) is added to the protein to ease its movement into the Rough Endoplasmic Reticulum (RER). The signal peptide is cleaved from the protein by an enzyme before

the translation process. and is inserted into the membrane channel. After completion of the translation process the protein has now been formed. To become functional the protein must be folded into its three-dimensional structure (Stelwagen, 2011).

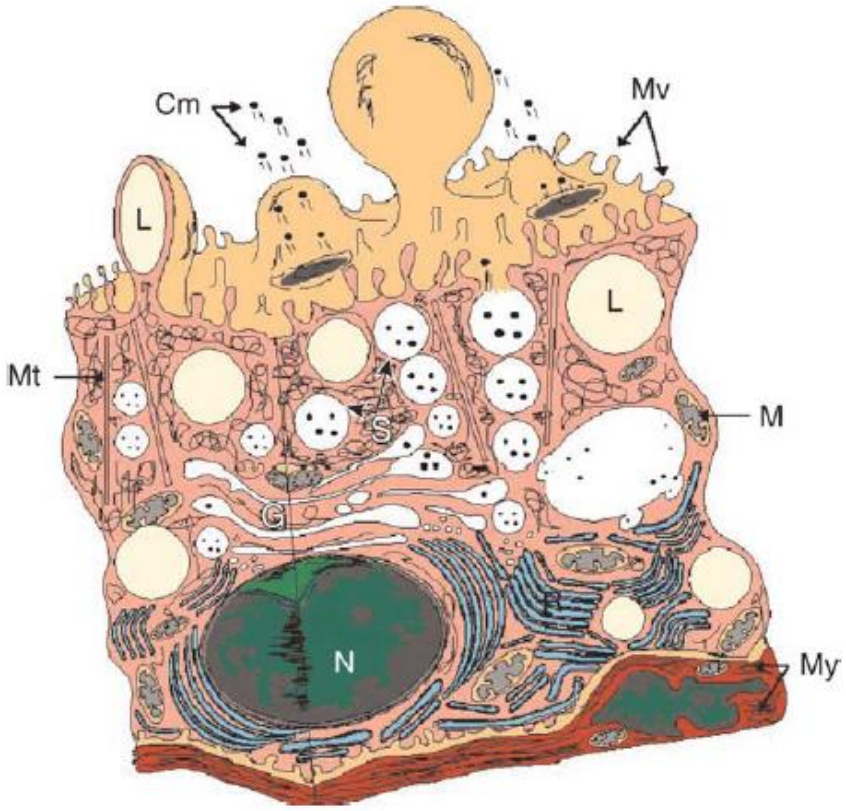


Figure 4**:** Diagram of an alveolar epithelial cell. Rough endoplasmic reticulum (R), secretory vesicles (S), Golgi secretory vesicles (G), mitochondria (M), microtubules (Mt), nucleus (N), microvilli (Mv), and myoepithelial cells (My). The casein micelles (Cm) and lipid droplets (L) are synthesized within the cell cytoplasm and released into the alveolar lumen for storage between milking (reprinted from Nickerson and Akers (2011) after permission from (Elsevier)

Proteins post-translational modifications through phosphorylation and glycosylation take place in the Golgi apparatus. The phosphate group for phosphorylation is provided by AdenosineTriPhosphate (ATP) and transfer is catalysed by casein kinases. The phosphate groups of the caseins are esterified as monoesters of serine or, to a very minor extent, of threonine. A specific sequence, Ser. X. A (where X is any amino acid

and A is an anionic residue, i.e., Glu, Asp or SerP), is required for phosphorylation. Most of the phosphoserine residues in the caseins occur in clusters. The phosphate groups per serine residue bind mainly calcium and smaller amounts of other cations as zinc. Glycosylation of proteins in the threonine residues that can contain galactose, galactosamine and N-acetylneuraminic (sialic) acid, which occur either as tri- or tetra-saccharides (Fox & Kelly, 2004). Total glycosylation potential is reported to be similar in bovine and camel κ-casein (Kappeler, 1998). Triglycerides are synthesized in the cytoplasm, forming small globules, which grow while they are transported to the apical end of the cell. Biosynthesis of lactose from glucose and galactose occurs in the membranes of the Golgi apparatus The Golgi vesicles grow while being transported through the cell and then open to release their contents in the lumen (Walstra et al., 2006).

In addition to proteins that are synthesized within the secretory cell of the mammary gland, the whey fraction of milk contains a large number of smaller proteins that are taken up from the blood and transported without further processing across the epithelial secretory cell into the milk, via either a transcellular route or a paracellular (i.e., between adjacent mammary epithelial cells) route. Some are taken up into the mammary cell by active transport mechanisms, whereas others enter by passive diffusion or by a process of internalization (Stelwagen, 2011). Generally, milk proteins are species specific (Walstra et al., 2006).

### 1.3.7 Proximate Composition of Dromedary Camel Milk

Konuspayeva et al. (2009) performed a meta-analysis for Dromedary camel milk proximate composition data for the years (1905-2006) and Alhag & Al Kanhal (2010) studied the data on Dromedary camel milk proximate composition for the years 1980

to 2009, their results are shown in Table 1. Konuspayeva et al. (2009) reported that the changes observed starting from 1983 can be explained by that the standard analytical methods for milk analysis were proposed at the beginning of the 1980s. The proximate composition of milk from Dromedary camels and other animals is shown in Table 2. camel, cow, and goat milk have similar composition.

Table 1: Proximate composition (mean ± SD) of Dromedary camel milk

| | Fat (%) | Total protein (%) | Dry matter (%) | Lactose (%) | Ash (%) |
|---|---|---|---|---|---|
| Western Asia | 3.31 ± 1.03 | 3.10 ± 0.62 | 11.62 ± 1.29 | 4.45 ± 0.40 | 0.78 ± 0.05 |
| East Africa | 4.14 ± 0.80 | 3.33 ± 0.52 | 12.69 ± 1.11 | 4.18 ± 0.72 | 0.76 ± 0.09 |
| North Africa | 3.50 ± 1.01 | 3.21 ± 0.60 | 12.53 ± 1.22 | 4.65 ± 0.67 | 0.84 ± 0.08 |
| Different areas in the world | 3.5 | 3.1 | 11.9 | 4.4 | 0.79 |

SD: Standard deviation. References: (Konuspayeva et al., 2009; Alhag & Al Kanhal, 2010).

Table 2: Proximate composition (g/kg) of milk from different mammals

| | Camel | Cow | Buffalo | Goat | Sheep | Yak |
|---|---|---|---|---|---|---|
| Dry matter | 130 | 127 | 169 | 132 | 178 | 167 |
| Protein | 36 | 34 | 42 | 36 | 57 | 49 |
| Fat | 43 | 38 | 72 | 43 | 74 | 64 |
| Lactose | 49 | 48 | 48 | 44 | 48 | 50 |
| Ash | 8 | 7 | 8 | 8 | 9 | 8 |

Bouhaddaoui et al. (2019) applied principal component analysis to camel milk data from different countries in Asia and Africa, the results have shown that camel milk from the North African countries (Morocco, Algeria, Tunisia, and Mauritania) formed pool 1 and was characterized by elevated levels of fats, proteins, and lactose. Pool 2

was formed by camel milk from Kingdom of Saudi Arabia, Pakistan, and Kazakhstan and was characterized by high levels of vitamin C. Konuspayeva et al. (2009) reported similar results.

Variations observed in camel milk composition could be attributed to genetic factors (breeds) and non-genetic factors, i.e., analytical measurement procedures, milk sampling procedures, geographical locations and regions, climate, season, environmental conditions (photo-period), water availability, feeding conditions, stage of lactation, age, calving number, calf sex, parity, physiological condition of animal, animal management, milking interval and machine milking (Khaskheli et al., 2005; Haddadin et al., 2008; Konuspayeva et al., 2009; Hammadi et al., 2010; AlHag & Al Kanhal, 2010; Abdalla et al., 2016; Nagy et al., 2017; Nagy et al., 2019).

Table 3**:** Chemical composition of Dromedary camel milk produced in different countries (g/L).

| | UAE | KSA | Morocco | Algeria | Tunisia | Sudan | Mauritania | Ethiopia |
|---|---|---|---|---|---|---|---|---|
| Proteins | 29.5 | 29 | 32.6 | 35.7 | 34.2 | 25.7 | 25.2 | 26.7 |
| Fats | 25.8 | 32 | 34.9 | 28 | 37.5 | 25.3 | 29.2 | 24.7 |
| Lactose | 41.9 | 44 | 37.8 | 43.1 | 42.78 | 39.1 | 49.1 | 46.7 |
| Ash | -- | 7.9 | 8.3 | 7.2 | 7.5 | 5.7 | 11.3 | -- |

References: (Nagy et al., 2017; Zeleke, 2007; Meiloud et al., 2011; Elobied et al., 2015; Bouhaddaoui et al., 2019). UAE: United Arab Emirates, KSA: Kindgom of Saudi Arabia.

### 1.3.8 Milk Fat

Camel milk fat was described as white in color because of the low amount of beta carotene (Vitamin A precursor). Camel milk has small fat globules compared to cow

milk (3.2 - 5.6 µm vs 4.3 - 8.4 µm). This might explain the easier digestibility of camel milk (Meena et al., 2014; Khalesi et al., 2017). The fat globules are surrounded by the Milk Fat Globule Membrane (MFGM) (Saadaoui et al., 2013). Triglycerides account for 96% of Dromedary camel milk fat (Gorban & Izzeldin, 2001). The cholesterol content in camel milk fat is less than bovine milk fat (Haddadin et al., 2008).

In camel milk samples collected from 8 locations in Jordan the content of long chain fatty acids (C14:0 - C22:0) was reported to average 95 g/100g of milk fat, while the content of short (C4:0 - C6:0) and medium (C8:0 - C14:0) chain fatty acids each averaged less than 3 g/100g. In the same study the saturated fatty acids content (g/100g) averaged 57.92 and the unsaturated 42.09, polyunsaturated fatty acids (C18:1 - C18:3) averaged 29.61 g/100 g (Ereifej et al., 2011). Similarly, Konuspayeva et al., (2008) reported that the short, medium, and long chain fatty acids content in camel milk fat was 1.5%, 16.38 % and 82.43%. Different results were reported in camel milk fat from three Sudanese ecotypes (Dowelmadina et al., 2018). The content (g/100 g) of medium chain fatty acids C13:0 - C16:1 averaged (74.25), short chain fatty acids C4:0 - C12:0 (49.25) and long chain C17:0 - C22:6n3 (16.73). The fatty acid composition of camel milk fat is affected by diet, stage of lactation, genetic differences, farming conditions, environmental conditions, and geographical location (Konuspayeva et al., 2008; Ereifej et al., 2011).

The low level of short and medium chain fatty acids may reduce the organoleptic property of camel milk (Ereifej et al., 2011). The ratio of unsaturated to saturated fatty acids is favorable in camel milk fat compared to other animals. The Atherogenicity Index (AI) which is highly associated with the onset of coronary heart diseases in consumers was estimated to be 2.75 in Dromedary camel milk (Konuspayeva et al.,

2008) while it was between 3.3 and 3.5 in cow milk with standard feeding (Chilliard et al., 2001).

### 1.3.9 Lactose

The lactose concentration in camel milk and cow milk is very similar (4.9 % vs 4.8%). However, camel milk is known to have less effect on lactose intolerance patients than cow milk. The lactose in camel milk is readily digested because it is more exposed to the action of lactase (Shori, 2015). Another hypothesis is linked to the type of lactate (D- or L-) which is the final metabolite of lactose fermentation in the digestive tract. In the human body the rate of metabolism of D-lactate by D-hydroxy-acid-dehydrogenase is one fifth the rate of L-lactate metabolism by L-lactate dehydrogenase (Ewaschuk et al., 2005). The content of total lactate (g/L) is 1.82 in camel milk and 2.49 in cow milk and the quantity of L-Lactate is 100 times more in camel milk (2.21% of the total lactate) than in cow milk (0.02%). The appearance of these products of lactose metabolism mainly depends on the microflora of Dromedary camel and cow milk (Konuspayeva et al., 2019; Konuspayeva, 2020).

### 1.3.10 Minerals

Haddadin et al. (2008) reported that the concentration of iron (4.4 mg/l), zinc (5.8 mg/l), and manganese (0.05 mg/l) in Dromedary camel milk can be valuable to the diet of urban populations. Camels show salt appetite because of the physiological requirement of very large amounts of sodium chloride that is addressed by feeding on halophytes which are salty pastures**.** Camel milk contains 15-20 mmol/l of sodium and reports on camels' salt requirement vary from equal to more than six times the amounts recommended for cows (Bekele et al., 2013; Dioli, 2018). This contributes to the

saltiness in camel milk. Faye & Seboussi (2009) reported that the selenium content in camel milk averaged 86.4 $\pm$ 39.1 ng/ml and in the group that took oral supplementation it averaged 167.1 $\pm$ 97.3 ng/ml. It was reported that maternal transfer of selenium to camel milk is more efficient than in cow milk (Faye et al., 2011).

### 1.3.11 Vitamins

Fat-soluble vitamins content was reported to vary according to the seasonal variation, decreasing in the summer with the decrease of fat concentration in milk (Haddadin et al., 2008). The contents of Niacin (Vitamin $B_3$), Vitamin C, and Vitamin D are higher in camel milk than bovine milk (Khalesi et al., 2017; Farah et al., 1992; Haddadin et al., 2008; Sboui et al., 2016). The contents of vitamin A, thiamine ($B_1$), riboflavin ($B_2$), folic acid and pantothenic are lower in camel milk than cow milk and the contents of pyrodixine, vitamin $B_{12}$ and vitamin E are the same in camel and cow milk (Farah et al., 1992). Camel milk distinctively has very high vitamin C content compared to other mammals' milk that contributes to lowering the pH and therefore stabilizing the milk from deterioration. Vitamin C concentration is 184 ± 21 mg/l and 53 ± 14 mg/l in camel and cow milk, respectively (Ahmed et al., 2017). Vitamin D was reported to be 8 times more in fresh camel milk (15.6 ± 2.01 ng/ml) than in cow milk (1.78 ± 0.99 ng/ml) (Sboui et al., 2016). The loss of vitamin C following pasteurization of camel milk is low (6.1%) which is an advantageous for the consumer (Wernery et al., 2005). β-carotene (precursor of vitamin A) in camel milk was reported to be below (<3.2 µg/l) while cow milk contained an average of 996 µg/l (Stahl et al., 2006; Faye et al., 2019).

### 1.3.12 Milk Proteins

Milk proteins are mainly divided into colloidal caseins and soluble whey proteins. The Milk Fat Globule Membrane (MFGM) contains mainly the proteins fatty acid synthetase, xanthin oxidase, butryophilin and lactoadherin (Saadaoui et al., 2013). Casein is suggested to convey high levels of calcium to the neonate in a way that prevents pathological calcification during its transport through the mammary gland (Holt, 1997). In Dromedary camel and cow milk the total protein concentration (g/100 ml) was reported by Hamed et al. (2012) as 2.8 and 3.3, respectively, the % casein in total protein was reported as 70.35 and 69.90, respectively. Salmen et al. (2012) reported that the percentage of casein in camel milk from three different Saudi breeds was 66%, 64% and 67%, while in cow milk it was 86 %. The percentage of nitrogen in casein and whey are similar in Dromedary camel and cow milk, while Dromedary camel milk has a slightly higher amount of non-protein nitrogen (Farah, 1993).

The ratios of essential to non-essential amino acids are rather similar in milks of different species, being 0.93, 1.00, 1.06, 1.02, 0.95, 0.99, 1.03, and 1.07 for camel, cow, buffalo, goat, sheep, ass, mare, and human milk, respectively (El-Agamy & Nawar, 2000).

#### 1.3.12.1 Caseins

##### 1.3.12.1.1 Structure and Characteristics of Caseins

The heterogeneous casein fraction comprises four main proteins, $\alpha_{s1}$-, $\alpha_{s2}$-, β-, κ-caseins and the γ-caseins and several minor proteins and peptides. Table 4 provides details on the characteristics of casein proteins ($\alpha_{s1}$-, $\alpha_{s2}$-, β- and κ-) from Dromedary camels (*Camelus dromedarius*) and bovine (*Bos taurus*). Caseins lack a fixed three-dimensional tertiary conformation. It is predicted that $\alpha_{s1}$- and $\alpha_{s2}$- caseins are unfolded

proteins with extended coil-like (or pre-molten globule-like) conformations, whereas β- and κ-caseins possess molten globule-like properties; they possess native secondary structures with little tertiary folds (Farrell et al., 2006; McMahon & Oommen, 2013). The high prolyl content of caseins tends to prohibit the formation of secondary structure and the protein molecules are small, amphipathic, randomly coiled, relatively open 'rheomorphic' structures (O'Regan et al., 2009). γ-caseins are produced by hydrolysis by plasmin (serine protease) which is the major milk proteolysis enzyme (Stelwagen, 2011). β-casein is very susceptible to plasmin, its cleavage results in the yielding of the peptides γ-caseins and proteose peptones. αs1-casein is also readily hydrolyzed by plasmin producing γ-caseins and proteose peptones (Aimutis & Eigel, 1982; Le Bars & Gripon, 1993; McSweeney et al., 1993; O'Flaherty, 1997). κ-casein is very resistant to hydrolysis by plasmin (Fox & Kelly, 2004). Kappeler (1998) identified proteins with molecular masses of 13.9, 15.7, and 15.9 kDa that belonged to one fraction VIII in the chromatogram and presumed that it belonged to hydrophobic γ-caseins.

#### 1.3.12.1.2 Micro-heterogeneity of the Caseins

Each of the $\alpha_{s1}$-, $\alpha_{s2}$-, β- and κ- caseins exhibits micro-heterogeneity that is due to genetic polymorphism and post translational modifications i.e., phosphorylation, glycosylation, formation of disulphide linked polymers and proteolysis by indigenous proteinases (plasmin). Kappeler (1998) was the first researcher to study the cDNA sequence of Dromedary camel caseins ($\alpha_{s1}$-, $\alpha_{s2}$-, β-, κ-) and their corresponding proteins, the amino acid sequence and the potentially phosphorylated and glycosylated residues in the proteins and genetic polymorphism. Table 4 provides details on the

micro-heterogeneity (genetic polymorphism and post-translational modifications) of casein proteins from camels (*Camelus dromedarius*) and bovine (*Bos taurus*).

The behavior of the milk proteins during milk processing is influenced by the microheterogeneity of caseins; genetic polymorphism results in differences in amino acid contents, different degrees of phosphorylation and variability in glycosylation of κ-casein contributes to variability in the protein net charge, hydrophilicity and metal binding. The presence of certain genetic variants in milk has a significant effect on protein content and profile, cheesemaking properties and heat stability (Frajman & Dovc, 2004; O'Regan et al., 2009).

Table 4 provides details on the genetic polymorphism of the caseins ($\alpha_{s1}$-, $\alpha_{s2}$-, β- and κ-). Kappeler (1998) reported that Somali camel breeds have two variants (A and B) of $\alpha_{s1}$-casein. Shuiep et al. (2013) reported variant A and C of $\alpha_{s1}$-casein in two Sudanese breeds. Erhardt et al. (2016) reported the presence of variant A, C and D of $\alpha_{s1}$-casein in camel milk of Sudanese breeds. Singh et al. (2019) studied Bikaneri Dromedary camel milk in India, they reported that the sequence revealed full similarity to $\alpha_{s1}$-casein variant A reported by Kappeler (1998). Ryskaliyeva et al. (2019) recently reported about a new variant of α-s2-casein in Dromedary camel milk from Kazakhstan. Kappeler (1998) suggested that variants of $\alpha_{s1}$-casein were a result of alternative splicing of the heterogeneous nuclear RNA transcribed from the $\alpha_{s1}$-casein gene rather than gene products of two different alleles. Kappeler (1998) suggested a minor peak in his chromatogram to represent a variant of (β-casein). Kappeler (1998) speculated that the fragment sequenced by Beg et al. (1986) belongs to a novel β-casein variant B; the gamma-casein sequence revealed a single exchange in the sequence Glutathione195 for Glycine195. No polymorphisms are yet reported in κ- casein.

Table 4 provides information about the phosphorylation of the camel milk caseins ($\alpha_{s1}$-, $\alpha_{s2}$-, β-and κ-) and provides details on the glycosylation of κ-casein, the only glycosylated casein. The glycosylation positions in camel κ-casein are predominantly towards the C-terminal end of the glyco-macropeptide, in bovine κ-casein it is high towards the N-terminal end (Kappeler et al., 1998). Table 4 also provides details on the disulphide linking of the caseins ($\alpha_{s1}$-, $\alpha_{s2}$-, β- and κ-). Dromedary camel milk αs2- and κ-caseins contain two cysteine residues, like bovine caseins αs1- and β-caseins are devoid of cysteine residues. The two cysteine residues in αs2- and κ-caseins do not undergo interchain bonding (Kappeler, 1998). In bovine caseins the two cysteine residues exist as intermolecular disulphide bonds; αs2-casein usually exists as disulphide-linked dimers and for κ -casein up to at least ten molecules may be polymerised by intermolecular disulphide bonds (O'Regan et al., 2009).

**1.3.12.1.3 Casein Micelles Structures and Stabilization**

Micelles are formed by the interaction of the nano clusters of calcium phosphate with serine-phosphate and some glutamate residues in $\alpha_{S1}$-and $\alpha_{S2}$-caseins, crosslinking the proteins resulting in the formation of the micelles. Caseins are susceptible to association due to regions of high hydrophobicity and the charge distribution arising from the amino acid sequence, phosphorylation and glycosylation. Micelles also contain magnesium, sodium, potassium, and citrate (O'Regan et al., 2009). Hydrogen bonding, hydrophobic interactions and electrostatic interactions are all important in maintaining micelle structure. Dromedary milk casein micelles have a salt plus citrate charge of about 98 versus 67 mg/g caseins for cow milk micelles.

Table 4: Characteristics of αs1-, αs2-, β-, κ- casein of camel (*Camelus dromedarius*) and bovine (*Bos taurus*) milks

| Protein | Length (no. of AA) | Molecular mass (da) | PTM description and position | Isoforms (Variants) | Highest AA % | Charge | Instability index | GRAVY | Aliphatic index | Theoretic -al pI |
|---|---|---|---|---|---|---|---|---|---|---|
| αs1-casein Camel<br><br>Gene: CSN1S1 | 230<br><br>Signal peptide (1-15)<br>Mature chain (16-230) | 26,861 | Phosphorylation in 6 serine positions 33,83,85,86,87,88 | Long (230)<br>Short (222), missing 8 amino acids (170-177) | 11.3 Glutamic acid<br>10.4 Leucine | (-) 38 Asp & Glu<br>(+) 25 Arginine & Lysine | 64.07 unstable | -0.661 | 84.30 | 4.96 |
| αs1-casein Bovine<br><br>Gene: CSN1S1 | 214<br><br>Signal peptide (1-15)<br>Mature chain (16-214) | 24,529 | Phosphorylation in 9 serine positions 56,61,63,79,81,82, 83,90,130 | A, missing amino-acids 29 - 41.<br>D, AA substitution position 68.<br>C, AA substitution position 207 | 11.7 Glutamic acid<br>10.3 Leucine<br>7.9 Proline | (-) 32 Asp & Glu<br>(+) 21 Arginine & Lysine | 56.03 unstable | -0.481 | 85.19 | 4.98 |
| αs2-casein Camel Gene: CSN1S2 | 193<br>Signal peptide (1-15)<br>Mature chain (16-193) | 22,964 | Phosphorylation in 9 serine positions 23,24,25,28,47,68, 123,125,128,136 | None | 11.4 Glutamic acid<br>10.4 Lysine | (-) 26 Asp & Glu<br>(+) 23 Arginine & Lysine | 58.11 unstable | -0.661 | 67.62 | 6.00 |
| αs2-casein Bovine Gene: CSN1S2 | 222<br>Signal peptide (1-15)<br>Mature chain (16-222) | 26,018 | Phosphorylation in13 serine residues in positions 23,24,25,28,46,71,72,73, 76,144,146,150,158. | A<br>D: Short maybe missing 9 AA 49-58, 50-59, or 51-60. | 11.3 Lysine<br>10.8 Glutamic acid<br>7.7 Serine | (-) 28 Asp &Glu<br>(+) 31 Arginine & Lysine | 44.68 unstable | -0.704 | 73.74 | 8.55 |

Table 4: Characteristics of αs1-, αs2-, β-, κ- casein of camel (*Camelus dromedarius*) and bovine (*Bos taurus*) milks (Continued)

| Protein | Length (no. of AA) | Molecular mass (da) | PTM description and position | Isoforms (Variants) | Highest AA % | Charge | Instability index | GRAVY | Aliphatic index | Theoretic -al pI |
|---|---|---|---|---|---|---|---|---|---|---|
| β-casein Camel<br><br>Gene: CSN2 | 232<br>Signal peptide (1-15)<br>Mature chain (16-232) | 24,900 | Phosphorylation in 4 serine residues in positions 30, 32, 33, 34 | None | 15.9 Proline<br>12.1 Leucine<br>10.8 Glycine | (-) 23 Aspartic acid & Glutamic acid<br>(+) 19 Arginine & Lysine | 96.58 (unstable) | -0.182 | 99.91 | 5.62 |
| β-casein Bovine<br><br>Gene: CSN2 | 232<br>Signal peptide (1-15)<br>Mature chain (16-224) | 25,107 | Phosphorylation in 5 serine residues in positions<br>30, 32, 33, 34 and in position 50 in variant A1, A2, A3, E, I, G, H | A1, A3, B, C, D, E, F, G, H, substitutions in the positions 33, 40, 51, 52,82,103, 108, 121, 132, 137, 152, 153, 167, 190 | 15.6 Proline<br>12.1 Iso-Leucine<br>9.4 Valine | (+) 23 Aspartic acid & Glutamic acid<br>(-) 19 Arginine & Lysine | 94.12 unstable | -0.154 | 97.37 | 5.26 |

Table 4: Characteristics of αs1-, αs2-, β-, κ- casein of camel (*Camelus dromedarius*) and bovine (*Bos taurus*) milks (Continued)

| Protein | Length (no. of AA) | Molecular mass (da) | PTM description and position | Isoforms (Variants) | Highest AA % | Charge | Instability index | GRAVY | Aliphatic index | Theoretic -al pI |
|---|---|---|---|---|---|---|---|---|---|---|
| κ-casein Camel<br><br>Gene: CSN3 | 182<br>Signal peptide (1-20)<br>Mature chain (21-182) | 20,417.56 | Glycosylation position 154, 178 O-linked (GalNAc...) threonine.<br><br>Glycosylation position 161, O-linked (GalNAc...) serine; alternate.<br><br>Phosphorylation position 161, phosphoserine; alternate.<br>Phosphorylation position 179, phosphoserine. | None | 12.6 Proline<br>10.4 Threonine | (-) 14 Aspartic acid & Glutamic acid<br>(+) 16 Arginine & Lysine | 44.72 unstable | -0.150 | 90.49 | 8.55 |
| κ-casein Bovine<br><br>Gene: CSN3 | 190<br>Signal peptide (1-21)<br>Mature chain (22-190) | 21,269 | Disulfide bond in positions 32 ↔ 109 (interchain).<br>Glycosylation in positions 142, 152, 154, 157, 163 O-linked (GalNAc...) threonine<br>Glycosylation 170 O-linked (GalNAc...) serine; alternate<br>Glycosylation 153 O-linked (GalNAc...) serine. | B, B2, E, F, G, H<br><br>Substitutions in the positions 31, 118, 156, 157, 169, 174, 176 | 11.1 Proline<br>8.9 Threonine<br>8.4 Alanine | (-) 16 Aspartic acid & Glutamic acid<br>(+) 15 Arginine & Lysine | 54.21 unstable | -0.287 | 81.63 | 6.29 |

Table 4: Characteristics of αs1-, αs2-, β-, κ- casein of camel (*Camelus dromedarius*) and bovine (*Bos taurus*) milks (Continued)

| Protein | Length (no. of AA) | Molecular mass (da) | PTM description and position | Isoforms (Variants) | Highest AA % | Charge | Instability index | GRAVY | Aliphatic index | Theoretic -al pI |
|---|---|---|---|---|---|---|---|---|---|---|
| κ-casein Bovine<br><br>Gene: CSN3 | 190<br>Signal peptide (1-21)<br>Mature chain (22-190) | 21,269 | Glycosylation 186 O-linked (GalNAc...) threonine; partial<br>Phosphorylation 187 phosphoserine by similarity.<br>Phosphorylation 148 phosphoserine<br>Phosphorylation 166 phosphothreonine<br>Phosphorylation 170 phosphoserine; alternate.<br>Modified residue position 22 , Pyrrolidone carboxylic acid. | B, B2, E, F, G, H<br><br>Substitutions in the positions 31, 118, 156, 157, 169, 174, 176 | 11.1 Proline<br>8.9 Threonine<br>8.4 Alanine | (-) 16 Aspartic acid & Glutamic acid<br>(+) 15 Argnine & Lysine | 54.21<br>unstable | -0.287 | 81.63 | 6.29 |

References: (UniProt, 2020; Expasy, 2020). PTM: Post translational Modification. AA: amino acid, Asp: Aspartic acid, Arg: Arginine, Lys: Lysine, Glu: Glutamic acid. GRAVY (Grand Average of Hydropathy). Instability index: Value < 40 protein predicted as stable, a value > 40 predicts that the protein may be unstable.

The difference is created because of the citrate content (mg/g caseins) which is 30 in Dromedary camel milk and 4 in cow milk. Micellar Mg, P and citrate proportions were reported to be higher than cow milk about 2/3, 2/3 and 1/3, respectively (Attia et al., 2000). As assembled casein is compact it remains stable in milk as a suspension, allowing the milk to have low viscosity that facilitates its flow (Cho & Jones, 2019). The protruding κ-casein hair coat on the micelle as well as the colloidal calcium phosphate salt bridges contribute to micelle stability, calcium binds to charged regions of the proteins and modulates hydrophobic interactions between proteins and between submicelles (O'Regan et al., 2009).

#### 1.3.12.1.4 Casein Micelle Size

Micelle size of camel milk was reported to range from (260-300 nm) vs. (120 -140 nm) in cow milk, in the same study the highest micelles size in camel milk was 500 nm while in cow milk it was 300 nm (Farah & Ruegg, 1989). Similarly, Attia et al. (2000) carried direct measurements on the screen of an electron microscope on 800 camel milk casein particles and estimated that 2/3 of the micelles have a size between 350 nm and 500 nm. The researchers reported that several characteristics of camel milk micelle contribute to its relatively large size. The micelles have a relatively higher mineral content and have a relatively low content of caseins (a similar reverse correlation was reported for caprine micelles), it has a relatively high hydration which is synonymous to voluminosity and has a relatively low content of κ-casein.

#### 1.3.12.1.5 Amino-acids Content/Mole in Camel Milk Caseins

The amino-acids residues in the peptide chains impart the properties caseins. The amino acids in the peptide chain can be positively or negatively charged, polar, aliphatic, or hydrophobic. Figure 5 shows a comparison between the amino-acid

content/mole in camel and bovine caseins (αs1-, αs2-, β- and κ-casein). The proline content/mole in camel caseins is to some extent higher than in bovine caseins except in αs2- casein. Kappeler (1998) reported that the higher proline content causes protein hydrophobicity and can lead to destabilization of the secondary structures in a noticeable way than it occurs in bovine caseins.

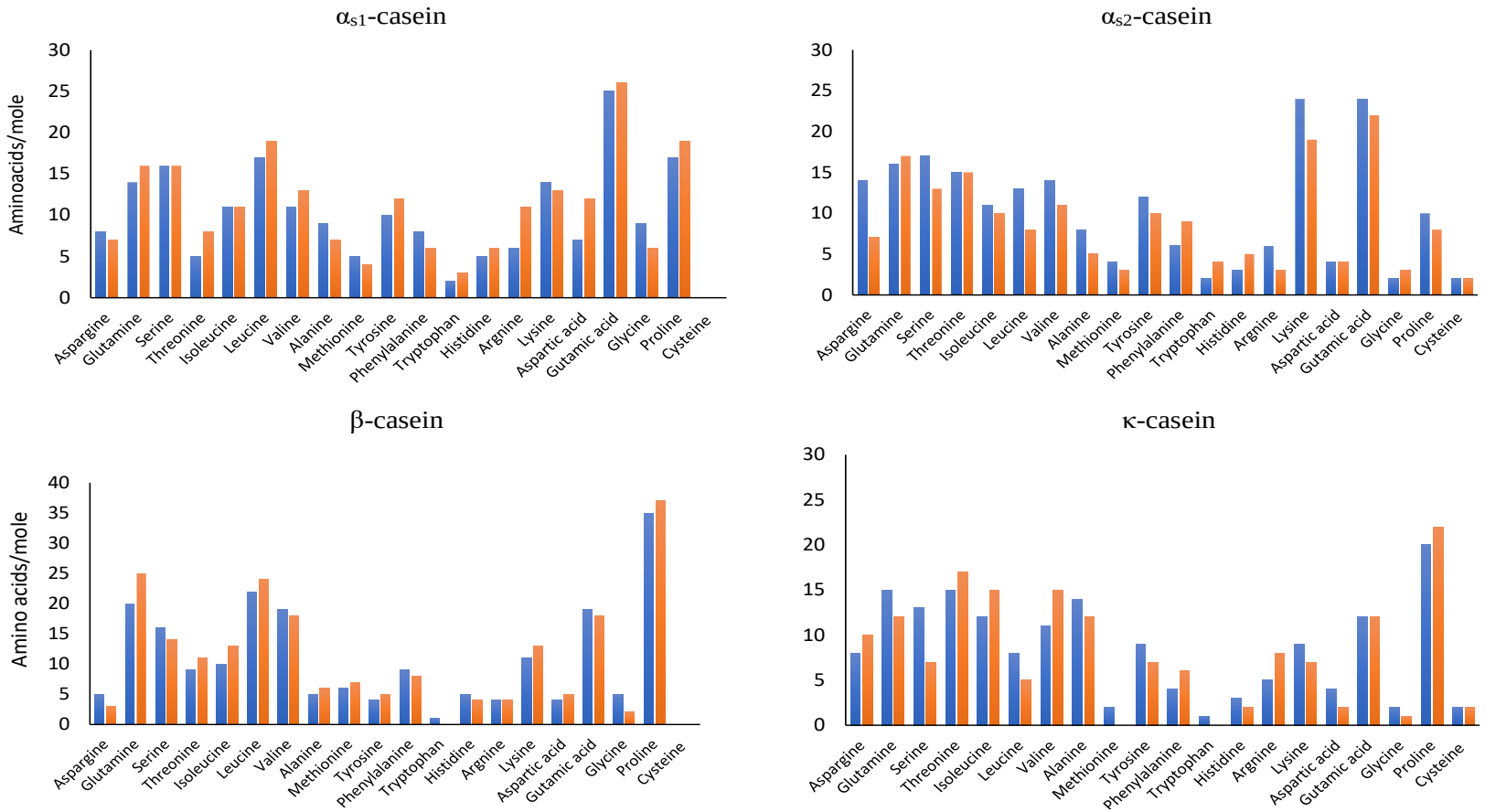


Figure 5**:** Amino-acid content per mole of $\alpha_{s1}$-, $\alpha_{s2}$-, β-, κ- caseins, α-lactalbumin and serum albumin from bovine *Bos taurus* and Dromedary camel *Camelus dromedarius*. Data to prepare the graphs from (UniProt, 2020; Expasy, 2020). Hydrophobic: Methionine, Phenylalanine, Leucine, Valine, Isoleucine, Alanine, Proline, Glycine, Cysteine, Tryptophan. Aliphatic: Valine, Isoleucine, Leucine. Aromatic, Phenylalanine, Tyrosine, Histidine, Tryptophan. Polar: Lysine, Serine, Threonine, Glycine, Glutamic acid, Aspargine, Aspartic acid, Arginine, Tyrosine, Histidine. Positively charged: Lysine, Arginine, Histidine. Negatively charged: Glutamic acid, Aspartic acid

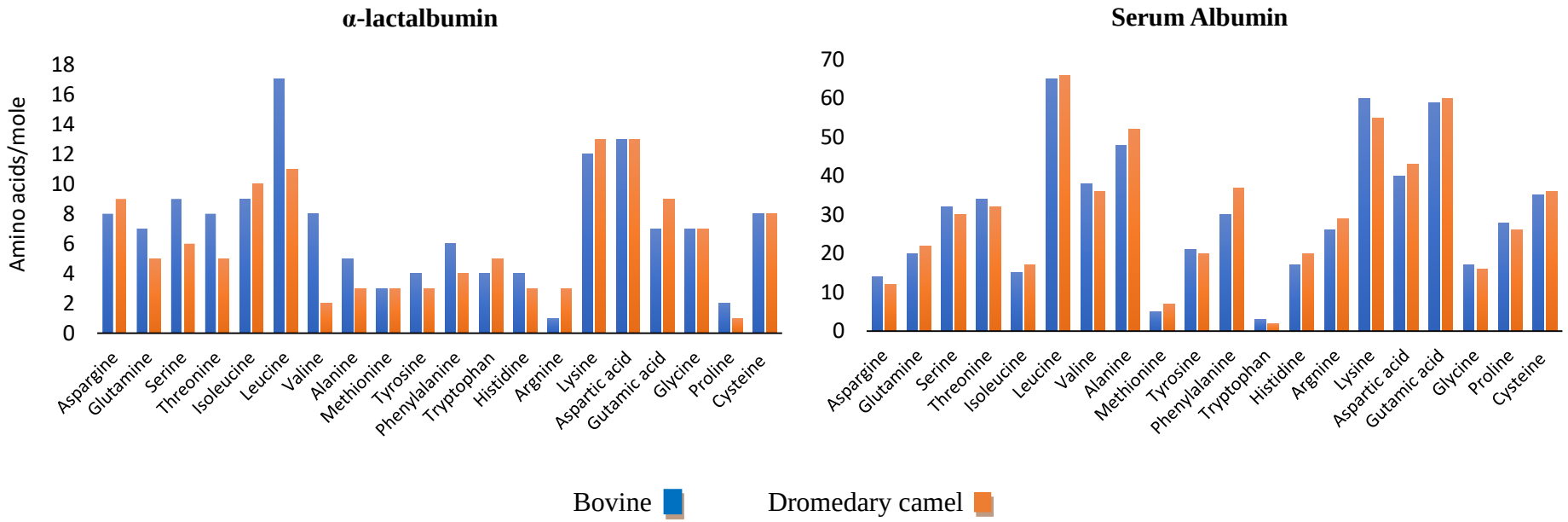


Figure 5: Amino-acid content per mole of $\alpha_{s1}$-, $\alpha_{s2}$-, β-, κ- caseins, α-lactalbumin and serum albumin from bovine *Bos taurus* and Dromedary camel *Camelus dromedarius.* Data to prepare the graphs from (UniProt, 2020; Expasy, 2020). Hydrophobic: Methionine, Phenylalanine, Leucine, Valine, Isoleucine, Alanine, Proline, Glycine, Cysteine, Tryptophan. Aliphatic: Valine, Isoleucine, Leucine. Aromatic, Phenylalanine, Tyrosine, Histidine, Tryptophan. Polar: Lysine, Serine, Threonine, Glycine, Glutamic acid, Aspargine, Aspartic acid, Arginine, Tyrosine, Histidine. Positively charged: Lysine, Arginine, Histidine. Negatively charged: Glutamic acid, Aspartic acid (Continued)

### 1.3.12.2 Whey Proteins

Whey is the fluid by-product resulting from the precipitation of proteins in milk. The precipitation can be facilitated by the growth of microorganism, addition of acid or enzymes. Wangoh et al. (1998) reported that the separation of casein and whey proteins of camel milk took place at pH 4.3, while for bovine milk the optimum pH for separation was 4.6.

The whey fraction of Dromedary camel milk is highly heterogeneous. Unlike the caseins, the whey proteins have globular conformations with high proportions of their sequences in ordered structures. Whey proteins display greater hydrophilicity, less amphipathicity and a more limited tendency for self-association; they have greater heat sensitivity but are less sensitive to changes in ionic strength and pH than caseins. Like caseins whey proteins also display micro-heterogeneity.

Table 5 shows the characteristics and microheterogeneity of whey proteins (α-lactalbumin, lactoferrin, insulin, insulin-like growth factor I, lysozyme C, lactoperoxidase and peptidoglycan recognition protein-1) from camels (*Camelus dromedarius*) and bovine (*Bos taurus*). Figure 6 shows the primary sequence of the whey proteins of camel (*Camelus dromedarius*) and bovine (*Bos taurus*) and their alignment.

#### 1.3.12.2.1 α-Lactalbumin

α-lactalbumin is the major whey protein in camel milk. α-Lactalbumin is a component of the enzyme lactose synthetase. In bovine milk, β-lactoglobulin is the major whey protein (55%) and α -lactalbumin is the second (20.25%). Camel whey proteins were separated by gel chromatography on sephadex G100 (Conti et al., 1985). Two different alpha-lactalbumins (A and B) were isolated and characterized. Although they have

equal MW (14 kDa), their iso-electric points, amino acid composition, and N-terminal sequence are different. Other whey proteins and their biological activities are discussed in Chapter 4.

Table 5: Characteristics of whey proteins **α**-lactalbumin, lactoferrin, insulin, insulin-like growth factor I, lysozyme C, lactoperoxidase and peptidoglycan recognition protein-1 of camel (*Camelus dromedarius*) and bovine (*Bos taurus*) milk

| Protein | Ligand | Length of chain | Molecular mass (da) | PTM description and position | Isoforms | Highest AA % | Charged AA | Instability index | GRAVY | Aliphatic index | Theoretical pI |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **α**-lact-albumin Camel<br><br>Gene: LALBA | Ca & Fe Ca (78 – 89) | Mature protein 123 | 14,430 | Disulphide bonds<br>6 ↔ 120<br>28 ↔ 111<br>61↔77<br>73 ↔ 91 | None | 10.6 Aspargine<br>10.6 Lysine<br>8.9 Leucine | Asp & Glu (-) 22<br>Arg & Lys (+) 16 | 32.8 stable | -0.678 | 73.74 | 5.1 |
| **α**-lact-albumin Bovine Gene: LALBA | Ca & Fe Ca (97 – 108) | 142<br>Signal peptide 1-19<br>Mature protein 20-142 | 16,247 | Glycosylation N-linked (GlcaseinAc...) asparagine 64<br>Disulphide bonds<br>25 ↔ 139<br>47 ↔ 130<br>80 ↔ 96<br>92↔ 100 | Substitution of R with Q position 29 | 12.0 Leucine<br>9.2 Aspargine<br>8.5 Lysine | Asp& Glu (-) 20<br>Arg & Lys (+) 13 | 27.58 stable | -0.169 | 91.27 | 4.92 |

Table 5: Characteristics of whey proteins α-lactalbumin, lactoferrin, insulin, insulin-like growth factor I, lysozyme C, lactoperoxidase and peptidoglycan recognition protein-1 of camel (*Camelus dromedarius*) and bovine (*Bos taurus*) milk (Continued)

| Protein | Ligand | Length of chain | Molecular mass (da) | PTM description and position | Highest AA % | Charged AA | Instability index | GRAVY | Aliphatic index | Theoretical pI |
|---|---|---|---|---|---|---|---|---|---|---|
| Lacto-ferrin Camel Gene: LTF | Iron binding sites: 79, 11, 211, 272, 414, 452, 545,614 Carbonate binding sites: 136,140, 142,143,478, 482, 484,485 | 708 Signal peptide chain 1-19 Mature protein 20-708. | 77,211 | Glycosylation N-linked (GlcaseinAc...) asparagine 385, 252,537,594 Disulphide bond 28 ↔ 64, 38 ↔ 55 134 ↔ 217, 176 ↔ 192, 179 ↔ 202, 189 ↔ 200, 250 ↔ 264, 367 ↔ 399 377 ↔ 390, 424 ↔ 703, 444 ↔ 666, 476 ↔ 551, 500 ↔ 694, 510 ↔ 524 521 ↔ 534, 592 ↔ 606, 644 ↔ 649 | 10.5 Leucine 9.6 Alanine 7.9 Glycine | Asp& Glu (-)72 Arg & Lys (+) 87 | 45.27 unstable | -0.245 | 79.64 | 8.66 |
| Lacto-ferrin Bovine Gene: LTF | Iron binding sites: 79, 11, 211, 272, 414, 452, 545,614 Carbonate binding sites: 136,140, 142,143,478, 482, 484,485 | 708 Signal peptide chain 1-19 Mature protein 20-708. | 78,056 | Glycosylation N-linked (GlcaseinAc...) asparagine 252,387,495, 564 Disulphide bond 28 ↔ 64, 38 ↔ 55 134 ↔ 217, 176 ↔ 192, 189 ↔ 200, 250 ↔ 264 367 ↔ 399, 377 ↔ 390 424 ↔ 703, 444 ↔ 666 476 ↔ 551, 500 ↔ 694 510 ↔ 524, 521 ↔ 534 592 ↔ 606, 644 ↔ 649 | 10.3 Leucine 9.9 Alanine 7.8 Glycine | Asp& Glu (-)72 Arg & Lys (+) 92 | 40.99 unstable | -0.289 | 78.6 | 8.69 |

Table 5: Characteristics of whey proteins **α**-lactalbumin, lactoferrin, insulin, insulin-like growth factor I, lysozyme C, lactoperoxidase and peptidoglycan recognition protein-1 of camel (*Camelus dromedarius*) and bovine (*Bos taurus*) milk (Continued)

| Protein | Ligand | Length of chain | Molecular mass (da) | PTM description and position | Highest AA % | Charged AA | Instability index | GRAVY | Aliphatic index | Theoretical pI |
|---|---|---|---|---|---|---|---|---|---|---|
| Insulin Camel Gene:INS | None | 51<br>1 – 30 B chain<br>31 - 51 A chain | 5,694 | Disulphide bond between B and A chains<br>7 ↔ 37,<br>19 ↔ 50,<br>36 ↔ 41 | 11.8 each Cystine and Leucine<br>7.8 each Tyrosine Alanine Glutamic acid Glycine Valine | Asp & Glu (-) 4<br>Arg & Lys (+) 2 | 8.8 stable | 0.263 | 84.12 | 5.39 |
| Insulin Bovine Gene: INS | None | 105<br>Signal peptide 1-24<br>Peptide 25-54<br>Pro-peptide 57-82<br>Peptide 85-105 | 11,393 | Disulphide bond between B and A chains<br>31 ↔91,<br>43 ↔ 104,<br>90 ↔ 95 | 15.2 Leucine<br>11.4 Glycine<br>10.5 Alanine | Asp & Glu (-) 8<br>Arg & Lys (+) 9 | 37.18 stable | 0.062 | 92.9 | 7.60 |

Table 5: Characteristics of whey proteins **α**-lactalbumin, lactoferrin, insulin, insulin-like growth factor I, lysozyme C, lactoperoxidase and peptidoglycan recognition protein-1 of camel (*Camelus dromedarius*) and bovine (*Bos taurus*) milk (Continued)

| Protein | Ligand | Length of chain | Molecular mass (da) | PTM description and position | Isoforms | Highest AA % | Charged AA | Instabilitity index | GRAVY | Aliphatic index | Theoretical pI |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Insulin-like Growth Factor I Camel Gene: Cadr_000 017117 | None | 113 | 12,759 | None | None | 14.2 Lysine 12.4 Arginine 8.8 Glycine | Asp & Glu (-) 10 Arg & Lys (+) 30 | 78.20 (unstable) | -1.615 | 26.02 | 10.53 |
| Insulin-like Growth Factor II Bovine Gene: IGF1 | None | 154 Signal peptide 1-? Propeptide ? – 49 Insulin growth factor 1 chain 50-119 E peptide 120 - 154 | 17,066 | Disulphide bond 55 ↔97 67 ↔110 96 ↔101 | 2 isoforms 188 and 172 AA | 9.7 each Serine, Leucine 8.4 Alanine 7.1 each Lysine Argnine | Asp & Glu (-) 11 Arg & Lys (+) 22 | 55.06 (unstable) | -0.249 | 65.32 | 9.36 |

Table 5: Characteristics of whey proteins **α**-lactalbumin, lactoferrin, insulin, insulin-like growth factor I, lysozyme C, lactoperoxidase and peptidoglycan recognition protein-1 of camel (*Camelus dromedarius*) and bovine (*Bos taurus*) milk (Continued)

| Protein | Ligand | Length of chain | Molecular mass (da) | PTM description and position | Highest AA % | Charged AA | Instability index | GRAVY | Aliphatic index | Theoretical pI |
|---|---|---|---|---|---|---|---|---|---|---|
| Lysozyme C (milk isozyme) Camel Gene: LYZ | None | 130 | 14,79 | Disulphide bonding 1↔130, 6↔ 128, 30 ↔116, 65↔ 81, 77↔ 95, | 8.5 each Aspargine, Valine 7.7 Glycine 6.9 each Aspartic acid, Alanine | Asp& Glu (-) 17 Arg & Lys (+) 15 | 14.82 stable | -0.599 | 67.46 | 5.90 |
| Lysozyme C (milk isozyme) Bovine Gene: N/A | None | 148 Signal peptide (1-18) Mature chain (19-148) | 16,783 | Disulphide bonding 24↔146,48↔ 134, 83↔99, 95↔113 | 10.1 Leucine 9.5 each Alanine, Argnine 8.1 each Lysine, Valine | Asp& Glu (-)10 Arg & Lys (+) 26 | 22.82 stable | -0.322 | 88.31 | 9.92 |
| Lacto-peroxidase Camels Gene: LPO | Ca in positions 226,300, 302, 306 | 711 Signal peptide (1-21) Mature chain (22-711) | 80,675 | None | 11.5 Leucine 6.8 Alanine 6.6 each Arginine, Proline | Asp& Glu (-) 71 Arg & Lys (+) 89 | 38.97 stable | -0.372 | 82.86 | 9.19 |

Table 5: Characteristics of whey proteins **α**-lactalbumin, lactoferrin, insulin, insulin-like growth factor I, lysozyme C, lactoperoxidase and peptidoglycan recognition protein-1 of camel (*Camelus dromedarius*) and bovine (*Bos taurus*) milk (Continued)

| Protein | Ligand | Length of chain | Molecular mass (da) | PTM description and position | Highest AA % | Charged AA | Instability index | GRAVY | Aliphatic index | Theoretical pI |
|---|---|---|---|---|---|---|---|---|---|---|
| Lacto-peroxidase. Bovine Gene: LPO | Binds one $Ca^{2+}$ ion per heterodimer, in positions (227, 301, 303, 305, 307). Binds 1 heme b (iron (II)-protoporphyrin IX) group covalently per heterodimer, in positions 225, 375. Iron (heme axial ligand) binds in position 468. | 712 Signal peptide (1-22) Propeptide (23-100) Mature chain (101-712) | 80,642 | Disulfide bond 132 ↔ 145, 246 ↔ 256, 250 ↔ 274, 354 ↔ 365, 573 ↔ 630, 671 ↔ 696.<br><br>Glycosylation 106,212, 322, 358, 449 (N-linked (GlcaseinAc...) asparagines.<br><br>Phosphorylation (phosphoserine 315) Modified residue 482,(nitrated tyrosine) | 11.1 Leucine 6.9 Alanine 6.5 Arginine | Asp& Glu (-)75 Arg & Lys (+) 86 | 36.85 stable | -0.378 | 82.21 | 8.83 |

Table 5: Characteristics of whey proteins α-lactalbumin, lactoferrin, insulin, insulin-like growth factor I, lysozyme C, lactoperoxidase and peptidoglycan recognition protein-1 of camel (*Camelus dromedarius*) and bovine (*Bos taurus*) milk (Continued)

| Protein | Ligand | Length of chain | Molecular mass (da) | PTM description and position | Isoform | Highest AA % | Charged AA | Instability index | GRAVY | Aliphatic index | Theoretical pI |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Peptido-glycan recognition protein 1 Camels Gene: PGLYRP1 | None | 193 Signal peptide (1-21) Mature chain (22-193) | 21,377 | Disulphide bonding 28 ↔ 152, 44 ↔ 89, 65 ↔ 71 | None | 10.4 Alanine 9.8 Leucine 9.3 Arginine | Asp& Glu (-)14 Arg& Lys (+) 20 | 42.88 unstable | -0.189 | 87.46 | 9.10 |
| Peptido-glycan recognition protein 1 Bovine Gene: PGLYRP1 | None | 190 Signal peptide (1-21) Mature chain (22-190) | 21,063 | Disulfide bonding 24 ↔ 148, 40 ↔ 85, 61 ↔ 67 Modified residues: position 22 pyrrolidne carboxylic acid. | One isoform With 179 AA | 10.5 Glycine 9.5 Alanine 8.9 Leucine | Asp & Glu (-)10 Arg & Lys (+) 20 | 40 unstable | -0.261 | 81.63 | 9.59 |

References: (UniProt, 2020; Expasy, 2020). PTM: Post translational Modification. AA: amino acid, Asp: Aspartic acid, Arg: Arginine, Lys: Lysine, Glu: Glutamic acid. GRAVY (Grand Average of Hydropathy). Instability index: Value < 40 protein predicted as stable, a value > 40 predicts that the protein may be unstable.

(a) α-lactalbumin, Similarity: 59.86%

```
LALBA_CAMDR ------------------- KQFTKCKLSDELKDMNGHGGITLAEWICIIFHMSGYDTETV 41
LALBA_BOVIN MMSFVSLLLVGILFHATQAEQLTKCEVFRELKDLKGYGGVSLPEWVCTTFHTSGYDTQAI 60
                          : *: *** :: **** :: *: ** :: * **: * ** ***** :::

LALBA_CAMDR VSNNGNREYGLFQINNKIWCRDNENLQSRNICDISCDKFLDDDLTDDKMCAKKILDKEGI 101
LALBA_BOVIN VQNNDSTEYGLFQINNKIWCKDDQNPHSSNICNISCDKFLDDDLTDDIMCVKKILDKVGI 120
        *. ** .. *************: * :: *: * ***: ************** **. *** *** **

LALBA_CAMDR DYWLAHKPLCSEKLEQWQCEKW 123
LALBA_BOVIN NYWLAHKALCSEKLDQWLCEKL 142
        : ****** ******: ** ***
```

(b) Insulin (INS), Similarity: 46.67%

```
P01320|INS_CAMDR ------------------------FANQHLCGSHLVEALYLVCGERGFFYTPK------- 29
P01317|INS_BOVIN MALWTRLRPLLALLALWPPPPARAFVNQHLCGSHLVEALYLVCGERGFFYTPKARREVEG 60
                                         *.**************************

P01320|INS_CAMDR ------------------------AGIVEQCCASVCSLYQLENYCN 51
P01317|INS_BOVIN PQVGALELAGGPGAGGLEGPPQKRGIVEQCCASVCSLYQLENYCN 105
                                         *********************
```

(c) Insulin-Like Growth Factor I (IGF1), Similarity: 33.51 %

```
A0A5N4DG25|A0A5N4DG25_CAMDR ------------------------------------------------------------
P07455|IGF1_BOVIN           MGKISSLPTQLFKCCFCDFLKQVKMPITSSSHLFYLALCLLAFTSSATAGPETLCGAELV 60


A0A5N4DG25|A0A5N4DG25_CAMDR ---------------KPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSAR 45
P07455|IGF1_BOVIN           DALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSAR 120
                                      ******************************************

A0A5N4DG25|A0A5N4DG25_CAMDR SVRAQRHTDMPKAQKYQPPSTNKKTKSQRRRKGGPKKHPGGEQKEGTEASQQMKGKKKEQ 105
P07455|IGF1_BOVIN           SVRAQRHTDMPKAQKEVHLKNTSRGS------AGNKNYR-------------M------- 154
                            ***************    ....: .      .* *::              *

A0A5N4DG25|A0A5N4DG25_CAMDR RRETGARN 113
P07455|IGF1_BOVIN           -------- 
```

(d) Insulin-Like Growth Factor II (IGF2), Similarity: 83.61 %.

```
A0A5N4BXU0|A0A5N4BXU0_CAMDR MPVGIPMEKSVLVLLAFLAFASCCFAAYRPSETLCGGELVDTLQFVCGDRGFYFSRPASR 60
P07456|IGF2_BOVIN           --MGITAGKSVLVLLAFLAFASCCYAAYRPSETLCGGELVDTLQFVCGDRGFYFSRPSSR 58
                              :**   ****************:*********************************:**

A0A5N4BXU0|A0A5N4BXU0_CAMDR MSRRSRGIVEECCFRSCDLALLETYCATPAKSERDVSTPPTVLPDNFPRYPVGKFFQYDT 120
P07456|IGF2_BOVIN           INRRSRGIVEECCFRSCDLALLETYCATPAKSERDVSASTTVLPDDVTAYPVGKFFQYDI 118
                            :.**********************************:  *****:.  **********

A0A5N4BXU0|A0A5N4BXU0_CAMDR WKQSAQRLRRGLPALLRARRGRTLAKELEVFREAKRHRPLIALPNQDPAAHGGASPEASS 180
P07456|IGF2_BOVIN           WKQSTQRLRRGLPAFLRARRGRTLAKELEALREAKSHRPLIALPTQDPATHGGASSKASS 178
                            ****:*********:**************.:**** ********.****:***** :***

A0A5N4BXU0|A0A5N4BXU0_CAMDR NRK 183
P07456|IGF2_BOVIN           D-- 179
                            :
```

Figure 6**:** Alignment of primary sequence of α-lactalbumin, lactoferrin, insulin, IGF1, IGF2, lysozyme C, lactoperoxidase, peptidoglycan recognition protein-1 of camel (*Camelus dromedarius*) and bovine (*Bos taurus*) and % similarity. (*) in the alignment shows that the amino acid and position is same in both sequences. *Camelus dromedarius* (CAMDR) and *Bos taurus* (BOVIN)

(e)Lactoferrin (LF), Similarity: 75.42%

```
Q9TUM0|TRFL_CAMDR MKLFFPALLSLGALGLCLAASKKSVRWCTTSPAESSKCAQWQRRMKKVRGPSVTCVKKTS 60
P24627|TRFL_BOVIN MKLFVPALLSLGALGLCLAAPRKNVRWCTISQPEWFKCRRWQWRMKKLGAPSITCVRRAF 60
                  ****.*************** :*.***** *  *  ** :** ****: .**:***:::

Q9TUM0|TRFL_CAMDR RFECIQAISTEKADAVTLDGGLVYDAGLDPYKLRPIAAEVYGTENNPQTHYYAVAIAKKG 120
P24627|TRFL_BOVIN ALECIRAIAEKKADAVTLDGGMVFEAGRDPYKLRPVAAEIYGTKESPQTHYYAVAVVKKG 120
                   :***:**: :**********:*::** *******:***:***::.*********:.***

Q9TUM0|TRFL_CAMDR TNFQLNQLQGLKSCHTGLGRSAGWNIPMGLLRPFLDWTGPPEPLQKAVAKFFSASCVPCV 180
P24627|TRFL_BOVIN SNFQLDQLQGRKSCHTGLGRSAGWIIPMGILRPYLSWTESLEPLQGAVAKFFSASCVPCI 180
                  :****:**** ************ ****:***:*.**   **** *************:

Q9TUM0|TRFL_CAMDR DGKEYPNLCQLCAGTGENKCACSSQEPYFGYSGAFKCLQDGAGDVAFVKDSTVFESLPAK 240
P24627|TRFL_BOVIN DRQAYPNLCQLCKGEGENQCACSSREPYFGYSGAFKCLQDGAGDVAFVKETTVFENLPEK 240
                  * : ******** * ***:*****:************************::****.** *

Q9TUM0|TRFL_CAMDR ADRDQYELLCPNNTRKPVDAFQECHLARVPSHAVVARSVNGKEDLIWKLLVKAQEKFGRG 300
P24627|TRFL_BOVIN ADRDQYELLCLNNSRAPVDAFKECHLAQVPSHAVVARSVDGKEDLIWKLLSKAQEKFGKN 300
                  ********** **:* *****:*****:**********:********** *******:.

Q9TUM0|TRFL_CAMDR KPSGFQLFGSPAGQKDLLFKDSALGLLRISSKIDSGLYLGSNYITAIRGLRETAAEVELR 360
P24627|TRFL_BOVIN KSRSFQLFGSPPGQRDLLFKDSALGFLRIPSKVDSALYLGSRYLTTLKNLRETAEEVKAR 360
                  *  .******* **:**********:*** **:**.*****.*:*::::***** **: *

Q9TUM0|TRFL_CAMDR RAQVVWCAVGSDEQLKCQEWSRQSNQSVVCATASTTEDCIALVLKGEADALSLDGGYIYI 420
P24627|TRFL_BOVIN YTRVVWCAVGPEEQKKCQQWSQQSGQNVTCATASTTDDCIVLVLKGEADALNLDGGYIYT 420
                   ::******* :** ***:**:**.*.*.*******:***.**********.*******

Q9TUM0|TRFL_CAMDR AGKCGLVPVLAESQQSPESSGLDCVHRPVKGYLAVAVVRKANDKITWNSLRGKKSCHTAV 480
P24627|TRFL_BOVIN AGKCGLVPVLAENRKSSKHSSLDCVLRPTEGYLAVAVVKKANEGLTWNSLKDKKSCHTAV 480
                  ***********.::* : *.**** **.:********:***: :*****:.********

Q9TUM0|TRFL_CAMDR DRTAGWNIPMGLLSKNTDSCRFDEFLSQSCAPGSDPRSKLCALCAGNEEGQNKCVPNSSE 540
P24627|TRFL_BOVIN DRTAGWNIPMGLIVNQTGSCAFDEFFSQSCAPGADPKSRLCALCAGDDQGLDKCVPNSKE 540
                  ***********: ::*.** ****:*******:**:*:*******:::* :******.*

Q9TUM0|TRFL_CAMDR RYYGYTGAFRCLAENVGDVAFVKDVTVLDNTDGKNTEQWAKDLKLGDFELLCLNGTRKPV 600
P24627|TRFL_BOVIN KYYGYTGAFRCLAEDVGDVAFVKNDTVWENTNGESTADWAKNLNREDFRLLCLDGTRKPV 600
                  :*************:********: ** :**:*:.* :***:*:  **.****:******

Q9TUM0|TRFL_CAMDR TEAESCHLAVAPNHAVVSRIDKVAHLEQVLLRQQAHFGRNGRDCPGKFCLFQSKTKNLLF 660
P24627|TRFL_BOVIN TEAQSCHLAVAPNHAVVSRSDRAAHVKQVLLHQQALFGKNGKNCPDKFCLFKSETKNLLF 660
                  ***:*************** *:.**::****:*** **:**::**.*****:*:******

Q9TUM0|TRFL_CAMDR NDNTECLAKLQGKTTYEEYLGPQYVTAIAKLRRCSTSPLLEACAFLMR 708
P24627|TRFL_BOVIN NDNTECLAKLGGRPTYEEYLGTEYVTAIANLKKCSTSPLLEACAFLTR 708
                  ********** *: ******* :******:*::************** *
```

Figure 6**:** Alignment of primary sequence of α-lactalbumin, lactoferrin, insulin, IGF 1, IGF2, lysozyme C, lactoperoxidase, peptidoglycan recognition protein-1 of camel (*Camelus dromedarius*) and bovine (*Bos taurus*) and % similarity. (*) in the alignment shows that the amino acid and position is same in both sequences. *Camelus dromedarius* (CAMDR) and *Bos taurus* (BOVIN) (Continued)

(f) Lactoperoxidase (LPO), Similarity: 83.85%

```
Q9GJW6|Q9GJW6_CAMDR MWVLLHLPVLLASLTLFQAAASDTNAQTT-AAAMSEAVRQVKVHVNKAFLDSRTRLKAAM 59
P80025|PERL_BOVIN   MWVCLQLPVFLASVTLFEVAASDTIAQAASTTTISDAVSKVKIQVNKAFLDSRTRLKTTL 60
                    *** *:***:***:***:.***** **:: ::::*:** :**::************:::

Q9GJW6|Q9GJW6_CAMDR SSEVPTTRQLSEYLKHAKGRTRTAIRNGQVWEESLKRLWQKVTQTNITDPSLDLTALSWE 119
P80025|PERL_BOVIN   SSEAPTTQQLSEYFKHAKGRTRTAIRNGQVWEESLKRLRRDTTLTNVTDPSLDLTALSWE 120
                    ***.***:*****:********************** :.* **:************

Q9GJW6|Q9GJW6_CAMDR VGCDVPVSVVKCDKNSPYRTITGDCNNGRHPALGAANQALARWLPAEYEDGLSLPFGWTR 179
P80025|PERL_BOVIN   VGCGAPVPLVKCDENSPYRTITGDCNNRRSPALGAANRALARWLPAEYEDGLALPFGWTQ 180
                    ***..** :****:************ * *******:**************:******:

Q9GJW6|Q9GJW6_CAMDR GKKRNGFPLPLAREVSNKIVGYLNEEGVLDQNRSLLFMQWGQIVDHDLDFPRDTELGSSE 239
P80025|PERL_BOVIN   RKTRNGFRVPLAREVSNKIVGYLDEEGVLDQNRSLLFMQWGQIVDHDLDFAPETELGSNE 240
                     *.**** :**************:************************  :*****.*

Q9GJW6|Q9GJW6_CAMDR YSKAQCDEHCIRGDNCFPIMFPRNDRKVMTQGKCMPFFRAGFVCPNPPYQSLAREQINAL 299
P80025|PERL_BOVIN   HSKTQCEEYCIQGDNCFPIMFPKNDPKLKTQGKCMPFFRAGFVCPTPPYQSLAREQINAV 300
                    :**:**:*:**:**********:** *: ***************.*************:

Q9GJW6|Q9GJW6_CAMDR TSFLDASLVYGSEPSLASSLRDLSSPLGLMAVNQEFWDHGLAYPPFVNKKPSPCEVINTT 359
P80025|PERL_BOVIN   TSFLDASLVYGSEPSLASRLRNLSSPLGLMAVNQEAWDHGLAYLPFNNKKPSPCEFINTT 360
                    ***************** **:************ ******* ** ********.****

Q9GJW6|Q9GJW6_CAMDR AQVPCFLAGDSRASEQILLATSHTLLLREHNRLARELKKLNPHWDGEKLYQEARKILGAF 419
P80025|PERL_BOVIN   ARVPCFLAGDFRASEQILLATAHTLLLREHNRLARELKKLNPHWNGEKLYQEARKILGAF 420
                    *:******** **********:**********************:***************

Q9GJW6|Q9GJW6_CAMDR MQIITFRDYLPIVLGDEMQKWIPPYRGYNKSVDPRISNVFTFAFRFGHLVVPSTMSRLDE 479
P80025|PERL_BOVIN   IQIITFRDYLPIVLGSEMQKWIPPYQGYNNSVDPRISNVFTFAFRFGHMEVPSTVSRLDE 480
                    :**************.*********:***:******************: ****:*****

Q9GJW6|Q9GJW6_CAMDR NYQPWGPEPELPLHTLFFNTWRIVKDGGIDPLVRGLLAKKSKFMSQKRMMTGELRNKLFQ 539
P80025|PERL_BOVIN   NYQPWGPEAELPLHTLFFNTWRIIKDGGIDPLVRGLLAKKSKLMNQDKMVTSELRNKLFQ 540
                    ******** **************:******************:*.*.:*:*.********

Q9GJW6|Q9GJW6_CAMDR PPYTIHGFDLAAIHIQRCRDHGMPGYNSWRGFCDLSQPQTLKELHAVLKNKKLAKKLLDL 599
P80025|PERL_BOVIN   PTHKIHGFDLAAINLQRCRDHGMPGYNSWRGFCGLSQPKTLKGLQTVLKNKILAKKLMDL 600
                    * :.*********::******************.****:*** *::***** *****:**

Q9GJW6|Q9GJW6_CAMDR YRTPDNIDIWLGGIAEPQVKRGRVGPLLACLLGRQFRQIRDGDRFWWENPGVFTKKQQKS 659
P80025|PERL_BOVIN   YKTPDNIDIWIGGNAEPMVERGRVGPLLACLLGRQFQQIRDGDRFWWENPGVFTEKQRDS 660
                    *:********:** *** *:****************:*****************:**:.*

Q9GJW6|Q9GJW6_CAMDR LQKLSFSRLVCDNTHITKVPLHPFQANSYPHGFVDCSAIDKLDLSPWASVEN 711
P80025|PERL_BOVIN   LQKVSFSRLICDNTHITKVPLHAFQANNYPHDFVDCSTVDKLDLSPWASREN 712
                    ***:*****:************ ****.***.*****::********** **
```

Figure 6**:** Alignment of primary sequence of α-lactalbumin, lactoferrin, insulin, IGF1, IGF2, lysozyme C, lactoperoxidase, peptidoglycan recognition protein-1 of camel (*Camelus dromedarius*) and bovine (*Bos taurus*) and % similarity. (*) in the alignment shows that the amino acid and position is same in both sequences. *Camelus dromedarius* (CAMDR) and *Bos taurus* (BOVIN) (Continued)

(g) Lysozyme C (LZ), Similarity: 60.81%

```
P37712|LYSC_CAMDR ------------------KVWERCALARKLKELGMDGYRGVSLANWMCLTKWESDYNTDA 42
Q6B411|LYSM_BOVIN MKALLIVGLLLLSVAVQGKKFQRCELARTLKKLGLDGYRGVSLANWVCLARWESNYNTRA 60
                                    * ::** ***.**:**:**********:**::***:*** *

P37712|LYSC_CAMDR TNYNPSSESTDYGIFQINSRYWCNNGKTPHAVNGCGINCNVLLEDDITKAVQCAKRVVRD 102
Q6B411|LYSM_BOVIN TNYNRGDKSTDYGIFQINSRWWCNDGKTPKAVNACRIPCSALLKDDITQAVACAKRVVRD 120
                  **** ..:***********:***:****:***.* * *..**:****:** ********

P37712|LYSC_CAMDR PQGVRAWVAWKNHCEGHDVEQYVEGCDL 130
Q6B411|LYSM_BOVIN PQGIKAWVAWRNKCQNRDLRSYVQGCRV 148
                  ***::*****:*:*:.:*:..**:** :
```

(h) Peptidoglycan Recognition Protein 1 (PGRP-1), Similarity:74.23%

```
Q9GK12|PGRP1_CAMDR MTRHCVLLVWALLALLSLGAAREDPPACGSIVPRREWRALASECRERLTRPVRYVVVSHT 60
Q8SPP7|PGRP1_BOVIN MSRRYTPLAWVLLALLGLGAAQ----DCGSIVSRGKWGALASKCSQRLRQPVRYVVVSHT 56
                  *:*: . *.*.*****.****:     ***** * :* ****:* :** :**********

Q9GK12|PGRP1_CAMDR AGSHCDTPASCAQQAQNVQSYHVRNLGWCDVGYNFLIGEDGLVYEGRGWNIKGAHAGPTW 120
Q8SPP7|PGRP1_BOVIN AGSVCNTPASCQRQAQNVQYYHVRERGWCDVGYNFLIGEDGLVYEGRGWNTLGAHSGPTW 116
                  *** *:***** :****** ****: ************************  ***:****

Q9GK12|PGRP1_CAMDR NPISIGISFMGNYMNRVPPPRALRAAQNLLACGVALGALRSNYEVKGHRDVQPTLSPGDR 180
Q8SPP7|PGRP1_BOVIN NPIAIGISFMGNYMHRVPPASALRAAQSLLACGAARGYLTPNYEVKGHRDVQQTLSPGDE 176
                  ***:**********:****  ******.*****.* * *  ********** ******.

Q9GK12|PGRP1_CAMDR LYEIIQTWSHYRA- 193
Q8SPP7|PGRP1_BOVIN LYKIIQQWPHYRRV 190
                  **:*** * ***
```

Figure 6**:** Alignment of primary sequence of α-lactalbumin, lactoferrin, insulin, IGF1, IGF2, lysozyme C, lactoperoxidase, peptidoglycan recognition protein-1 of camel (*Camelus dromedarius*) and bovine (*Bos taurus*) and % similarity. (*) in the alignment shows that the amino acid and position is same in both sequences. *Camelus dromedarius* (CAMDR) and *Bos taurus* (BOVIN) (Continued)

### 1.3.14 Analytical Methods used in the Determination of Camel Milk Proximate Composition

Primary chemical methods used for milk analysis are: Kjeldahl for protein content, ether extract for determination of fat content, polarimetry for lactose determination, gravimetry and forced air oven drying method for total solids determination. Camel milk composition was determined by primary chemical methods approved by the American Association of Analytical Chemists as in Mehaia et al. (1995) and Elamin & Wilcox (1992). Zia-ur-Rahman and Straten (2000) used fat milko-tester for fats

determination, protein determination was done by the pro-milk dye binding method, solid non-fat was determined by hydrometer. Musaad et al. (2013) used an ultrasonic analyzer (Lactoscan Milk Analyzer, Milkotronic Ltd, Europe). Mid infrared (MIR) spectroscopy has recently been applied for camel milk proximate composition determination (Zeleke, 2007; Ahmad et al., 2012; Nagy et al., 2019).

#### 1.3.14.1 Near and Mid InfraRed Spectroscopy

A shift from marketing commodity dairy products to the manufacture of value-added products in an increased scale and the concomitant need for process quality control as well as rapid decision has driven the development of instrumental and faster methods of analysis. However, the results from validated chemical methods (primary methods of analysis) form the basis for the calibration of rapid instrumental methods (electronic secondary methods of analysis) (Barbano & Lynch, 2006).

Near and Mid infrared spectroscopy instruments are nowadays commonly used for determining the composition of milk. Infrared spectroscopy measures the absorption of radiation in the near ($\lambda$ = 0.8 – 2.5 μm) or mid ($\lambda$ = 2.5 – 15 μm) infrared regions by functional groups in the molecules of milk, different functional groups absorb different frequencies of radiation. Infrared radiation is absorbed as molecules change their vibrational energy levels. By using multivariate statistical techniques, NIR and MIR instruments can be calibrated to measure the composition of milk based on the amount of IR radiation absorbed at specific wavelengths (Wehling, 2014). To achieve the performance potential of infrared spectroscopy equipment the accuracy of the reference values and the design of the calibration sample set (range and distribution of component concentrations, lack of correlation between individual component

concentrations, and the number of samples) are important determinants of the actual method performance (Barbano & Lynch, 2006).

### 1.3.15 Analytical Methods used for the Quantification of Dromedary Camel Milk Proteins

Dromedary camel milk casein and whey proteins were quantified by a couple of researchers using different analytical methods. Table 6 summarizes the published research on camel milk casein concentrations, relative proportions (%) and the methods of analysis used.

#### 1.3.15.1 One Dimensional Sodium Dodoecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS PAGE)

The separation of proteins by electrophoresis is based on the migration of charged molecules through a polyacrylamide gel matrix upon application of an electric field that is usually provided by immersed electrodes. The polyacrylamide gel prepared in vertical slabs is used as a molecular sieve for the quantitation of protein, estimation of protein size, purity, monitor protein integrity, comparison of the polypeptide composition of different samples, analysis of the number and size of polypeptide subunits. The polyacrylamide gels are formed by polymerization of monomeric acrylamide by the action of a cross-linking agent, N, N'-methylene-bisacrylamide, in the presence of ammonium persulfate as an initiator and N, N, N, N TetraMethyleneDiamine (TEMED) as the catalyst. The ratio between acrylamide/bisacrylamide as well as the total concentration of both components, affects the pore size and rigidity of the final gel matrix. That accordingly affect the range of protein sizes that can be resolved by the gel.

Sodium dodecyl sulfate (SDS)-polyacrylamide discontinuous gel electrophoresis was described by Laemmli (1970), in this type proteins are denatured and separation of

proteins is according to their molecular weight. In SDS-PAGE the protein mixture is denatured by heating at 100°C in the presence of excess SDS and a thiol reagent (dithiothreitol). Proteins are dissociated into their individual polypeptide subunits that bind SDS in a constant weight ratio (1.4g SDS/g polypeptide) and form complexes which are negatively charged. Due to their negative charge and similar charge densities the protein complexes migrate according to their size to the positive rod (Shi & Jackowski, 1998). Densistometric analysis of stained band intensities is applied to evaluate proteins molecular weights and quantities.

**1.3.15.2 Capillary Electrophoresis**

Capillary electrophoresis is an electrochemical process in which macromolecules or colloidal particles with a net electric charge migrate in a capillary column under the influence of an electric current. It offers simultaneous separation of caseins and whey proteins with high resolutions and possibilities of good quantification. It also provides a good opportunity to determine genetic variants, glycosylation and phosphorylation states of milk proteins (de Jong et al., 1993; Heck et al., 2008; Johansson et al., 2013; Gustavsson et al., 2014). The mobility of a molecule in the capillary column is dependent on its charge-to-size ratio, the size being determined by molecular weight, three-dimensional structure, and degree of slovation; charged molecules will be separated in an electric field according to their intrinsic mobility (Lindeberg, 1996).

Capillary electrophoresis is performed in fused silica tubing (Figure 7) which has good thermal properties, is transparent to ultraviolet and visible light and can be made with internal diameters smaller than 100 µm. Due to the fragility of naked fused silica, the flexibility of the capillary is improved with a polyimide coating of the outer wall (Lindeberg,1996). When an electric field is applied a double layer is formed at the

silica-solution interface, the solvated cationic species will migrate toward the negative pole causing bulk solvent molecules to move in the same direction. This Electro-Osmotic Flow (EOF) originates at the inner wall of the capillary. For a wide range of pH-values, the inner wall of a fused-silica capillary is negatively charged due to ionized silanol groups and therefore it attracts proteins from the bulk solution. To suppress the negative charge of the silanol groups on the capillary walls and prevent proteins attraction to the wall, a cationic surfactant (cellulose additive) is added to the running buffer that coats the silanol groups on the capillary wall this leads to the movement of protein molecules by their electrophoretic mobility rather than the electrosmotic flow (de Jong et al., 1993; Suratman, 2008).

Based on a balance between electromotive and frictional forces, the electrophoretic mobility ($\mu_{ep}$) of proteins can be expressed as (Lindeberg, 1996):

$$\mu_{ep} = q/6\pi\eta r$$

q = charge

n = viscosity

r = radius

Separation by electrophoresis is based on differences in solute velocity in an electric field. The velocity of an ion can be given by:

$v = \mu_{ep} \times E$

v = ion velocity

$\mu_{ep}$ = electrophoretic mobility

E = applied electric field (function of the applied voltage and capillary length (in volts/cm).

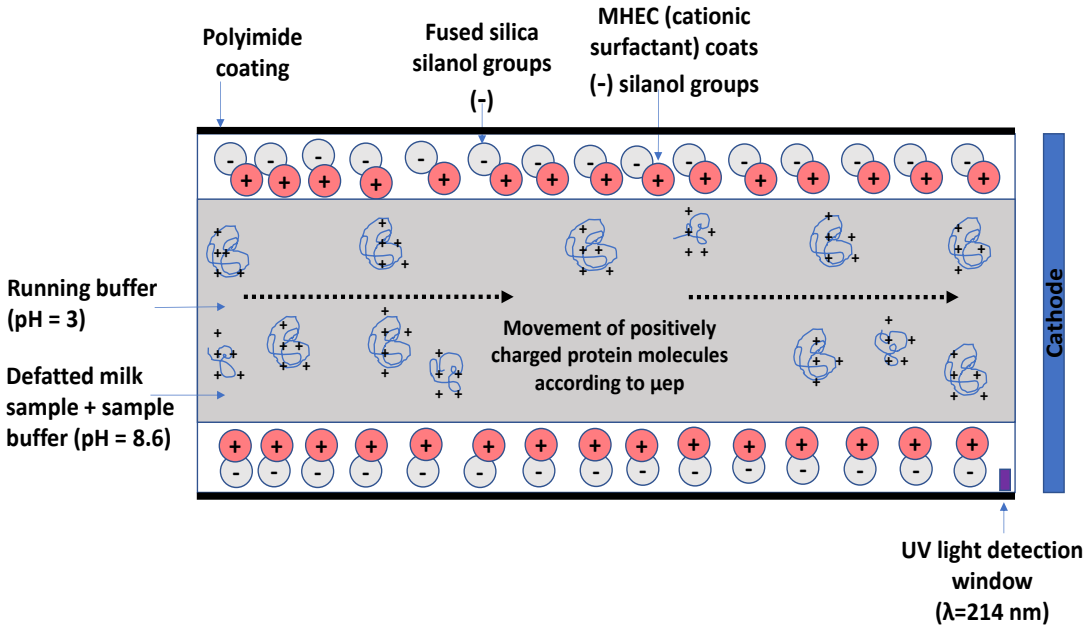


Figure 7: Inside a capillary column, elimination, and reversal of electro-osmotic flow by using a cationic surfactant. µep: Electrophoretic mobility

### 1.3.15.3 Enzyme-Linked Immuno-Sorbent Assay (ELISA)

Immunoassays are generally based on the specific and high affinity binding of antibodies with antigens (Hsieh, 2014). ELISA assays are based on the reaction of antigen (protein) and enzyme labelled antibody that allow quantitative determinations by UV-Visible spectrophotometers (Sakamoto et al., 2018). The enzyme conjugated to the antibody converts a colorless substrate to a colored soluble product in the solution. The color generated is used to determine the result in a qualitative assay or can be quantified by microplate readers (spectrophotometers). The enzymes horseradish peroxidase and alkaline phosphatase are commonly used to label antibodies (Hsieh, 2014). ELISA can be divided into four categories: direct, indirect, sandwich and competitive. Figure 8 is an illustration of a direct sandwich ELISA. ELISAs are quick and simple to carry and allow to handle a large number of samples in parallel in the same polystyrene multi-well plate.

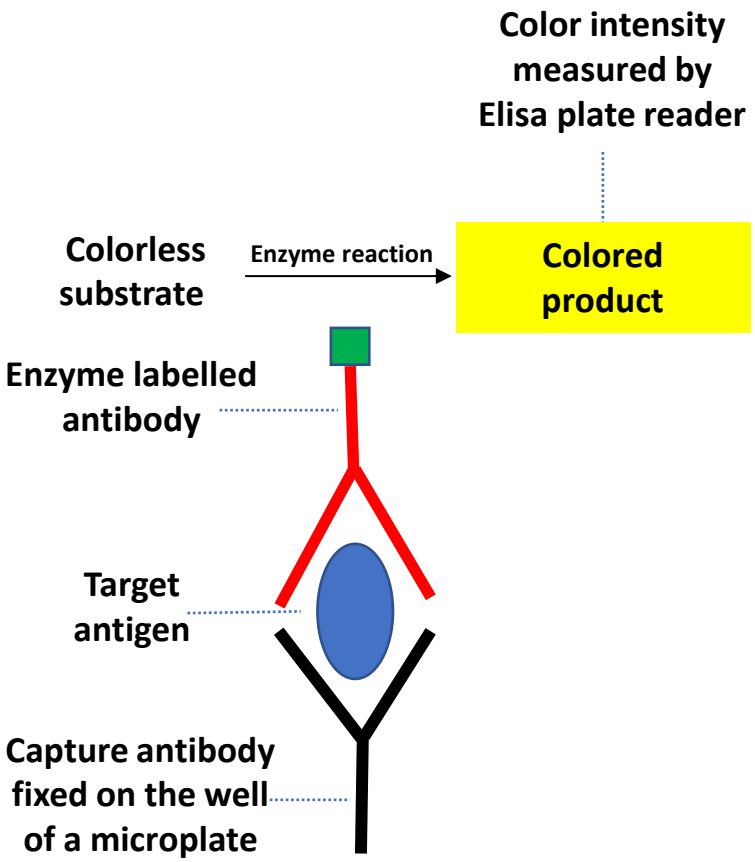


Figure 8: An illustration of direct sandwich ELISA

### 1.3.16 Challenges of Processing Dromedary Camel Milk

The challenges of processing camel milk limit the opportunity to process and add value to this milk. Challenges of transforming Dromedary camel milk to different fermented dairy products (cheese and yoghurt) and Ultra-high treatment processing of milk are well reported (Berhe et al., 2017; Hailu et al., 2016a). Efforts to overcome those challenges were exerted by many researchers (Ramet, 1989; Ramet, 2001; Farah & Bachmann, 1987; El Zubeir & Jabreel, 2008; Hailu et al., 2014; Qadeer et al., 2015; Hailu et al., 2016b). Differences between the relative proportions of the individual caseins ($\alpha_{s1}$-, $\alpha_{s2}$-, β-, κ-) compared with cow milk rather than the structural variations within the proteins was suggested by Kappeler (1998) as the reason for the difficulty

in Dromedary camel milk processing to cheese. Accordingly, the researcher suggested that a lower ratio of β-casein to κ-casein would be favorable for curd coagulation and heat sterilization. Kappeler (1998) also mentioned that the lack of selective breeding of camels for milk with favorable cheese-making properties is responsible for the high β-casein and the low κ-casein content. Other researchers attributed the inferior quality of the camel milk coagulum to the composition of the casein micelle and that the κ-casein which reacts with the clotting enzyme has a different electro-potential from cows' milk which causes lower electrophoretic mobility (Farah & Farah-Riesen, 1985; Mohamed & Larsson-Raznikiewicz, 1990; Farah, 1993; El Zubeir & Jabreel, 2008). Processing camel milk to cheese yields a soft and weak coagulum in a long coagulation time and the yield is low because a significant amount of the dry matter is lost with the whey (Ramet, 1989). There are also challenges encountered in the processing of camel milk yoghurt. Camel milk yoghurt curd is fragile, heterogeneous and consists of dispersed flakes (Attia et al., 2001; Berhe et al., 2017). Shelf-life extension of camel milk through Ultra High temperature Treatment (UHT) is impossible for camel milk due to heat coagulation, the heat stability of camel milk at 140℃ is significantly lower than cow milk (1807.4 sec vs. 133.6 sec) (Sagar et al., 2016).

The information on the protein composition of camel milk is very scarce. The peculiar processing behavior of camel milk that is affected by inherent proteins escalates the need to study the protein composition of camel milk proteins in a larger number of samples from individual animals. Table 6 shows information from previous reports about the concentration of different proteins in camel milk, number of samples analyzed and methods of analysis.

Table 6: Reported studies on the concentration of caseins ($\alpha_{s1-}$, $\alpha_{s2-}$, β- and κ- caseins) in Dromedary camel milk (*Camelus dromedarius*)

| No. of samples | Sample description | Analytical method | Quantification performed | αs1-casein | αs2-casein | α-casein | β -casein | κ-casein | References |
|---|---|---|---|---|---|---|---|---|---|
| 8 | 8 locations in Jordan | SDS-PAGE & Densistometry | relative proportions (%) of caseins | | | 27% - 54.58% | 12.56 - 33.95% | ND - 7.79% | Ereifeij et al. (2011) |
| 1 | Kazakhstan | SDS-PAGE& Densistometry | relative proportions (%) of caseins | | | 31.50 % | 64.50 % | 4% | Yelubaeva et al. (2017) |
| 1 | Pooled sample | CE | Conc. (g/L) | | | 2.89 | 12.78 | 1.67 | Omar et al. (2016) |
| No inf. | Somali breed | C18 RP-HPLC | relative proportions (%) of caseins & conc. (g/L) | 22% (5.3) | 9.5% (2.3) | | 65% (15.6) | 3.5% (0.8) | Kappeler (1998) |
| 36 | Samples from individual Maghrebi breed | RP-HPLC | relative proportions (%) of caseins | 23.9%± 0.7 | 13.2%± 0.5 | | 59.4%± 1 | 3.5% ± 0.3 | Hamed et al. (2012) |
| 1 | Pooled from 20 camels, Tunisia | C4 HPLC | Conc. (g/L) | 57 | 6 | | | | Felfoul et al. (2017) |
| 10 | Arvana breed | RP-HPLC | relative proportions (%) of caseins | 37.39% ± 3.89 | 5.79 % ± 0.98 | | 53.19% ± 3.46 | 3.63% ± 2.13 | Ryskaliyeva et al. (2018) |

SDS PAGE: Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis. CE: Capillary Electrophoresis, RP-HPLC: Reversed phase High performance Liquid Chromatography.

# Chapter 2: Use of Near and Mid InfraRed Spectroscopy for Analysis of Protein, Fat, Lactose and Total Solids in Raw Cow and Camel Milk

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**Abstract**

Milk samples (150 cow and 217 camel milk samples) were analyzed for protein, fat, lactose and total solids by near and mid infrared transmission spectroscopy. Excellent positive correlations between the two methods were obtained for both types of milk ($p<0.001$); for protein ($r \geq 0.96$), fat ($r \geq 0.99$), lactose ($r = 0.82$) and total solids ($r = 0.90$). The mean of the relative difference ((MIR value – NIR value) / 0.5 (MIR values + NIR values) x 100%) for cow and camel milk were, for protein (+8.2 & +13.4%), fat (-9.3 & +0.9%), lactose (-5.4 & -0.7%) and total solids (-2.2 & -3.4%), respectively. The difference between the two methods may be due to the effects of differences in milk homogeneity, especially with respect to casein micelles and fat globules.

**Keywords**: Milk, protein, fat, lactose, total solids, infrared transmission spectroscopy, NIR, MIR.

## 2.1 Introduction

Camel *(Camelus dromedarius)* milk is currently receiving much interest because of several nutritional and health benefits including anti-diabetic, hypo-allergenic, and anti-carcinogenic properties (Alhag & Al Kanhal, 2010; Malik et al., 2012; Mati et al., 2017). This milk has many differences compared to cow milk, mainly related to its protein composition, casein micelle and fat globule sizes (Khalesi et al., 2017). However, camel milk is not highly exploited because of lack of large scale production and processing, lack of customer demand, and difficulties facing its processing into fermented products (Berhe et al., 2017). The proximate composition (protein, fat, lactose, and total solids) of milk from 1500 camels over five years was studied using Mid InfraRed (MIR) spectroscopic method calibrated with 25 camel milk samples that were analyzed by reference methods (Nagy et al., 2019). Alhag & Al Kanhal (2010) reviewed literature from 1980 to 2009 and found average contents of protein, fat, lactose, and total solids to be 3.1%; 3.5%; 4.4%; and 11.9%, respectively. Similarly, Konuspayeva et al. (2009) conducted a meta-analysis of literature data on camel milk composition covering five regions of the world.

Infrared spectroscopy provides high throughput, non-destructive, environmentally friendly methods for food analysis. The widespread use of these methods in the food industry is justified by the rapid analytical results that lead to the early detection of defects in the intermediate and end products. Two infrared spectral ranges are available for the analysis of milk, namely near infrared (800 – 2500 nm) and mid infra (2500 – 15000 nm) spectroscopy. These methods were calibrated and validated using milk samples with known analytical values established by reference analytical methods (Jankovska & Sustova, 2003; Barbano & Lynch, 2006). Differences in composition

between milk from cows and other species might affect the calibration and validity of the calibration models when applied to other animal's milk. For example, the IR absorption of milk components might be affected by the concentration and size of fat globules in milk, which cause light scattering (Cattaneo et al., 2009; Holyrod, 2013). Moreover, the difference in milk homogeneity might also affect the accuracy of the results (Kaylegian et al., 2006). Camel milk has smaller fat globules and larger casein micelles (Khalesi et al., 2017) but it is not known how these differences might affect the accuracy of the analysis of protein, fat, lactose and total solids in the camel milk using calibrations made using cow milk.

The accuracy of analytical methods may be investigated by comparing two independent analytical methods (Melenteva et al., 2013; Parat et al., 2017). The purpose of this study was to evaluate the validity of the cow milk calibration models built-into commercial mid infrared instrument (Foss MilkoScan FT-120) and near infrared instrument (Bruker's Multipurpose Analyzer, MPA) for the analysis of raw camel milk samples in comparison with raw cow milk samples. Both instruments use Fourier transformation for measurement of milk parameters using a full spectral calibration mode.

## 2.2 Materials and Methods

### 2.2.1 Milk Samples

Milk samples used in this study were collected from the farm animals at Al Rawabi Dairy Factory (raw cow milk) and Emirates Industry for Camel Milk and Products – EICMP (raw camel milk), located in Dubai, United Arab Emirates. A total of 150 cow milk samples were collected from 150 cows, and a total of 217 camel milk samples were collected from 109 camels. All samples were collected in sterile bottles and

immediately placed in a thermo cool box with frozen gel packs and ice. Samples collected were shaken for homogenization and were divided into two parts for analysis by the two methods described below. Samples were transported in a thermo cool box and stored at 4ºC and analyzed on the same day.

### 2.2.2 Instrumental Analysis

Lactose, protein, fats and total solids contents (%) were determined in samples by two rapid infrared devices that have built-in models for milk components determination; namely, Mid InfraRed, MilkoScan FT-120, Foss, (Foss A/S, Hillerød, Denmark) and Near InfraRed Multipurpose Analyzer (MPA), Bruker Optik Gmbh, (Ettlingen, Germany). Analysis of each milk sample on both devices was performed on the same day. Each sample was analyzed in duplicate and mean values were used in the method comparison.

### 2.2.3 Statistical Analysis

Minitab statistics package (version. 18, Minitab, Inc, State College, Pennsylvania, U.S.A) was used to test the correlation between the values of the Foss Milkoscan FT-120 and Bruker MPA. The agreement of the values received from the two instruments was assessed by Bland and Altman's plots prepared by Minitab. Minitab was used to apply the paired sample t-test. Minitab was also used to determine the frequency distribution of the results of protein, fat, lactose, and total solids concentrations (%).

## 2.3 Results and Discussion

### 2.3.1 Theoretical Background

InfraRed (IR) spectroscopy, measuring vibrations of covalent bonds in the near infrared (800 - 2500 nm) or mid infrared (2500 - 15000 nm), are used for the qualitative and quantitative analysis of different molecular species in food (Etzion et al., 2004). Figure 9 summarize the Near InfraRed (NIR) and Mid InfraRed (MIR) absorption wavelengths characteristic of the chemical bonds within milk components, e.g. –CH groups within the chains of fatty acid molecules, carbonyl groups in ester linkages of fat molecules, peptide linkages between amino acids of protein molecules, and –OH groups in lactose. The two IR spectroscopic techniques rely on different energy states with NIR (780-2500 nm) using excitations of higher quanta transitions, i.e. first overtones and binary combinations of fundamental vibrations and electron transitions, and MIR (2500-25,000 nm) utilizing chemical information only from the fundamental vibrations. Therefore, there are considerable differences in the sensitivity and sample penetration level for each technique. In addition, the food matrix composition and structure may cause noise and interfere differently with the absorption of IR radiations by target analytes. In NIR, for example, characteristic absorption bands of fat and other milk components may be affected by the high absorption by water in combination with the strong light scattering by the fat globules in the milk (Aernouts et al., 2011a). Milk contains about 88% water, which produces very strong bands in NIR around 960, 1440, 1950, and 2076 nm, which overlap with some bands of interest creating noise (Tsenkova et al.,1999; Socrates, 2001; Coppa et al., 2012). In MIR, the first water band overlaps with much smaller bands characteristic of amide I and amide II bands of proteins located at 5882 - 6250 nm and 6369 - 6451 nm ranges, respectively (Etzion

et al., 2004). IR absorption by the target analytes is also affected by the concentration and size of fat globules in milk, which cause light scattering contributing up to 50% of the total absorbance in NIR at 1454, 1894, 1953, 2048, 2100, 2174, and 2230 nm (Cattaneo et al., 2009; Holyrod, 2013). These interferences may affect the precision and/or accuracy of the analytical results.

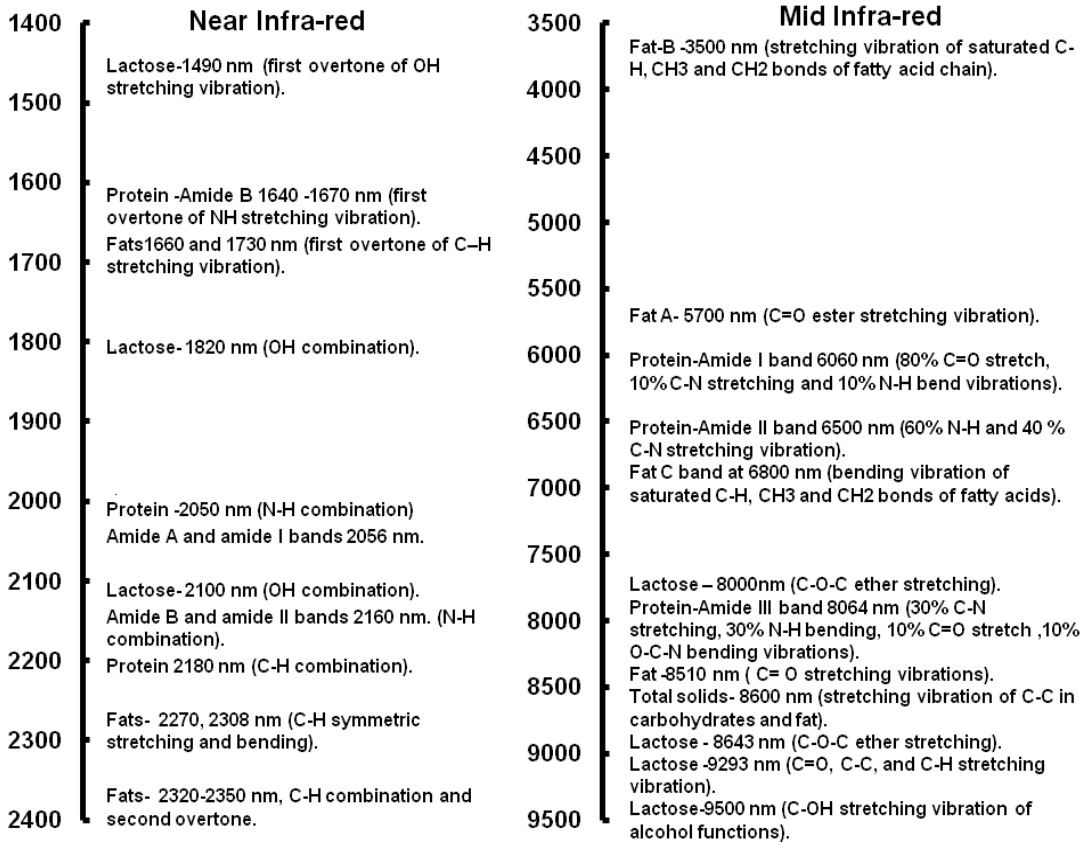


Figure 9: Bands assignment in near infrared (NIR) and mid infrared (MIR) spectra used for milk composition analysis, scale in wavelength (nm). Sources: (Robert et al., 1987; Stuart & Ando, 1997; Sasic & Ozaki, 2000; Foss Analytics, 2007; Brandao et al., 2010; Aernouts et al., 2011a, b; Coppa et al., 2012; Grelet et al., 2015 and Mabood et al., 2017)

Figure 10 presents the absorption bands in the NIR and MIR spectra of camel and cow milk. Multivariate calibration models are developed by chemometrics to establish the

relation between analyte concentrations and to overcome the noise in the IR light absorption of analytes resulting from interface from other matrix compounds.

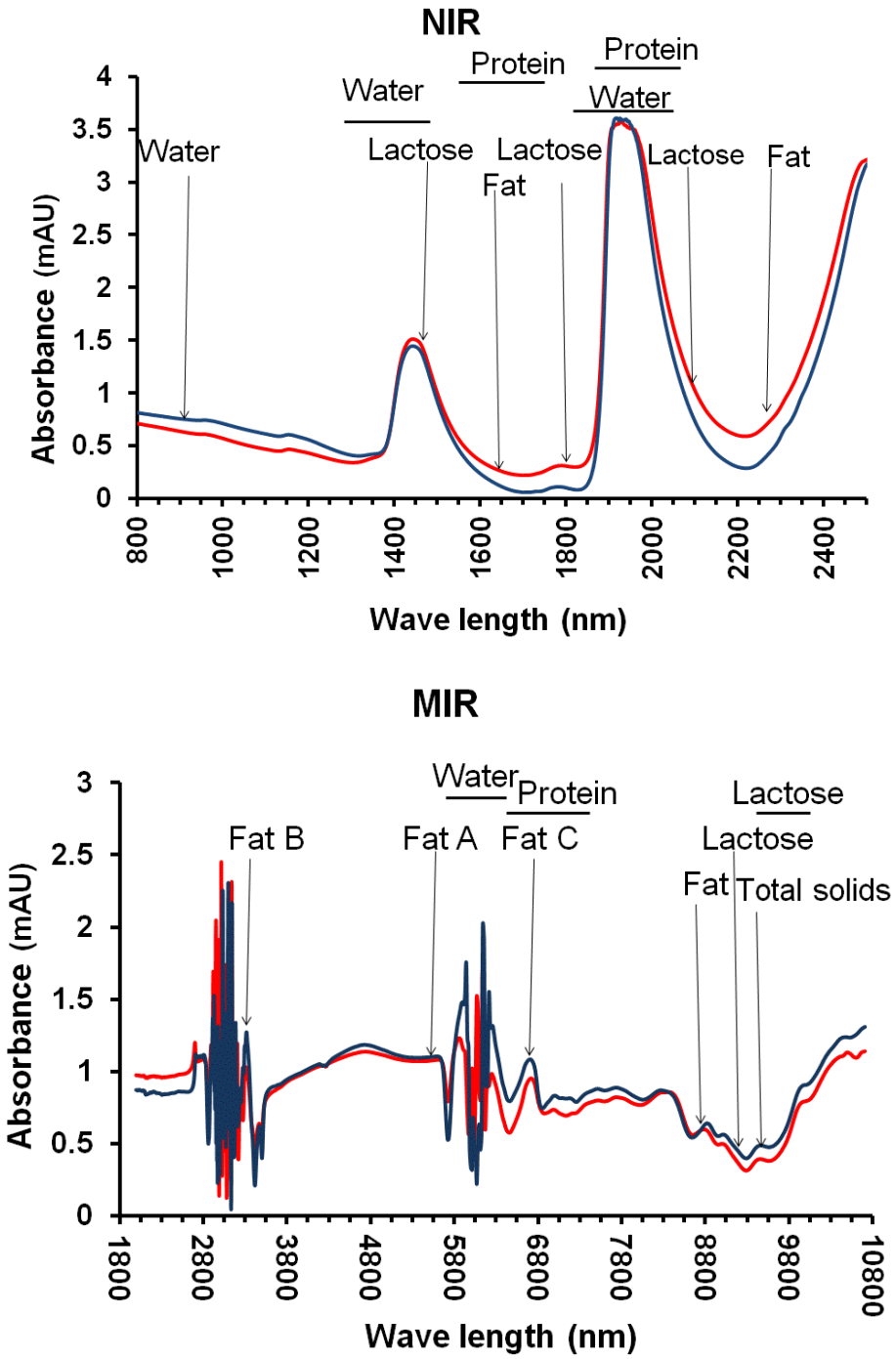


Figure 10**:** Near infrared (NIR) and mid infrared (MIR) spectra of raw cow and camel milk acquired from Bruker MPA and Foss MilkoScan FT-120. Graphs, blue: cow milk, red: camel milk

Compared to cow milk, camel milk has smaller fat globules (3.2 - 5.6 um) vs. (4.3 - 8.4 um) and larger casein micelle (260 - 300 nm) vs. (100 - 140 nm) (Farah & Ruegg, 1989; Meena et al., 2014; Khalesi et al., 2017). It is not known how these, and possibly other compositional and structural differences, might affect the quantitative models for the analysis of protein, fat, lactose, and total solids in these two types of milk.

### 2.3.2 Comparison of NIR and MIR Methods for the Analysis of Protein, Fat, Lactose, and Total Solids in Cow and Camel Milks

Correlation analysis was applied to assess the relationship between MIR analysis by (Foss MilkoScan FT-120) and NIR analysis by (Bruker MPA) for protein, fat, lactose and total solids concentrations (%) in cow and camel milk samples. The results presented in Figure 11 show a strong positive linear correlation between the two instrumental values ($p<0.001$). The agreement between the two methods was assessed using Bland-Altman scatter plots (Figure 11), in which the y-axis shows the difference between the values obtained from the two methods, and the x-axis represents the mean of these measurements (Altman & Bland, 1983; Giavarina, 2015). For a perfect agreement, the mean difference between any two methods should be as close as possible to zero. Both Bland-Altman and correlation plots confirm that the mean difference between the MIR and NIR methods is slightly positive for protein and slightly negative for lactose and total solids. The Bland-Altman plots also showed the upper and lower limits of agreement (ULA, LLA) that comprise 95% of the data points within $\pm$1.96 standard deviations of the mean difference. Excluded samples (marked red) in the Bland-Altman plot (Figure 11) are lying on both sides of upper and lower limits of agreement in the case of protein and total solids, above the upper limit for fat and below the lower limit for lactose. Correlation analysis was applied to study the relation between the mean of each determination (MIR value + NIR value) / 2 and the

difference (MIR value – NIR value). The correlation coefficient (r) for protein, fat, lactose, total solids where 0.11, 0.76, 0.74, 0.46, respectively, for cow milk and 0.72, 0.20, 0.36, and 0.16, respectively for camel milk, all these correlation coefficients (r) were significant ($p<0.05$) except for the correlation for protein concentration in cow milk ($p>0.05$). The correlation was moderately strong for fat and lactose concentrations in cow milk and protein in camel milk.

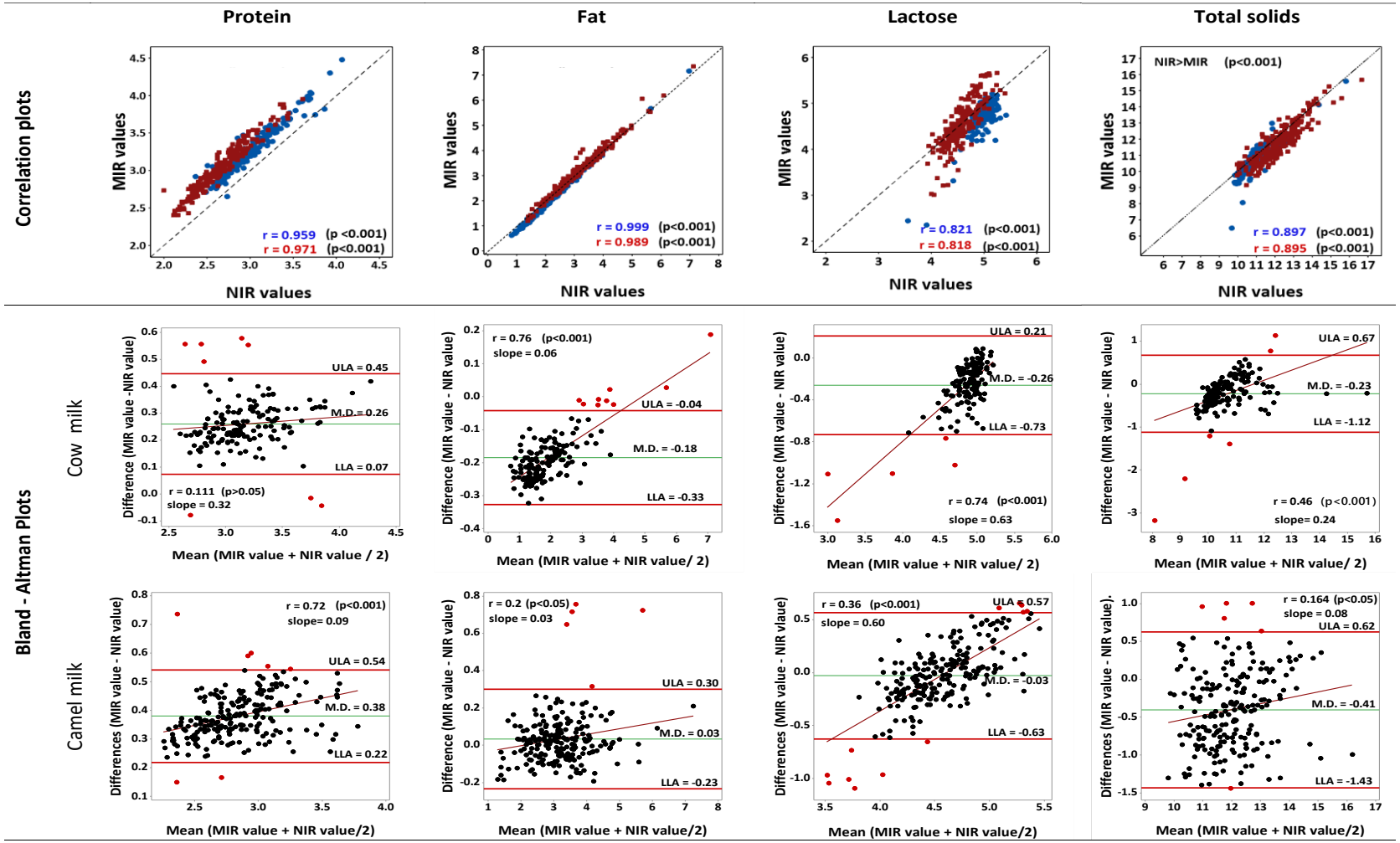


Figure 11**:** Correlation plots (Blue: cow milk, n =150, Red: camel milk, n =217) and Bland-Altman plots for values of protein, fat, lactose and total solids concentration (%) in raw cow and camel milk measured by near infrared (NIR, Bruker-MPA) and mid infrared instrument (MIR, Milkoscan FT-120). Samples with values above the ULA or below the LLA are marked red

Table 7 shows the mean of the relative difference ((MIR value – NIR value) / 0.5 (MIR value + NIR value) x 100%) for protein, fat, lactose, and total solids concentrations in raw cow and camel milk. The differences between the MIR and NIR results for fat concentrations that are evident in cow milk but not in camel milk may be attributed to the differences in the sizes of fat globules. Compared to cow milk, camel milk has smaller fat globules (3.2 - 5.6 vs. 4.3 - 8.4 um) (Meena et al., 2014; Khalesi et al., 2017). The large fat globules in cow milk cause the light to be scattered; this leads to decreased transmittance and false-positive absorbance (Foss Analytics, 2007; Cattaneo et al., 2009; Holyrod, 2013). While measuring transmittance, the infrared detector can't distinguish between light lost inside the cell by absorbance and scattering (Figure 12).

Table 7: Mean (%) ± standard deviation and mean relative difference (%) * for the concentrations of protein, fat, lactose, and total solids in raw cow and camel milk samples analyzed by MIR and NIR

| Parameter | Cow milk (n =150) | | | Camel milk (n =217) | | |
|---|---|---|---|---|---|---|
| | MIR | NIR | Mean relative diff. (%) | MIR | NIR | Mean relative diff. (%) |
| Protein | $3.3 \pm 0.33^{a}$ | $3.0 \pm 0.32^{b}$ | + 8.2 | $3.0 \pm 0.33^{a}$ | $2.7 \pm 0.30^{b}$ | +13.4 |
| Fat | $1.8 \pm 0.90^{b}$ | $2.0 \pm 0.86^{a}$ | - 9.3 | $3.2 + 0.91^{a}$ | $3.2 \pm 0.88^{b}$ | + 0.9 |
| Lactose | $4.7 \pm 0.38^{b}$ | $5.0 \pm 0.21^{a}$ | - 5.4 | $4.5 \pm 0.49^{a}$ | $4.6 \pm 0.28^{a}$ | - 0.7 |
| Total solids | $10.6 \pm 1.01^{b}$ | $10.8 \pm 0.81^{a}$ | - 2.2 | $11.8 \pm 1.2^{b}$ | $12.2 \pm 1.09^{a}$ | - 3.4 |

*Mean relative difference (%) = (MIR values – NIR values) / 0.5 (MIR values + NIR values) x 100%.
** (MIR): Mid Infrared, Foss MilkoScan FT -120. (NIR): Near Infrared, Bruker Multipurpose Analyzer (MPA).
-For each type of milk, values within a raw having different superscripts are significantly different (p-value <0.05).

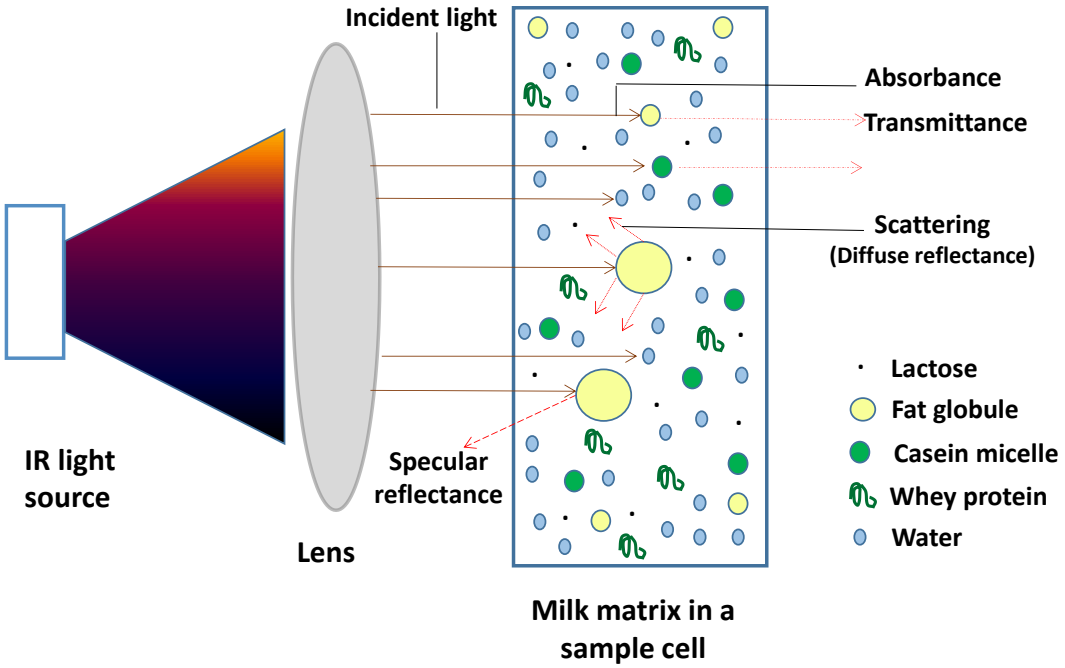


Figure 12: Absorbance, transmittance, scattering, and specular reflectance responses of milk to incident infrared light. In the NIR and MIR methods used in this study, transmittance is measured, and apparent absorbance is used in model building. Differences in matrix effects on scattering and specular reflectance may contribute secondary effects on the validity of the models based on slightly different matrices

The lack of a milk homogenizer in the NIR instrument used in this study might have contributed to these differences in cow milk. For protein concentrations, the mean relative difference (%) between the MIR and NIR methods for cow and camel milk can't be explained.

In this study, comparative validation of two ready-to-use infrared spectroscopic methods (Bruker's Multipurpose NIR Analyzer (MPA) and Foss MIR MilkoScan FT-120) were performed. The two methods are used world-wide in dairy laboratories for quick analysis of industrial samples, mainly cow milk samples. The mean relative difference (%), i.e. (MIR values – NIR values) / 0.5 (MIR values + NIR values) x 100%) was used to evaluate the similarity in the performance of the built-in calibration

models of the two methods. Although these methods are mainly calibrated for the analysis of cow milk, their application to camel milk gave results pointing to the same direction, i.e., MIR gives higher values than NIR for protein content and lower values for lactose and total solids contents for both cow and camel milk samples. The results for fat content are different, with NIR giving higher values for cow milk and slightly lower values for camel milk. This study suggest that it is important to run these two analyses on sets of cow, camel, and possibly other milk samples analyzed by reference methods to investigate the nature of any bias in these methods. An important limitation for this study relates to the fact that no idea about the models operating in any of the two commercial equipment (Bruker MPA and Foss Milkoscan FT-120) is available. This lack of knowledge makes it difficult to evaluate the nature and magnitude of bias in each method. Thus, it is important in the future to compare the performance of these methods against analytical data from reference methods.

### 2.3.3 Variability of Milk Composition Data in Raw Cow and Camel Milk Samples

Figure 13 shows the variability of protein, fat, lactose, and total solids concentrations (%) in the 150 raw cow and 217 raw camel milk samples collected from individual animals. This wide range of samples and the variability in their composition is necessary for the comparison of the tested methods. The mean values for the protein, fat, lactose, and total solids concentrations in cow and camel milk, as analyzed by MIR and NIR are presented in Table 7. The mean values for protein fat, lactose, and total solids in the cow milk samples analyzed in this study is in agreement with reported values suggesting that variability might be affected by breed, genetics, diet and unknown environmental factors (Kabil et al., 2015). Results of camel milk are also in agreement with previous studies (Nagy et al., 2019).

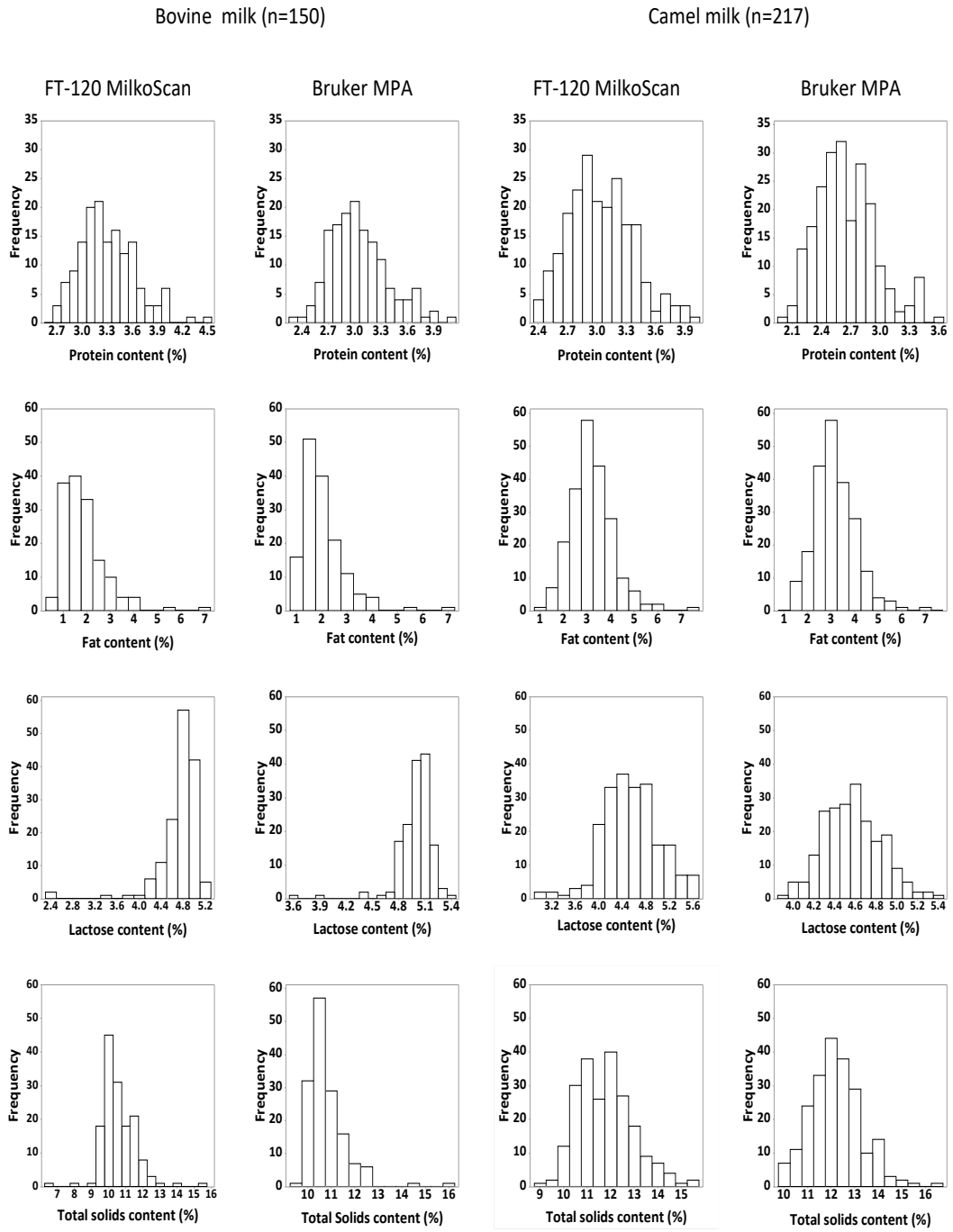


Figure 13**:** Variability in the protein, fat, lactose and total solids concentration (%) in raw cow and camel milk samples analyzed by MIR (Foss MilkoScan FT-120) and NIR (Bruker MPA)

The variation is attributed to breed, geographic region, month of the year, season, level of production, age, lactation stage, lactation number, feeding, physiological condition

and analytical and sampling procedures (Alhag & Al Kanhal 2010; Hamed et al., 2012; Nagy et al., 2019).

## 2.4 Conclusions

Near and mid infrared spectroscopy methods are both valuable and provide comparable results for raw milk analysis. However, differences between the two methods were evident in this study, especially for protein and fat concentrations. The difference between the two methods may be due to the effects of differences in milk homogeneity, especially with respect to casein micelles and fat globules. It is suggested that these two analytical methods need to be compared again together with the reference methods using sets of cow, camel, and possibly other milk samples to investigate the nature of any bias.

# Chapter 3: 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 compared to other milks especially bovine. Because proteins confer many of the properties of milk and its products, this study aims to determine the proteins of camel milk, their correlations and relative distribution. Raw milk samples were collected from 103 Dromedary camels, morning and evening. Capillary electrophoresis results showed wide variation in the concentrations (g/L) of proteins between samples, as follows: α-lactalbumin, 0.3 - 2.9; α-s1-casein, 2.4 - 10.3; α-s2-casein, 0.3 - 3.9; β-casein, 5.5 - 29.0; κ-casein, 0.1 - 2.4; unknown casein protein 1, 0.0 - 3.4; and unknown casein protein 2, 0.0 - 4.6. The range in percent composition of the four caseins were as follows: α-s1, 12.7 - 35.3; α-s2, 1.8 - 20.8; β, 42.3 - 77.4; and κ-, 0.6 - 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 two milks with respect to technical and nutritional properties.

**Keywords**: Camel milk, proteins, α-lactalbumin, caseins, capillary electrophoresis.

## 3.1 Introduction

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 anti-diabetic and anti-allergic effects (Izadi et al., 2019). However, difficulties are encountered in the processing of CM into fermented products and ultra-high-temperature treatment (Berhe et al., 2017). CM proteins are mainly composed of caseins (50-88%) and whey proteins (20-25 %) (Shuiep et al., 2013; Mati et al., 2017). CM is rich in α-lactalbumin but is devoid of the whey protein β-lactoglobulin, 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, capillary electrophoresis was used 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-lactalbumin. In addition, the variability in the relative proportions of the different caseins was investigated, which might affect the properties of CM with respect to commercial processing and health benefits (Ghnimi & Kamal-Eldin, 2015).

## 3.2 Materials and Methods

Fresh camel milk samples were collected from 103 Dromedary camels in the evening and morning of consecutive days (Total 206 milk samples). 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). Data about the animals breed, age,

parity and lactation number is shown in Appendix A. 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 was then mixed manually before aliquots were collected in sterile bottles (250 mL). The samples were transported to the laboratory in a thermo cool box and were aliquoted and frozen at -20ºC. The total protein concentrations in the CM samples (g/L) were determined using a mid-infrared spectroscopy instrument (Foss Milkoscan FT-120, Foss A/S, DK-3400 Hillerød, Denmark). Somatic cell count/ml was determined by a Fossomatic Minor instrument (Foss A/S, DK- 3400 Hillerød**,** Denmark).

Milk proteins were separated by capillary electrophoresis (7100 A, Agilent Technologies, Palo Alto, California, USA) 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). Sample buffer, running buffer, and milk samples preparation 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 minutes with water and 5 minutes with running buffer. Parameters included a voltage of 25 kV and injection pressure of 5 kPa. The column was washed with NaOH (0.1 M) after running four samples to remove any adsorbed contaminants from the capillary walls. Separated peaks were detected via UV light absorbance at 214 nm. Sigma (St. Louis, Missouri, USA) bovine protein standards (α-casein (>85%), β-casein (>80%), κ-casein (>80%), α-lactalbumin (>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 vs. 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:

Concentration (mg/mL) = Peak area (mAU) / Slope of standard curve of bovine protein x dilution factor.

### 3.3 Results and Discussion

Figure 14 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 αs1-, αs2-, β-, and κ- caseins, and the whey protein α–lactalbumin. 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. Buffer additives were used to optimize the selectivity and fine tune protein separation by stabilizing the proteins and preventing their adsorption onto the capillary wall (Schwartz & Pritchett, 1994). In this study, the separation of milk proteins, especially the caseins, was improved over that obtained by Omar et al. (2016). The results have shown that the CM samples were devoid of the whey protein β-lactoglobulin in agreement with others (Hinz et al., 2012; El-Hatmi et al., 2015).

Lactoferrin was not detected in the electropherograms of this study but was detected by Omar et al. (2016) when the whey proteins were separated from caseins.

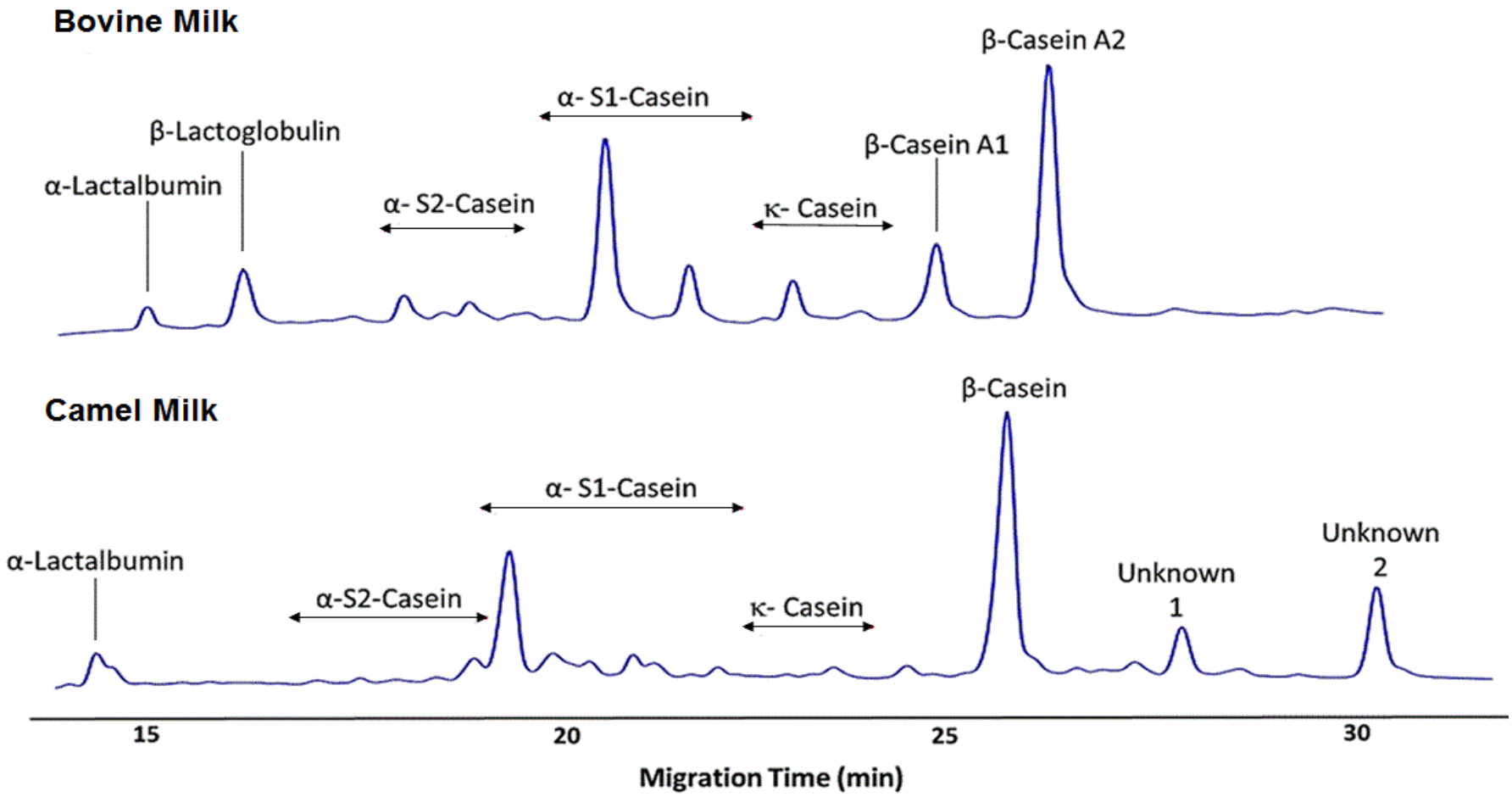



Figure 14**:** Representative electropherogram of bovine and Dromedary camel milk samples determined by capillary electrophoresis

It is possible that 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 improved resolution from interfering proteins.

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

The concentrations of αs1-, αs2-, β-, and κ- caseins and α-lactalbumin in CM samples (n = 206) are shown in Figure 15. The ranges of protein concentrations (g/L) were as follows: α-lactalbumin (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 15. 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-lactalbumin (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 lead to some uncertainty in the quantifications (Kappeler et al., 1998; Omar et al., 2016; Ryskaliyeva et al., 2018). However, since all researchers used the same standards and agreement between results was obtained for CM samples (using high performance liquid chromatography or capillary electrophoresis), this gives validity to the results. Protein's concentration by animals breed, age, parity, and lactation stage are shown in Appendix B.

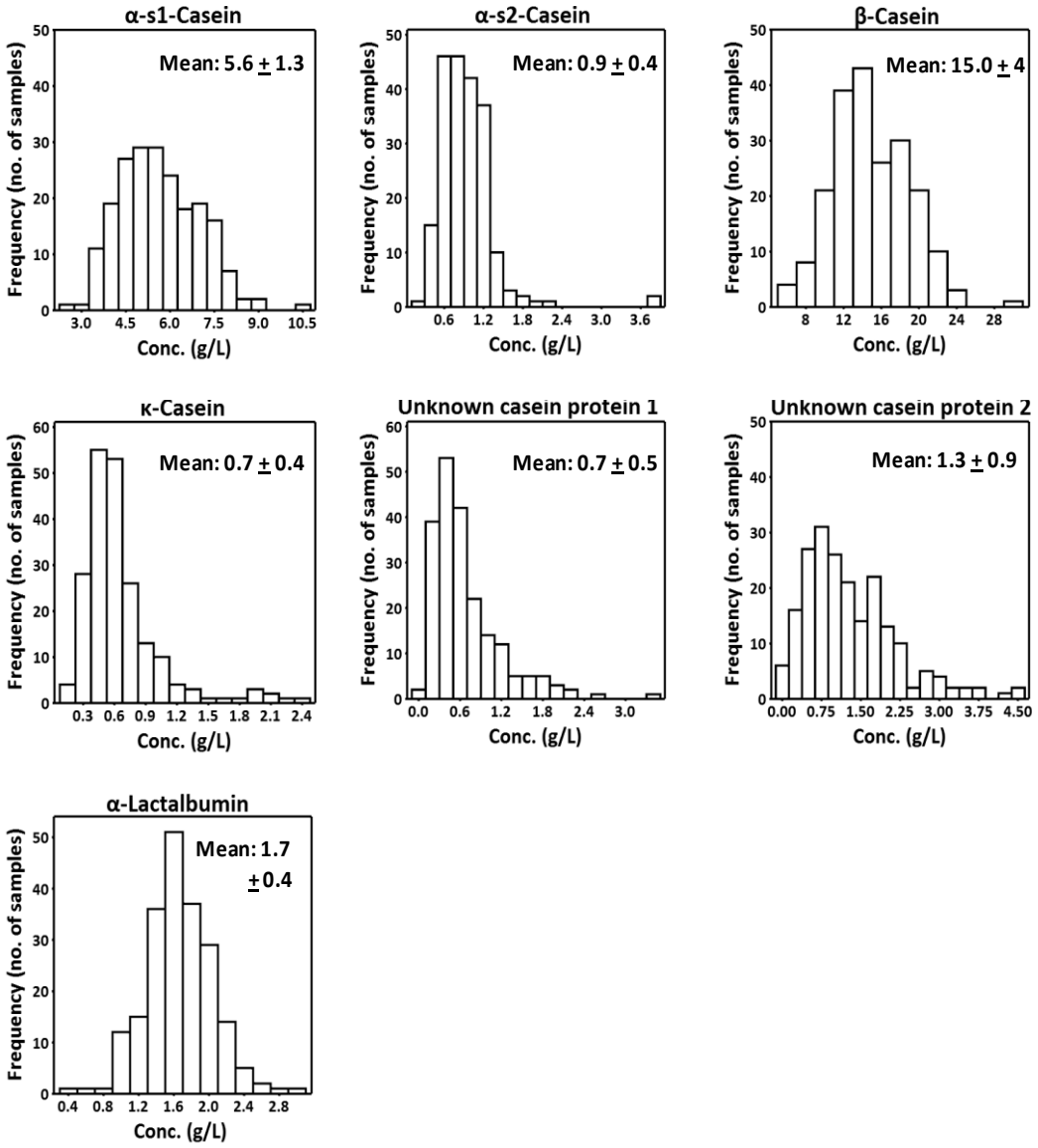



Figure 15**:** Histograms of concentration (g/L) of α-s1-casein, α-s2-casein, β-casein, κ–casein, unknown casein protein 1, unknown casein protein 2, and α-lactalbumin in morning and evening Dromedary camel milk samples (n = 206)

Pearson correlation test were applied by using Minitab statistics package (version. 18, Minitab, Inc, State College, Pennsylvania, U.S.A). Table 8 presents Pearson correlation coefficients (r) between the different CM proteins, percentage caseins in total proteins, and somatic cell count. The results showed a weak positive correlation ($r = 0.266$, $p < 0.01$) between somatic cell counts (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 decrease in casein content, proteolysis, and changes in the protein fraction distribution in bovine milk (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 ($r = - 0.39$, $p<0.01$) while 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 bovine milk. Subclinical and clinical mastitis is 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, 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 the casein proteins (g/L), a correlation that cannot be explained.

Table 8: Pearson correlation coefficients (r) for Dromedary camel milk proteins and somatic cell count (SCC)

| | Total protein (g/L) | SCC (cell number/ml) | Casein/ Total protein (%) | α-Lactalbumin (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-Lactalbumin (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 ** |

Correlations are (**) significant p-value < 0.01, (*) significant at p-value < 0.05, and NS: Nonsignificant. UCP 1: Unknown Casein Protein 1. UCP 2: Unknown Casein Protein 2.

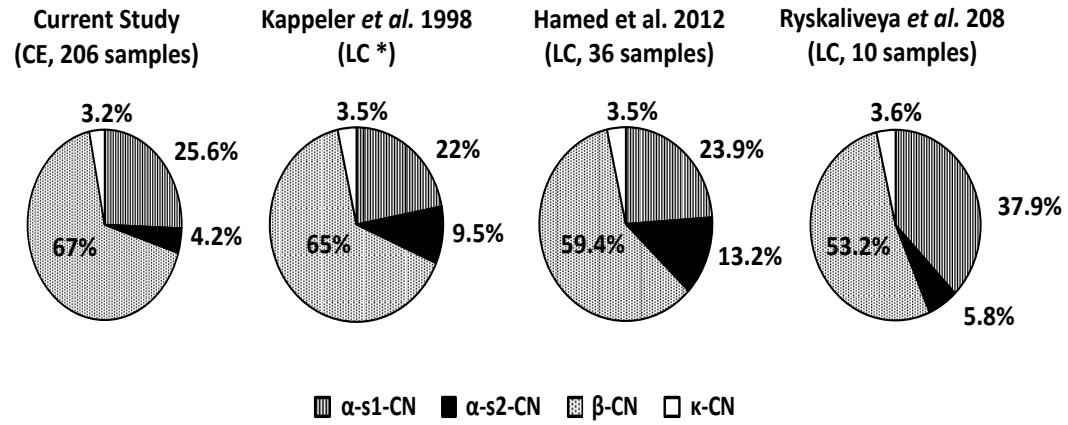



Figure 16: Relative proportion (%) of α-s1-, α-s2-, β-, and κ- caseins in Dromedary camel milk as determined in the current and previous studies. "CE, capillary electrophoresis; LC, liquid chromatography; CN, caseins, *Number of samples not given"

The relative percentage of the four caseins in the CM samples (n = 206) is shown in Figure 16. β-Casein was the major casein in camel milk (67%) in agreement with previous studies, (Kappeler et al., 1998; Hamed et al., 2012; Ryskaliyeva et al., 2018). It is observed that the range of the relative percentage was very wide for all the caseins (α-s1, 12.7 - 35.3%; α-s2, 1.8 - 20.8%; β, 42.3 - 77.4%; and 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.

The results of this study 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 this study (35.3%).

The results of this study suggest that the relative ratio of αs1-, αs2-, 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 & Kelly, 2004). This difference and the dominance of β-casein in

camel milk may be important in explaining some of the special properties of this milk. When processing CM to cheese a weak coagulum is formed in a long coagulation time and the yield is low because a significant amount of the dry matter is lost with the whey (Ramet, 2001; Berhe et al., 2017). Camel milk yoghurt curd is fragile, 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 non-coagulating BM (Wedholm et al., 2006). It was recently reported that non coagulating property of milk from red cattles significantly correlated with higher relative concentrations of α-lactalbumin and β-casein and lower relative concentrations of β-lactoglobulin and κ-casein (Nilsson et al., 2020). The anti-coagulation properties of β-casein can be explained by its chaperone-like activity (Zhang et al., 2005).

### 3.4 Conclusion

A wide variation in the concentrations of the four caseins (αs1-, αs2-, β- and κ-) and α–lactalbumin in 206 CM samples was observed, the variation might be attributed to that the samples were collected from individual animals from different breeds and physiological conditions. The relative proportion of the casein proteins in CM is different than in BM, this disparity is likely responsible for a number of peculiarities of camel milk.

# Chapter 4:Variability of Bioactive Proteins in Camel Milk (*Camelus dromedarius*): Insulin, Insulin-like growth factors, Lactoferrin, Immunoglobulin G, PGRP1, Lysozyme, and Lactoperoxidase


## Abstract

Dromedary camel milk whey protein includes several bioactive proteins with potential health effects. The aim of this research was to study the variability in the concentrations of several whey proteins in milk collected from Dromedary camels reared in the UAE. Milk samples (n =140) were collected from individual camels reared under intensive management. The concentrations of Insulin (IN), Insulin-Like Growth Factor-I (IGF1), Insulin-Like Growth Factor-II (IGF2), Lactoferrin (LF), Immunoglobulin G (IgG), Peptidoglycan Recognition Protein-1 (PGRP1), Lysozyme (LZ), and Lactoperoxidase (LPO), were determined in each of the 140 samples using camel-specific quantitative sandwich Enzyme Linked Immuno-Sorbent Assay (ELISA) kits. The range of concentration of the studied proteins were: IN (17.8-51.1 mIU/L), IGF1 (1.4 - 736.1 ng/ml), IGF2 (13.7 - 82.6 ng/ml), LF (639.4 - 2,094.9 ug/ml), IgG (7.3 - 17.9 mg/ml), PGRP1 (1.6 - 22.3 ng/ml), LZ (23.3 - 71.4 ug/ml), and LPO (7.1-15.5 ng/ml). Significant Pearson correlations ($p<0.05$) were observed between IN & LZ ($r = 0.759$), IN & IgG ($r = 0.502$), IN & PGRP1 ($r = 0.6702$), LZ & PGRP1 ($r = 0.641$), IgG & LPO ($r = 0.698$) and IgG & PGRP1 ($r = 0.398$). There is a wide variability in the concentrations of the studied bioactive whey proteins in Dromedary camel milk. IGF1 and IGF2 are present in concentrations much higher than reported values in bovine and human milk shedding a light on possible importance in human nutrition.


**Keywords**: Camel milk, insulin, insulin-like growth factors, lactoferrin, immunoglobulin, peptidoglycan recognition protein-1, lysozyme, lactoperoxidase.

## 4.1 Introduction

Dromedary camel milk is traditionally valued for its medicinal properties including a number of beneficial health effects (Singh et al., 2017; Wernery, 2006). *Inter alias*, camel milk was reported to be beneficial for patients with diabetes (Agrawal et al., 2011; Ayoub et al., 2018; Mihic et al., 2016; Shori, 2015), autism (Al-Ayadhi et al., 2015; Al-Ayadhi & Elamin, 2013; Bashir & Al-Ayadhi, 2014), and allergy (Navarrete-Rodríguez et al., 2018; Talarico et al., 2019). Camel milk has also demonstrated efficacy in hepatitis C patients; where the viral load in most patients' sera was reduced after consumption of camel milk (El-Fakharany et al., 2017). Camel milk promoted the recovery from chronic hepatitis B possibly by enhancing the cellular immune response and inhibiting the replication of the virus DNA (Saltanat et al., 2009).

Generally camel milk proteins are divided into colloidal caseins and soluble whey proteins. Recently, the major proteins in 206 camel milk samples were quantified as (g/L): α-lactalbumin (0.3 - 2.9); α-s1-casein (2.4 - 10.3); α-s2-casein (0.3 - 3.9); β-casein (5.5 - 29.0); κ-casein (0.1 - 2.4); and two unknown casein proteins (0.0 - 3.4 and 0.0 - 4.6) (Mohamed et al., 2020). The exact components and mechanisms responsible for the health benefits of camel milk are not yet known but whey proteins were suggested as the source for these benefits (Izadi et al., 2019; Mati et al., 2017). The whey fraction of camel milk is highly heterogeneous and is a rich source of proteins with biological and protective activities. These proteins include Insulin (IN), Insulin-Like Growth Factors (IGFs), Lactoferrin (LF), Lysozyme (LZ), Lactoperoxidase (LPO), Serum Albumin (SA), Whey Acidic Protein (WAP), Whey Basic Protein (WBP), Lactophorin (LP), and Peptidoglycan Recognition Protein-1 (PGRP1), various classes of Immunoglobulins

(IG), and other small peptides (Assaf & Ruppanneb, 1992; El-Hatmi et al., 2007, 2015; Mati et al., 2017; Park et al., 2017; Singh et al., 2017; Izadi et al., 2019).

Milk proteins are either synthesized in the epithelial cells of the mammary gland or are sourced from the blood and then secreted into the milk pool within the alveolar lumen. The whey fraction of milk contains a large number of soluble proteins that are taken up from the blood and transported across the secretory cell into the milk without further processing, through either a paracellular or a transcellular route (Stelwagen, 2011). LF, LZ, LPO, PGRP, lactophorin and α-lactalbumin are synthesized in the epithelial cells of the mammary gland, while Insulin, IGF-1, IGF-2, Ig (A, M, G), and serum albumin are sourced from the blood. The protective proteins (LF, IgG, LZ, and LPO) have a main role in the protection of the mammary gland and passive immunization of the new born (Kappeler et al., 2004).

It has been suggested that camel milk IN is not degraded by the acidic environment of the stomach because it is protected by inclusion in nano-capsules (Malik et al., 2012). It has also been hypothesized that camel milk whey proteins and some hydrolysates of camel milk whey proteins synergize with insulin by stimulating its receptor (Ashraf et al., 2021; Ayoub et al., 2018). Camel milk LF exhibits hypoglycemic, antidiabetic, anti-inflammatory, and immunomodulatory effects (Mohamed & Schaalan, 2018). While human IgG failed, IgG from camel milk showed capability to recognize and inactivate hepatitis C virus peptides with a significant titre (El-Fakharany et al., 2012; Mullaicharam, 2014). In comparison with bovine species, camel whey contains higher levels of LZ, LF, and Ig to which antibacterial and antiviral properties have been attributed (Assaf & Ruppanneb, 1992; Elagamy, 2000).

The aim of this research was to study the variability in the concentrations of a number of whey proteins, namely, IN, IGF1, IGF2, LF, IgG, PGRP1, LZ, and LPO in 140

camel individual milk samples using camel specific sandwich Enzyme-Linked Immune-Sorbent Assay (ELISA) kits. There are few reports on the concentrations of some of these proteins in camel milk mostly including few samples from individual animals or pooled samples. Studying the concentrations of camel milk whey proteins may lead to better inferences regarding exploitation of their bioactivities and the use of camel milk as a nutraceutical component of the diet.

## 4.2 Materials and Methods

### 4.2.1 Chemicals and Reagents

Sodium monobasic phosphate was sourced from Riedel-deHaén (Seelze, Germany), sodium dibasic phosphate was sourced from Sigma Aldrich (St. Louis, Missouri, USA). Camel specific, ready-to-use, quantitative sandwich ELISA kits (Table 9) were purchased from MyBioSource Inc. (San Diego, CA, USA).

### 4.2.2 Milk Samples Collection

Raw camel milk samples (n = 140) were collected from individual Dromedary camels reared in the farm of the company Emirates Industry for Camel Milk and Products (EICMP), Dubai, United Arab Emirates. Samples were collected during the morning milking in three consecutive days. The animals were milked in the automated milking system adapted to Dromedary camels (Fullwood Ltd., Ellesmere, UK and Agromilk Ltd., Székesfehérvár, Hungary) (Nagy et al., 2013). The udder and teats of the camels were cleaned and disinfected prior to automatic milking. To collect representative milk samples, an International Committee for Animal Recording (ICAR) approved sampling device connected to a milk meter was used.

Table 9: Description of the ELISA kits used in the analysis and information about their detection range, recovery (%), and intra- and inter assay precision (CV, %) *

| Name of kit | ELISA Kit (Catalogue #) | Detection range | Recovery (%) | Precision (CV, %)** | |
|---|---|---|---|---|---|
| | | | | Intra-assay | Inter-assay |
| Camel Insulin (IN) | MBS060615 | 3.12 - 100 mIU/L | 76 - 92 | 2.3 - 3.0 | 2.1 - 5.3 |
| Camel Insulin- Like Growth Factor 1 (IGF1) | MBS077229 | 15.6 - 500 ng/mL | 75 - 99 | 4.4 - 5.0 | 4.0 - 7.9 |
| Camel Insulin- Like Growth Factor 2 (IGF2) | MBS058122 | 6.25 - 200 ng/mL | 71 - 96 | 2.0 - 4.1 | 2.1 - 5.0 |
| Camel Lactoferrin (LF) | MBS779163 | 50 - 3200 µg/mL | 79 - 95 | 2.0 - 4.1 | 1.9 - 5.0 |
| Camel Immunoglobulin G (IgG) | MBS107777 | 1.56 - 50 mg/mL | 75 - 90 | 4.0 - 5.0 | 4.4 - 8.9 |
| Camel Peptidoglycan Recognition Protein-1 (PGRP1) | MBS089055 | 1.56 - 50 ng/mL | 80 - 91 | 4.0 - 4.9 | 4.2 - 9.0 |
| Camel Lysozyme (LZ) | MBS063733 | 1.56 - 50 µg/mL | 73 - 89 | 4.0 - 4.9 | 4.5 - 6.5 |
| Camel Lactoperoxidase (LPO) | MBS073926 | 0.625 - 20 ng/mL | 76 - 93 | 4.0 - 4.9 | 2.4 - 4.7 |

*Validation data obtained from the manufacturer; MyBioSource Inc. (San Diego, CA, USA).

** Intra-assay coefficient of variability (%) is a measure of the variance between sample replicates ran within the same plate. Inter-assay CV (%) is a measure of the variance between the sample replicates run on different plates. CV%= (standard deviation/mean) x 100 %.

Samples were stored in sterile bottles, immediately placed in a thermo cool box filled with ice, and directly delivered to the lab at the Department of Food Nutrition and Health, College of Food and Agriculture, United Arab Emirates University.

### 4.2.3 Separation of Whey from Caseins

Sodium phosphate buffer (1M sodium monobasic phosphate and 1M sodium dibasic phosphate (51:49, v/v), pH 6.8) was used to precipitate the casein from the milk according to the method patented (US 7,943,739 B2) by Yen et al. (2011). In 1.5 ml microcentrifuge tubes, sodium phosphate buffer (0.25 ml) was added to milk (1 mL), mixed, and frozen at -20℃ overnight. This was followed by thawing at room temperature, centrifugation (4℃, 12000 rpm, 16 minutes) using Z 216 MK centrifuge (Hermle Labortechnik Gmbh, Wehingen, Germany). The supernatant layer was carefully removed using a 3 ml syringe with needle. The mix was centrifuged again (4 ℃, 12000 rpm, 16 minutes) and the supernatant layer was carefully removed using a 3 ml syringe with needle.

### 4.2.4 Determination of the Concentration of Proteins

The concentrations of IN, IGF2, LF, IgG, PGRP1, LZ and LPO were determined in the separated milk serum of the 140 samples, while IGF1 was determined in 128 samples. The analyses using the ready-to-use camel specific quantitative sandwich ELISA kits was performed according to the supplier protocols. The kits contained a 96 microwell plates coated with antibodies and chemicals supplied with the kit were horse radish peroxidase antibody conjugate, chromogen A, chromogen B, stop solution, washing solution, and six concentrations of calibrant standards for each protein. Serum (50 µl) and horse radish peroxidase (100 µl) were added to all the wells except the blank well.

The plates were covered with a closure plate membrane and incubated at 37°C for 60 minutes. All wells (including blank and standards wells) were washed 4 times with the wash solution (20 x) using a microplate's washer before Chromogen A solution (50 µl) followed by Chromogen B solution (50 µl) were added to all wells. The plates were incubated (37°C, 15 minutes) and then the reaction was stopped by the adding stop solution (50 µl) of to all wells. The optical density was measured at 450 nm using an $E_{max}$ Plus microplate reader (Molecular Devices LLC, San Jose, California, USA). Data acquisition and analysis software (SoftMax Pro, version 7) was used to control the $E_{max}$ Plus microplate reader, prepare standard curves, and calculate the concentration of the proteins in the samples.

### 4.2.5 Statistical Analyses

Minitab statistics package (version 19, Minitab, Inc., State College, Pennsylvania, USA) was used to prepare the histograms of the protein's concentrations and to apply the Pearson correlation analysis to test the association between the protein's concentrations and test the significance of the correlation, $p \leq 0.05$ was considered significant.

## 4.3 Results

### 4.3.1 Variability in the Concentrations of Studied Whey Proteins

Camel specific quantitative sandwich (ELISA) kits were used for the analysis of the camel milk whey bioactive proteins and their validation by the manufacturer is shown in Table 9. The concentrations of the studied proteins in camel milk are presented in Figure 17 as histograms show their variability in terms of ranges, means, and standard deviations. Table 10 compares from the findings of this study with previous studies quoting sample description, methods of quantitation, and protein concentrations.

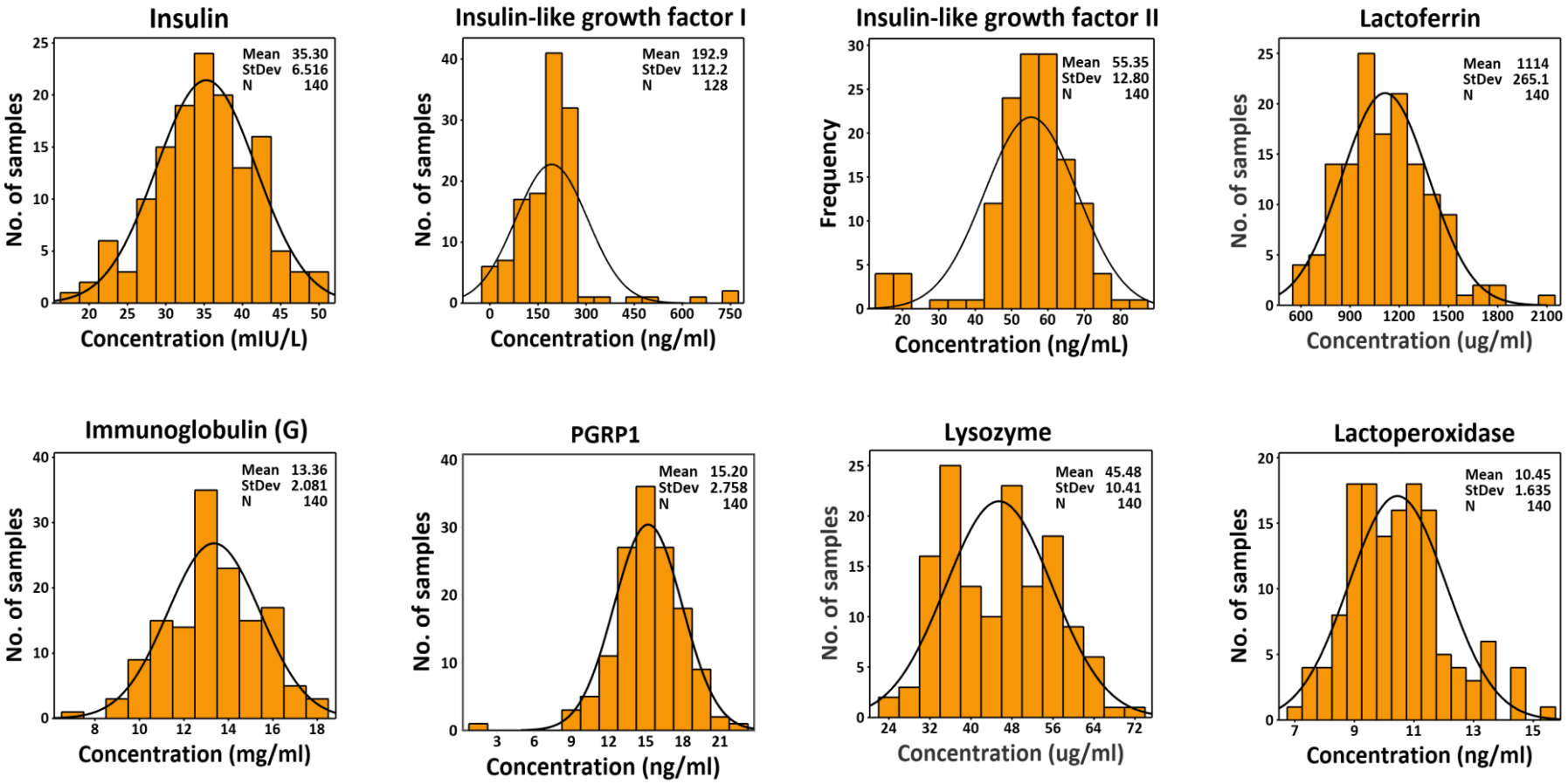



Figure 17**:** Histograms of the concentrations of insulin, insulin-like growth factor I, insulin-like growth factor II, lactoferrin, immunoglobulin G, Peptidoglycan recognition protein-1 (PGRP1), lysozyme and lactoperoxidase, in Dromedary camel milk samples.

In summary, the values for IN fall within the reported range, for IGF1, LF, LZ, and IgG were much higher than the reported values, while the values for PGRP1 in ng/ml were extremely less than the value of 120 mg/ml reported by (Kappeler et al., 2004).

The differences in the concentrations of some of the proteins are very high (Table 10) suggesting the need for further studies that takes into consideration all the factors that might affect the levels of these proteins. Based on published research, no values have been reported in literature for the levels of IGF2, and LPO in camel milk making the results of this study the first to be presented. The amino acid sequences of camel milk IN, IGF1, and IGF2 and their alignment with human and bovine proteins as per UniProt (2020) are shown in Figure 18.

### 4.3.2 The Effect of Lactation Stage (4-8 months)

Figure 19 shows the effect of lactation stage (4 to 8 month) on the concentrations of the studied proteins. The samples were grouped into three groups A (4 - 5 months), B (6 months), and C (7 - 8 months). The variation within the groups was too large to allow statistical comparisons but no clear trend was identified.

### 4.3.3 Correlation between Proteins Concentrations

Figure 20 shows the scatter plots for the studied protein-protein correlations and the Pearson's correlation coefficients and their significance. Significant correlations ($p<0.05$) were observed between several of the studied whey protein's concentrations. For example, IN correlated with LZ ($r = 0.759$), IgG ($r = 0.502$), and PRGP-1 ($r = 0.6702$). LZ correlated with PGRP1 ($r = 0.641$). IgG correlated with LPO ($r = 0.698$) and PGRP1 ($r = 0.398$).

Table 10: Samples description, analysis methods and concentrations of camel whey proteins from the current and previous studies

| References | Samples description | Analytical method* | Concentrations |
|---|---|---|---|
| **Insulin (mIU/L)** | | | |
| This study | 140 samples from 140 animals | Camel IN sandwich ELISA | 35.3 ± 6.5 (mean±SD) |
| Abou-Soliman &Elmetwaly (2018) | 60 samples from 34 animals | Human IN ELISA | 55.1 ± 33.2 (mean±SD) |
| Wernery et al. (2006a) | 126 samples from 7 animals | RIA (Human IN kit) | 40.5 ± 10.7 (mean±SD) |
| Wernery et al. (2006b) | 57 samples from 19 animals | RIA (Human IN kit) | 41.9 ± 7.4 (mean±SE) |
| Royatvand et al. (2013) | 10 samples from 10 animals | UV/Vis spectroscopy (276 nm) | 18 ± 0.4 (mean±SD) |
| Alkaladi et al. (2014) | 50 samples from 50 animals | UV/Vis spectroscopy (276 nm) | 41.2 ± 5.7 (mean±SE) |
| **Insulin-like growth factor I (ng/ mL)** | | | |
| This study | 128 samples from 128 animals | Camel IGF1 sandwich ELISA | 192.9 ± 112.2 (mean±SD) |
| El-Khasmi et al. (2002) | Samples from 4 animals | RIA | 7.3 ± 1.4 (mean±SE) |
| **Lactoferrin (μg/mL)** | | | |
| This study | 140 samples from 140 animals | Camel LF sandwich ELISA | 1114 ± 265 (mean±SD) |
| Kappeler et al. (2004) | 29 samples | UV spectroscopy (280 nm) | 95 ± 7 (mean±SD) |
| Elagamy (2000) | 3 bulk samples | RID | 170 ± 21 (mean±SD) |
| Al-Majali (2007) | 180 samples from 180 animals | RID | 20 – 2100 (range) |
| Konuspayeva et al. (2007) | 42 samples | RID | 209 ± 131 (mean±SE) |
| Kappeler et al. (1999) | One pooled sample | UV spectroscopy (280 nm) | 220 (single value) |

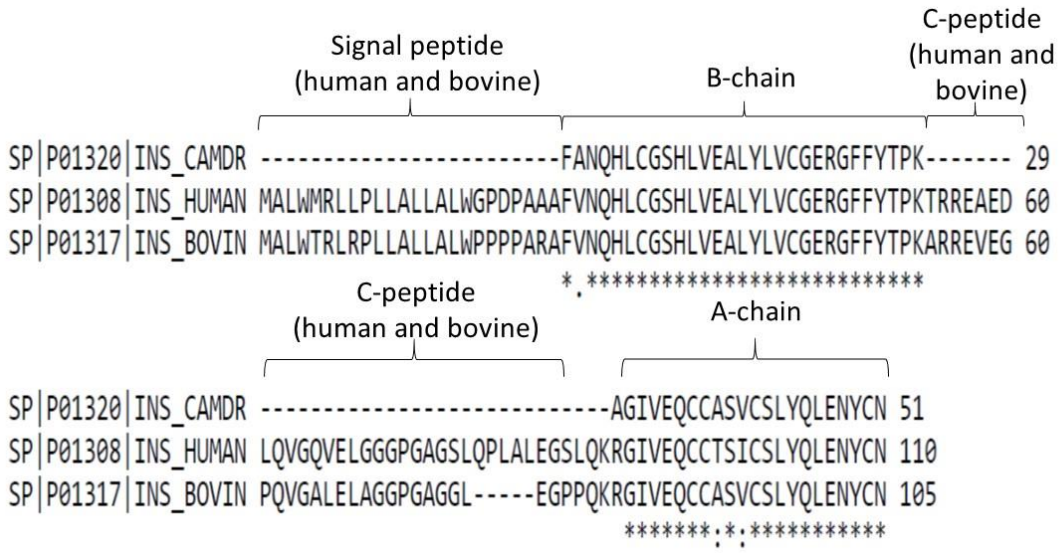
*RID: Radial Immuno-Diffusion Assay, RIA: Radioimmunoassay, ELISA: Enzyme Linked Immuno-Sorbent Assay

Table 11: Samples description, analysis methods and concentrations of camel milk whey proteins from the current and previous studies (Continued)

| References | Samples description | Analytical method* | Concentrations |
|---|---|---|---|
| **Immuno-globulin G (mg/mL)** | | | |
| This study | 140 samples from 140 animals | Camel IgG sandwich ELISA | 13.36 ± 2 (mean±SD) |
| Elagamy (2000) | 3 bulk samples | RID | 2.227 ± 0.153 (mean±SD) |
| Konuspayeva et al. (2007) | 42 samples | RID | 0.833 ± 0.375 (mean±SE) |
| **PGRP1** | | | |
| This study | 140 samples from 140 animals | Camel PGRP1 sandwich ELISA | 15.2±2.8 ng/ml |
| Kappeler et al. (2004) | 29 samples | UV spectroscopy (280 nm) | 120 µg/mL |
| **Lysozyme (µg/mL)** | | | |
| This study | 140 samples from 140 animals | Camel LZ sandwich ELISA | 45.48 ± 10.4 (mean±SD) |
| Elagamy (2000) | 3 samples | Lysoplate method | 1.32 ± 0.088 (mean±SD) |
| Elagamy et al. (1996) | One sample pooled from 90 | Lysoplate method | 15 (single value) |
| Barbour et al. (1984) | 58 samples | Turbidimetry and spectroscopy | 0.62 - 6.48 (range) |

*RID: Radial Immuno-Diffusion Assay, RIA: Radioimmunoassay, ELISA: Enzyme Linked Immuno-Sorbent Assay.

Insulin (IN)




Insulin-Like Growth Factor I (IGF1)

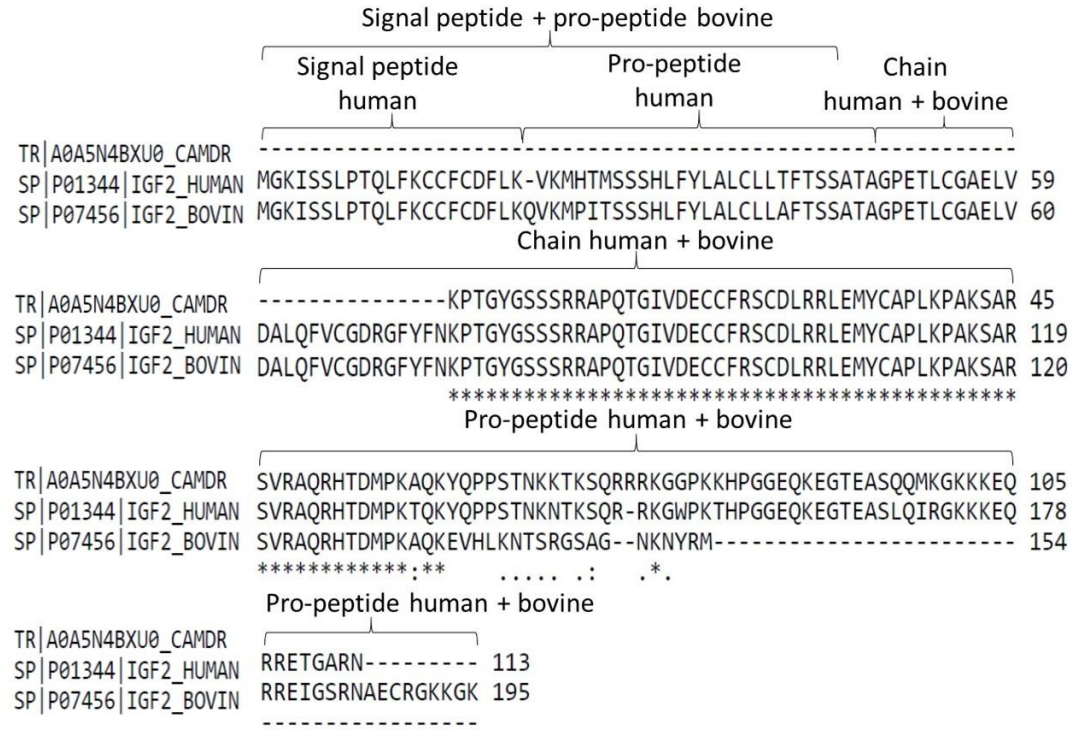



Figure 18**:** Sequence alignment of insulin, IGF1 and IGF 2 from Dromedary camel-CAMDR (*Camelus dromedarius*), Human and Bovine (*Bos taurus*). Source: UniProt (2020). Insulin homology: Camels/Humans: 47%, Camels/Bovine: 46%, Bovine /Humans: 80%. IGF1 homology: Camels /Humans (52.5%), Camels/Bovine (33.5%), Bovine /Humans (66.8%). IGF2 homology: Camels/Humans (90.7%), Camels/ Bovine (83.6%), Bovine /Humans (82.8%)

Insulin-Like Growth Factor II (IGF2)

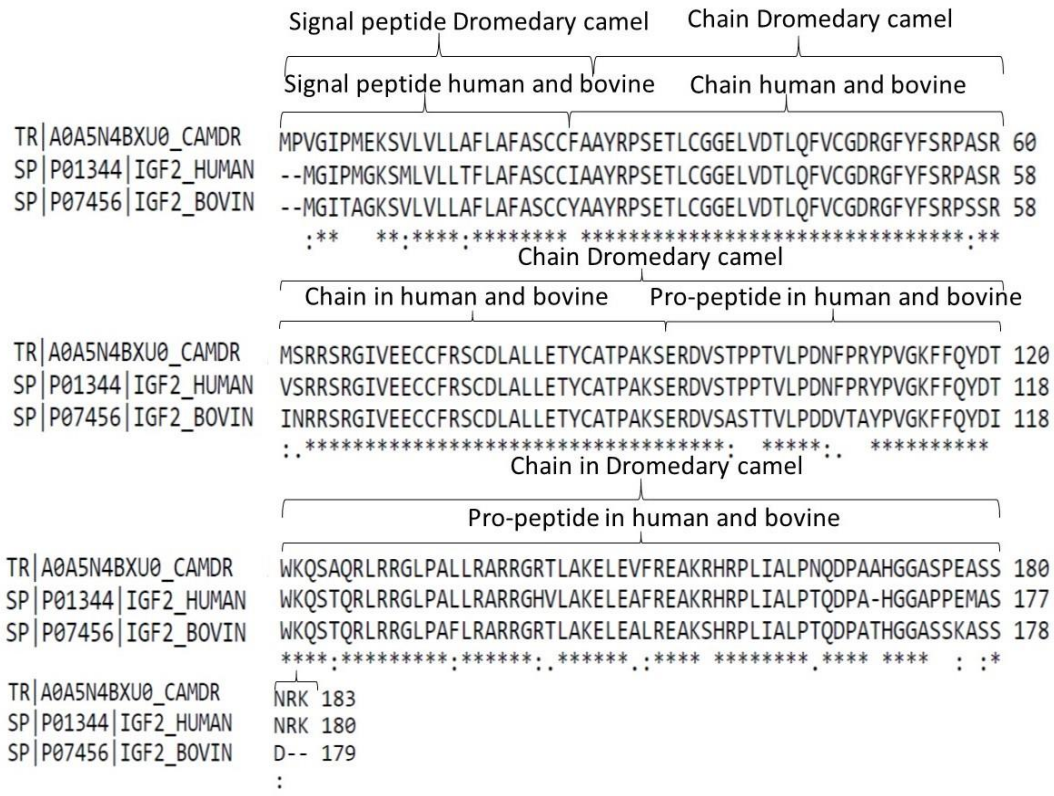



Figure 18: Sequence alignment of insulin, IGF1 and IGF 2 from Dromedary camel-CAMDR (*Camelus dromedarius*), Human and Bovine (*Bos taurus*). Source: UniProt (2020). Insulin homology: Camels/Humans: 47%, Camels/Bovine: 46%, Bovine /Humans: 80%. IGF1 homology: Camels/Humans (52.5%), Camels/Bovine (33.5%), Bovine/Humans (66.8%). IGF2 homology: Camels/ Humans (90.7%), Camels/Bovine (83.6%), Bovine/Humans (82.8%) (Continued)

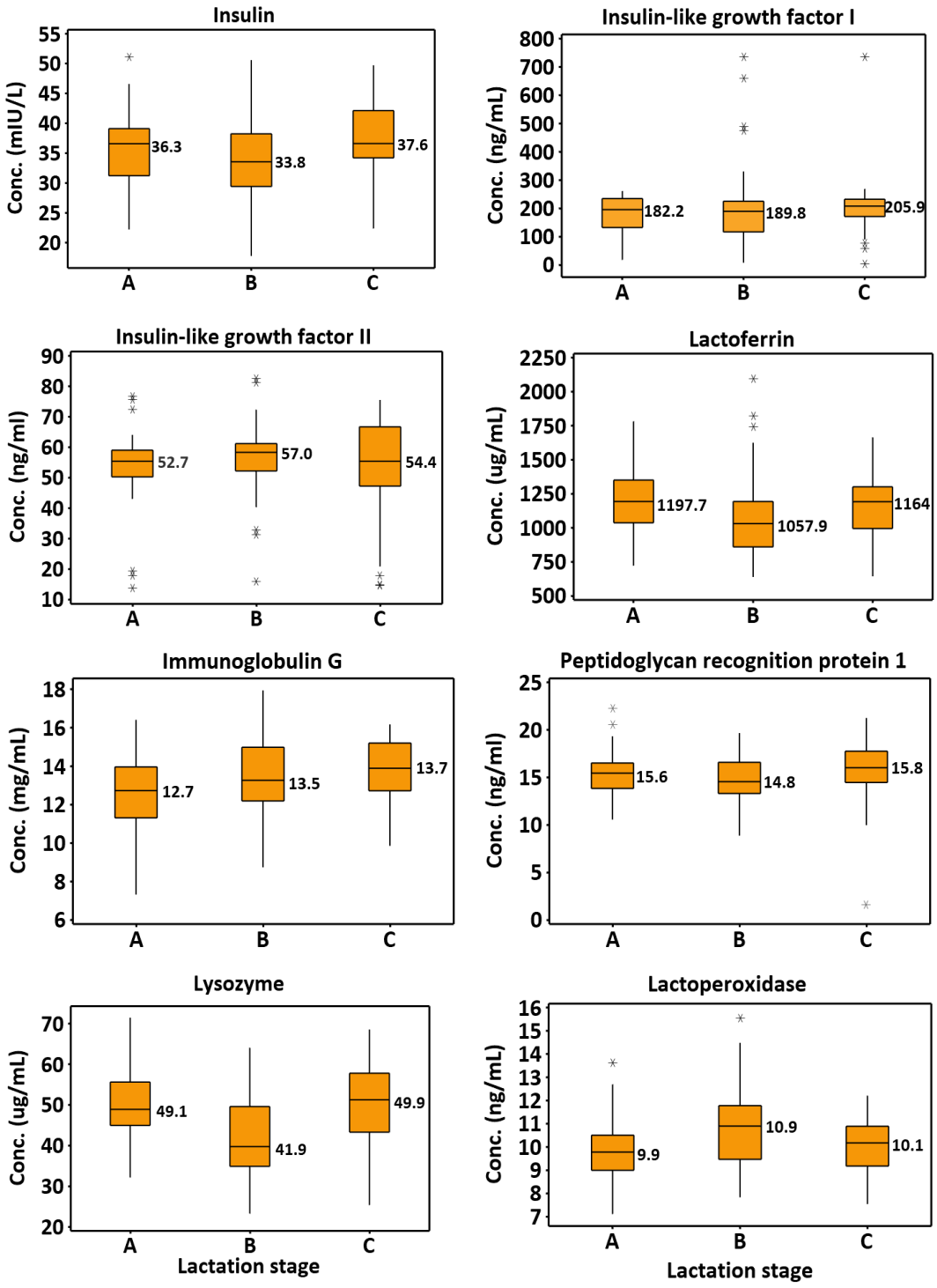



Figure 19**:** The effect of lactation stage on the concentration of whey bioactive proteins in Dromedary camel milk. (A) 4 - 5 months, 29 samples, (B) 6 months, 75 samples and (C) 7 - 8 months, 36 samples. For IGF1: group A:30, group B:59, group C: 39. Means are represented by a black horizontal line in the boxes

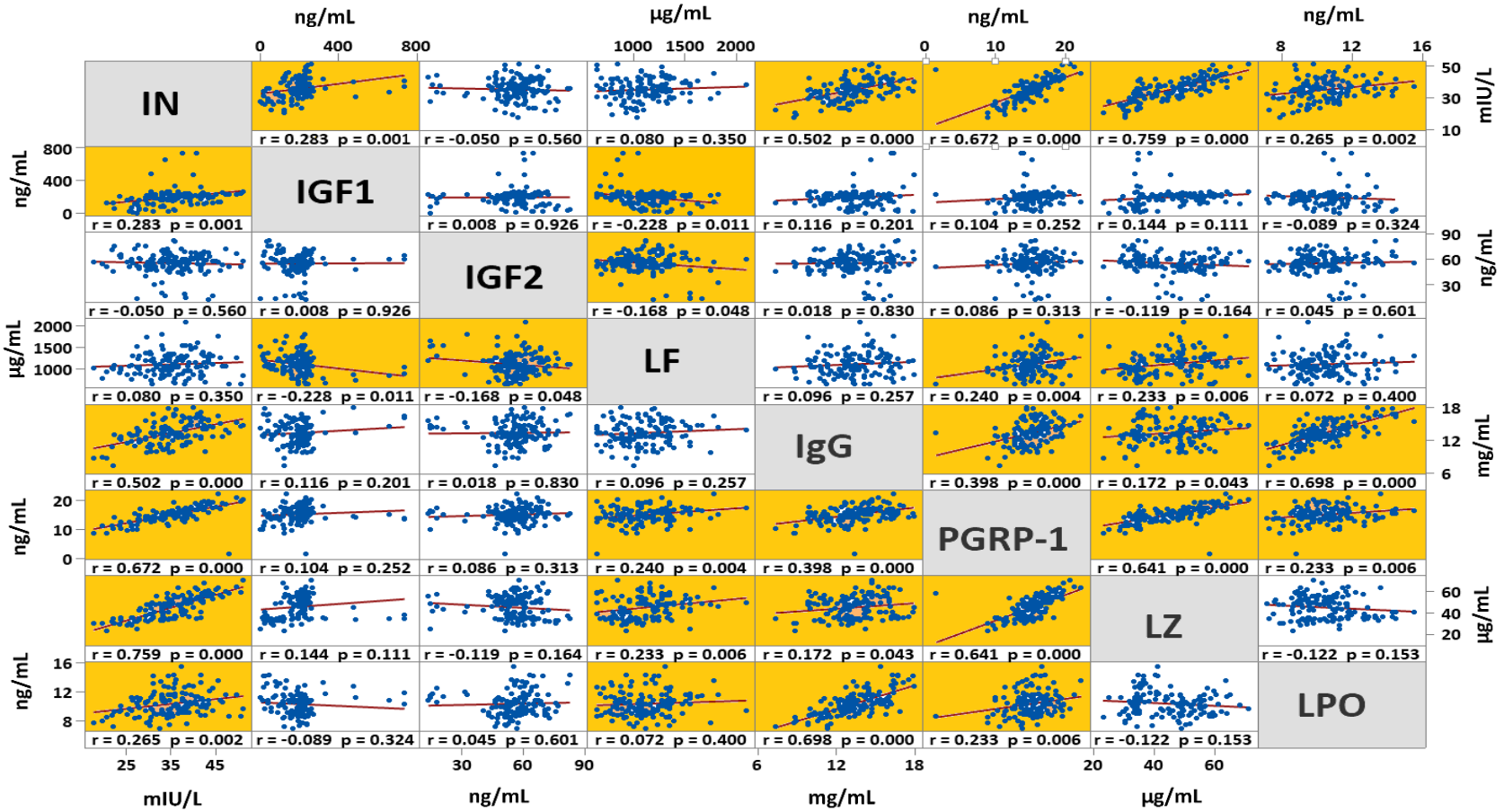



Figure 20**:** Pearson correlation (r) between the concentrations of Insulin (IN), Insulin-Like Growth Factor I (IGF1), Insulin-Like Growth Factor II (IGF2), Lactoferrin (LF), Immunoglobulin G (IgG), Peptidoglycan Recognition Protein-1 (PGRP1), Lysozyme (LZ), and Lactoperoxidase (LPO) in Dromedary camel milk. Orange plots indicate significant correlations ($p<0.05$)

## 4.4 Discussion

### 4.4.1 Methodological Considerations

Milk samples were collected during the milk let down by using an ICAR approved milk meter with a connected sampling device. This method is optimum to obtain representative samples of milk from the individual animals. The method of Yen et al. (2011) presented in the patent US 7,943,739 B2, for precipitating milk caseins under neutral or weakly acidic conditions was used, as the low pH of 4.6, commonly used for casein precipitation leads to significantly poor yields, damaged protein structures, low biological activities, inconveniences, and difficulties in operation. The patented method is based on adding a phosphate solution to milk, mixing, freezing the mixture, thawing, and then centrifugation to obtain a supernatant whey fraction with more than 90 % yield of the target proteins. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) confirmed that the supernatant whey fraction was free of casein bands while the supernatant separated by other methods contained caseins.

For the bioactive whey proteins analysed in this study, camel specific quantitative sandwich ELISA kits were used as they are selective, sensitive, and quick methods that allow handling of many samples in parallel. The procedure for a sandwich ELISA requires that the wells of the micro-plate be coated with a capture antibody. The sample is then added, followed by the addition of a detection antibody conjugated to the enzyme horse radish peroxidase. Sandwich ELISA methods are particularly selective and are suitable for the analysis of complex samples; the antigen does not need to be purified before the assay. The validation data provided by the manufacturer (Table 9), confirm acceptable values for detection ranges, recovery percentages, and intra and inter assay

precision for the different proteins. This information is necessary for future comparisons and applications of the obtained data.

### 4.4.2 Variability in the Concentrations of Camel Milk Whey Bioactive Proteins

Insulin (IN) and Insulin-Like Growth Factors (IGF1 & IGF2)

The concentration range of IN (17.8 - 51.1 mIU/L) and the mean (35.5 $\pm$ 6.5 mIU/L) (Figure 17) fall within the ranges reported by other researchers (Table 10). According to Abou-Soliman & Elmetwaly (2018), the IN content of camel milk is very high in colostrum (1857 ± 804 mIU/L) compared to mature milk (55.1 ± 33.2 mIU/L). Variability in the IN concentration in milk can be affected by animal breed, quantity of milk produced, and diet, e.g. the highest levels of IN were found in milk of camels that receive concentrate diet than camels grazing native pastures (Abou-Soliman & Elmetwaly, 2018). Camel IN is considerably shorter and have low homology with human and bovine IN (Figure 18). The differences between the human, bovine, and camel insulins are due to the signal peptide and the pro-peptide but they are rather similar with respect to the A chain (21 amino-acids) and B chain (30 amino-acids). Since the signal and pro-peptides are removed before insulin secretions into milk, camel milk insulin can be considered as identical to bovine insulin and differs from human insulin only in Thr54Ala, Thr 97Ala, and Ile99Valine (Malik et al., 2012). Thus, the camel milk insulin as such cannot explain the antidiabetic effect of this milk unless it is protected from degradation by acid in stomach such as encapsulation in nanoparticles (Malik et al., 2012). Camel milk IN was thought to resist acidity and proteolysis in the stomach due to encapsulation in nanoparticles (lipid vesicles) that make possible its passage through stomach and entry into circulation (Malik et al., 2012). It is still not known if

camel milk IN contributes to the anti-diabetic properties of this milk (Agrawal et al., 2011; Mihic et al., 2016; Shori, 2015), a possibility that needs to be studied further.

The concentration of IGF1 in Dromedary camel milk was reported in only one study and the concentration of IGF2 was not reported before. In this study, the concentration ranges for IGF1 and IGF2 were 1.4 - 736 and 13.7 - 82.6 ng/ml and the means were 192.9 $\pm$ 112.2 and 55.4 $\pm$ 12.8 ng/ml, respectively. El-Khasmi et al. (2002), in a study conducted on 4 animals reported that IGF1 concentration in colostrum was 13.7 $\pm$ 2.1 ng/ml during parturition and decreased to 7.3 $\pm$ 1.4 ng/ml by reaching day 21 of lactation. Corresponding values of 10-50 and 32 ng/ml, respectively were reported for IGF1 and IGF2 in bovine milk (Meyer et al., 2017). Similar to IN, concentrations of IGF1 and IGF2 in Holstein cows and buffalos are higher around parturition and decline at later time-points of lactation (Abd El-Fattah et al., 2012; Meyer et al., 2017). The homology of IGF1 between Dromedary camel and human is 52.5% while the homology of IGF2 is 90.2% (UniProt, 2020). IGF1 and IGF2 are transmitted from the blood serum to the milk and they impact the cell physiology, growth, and metabolism throughout the body. Milk IGF-I can be absorbed intact and affect the liver and other body tissues as suggested by a study in rats (Philipps et al., 2000). The IGF system contributes to intestinal development and metabolism in children as one study have suggested that milk consumption increases serum IGFI levels by 9-20% in 10-12 years old children (Hoeflich & Meyer, 2017). On the other hand, IGF1 is a dominant growth factor with higher mitogenic activity than IN and is known to modulate the cell cycle, upregulate cell proliferation, inhibit apoptosis postnatally (Donovan et al., 1994; Kang et al., 2006; Philipps et al., 2000). The expression of IGF2 is increased in malignant conditions and in the case of metabolic dysfunction including obesity and diabetes

(Meyer et al., 2017). Thus, the significance of camel milk and other milks IGFs in human nutrition requires further studies.

### 4.4.3 Immuno-Modulatory and Protective Proteins (LF, IgG, PGRP-1, LZ, and LPO)

Several milk proteins serve as multifunctional components acting as immune-modulators, antioxidants, anti-inflammatory elements, antimicrobial proteins/peptides, enzymes, *etc*. The concentration range of LF in the camel milk samples analysed here varied 639 – 2095 µg/ml and averaged 1114 $\pm$ 265.1 µg/ml, which is in line with the values of 20 - 2200 µg/ml reported in samples from 180 individual camels (Al-Majali et al., 2007) but very much higher than the values 95 - 220 ug/ml reported by others (Kappeler et al., 1998; Kappeler et al., 1999; Elagamy, 2000; Konuspayeva et al., 2007). The concentration of milk from Dromedary camels with mastitis was 44 - 3100 µg/ml (Al-Majali et al., 2007). In bovine milk, it was reported that LF concentration is 76.7 (Elagamy, 2000) and 140 µg/ml (Kappeler et al., 1999). LF belongs to the transferrin family and is an essential component of non-specific innate immunity of humans and other mammals (Legrand et al., 2008). In an *in-vitro* assay, concentrations of 5 mg/ml of LF caused a 56% decline in the growth of colon cancer cell line (HCT-116) in 48 hours (Habib et al., 2013). The activity of camel LF measured by five different assays showed that LF exerted different antioxidant activity including scavenging of nitric oxide (Habib et al., 2013).

The range of IgG in the milk samples, i.e. 7.3 - 17.9 mg/ml (mean 13.4 $\pm$ 2 mg/ml), is much higher than the values of 2.22 and 0.83 mg/ml, reported by Elagamy (2000) and Konuspayeva et al. (2007). The levels of IgG in bovine milk were reported as 2.05 $\pm$ 0.83 mg/ml (Kociņa et al., 2012), 0.67 mg/ml (Elagamy, 2000), and 0.15 – 0.8 mg/ml (Claeys et al., 2014). In the milk of ruminants, IgG is the dominant immunoglobulin.

Camel IgG consists of three main sub-classes IgG1, IgG2, and IgG3 (Azwai et al., 1996) and has an exceptional immunological system because IgG2, and IgG3 are devoid of light chains and are made of heavy chains with molecular mass of 42 and 45 kDa, respectively (Hamers-Casterman et al., 1993; El-Hatmi et al., 2007). The functional domain (VH) of the heavy chain antibodies was suggested to interfere with numerous biological processes making them good candidates for human therapy (Holt et al., 2003). Camel immunoglobulins are called nano-antibodies because they are significantly smaller than the corresponding human and bovine antibodies (Mullaicharam, 2014). As these milk immunoglobulins are small in size they can cross the intestine and enter the bloodstream (El-Hatmi et al., 2007).

The PGRPs are antibacterial proteins of the innate immune system**.** Pathogens are probably inactivated by binding to peptidoglycan in bacterial cell walls. In this study, the range of PGRP1 in the milk samples was 1.6 - 22.3 ng/ml and the mean was 15.2 $\pm$ 2.8 ng/ml. These values are very much lower than the value of 120 mg/ml for PGRP reported by Kappeler et al. (2004) who also reported that the mean concentration of PGRP in Dromedary camel milk decreased by 19% during lactation and increased by 45% in the incident of severe mastitis. Kappeler et al. (2004) did not name this protein as PGRP1 but the N-terminal sequence of their protein is exactly same as PGRP1 in the Universal Protein database (UniProt). The molecular weight reported by the researchers is 19.1 *versus* 21.3 kDa in UniProt (2020). The isoelectric point reported by Kappeler et al. (2004) is 9.02 compared to 9.1 in UniProt (2020). PGRP1 was isolated in major amounts from milk at the end of the lactation stage that indicates continuous expression of the protein in camel milk during the lactation period (Kappeler et al., 2004; Park et al., 2017).

The range of variation for LZ in the samples was 23.3 - 71.4 µg/ml and the mean was 45.5 ± 10.4, which is much higher than the values reported in previous studies (Table 10). Elagamy et al. (1996) reported a LZ concentration in bovine milk of 7 µg/ml. Barbour et al. (1984) reported that LZ concentrations in camel milk samples (6.48 µg/ml) inhibited the growth of pathogenic bacteria while sample containing 0.626 µg/ml had no inhibitory effect. LZ was suggested to contribute to the antibacterial properties of camel milk including inhibition of milk fermentation bacteria (Attia et al., 2001).

The concentration range of LPO in the milk samples was 7.1 - 15.5 ng/ml and the mean was 10.5 ± 1.6 with no previous data reported in this milk. Reiter (1985) reported LPO concentration of 30 mg/ml in bovine milk. Isobe et al. (2011) found a correlation between LPO activity and the somatic cell count in bovine milk and proposed that LPO can potentially be used as indicator for somatic cell count in milk. Amenu et al. (2017) suggested that activation of the LPO system helps to extend the shelf life of fresh milk up to 6 and 12 hours in cow and camel milk, respectively.

### 4.4.4 The Effect of Lactation Stage (4 - 8 months) on the Concentrations of the Bioactive Proteins in Camel Milk

Since data on the delivery dates of the milked camels was available, the samples analysed were divided into three groups according to the lactation stage, group A (4 - 5 months, 29 samples), group B (6 months, 75 samples), and group C (7 - 8 months, 36 samples) (Figure 19). The farm animals included in this study were reared under intensive management and according to Nagy et al. (2013), the mean length of lactation of Dromedary camels under intensive management is 586 days, equivalent to approximately 20 months. Due to the limited lactation span and the large variability within groups, it was not possible to perform meaningful statistical comparisons. In

literature, LZ concentrations was mentioned to be negatively correlated with lactation stage (up to 210 days) (Barbour et al., 1984), while lactation stage (beginning, middle and late) were found to have no significant effect on the concentration of LF (Al-Majali et al., 2007). A study on the variability in LF and IgG contents in milk from *C. dromedarius, C. bactrianus* and their hybrids under different seasonal and geographic conditions found that these proteins show higher levels in Spring (Konuspayeva et al., 2007). Abou-Soliman & Elmetwaly (2018) reported that the concentration of IN in camel milk was nearly stable between the second week and the fifth month of lactation. In caprine milk, LF concentration varied between 10 and 28 μg/ml until 32 lactation weeks and reached over 100 μg/ml in week 44 (Hiss et al., 2008). LF concentration in bovine milk in the $10^{th}$ month of lactation was 5 times the concentration in the first month (Wielgosz-Groth et al., 2009; Król et al., 2010). For bovine milk a study done on 423 cows from 4 breeds showed that the highest concentrations of LF, LZ, and IgG was in milk from multiparous cows of Jersey and Simental breed at the late stage of lactation (Król et al., 2010). Detailed studies on the effect of animal breed, age, lactation stage, *etc*. on the concentrations of the different proteins in camel milk need to be conducted.

### 4.4.5 The Correlations between Bioactive Proteins in Camel Milk

The correlation between the concentration of the antimicrobial and protective proteins shown in Figure 20 are expected as they might be affected similarly by regulating factors to function in synergism in protecting the host. For example, Kappeler et al. (2004) reported that in cases of mastitis, PGRP was upregulated concurrently with LF suggesting its role in the protection of the udder. Correlations between milk proteins are not widely discussed in literature and deserve elaborative studies.

Nowadays, the recognition of the potential of camel milk and, therefore, the significant roles of bioactive whey proteins lead to increased research on the nutritional significance of camel milk consumption. Determining the concentrations of the bioactive whey proteins in camel milk is important for research concerning the use of this milk in nutrition and therapy. Further studies are required to evaluate how the observed variability is affected by factors such as animal breed, age, nutrition and health, stage of lactation, etc. as well as the significance of these proteins in human nutrition.

# Chapter 5: Summary and Conclusions

The general objective of this thesis was to explore the variability in the proximate composition and protein composition of Dromedary camel milk collected from a large number of individual animals. Determining the protein composition of camel milk and the concentration of the different proteins is valuable for the coagulation process which is vital in processing milk to cheese and fermented products. Only very few research (presented in Table 8 and Table 10) provided information about the concentrations of the proteins in a very limited number of samples. To conduct the study in a large sample size using optimum milk sampling procedures, a collaboration was successfully done with the largest camel milk processing plant in the world (Emirates Industry for Camel milk and products, Dubai, UAE) that has well-established experiences and facilities for animal management and milking.

## 5.1 Summary of Research Findings

Generally, the results obtained showed that there is a wide variation in all the studied parameters: proximate composition, heterogeneous casein fraction, bioactive whey proteins concentrations and relative proportions of caseins. Variations observed in camel milk proximate and protein composition can be attributed to genetic factors (breeds) and non-genetic factors, i.e stage of lactation, age, parity and physiological condition of animal (Khaskheli et al., 2005; Haddadin et al., 2008; Konuspayeva et al, 2009; Alhag & Al Kanhal, 2010; Aljumaah et al, 2012; Nagy et al., 2017, 2019; Ryskaliyeva et al., 2018).

It was interesting to see that the electropherograms of milk collected from individual animals looked different as if carrying a fingerprint for each animal and showing the

actual variability in the protein composition. The caseins concentrations and the relative proportions of the caseins are very critical to the milk coagulation process. The average approximate relative proportion of the caseins (αS1-: αS2-: β-: κ-caseins) in camel milk (26:4:67:3, wt/wt) is very different from that of bovine milk (38:10:36:12, wt/wt) this disparity is likely responsible for a number of peculiarities of camel milk. Camel milk contains a unique mixture of bioactive whey proteins in considerable concentrations. This transforms camel milk to be a candidate with promising functional and health potentials. This also by some means supports the empirical observations on the successful use of camel milk in adjunctive therapy for different diseases.

**5.2 Significance of the Research**

1-Camel milk is a suitable and optimum staple food for people living in semi-arid and arid areas including the U.A.E., researching the protein composition is a prerequisite to promote this staple food and add value it.

2-Information on the concentration of the casein and whey proteins are a prerequisite to understand and resolve the technological challenges of camel milk.

3-Bioactive whey proteins are a suggested source of the medicinal properties of camel milk, data on their concentration is an important input for future research on nutraceuticals and functional foods.

4-Contribution to achieve sustainability goals.

## 5.3 Recommendations for Future Research

During this study a wide variation in the proximate composition was discovered, protein concentrations (caseins and whey) and relative proportions of caseins, a variation not reported before.

The following can be explored in future research:

1. Study the effect of milk protein composition on casein micelle stability and functionality during processing.
2. Study the effect of milk protein composition on the coagulation properties of milk at chymosin and acid induced coagulation.
3. Explore the two unknown casein proteins that appeared in the capillary electrophoresis electropherograms.
4. Study the effect of bioactive proteins on the nutritional and medicinal properties of camel milk.
5. Study the genetic and non-genetic factors that contribute to the variation in protein composition while collecting samples from individual animals.

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## List of Publications

1- Mohamed, H., Nagy, P., Agbaba, J. and Kamal-Eldin, A. (2021). Use of Near and Mid-infra spectroscopy for analysis of protein, fat, lactose and total solids in raw cow and camel milk. *Food Chemistry*. 334, 127436. https://doi.org/10.1016/j.foodchem.2020.127436

2- Mohamed, H., Johansson, M., Lundh, A. and Kamal-Eldin, A. (2020). Short communication: Casein and **α**-lactalbumin content of camel milk (*Camelus dromedarius*) determined by capillary electrophoresis. *Journal of Dairy Science*. 103,11094–11099. https://doi.org/10.3168/jds.2020-19122

# Appendices

## Appendix A: Animals Data (Data of Chapter 3)

- Breed:

| Breed/ecotype | Count of animals |
|---|---|
| A: Emirate and Emirate mix | 26 |
| B: Black camel | 5 |
| C: Cross-Emirati, Black/Cross-Emirati | 15 |
| D: Pakistan | 23 |
| E: Saudi, Sudan | 23 |
| F: Saudi-Sudan mix | 11 |
| Total | 103 |

-Age of animals:

| Age | Count of animals |
|---|---|
| A: 3 to 6 years | 31 |
| B: 7 to 12 years | 41 |
| C: 13 to 18 years | 31 |

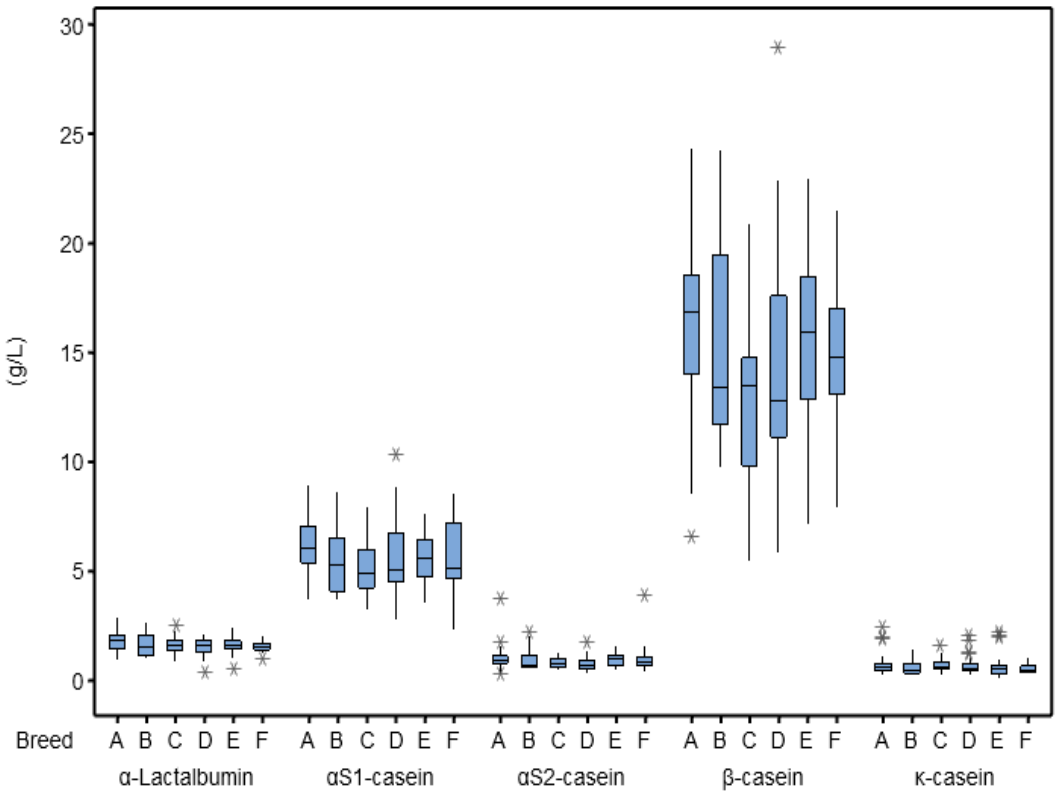
-Parity:

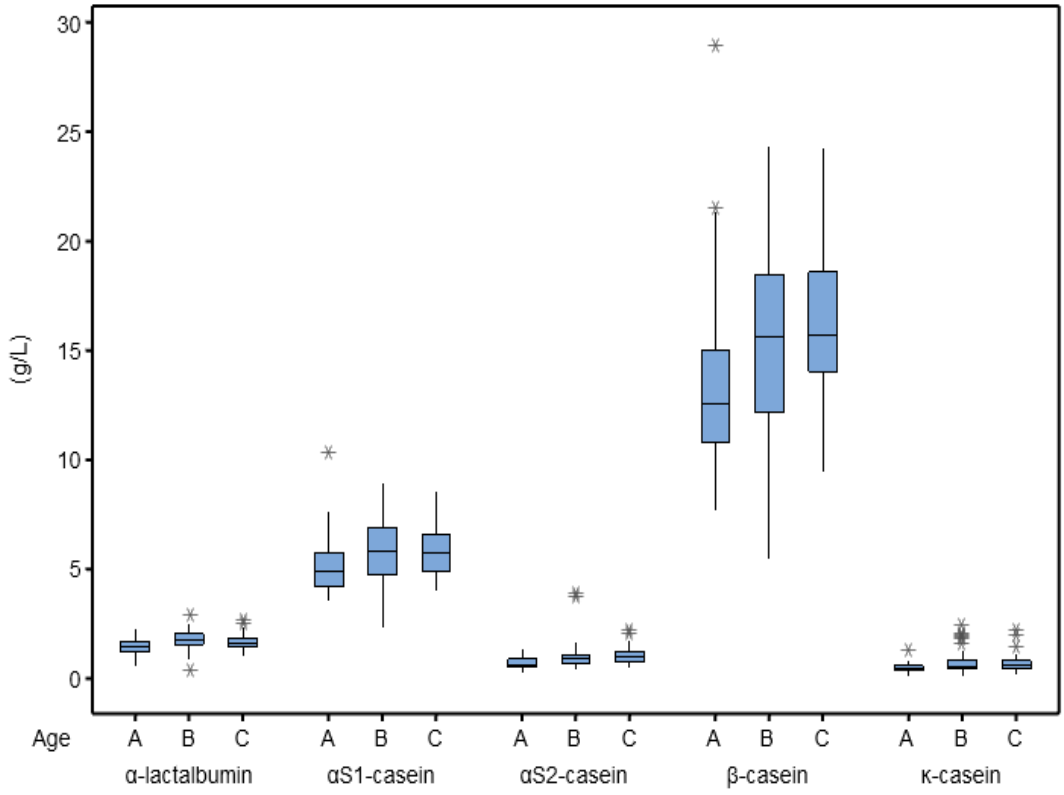
| Parity | Count of animals |
|---|---|
| A: 1st | 34 |
| B: 2nd | 26 |
| C: 3rd | 27 |
| D: 4th | 6 |
| E: 5th | 8 |
| F: 6th | 2 |

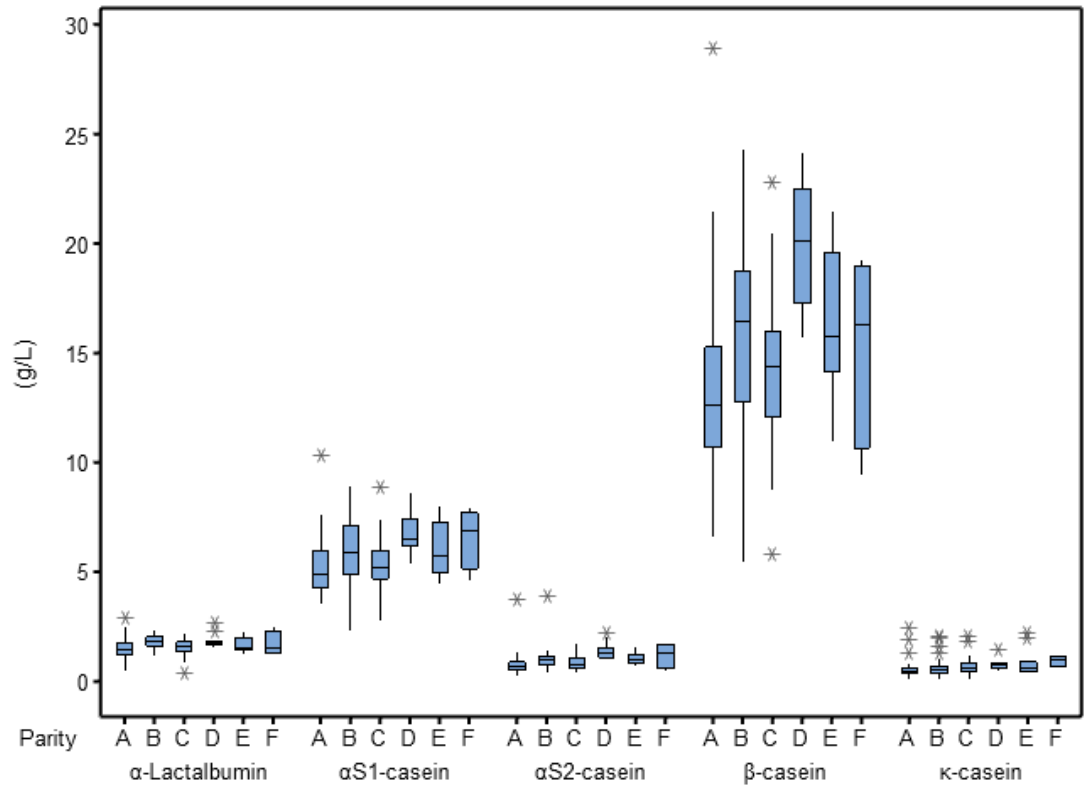
-Lactation stage:

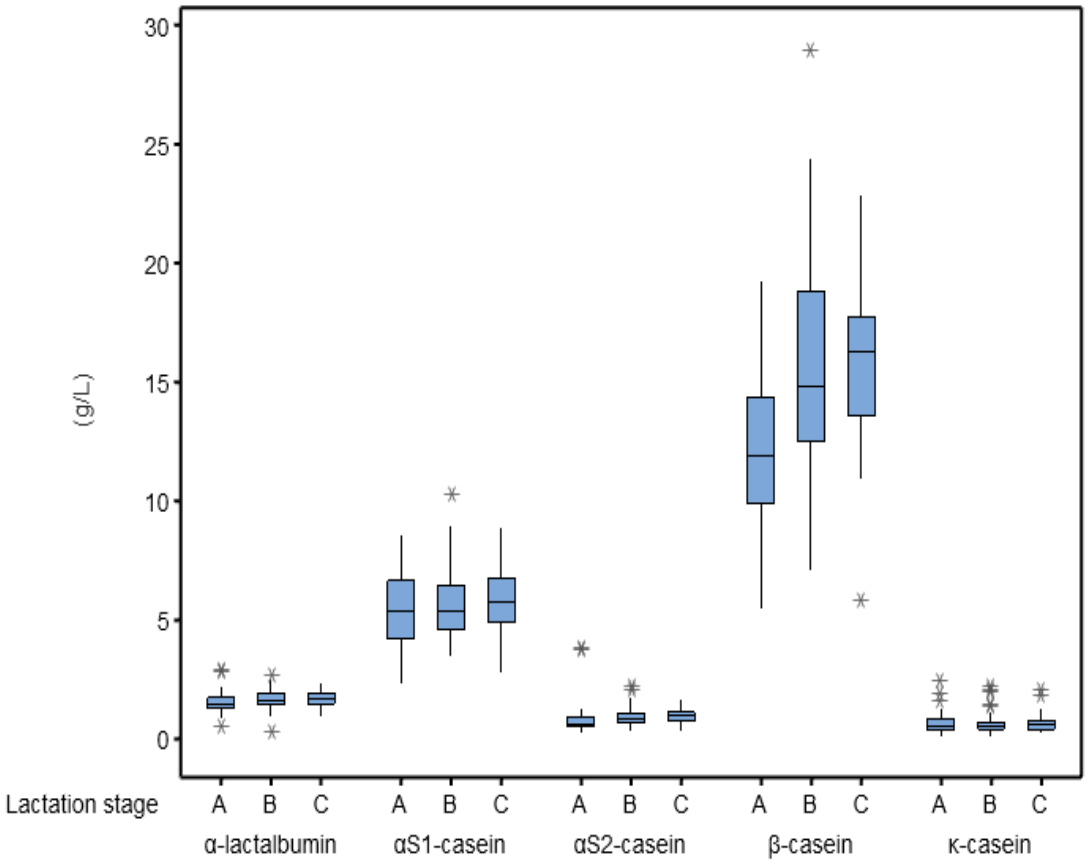
| Lactation stage | Count of animals |
|---|---|
| A: > 6 months | 20 |
| B: 7 to 12 months | 64 |
| C: 13 to 18 months | 19 |

**Appendix B: Protein's concentration by breeds, age, parity, and lactation stage**






- The description of the letters in each graph is shown in Appendix A (page 129).