Characterization of llama (Lama glama) milk proteins

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Abstract

Llamas belong to the Camelidae family along with camels. While camel milk has been broadly characterized, data on llama milk proteins are scarce. Previously released studies were only limited to the analysis of gross composition of milk (i.e. total fat, protein, or lactose content). The objective of this study was thus to investigate the protein composition of llama milk. Data were compared with those from dromedary milk, a closely related species. First, the protein concentration of llama and dromedary milk was determined. Surprisingly, the average value of protein concentration was roughly twice higher in llama milk compared with dromedary milk. Skimmed llama milk proteins were further characterized by a two dimensional separation technique coupling Reverse Phase High Pressure Liquid Chromatography (RP-HPLC) in the first dimension with sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) in the second dimension. Identification of proteins was achieved using peptide mass fingerprinting. This proven methodological approach allowed us to identify the major proteins in llama milk, namely caseins (αs1-, αs2-, β- and κ-caseins), α-lactalbumin, lactoferrin, lactophorin and serum albumin. Significant quantitative and qualitative differences were observed between camel and lama milk samples. Finally, we characterized proteins of the Milk Fat Globule Membrane (MFGM), the membrane surrounding fat in milk, in the llama species. The MFGM protein profile from llama was found to be highly similar to the MFGM protein profile from camel milk. Taken together, these data provide for the first time a thorough description of the milk protein fraction from llama, a new-world camelid.

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Équipe : « Lait, Génome & Santé »
Jouy-en-Josas, France

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Family-*Camelidae*

Order: Artioadactyla/Suborder: Tylopoda
Old World (Camelini) - New World (Lamini)

Genus *Camelus*

Camelus dromedarius

Camelus bactrianus

Camelus ferus

Genus *Lama*

Lama glama

Lama guanicoe

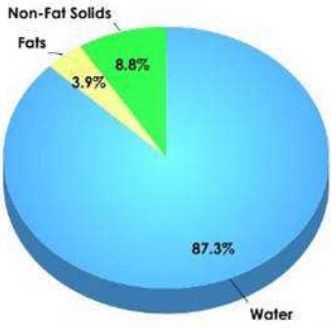
Genus *Vicugna*

Lama pacos

Vicugna vicugna

Llama: domestication for transport and animal products (wool, meat and milk)

Milk: the primary source of nutrition for the offspring

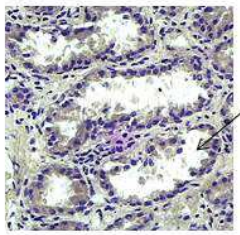


Non fat solids: Proteins, Immune Factors, Oligosaccharides (lactose, etc) Minerals (Calcium, etc) Vitamins (Riboflavin, Vitamin C etc…).

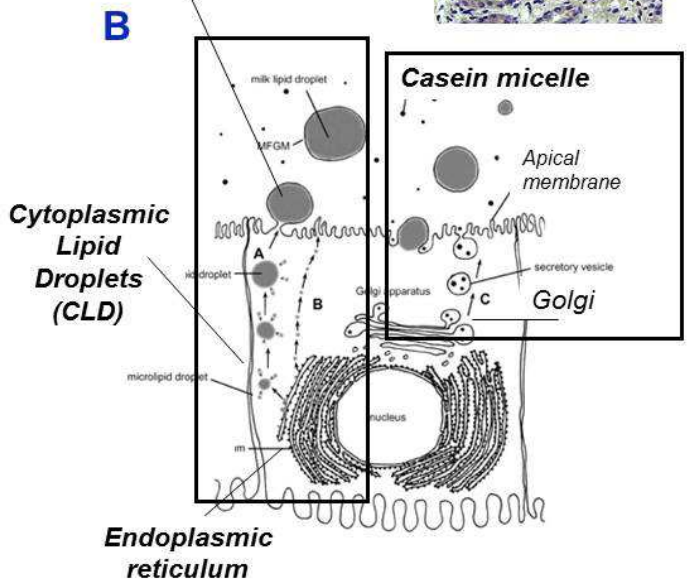
Milk composition fits with nutrition requirements of the young mammals.

- Milk as a source of macro nutrients: sugars (lactose), fat and proteins
- **Llama milk** is higher in protein (4.2%) and sugar (6.5%) and lower in fat (2.7%) than milks of other domestic ruminants

Milk is synthesized by the Mammary Epithelial Cell



Mammary acini



A - Secretion of caseins and whey proteins

A

B- Secretion of the lipid fraction: the milk fat globules

Dromedary and bactrian milks are consumed in dry regions of the world : Food "Fresh, Shubat" and medicinal treatments.

Llama : important for transport.
No llama milk production for human consumption.

Dromedary and Llama: ability to digest the poor quality grasses.

Farming systems for llama limited in remote mountainous in South America (Beneficial for human health in western countries).
The milking of the llama is occasional: Low developed market by comparison with other ruminant milks.

Dromedary milk has been largely characterized with regard to:

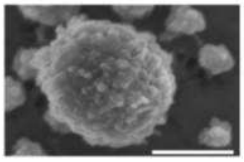
- Casein and whey protein fractions (Kappeler, 1998, Alhaider et al., 2013)
- Milk Fat globule membrane proteins (Saadaoui et al., 2013)

Data on llama milk proteins are scarce

Milk proteins

Colloidal fraction

(Caseins)



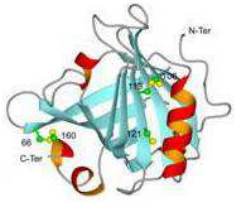
(~80%)

Milk Fat Globules

(~1-2%)

Soluble fraction

(Whey proteins)



(~20%)

INRA SCIENCE & IMPACT

Aim and objectives

Characterization of llama milk proteins:

- Major proteins from skimmed milk by combining different proteomic tools and comparison with dromedary milk proteins.

- Milk fat globule membrane proteins by SDS-PAGE and comparison with our previous published data for dromedary milk (Saadaoui et al., 2013).

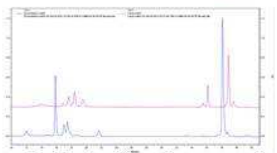
INRA
SCIENCE & IMPACT

Materials & Methods

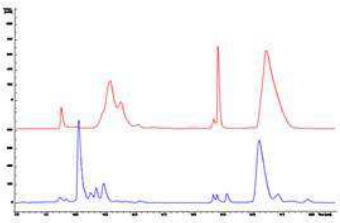
Skimmed milk (casein and whey) ← Milk samples (4 llamas and 10 dromedaries) → Milk fat globule membrane proteins (SDS-PAGE 10%)

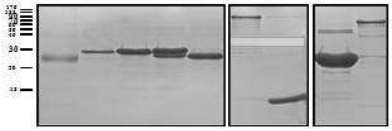
I- Characterization of proteins by Reverse-Phase High Pressure Liquid Chromatography coupled with Sodium Dodecyl Sulphate Polyacrylamide Electrophoresis (RP-HPLC/SDS-PAGE) (Miranda et al., 2004).

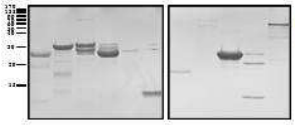
II- Determination of precise molecular masses by Reverse-Phase High Pressure Liquid Chromatography coupled with Electrospray Ionisation/Mass spectrometry (RP-HPLC/ESI-MS)



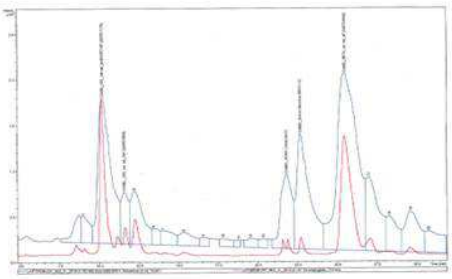
Collected fractions resolved by SDS PAGE



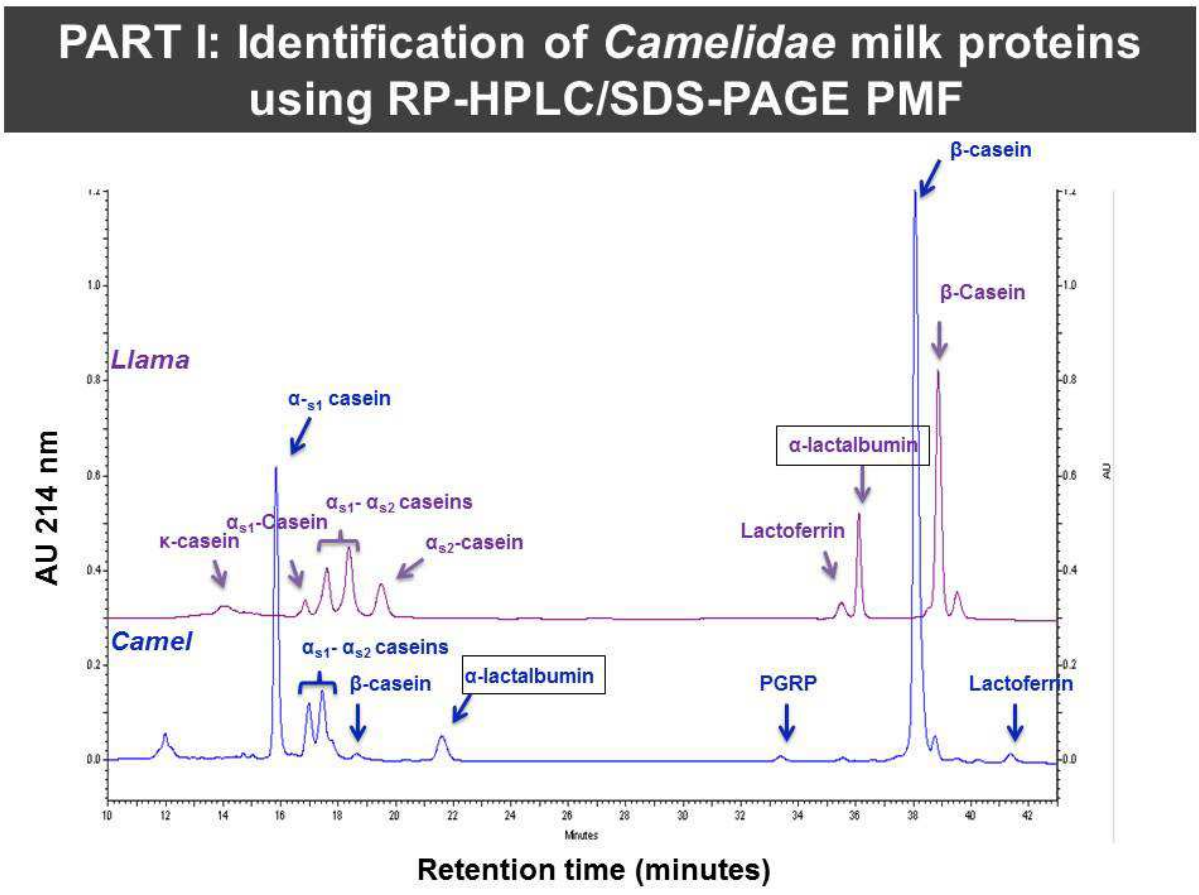


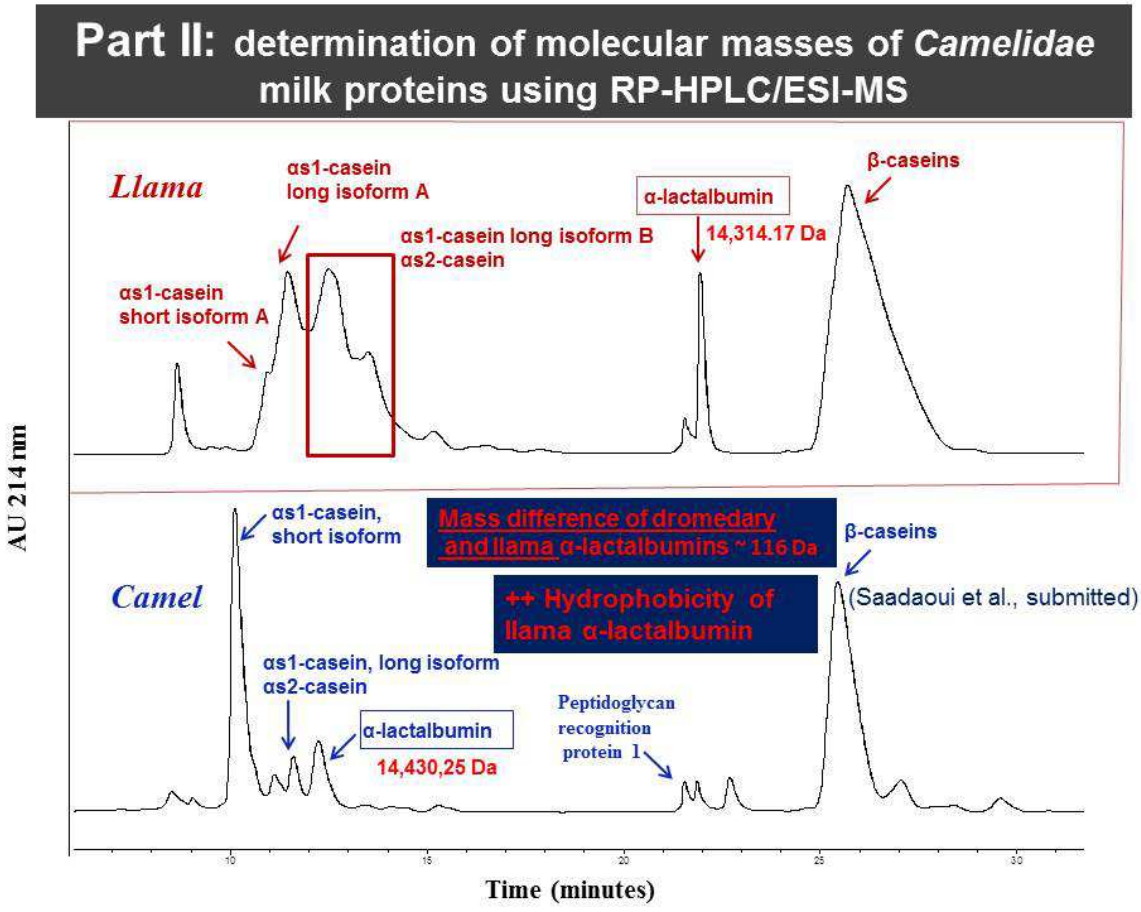


Trypsin digestion of protein bands

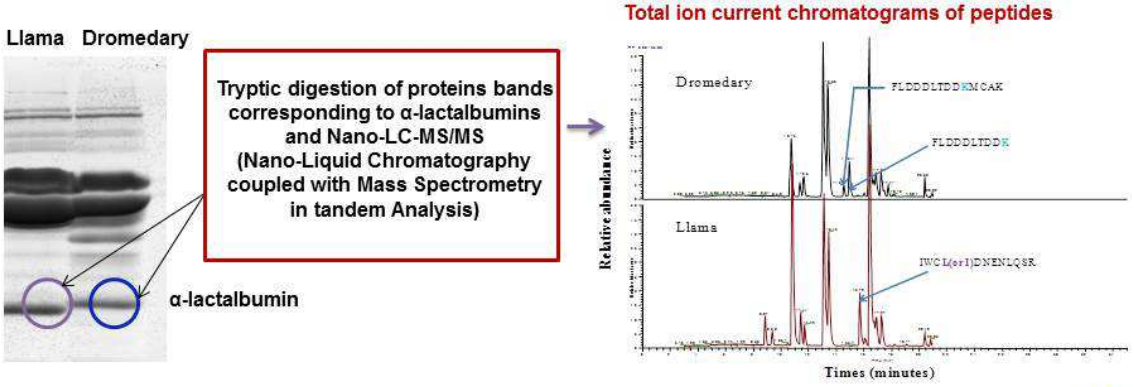


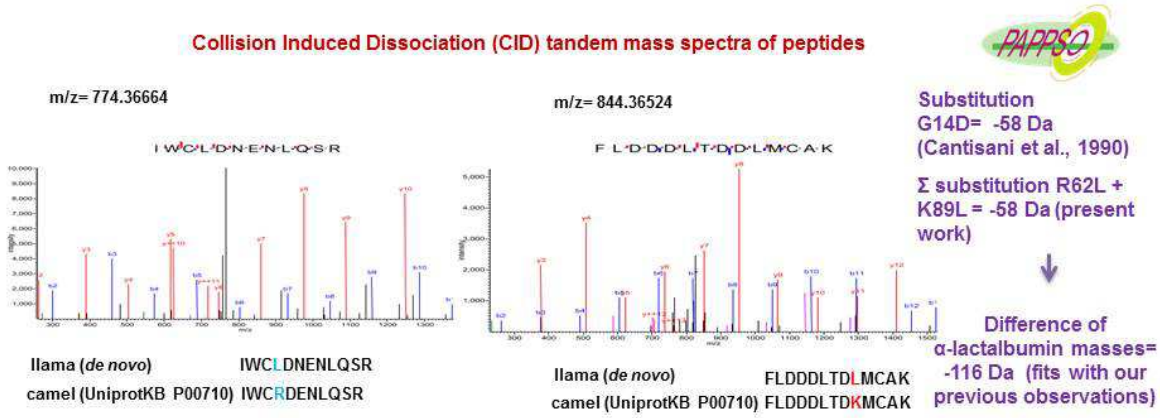
Peptide Mass Fingerprinting (PMF)
(database *Camelus dromedarius*+ Mammalia)


PART I: Identification of Camelidae milk proteins using RP-HPLC/SDS-PAGE PMF
β-casein
β-Casein
Llama
α-s1 casein
α-lactalbumin
αs1- αs2 caseins
αs1-Casein
κ-casein
αs2-casein
Lactoferrin
Camel
αs1- αs2 caseins
β-casein
α-lactalbumin
PGRP
Lactoferrin
AU 214 nm
Minutes
Retention time (minutes)

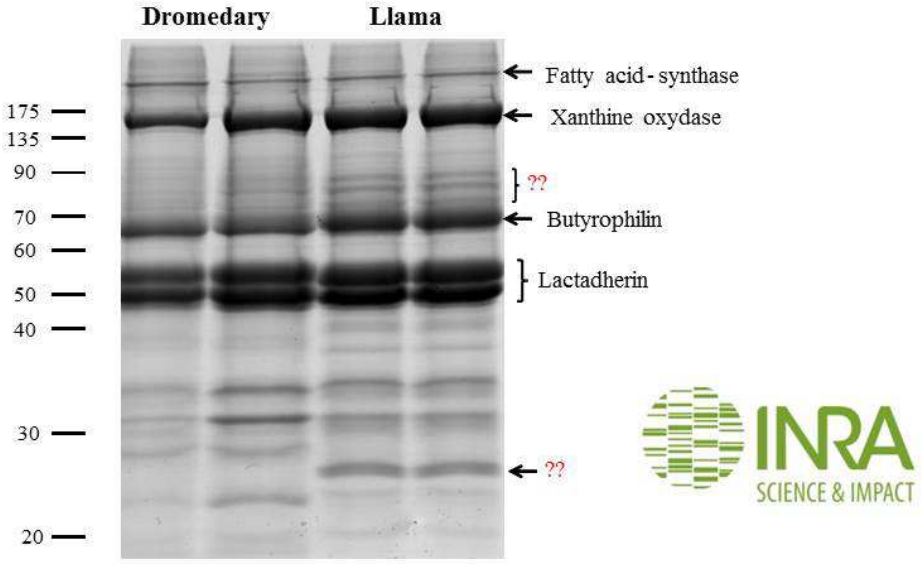

Part II: determination of molecular masses of Camelidae milk proteins using RP-HPLC/ESI-MS
Llama
αs1-casein long isoform A
α-lactalbumin
β-caseins
αs1-casein long isoform B αs2-casein
14,314.17 Da
αs1-casein short isoform A
AU 214 nm
αs1-casein, short isoform
Mass difference of dromedary and llama α-lactalbumins ~ 116 Da
β-caseins
Camel
++ Hydrophobicity of llama α-lactalbumin
(Saadaoui et al., submitted)
αs1-casein, long isoform αs2-casein
α-lactalbumin
14,430,25 Da
Peptidoglycan recognition protein 1
Time (minutes)

Part III: Nano LC-MS/MS and *De novo* sequencing of llama α-lactalbumin





Part VI: *Camelidae* Milk Fat globule membrane protein analysis



10 % SDS-PAGE of MFGM proteins

Conclusions & perspectives

1

Identification of the four caseins and some whey proteins in llama milk

2

Two amino acid substitutions (R62L and K89L) in amino acid sequence of llama α-lactalbumin compared with its dromedary counterpart: higher hydrophobicity of llama α-lactalbumin

3

Highly similar MFGMP profiles between the llama and the camel species.
Some differences which remain to be characterized !

To help the annotation of llama milk proteins-encoding genes

Future directions:

- Using genomic tools to amplify milk proteins-encoding genes in the llama species in order to determine the full-lenth amino acid sequence of llama milk proteins.
- Studying the impact of amino acid substitutions of llama α-lactalbumin with regard to the lactose content in milk.

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Thank You for Your Time and Attention!