Lactoferrin and immunoglobulin content in camel milk from Bactrian, Dromedary and hybrids in kazakhstan

G. Konuspayeva[1,2], G. Loiseau[2], D. Levieux[3], B. Faye[2]

[1]Kazakh State University Al Farabi, 71 av. Al-Farabi, 050013Almaty, Kazakhstan

[2]Centre de Coopération International en Recherche Agronomique pour le Développement, Campus international de Baillarguet TA 30/A, 34398 Montpellier cedex, France

[3]Institut National de la Recherche Agronomique, Qualité des produits animaux -Immunochimie, Theix, 63122 Saint-Genest-Champanelle, France

ABSTRACT

Milk of *Camelidae* is well-known for there health effects on human. This effect is partially attributed to their antibacterial properties. These properties could be linked to some substances, like proteins, lipids and vitamins. Among the milk proteins there are lactoferrin (LF), immunoglobulins (IgG), lactoperoxydase, lyzozyme and some peptides. In the present study, lactoferrin and Immunoglobulins were estimated in camel milk from Kazakhstan where 3 species cohabit: *Camelus bactrianus*, *Camelus dromedarius* and their hybrids. It was estimated the quantity of LF and IgG according 3 variation factors: regions (n=4), seasons (n=4) and species (n=3). LF and IgG concentrations varied between 55 – 659 µg/mL and 241 – 1000 µg/mL respectively. By taking in account all variation factors the variability of the LF and IgG was optimal but it is not possible to identify the rule of region, season or species in this variability.

1. Introduction

The actual camel population in Kazakhstan is around 130 thousands head (Anon., 2006). This country is well known for the cohabitation of the two species of camels (*Camelus bactrianus* and *Camelus dromedarius)* and their hybrids. Within the Kazakh Bactrian breed, three types were described according their geographical distribution: uralobukeevskii, kyzylordinskii and yuzhnokazakhstanskii (Konuspayeva and Faye, 2004).

Consumption of camel milk in Kazakhstan is very popular. Traditionally, peoples give many benefice properties for camel milk and fermented product shubat. They use for TB treatment, in gastro-enteritis, any infectious and also like tonic drunk for old peoples (Sharmanov and Dzhangabylov, 1991). This effect is partially attributed to their antibacterial properties (Konuspayeva et al., 2004). These properties could be linked to some substances, like proteins, lipids and vitamins (Faqrah, 1993). Among the milk proteins lactoferrin (LF), immunoglobulins (IgG), lactoperoxidase, lyzozyme and some peptides are the main incriminated components. However, the quantity of those components and overall their variability in camel milk are not well documented. In the present study, we have estimated lactoferrin and immunoglobulins in camel milk from *Camelus bactrianus, Camelus dromedaries* and there hybrids, in different seasonal and geographical conditions.

2. Materials and methods

2.1. Sampling procedure

In order to get the maximum of variability, the milk was sampled in 4 whole different regions of Kazakhstan in 4 seasons. The milk samples were collected at the milking time in Bactrian, dromedary and hybrid animals. As a whole, 111 samples was used for quantitative and qualitative content of LF and IgG camel milk (Tables 1 and 2). Bactrian camel milk samples were from Kazakh breeds as described above. Milk samples from dromedary camel were from Turkmen Arvana breed. Hybrid samples involved animals from F1 or F2 crossbred. Samples were obtained by manual milking and kept frozen at –20 °C until analysis.

Table 1. Sampling design by region x species x season.

	Almaty				***Atyrau***			
	winter	**spring**	**summer**	**autumn**	**winter**	**spring**	**summer**	**autumn**
Bactrian	2	5	4	3	7	3	8	6
Dromedary	4	9	12	2	2	2	2	1
Hybrid							1	
	Aralsk				**Shymkent**			
	winter	**spring**	**summer**	**autumn**	**winter**	**spring**	**summer**	**autumn**
Bactrian	2	1	1			2	2	1
Dromedary	2	1	1			2	5	5
Hybrid		3	1		1	4	4	

Table 2. Sampling design by Region and Species.

	Bactrian	Dromedary	Hybrid	**Total**
Almaty	14	27		41
Atyrau	24	7	1	32
Aralsk	4	4	4	12
Shymkent	5	12	9	26
Total	47	50	14	**111**

2.2. Reference materials for laboratory analysis

Agar Noble was supplied by Difco (Detroit, MI). Bovine serum albumin, Trichlo-trifluoroethane, Freund's complete and incomplete adjuvants were purchased from Sigma Aldrich (Saint Quentin Fallavier, France).

Individual colostrums samples, using for protein purification, were obtained from lactating camels (*Camelus dromedarius*) at the Experimental Station of the Arid Land Institute of Medenine (Tunisia).

For protein purification, colostrum was firstly defatted by centrifugation at 2500 g for 30 min and diluted 3.5 fold (v/v) with distilled water. Caseins were then precipitated by decreasing the pH to 4.2 with 1 m-HCl. After centrifugation at 20 000 g and 4 °C for 20 min, the supernatant was dialyzed overnight against 0.02 m-Tris-HCl buffer, pH 8.4 and centrifuged at 20 000 g and 4 °C for 30 min.

2.3. Purified proteins

Immunoglobulins G (IgG) were purified as previously described (Levieux et al.,

2005). Briefly, IgG were obtained from camel colostrum by a combination of gel permeation chromatography on Sephadex G200 (Amersham-Biosciences, Orsay, France) and ion exchange chromatography on Q-Sepharose Fast Flow (Amersham).

For the lactoferrin purification, the third peak obtained by gel permeation chromatography of colostral whey on Sephadex G200 equilibrated in 0.02 M-Tris-HCl buffer pH 8.6 was passed through a 5 ml Hitrap-heparin column (Amersham-Biosciences, Orsay, France) and equilibrated in the same buffer. Elution was performed at 2 ml/min over a 0-1 M-NaCl gradient (60 ml) using HPLC equipment (Pump 420, detector 430; Kontron Instrument, St-Quentin-en-Yvelines, France). Lactoferrin eluted as a single peak at 0.3 M-NaCl.

Purity was checked by polyacrylamide gel electrophoresis (12.5 % acrylamide) with or without denaturing agents (SDS and mercapto-ethanol with heating for 5 min in a boiling water bath).

2.4. Polyclonal antibodies

Rabbits were immunized at monthly intervals by multiple intradermal injections of antigen-adjuvant mixture (Vaitukaitis et al, 1971) prepared by emulsifying one volume saline containing 0.5-1 mg purified protein/ml and one volume complete (first injection) or incomplete (booster injections) Freund's adjuvant. Each rabbit received 2 ml of the emulsion. Animals were bled 7 d after each booster injection and the sera were analysed for antibody activity and specificity by immunoelectrophoresis (Scheidegger, 1955) and single radial immunodiffusion assay (SRID) (Mancini et al., 1955). Immunogens used were purified IgG and lactoferrin.

2.5. Immunochemical assay of proteins

Concentrations of IgG and lactoferrin in individual colostrum and milk samples were determined by SRID assay using 1.9-mm-thick agar plates containing 1.2 % agar Noble in 0.005 M-barbital buffer, pH 7.3 and suitable quantities of each specific antiserum. Circular wells (1.5 mm diameter) were punched out in the gel and filled with 3 µl aliquots of the adequately diluted samples or 3 µl of purified proteins of known concentrations as standards. The purified proteins and samples were diluted in the barbital buffer containing 1 % normal rabbit serum and 1 mg sodium azide/ml. Plates were incubated in a moist box at 37 °C for 15–20 h and the diameter of the ring-shaped precipitates was measured using a magnifying video camera system (Levieux, 1991). Standard curves were constructed by plotting the diameter of the precipitating ring *vs* the square root of the protein concentration. With the diffusion time used, a linear regression was always obtained. Samples and standards were plated in duplicate. The CVs of the assays were 3–5 %.

2.6. Statistical analysis

Data were treated with R software. As the main objective was to evaluate the differences according to the variation factors (season, region and breeds), ANOVA was used and the p value below 5% was retained for signification level.

3. Results

The main interesting variation factor being the breeds, the results are presented by focusing the comparison between species: *C.bactrianus*, *C.dromedarius* and hybrids.

Including all the samples, the correlation between LF and IgG content was not significant (r = 0,121).

3.1. Camelus bactrianus

For *C. bactrianus* by region, some tendencies of LF and IgG content were observed (Fig.1). In Aralsk, concentration of LF (247 µg/mL) and IgG (756 µg/mL), is higher than in other regions, but p value is not significant.

Concerning the seasonal variation (Fig.2), it was observed that IgG content decreased in summer compared to spring, at Almaty (534µg/mL *vs* 621µg/mL), at Atyrau (516µg/mL *vs* 827 µg/mL), at Aralsk (386 µg/mL *vs* 947 µg/mL), at Shymkent (443µg/mL *vs* 653µg/mL).. For LF content no systematic changes was observed.

3.2. Camelus dromedarius

C.dromedarius samples had different changes in LF and IgG content than *C.bactrianus* (Fig.1). For dromedary, camel IgG content was under 550 µg/mL in Atyrau, contrary to other regions.

The seasonal variation of IgG was similar to Bactrians (Fig.3). For LF content seasonal changes were not significant. Concentration was between 100 µg/mL and 300 µg/mL values. Only Aralsk region had high level of LF, more than 300 µg/mL.

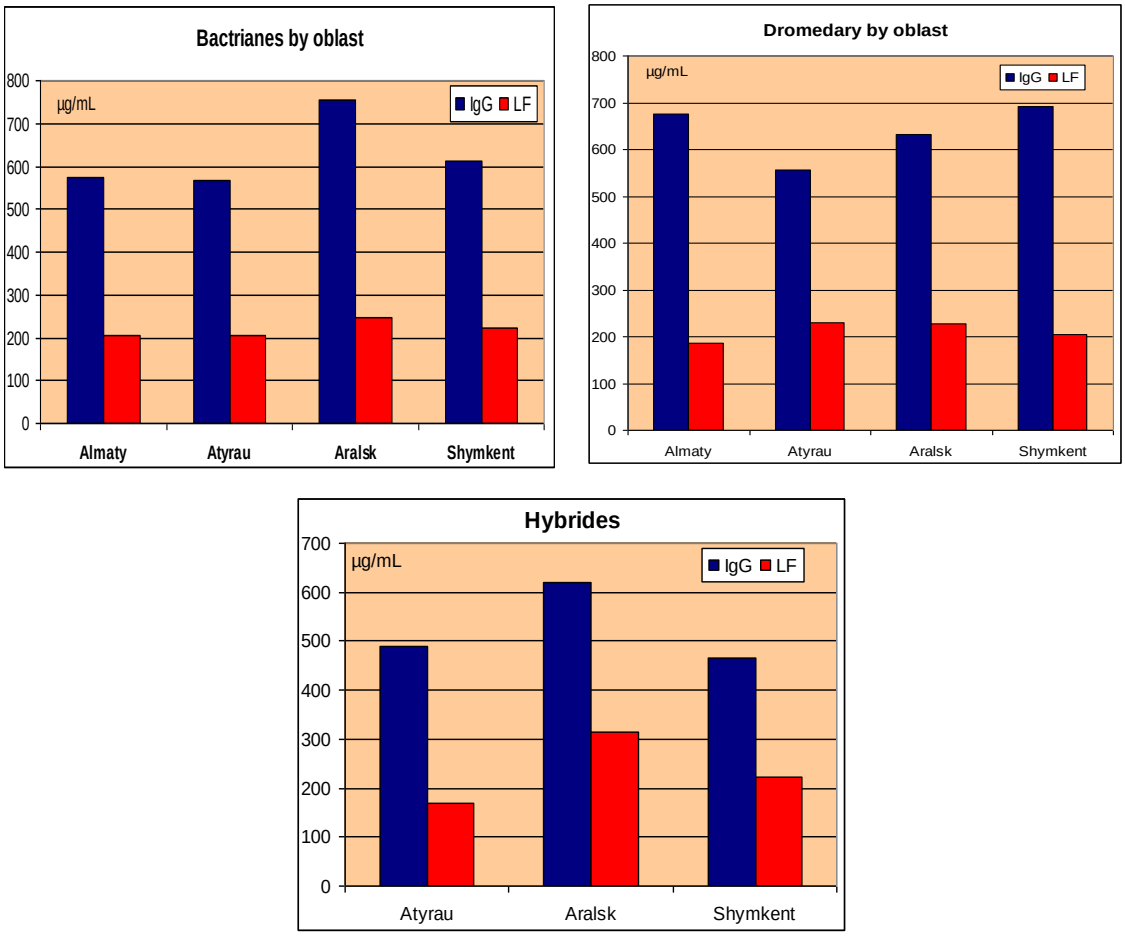

Figure 1. LF and IgG content in *C.bactrianus*, *C.dromedarius* and hybrid milk samples by regions.

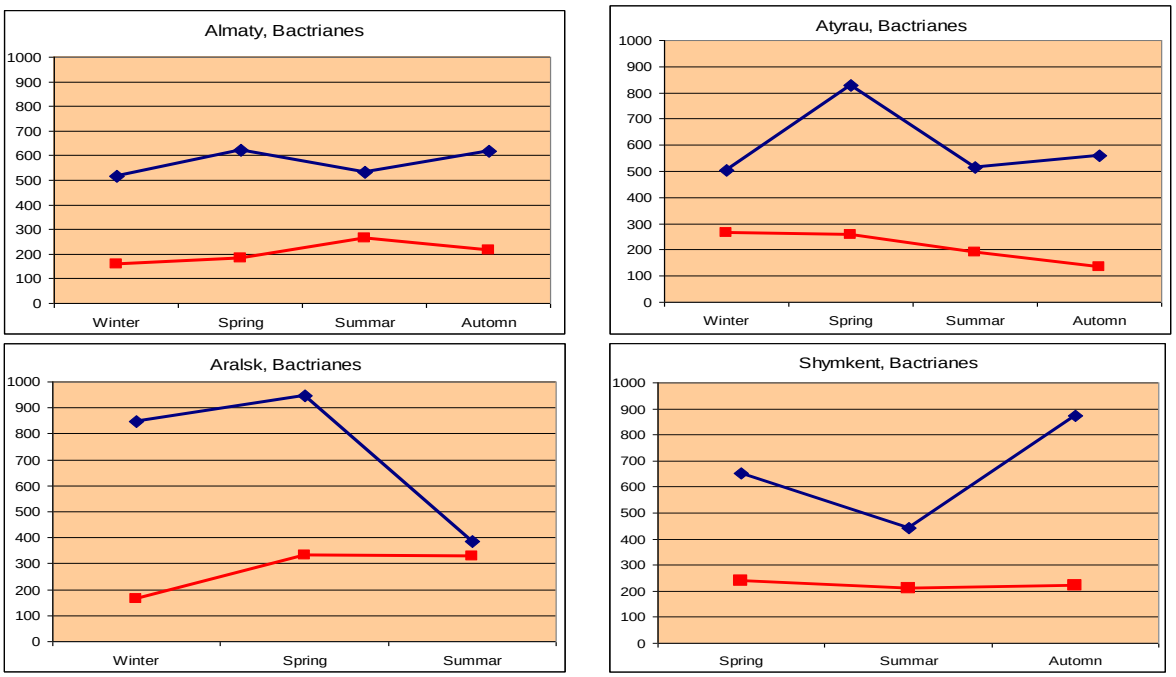

Figure 2. Content of LF and IgG at *C.bactrianus* milk samples by seasons at each region

3.3. Hybrids

In the present study, hybrids camels were not present in Almaty region (Fig.1). Hybrids milk samples from Aralsk region had higher concentration of proteins: LF was around 300 µg/mL, and IgG , 600 µg/mL. There was no significant difference in the other regions for LF content. By seasons it was not easy to observe clear differences (Fig.4), especially because the number of hybrid milk samples from 4 seasons was few.

At Aralsk, level of proteins was higher than in others cases, LF content 300-400 µg/mL and IgG, 600-800 µg/mL.

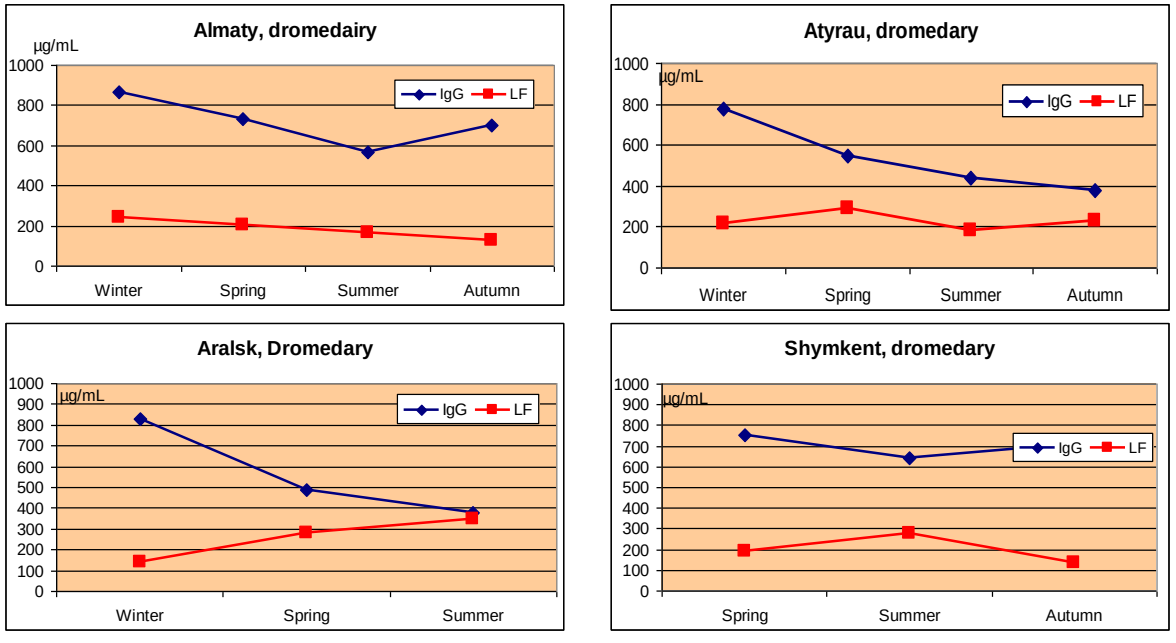

Figure 3. Content of LF and IgG at *C.dromedarius* milk samples by seasons at each region.

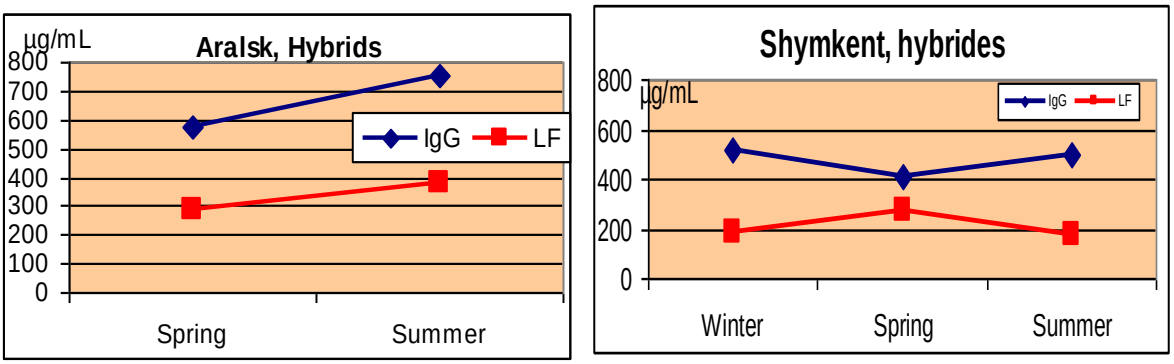

Figure 4. Content of LF and IgG at hybrid milk samples by seasons at each region

3.4. Within region comparison

Comparison of proteins contents between three species within region (Fig.5) showed that level of LF was around 200 µg/mL for all regions and 450-700 µg/mL for IgG. It was rather difficult to find regional effect.

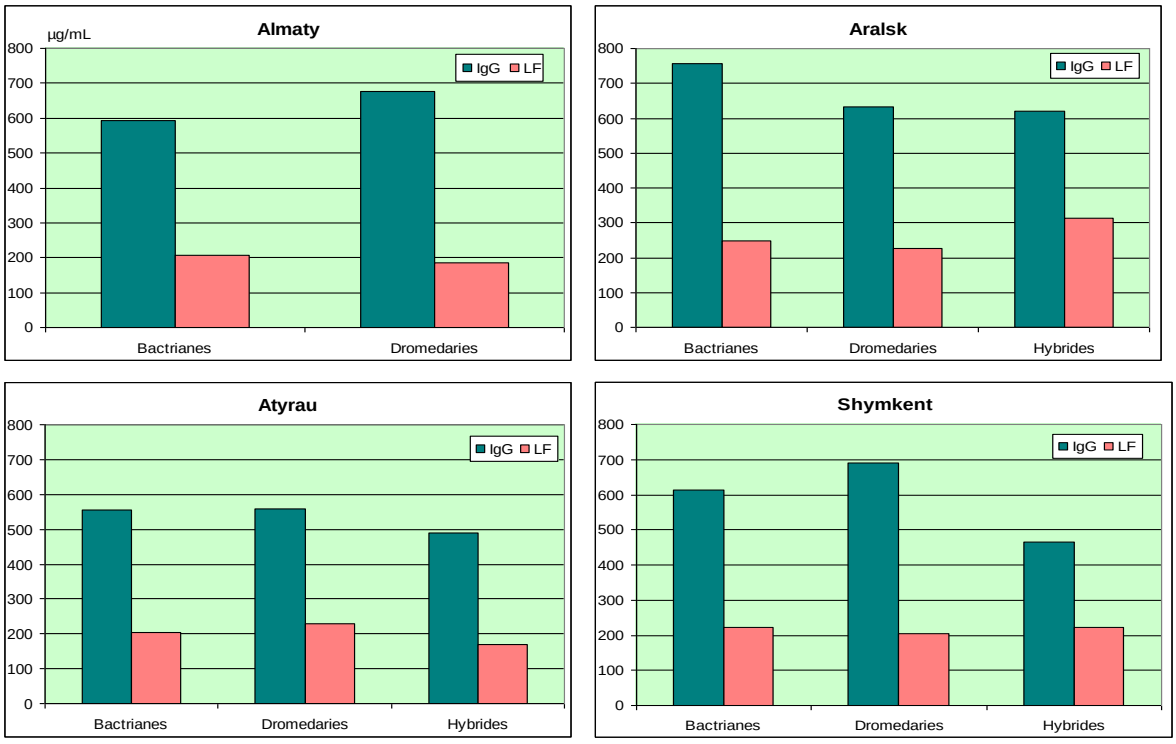

Figure 5. Changes of LF and IgG content of species by region.

3.5. Within season comparison

Comparison of protein levels by season was more significant (Fig.6). LF content was more stable within season between 3 species with a mean of 200 µg/mL. Only in spring, the LF level in hybrids was more than 250µg/mL.

For IgG content at winter time dromedary camel have more proteins than other species. At spring LF and IgG content for Bactrian and dromedary were

similar, IgG and LF for hybrids were higher. For summer and autumn protein levels, the differences were not significant.

Finally in all samples by species LF and IgG content was: 211 µg/mL and 590 µg/mL for Bactrian, 200 µg/mL and 660 µg/mL for dromedary, 244 µg/mL and 511 µg/mL for hybrid.

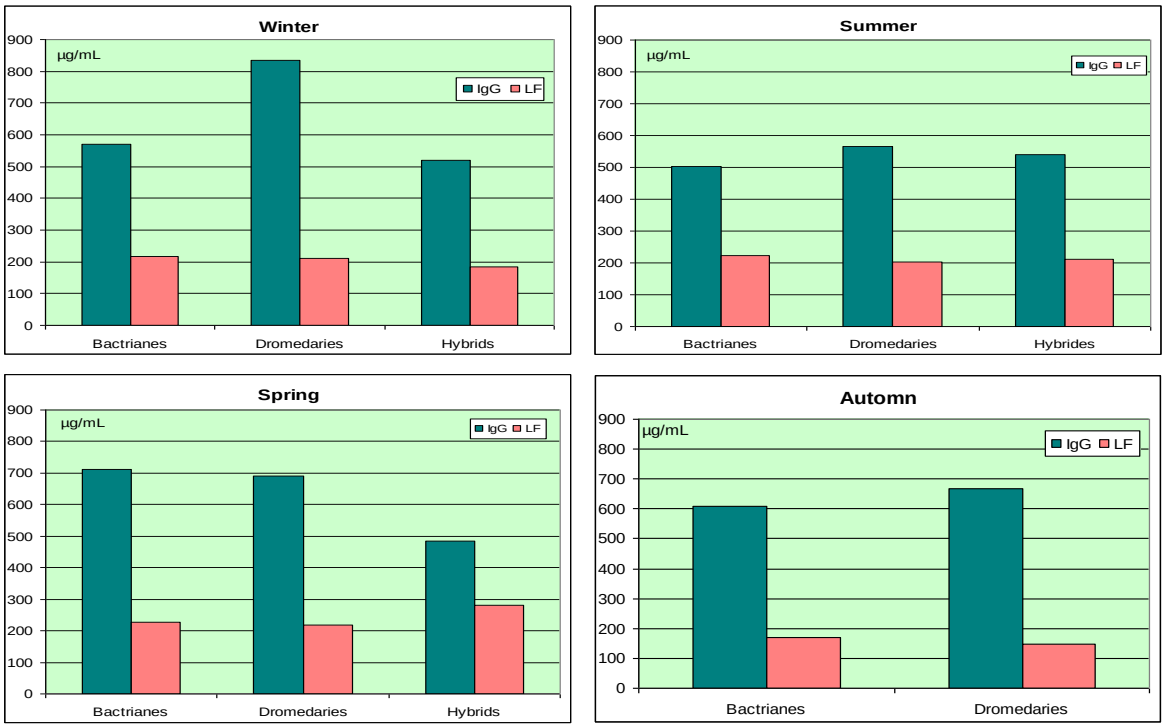

Figure 6. Changes of LF and IgG content of species by seasons.

4. Discussion

Lactoferrin was found in all exocrine liquids (Table 3), but only milk has important level of LF. In literature, there are some data about LF content in milk (Table 4, 5) (Mason and Heremans, 1971; Qian et al., 1995; El-awad et al., 1996). About content of LF in camel milk there are some studies showed different level of this protein (Table 6). However, the authors used different methods for quantitative analyses and different units. So, the comparison is difficult. Some of these results could be debatable. In most of the time the analytical methods were not clearly described

Table 3. Concentration of lactoferrin in different secretion of organisms.

Tears	*0,5 – 1,5 mg/mL*	Nasal excretion	*0,2 mg/mL*
Saliva	*5 – 11 µg/mL*	Urine	*1,5 µg/mL*
Uterine secretions	*0,5 – 1,0 mg/mL*	Amniotic fluid	*2 – 30 µg/mL*
Blood	*0,30 µg/mL*	Neutrophiles	*0,10 µg/10^6 celles*

Table 4. Interval of LF concentration in different animals (in mg per mL) (Qian et al., 1995; El-awad et al., 1996).

	Camel	*Mare*	*Co w*	*Goat*	*Ewe*	*Sow*	*Mice*	*Rabbit*	*Dog*
LF	*2-6*	*0,2-2,0*	*0,02-0,2*	*0,02-0,2*	*0,02-0,2*	*0,2-2,0*	*<0,05*	*<0,05*	*<0,05*

Table 5. Concentrations of total proteins (TP, %) and lactoferrin (LF, mg/mL) in milk and colostrum from different species.

	Camel		***Cow***		***Goat***		***Ewe***		***Buffalo***	
	LF	TP	LF	TP	LF	TP	LF	TP	LF	TP
Colostrum*	5.10	8.26	0.84	12.68	3.09	9.50	1.56	18.89	2.1	14.57
Milk**	2.48	2.79	0.08	4.00	0.17	3.50	0.14	6.26	0.05	4.10

*0–2 day after debut of lactation; **15-30 day after debut of lactation

Table 6. Content of LF in camel milk by literature.

References	***Min value***	***Max value***
*Qian et al. (1995)	2,000	6,000
*El-Gawad et al. (1996)	2,480	
*Elagamy (1998)	0,020	0,080
**Zhang (2005)	2,350	7,280
*Levieux (2006)	0,140	0,420
*Present results	0,055	0,659

However, according the recent results (El-Hatmi, 2006) got with similar method than us, it appeared that LF and IgG concentration in camel milk was slightly higher than in cow's milk.

However, this difference in favour of camel is probably not enough to explain the medical properties attributed to camel milk. Further investigations are necessary to deepen the antibacterial activity of camel lactoferrin and to compare such activity to bovine LF.

5. References

Anonymous. Short Review of Statistic Agency of Republic of Kazakhstan. 2006. Social-economic development of Kazakhstan, Almaty, pp.21-22.

Elagamy E.I., Ruppanner R., Ismail A., Champagne C.P., Assaf R. (1996). Purification and characterization of lactoferrin, lactoperoxydase, lysozyme and immunoglobulins from camel's milk. Int Dairy J., 6, 129-145.

El-Gawad I.A.Abd, El-Sayed E.M., Mahfouz M.B., El-Salam A.M. Abd. (1996). Changes of lactoferrin concentration in colostrum and milk from different species. Egyptian Journal of Dairy Sciences, 24:297-308.

El-Hatmi H., Levieux A., Levieux D. 2006. Camel (*Camelus dromedarius*) immunoglobulin G, α-lactalbumin, serum albumin and lactoferrin in colostrum and milk during the early *post partum* period. Journal of Dairy Research 73, 1-6.

Farah Z. 1993. Composition and characteristics of camel milk. Journal of Dairy Research, 60, pp.603-626.

Konuspayeva G., Faye B. 2004. A better knowledge of milk quality parameters: A preliminary step for improving the camel milk market opportunity in a transition economy – The case of Kazakhstan. Proc. of Int. Conference "Saving the Camel and Peoples' Livelihoods". Sadri, Rajasthan, India 23-25 November, 2004, p.28-36.

Konuspayeva G., Loiseau G., Faye B. 2004. La plus-value « santé » du lait de chamelle cru et

fermenté : l'expérience du Kazakhstan. Proc. 11 Rencontre autour des Recherches sur les Ruminants, INRA, Paris 8-9 décembre, 2004.

Levieux D. 1991 Dosage des IgG du lait de vache par immunodiffusion radiale semi-automatisée, pour la détection du colostrum, des laits de mammites ou de fin de gestation. I. Mise au point du dosage. Lait 71 327-338

Levieux D, Levieux A, El-Hatmi H & Rigaudière JP 2005 Immunochemical quantification of heat denaturation of camel (*Camelus dromedarius*) whey proteins. Journal of Dairy Research, 72, pp.1-9

Mancini G, Carbonara AO & Heremans JF 1965 Immunochemical quantification of antigens by single radial immunodiffusion. Immunochemistry 2 235-254

Mason P.L., Heremans J.F. (1971). Lactoferrin in milk from different species. Comp. Biochem. Physiol., , 39, 119 – 129.

Qian Z.-Y., Jollès P., Migliore-Samour D., Fiat A.-M. (1995). Isolation and characterization of sheep lactoferrin, an inhibitor of platelet aggregation and comparison with human lactoferrin. Biochimica et Biophysica Acta. 1243 25-32.

Sharmanov T. Sh., Dzhangabylov A.K. 1991. In « Лечебные свойства кумыса и шубата. » Алматы : Гылым, 173 p.

Scheidegger JJ 1955. Une microméthode de l'immuno-électrophorèse. International Archives of Allergy and Applied Immunology 7 103-110

Vaitukaitis J, Robbins JB, Nieschlag E & Ross GT 1971 A method for producing specific antisera with small doses of immunogen. Journal of Clinical Endocrinology 33 988-991

Zhang H., Yao J., Zhao D., Liu H., Li J. And Guo M. (2005) Changes in Chemical Composition of Alxa Bactrian Camel Milk During Lactation. Journal of Dairy Science, 88, No 10, pp.3402-3410.