Therapeutic potential of whey proteins of camel colostrums

HALIMA EL-HATMI[1*], JEAN-MICHEL GIRARDET[2], JEAN-LUC GAILLARD[2], TOUHAMI KHORCHANI[1] AND HAMADI ATTIA[3]

[1] Institut des Régions Arides, Laboratoire d'Elevage et de Faune Sauvage, 4119 Medenine, TUNISIE.
[2] Laboratoire des BioSciences de l'Aliment, U.S.C. INRA 885, Université Henri Poincaré – Nancy 1, B.P. 239, 54506 Vandœuvre-lès-Nancy CEDEX, FRANCE.[3] Ecole Nationale d'Ingénieurs de Sfax, Unité d'Analyses Alimentaires, B.P. W, 3038 Sfax, TUNISIE.

ABSTRACT

Camel's colostrum whey and milk whey proteins were studied from parturition to the tenth day (192 h) of lactation with emphasis on protective proteins. After discarding insoluble materials by acidification at pH 4.6, the major whey proteins of colostrum (from parturition to 96 h) and milk (from 96 to 192 h) were characterized by cation-exchange fast protein liquid chromatography and polyacrylamide gel electrophoresis. They corresponded to immunoglobulins G (IgG), serum albumin, α-lactalbumin, lactoferrin, proteose peptone component-3 (PP3), and camel whey basic protein (CWBP). The two latter were detected in the colostrum only from 48 h and increased in milk to reach 4.9 and 3.1 g/l of whey at 192 h, respectively. The lactoferrin concentration increased from 24 to 48 h (2.3 g/l at 48 h), and then decreased to 0.7 g/l at 120 h. α-Lactalbumin displayed a mean concentration of 2.2 g/l from 1 h after parturition to 192 h. The serum albumin concentration decreased from 20.8 to 12.5 g/l during the first day and was stabilised to an average value of 10.8 g/l (for the four last days). The main proteins in colostrum were IgG1 and enzyme inhibitory antibodies IgG2+IgG3. Their respective concentrations were 43.4 and 58.4 g/l at 1 h after parturition. At 48 h, the total IgG concentration fell sharply and reached 7.9 g/l in milk at 192 h, whereas that of PP3 increased with a degree of magnitude of two. PP3 might complete the antibacterial function of IgG when milk replaces colostrum, as far as PP3 might display immune functions. Camel's colostrum was revealed to be a potential source of inhibitory antibodies promised to fine prospect in human therapy.

Keywords Camel, colostrum, antibacterial protein, IgG_1, IgG_2, IgG_3

RÉSUMÉ

Potentiel thérapeutique des protéines solubles du lait de chamelle.
L'identification et la quantification des protéines solubles antibactériennes du colostrum et du lait de chamelle ont été réalisées par FPLC et électrophorèse en SDS-PAGE couplée à la densitométrie. Elles ont été identifiées aux immunoglobulines (IgG_1, IgG_2, IgG_3), à la lactoferrine, au sérum albumine, au *Protéose Peptone 3* (PP_3), à l'α-lactalbumine, et au CWBP (*Camel Whey Basic Protéin*). La concentration en lactoferrine est de 2,3 g/l au terme du deuxième jour de lactation. L'α-lactalbumine est présente dans le colostrum et le lait à une concentration moyenne de 2,2 g/l entre la première et la 192ème heure après mise-bas. Les protéines majeures du colostrum sont l'IgG_1 et les immunoglobulines inhibitrices d'enzymes (IgG_2, IgG_3). Leurs concentrations à 1 h *post-partum* sont respectivement de 43,4 et 58,4 g/l. Le PP_3 jouerait un rôle dans la fonction immunitaire. Le colostrum camelin serait une source potentielle d'immunoglobulines inhibitrices applicables en fine thérapie humaine grâce notamment à ses deux spécificités : faible masse moléculaire (IgG_1-H55, IgG_2-H45, IgG_3-H-42) et absence de chaînes légères. Les protéines majeures du colostrum sont l'IgG_1 et les immunoglobulines inhibitrices d'enzymes (IgG_2 + IgG_3). Cependant, la concentration en lactophorine (PP_3) croit en passant du simple au double lorsque celle des IgGs chute, le PP_3 semble jouer le rôle de la fonction antibactérienne des IgGs quand le lait remplace le colostrum. Le PP_3 pourrait contribuer à la fonction immunitaire.

Key words: chamelle, colostrum, protéines antibacteriennes, IgG_1, IgG_2, IgG_3

INTRODUCTION

The colostrum contains mainly immunoglobulins G (IgG), which are needed to provide the new-born with immunity. Camel's milk contains all essential nutrients as cow's milk; however, milk is not only a source of nutrients for the neonate, as it also contains molecules with specific biological activities.

* Correspondance: **HALIMA EL-HATMI,** Institut des Régions Arides, Laboratoire d'Elevage et de Faune Sauvage, 4119 Medenine, TUNISIE.

It is assumed that the immunological challenges, which face these two ruminating species in their respective natural environment, have led to adaptation in the milk composition (Kappeler *et al.*, 2003).

The protein composition of camel's and cow's milks differs in some fundamental aspects. For example, β-lactoglobulin and lysozyme C, which are important proteins of bovine milk, are not found in camel's milk; at the opposite, the whey acidic protein (Beg *et al.*, 1986) or the camel whey basic protein (CWBP; Ochirkhuyag *et al.*, 1998) are not detected in bovine milk. The different distribution of these factors in cow's and camel's milks probably results of the harsh conditions in the natural habitat of the camels (Kappeler *et al.*, 2003). Changes in protein composition from camel's colostrum to milk have been the subject of only few studies (Abd El-Gawad *et al.*, 1996; Merin *et al.*, 2001).

In the present work, colostrum of camels was analysed from 1 h *post partum* up to 192 h of lactation, for concentration of total proteins, IgG1, IgG2 + IgG3, lactoferrine (Lf), camel serum albumin (CSA), α-lactalbumin (α-LA), CWBP, and proteose peptone component-3 (PP3) with emphasis on protective proteins.

MATERIALS AND METHODS

Colostrum and milk samples

Camel's colostrum or milk were collected from a multiparous animal (8 years aged) of a local herd of camels (*Camelus dromedarius*) bred in the experimental farm at the Arid Land Institute, Livestock Widelife Laboratory, in Tunisia. The female was milked manually at the same time in the morning every day from 1 h *post-partum*, after that the new-born started to suckle, and the camel was milked several times between 4 and 24 h, and every day from 48 h to 192 h *post-partum*.

Preparation of whey proteins

After removal of fat by centrifugation at 5000 *g* at 10°C for 30 min, whey was obtained by acid precipitation of caseins at pH 4.6 by adding 1 ml of 10% (v/v) acetic acid and 1 ml of 1 M sodium acetate to 10 ml of colostrum or milk. After centrifugation at 5000 *g* at 10°C for 30 min, the supernatant was dialyzed for 48 h and freeze-dried.

Fast protein liquid chromatography

Fractionation of the whey proteins was performed by cation-exchange fast protein liquid chromatography (FPLC; Amersham Pharmacia, Uppsala, Sweden) on a Protein Pak SP 5PW column (7.5 x 75 mm^2; Waters, Milford, MA, USA) according to the method of Ekstrand and Björck (1986). The buffer used for equilibration was 0.01 M imidazole-hydrochloric acid, pH 7.0. Volumes of 500 µl of proteins (5 mg/ml) were loaded onto the column. Proteins were eluted with a 0–1 M NaCl gradient from 5 to 30 min and detection was recorded at 280 nm. The flow rate was 1 ml/min.

Electrophoresis

Camel's whey samples and the FPLC fractions (F1 to F4) were characterized by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli and Favre (1973). In the presence of 1.1% (w/v) SDS and 5% (v/v) 2-mercaptoethanol, SDS-PAGE was performed with a 4.9% polyacrylamide staking gel in 0.125 M Tris-HCl buffer, pH 6.8, and with a 15.4% polyacrylamide resolving gel in 0.38 M Tris-HCl buffer, pH 8.8. Volumes of 20 µL of whey samples or FPLC fractions (at 2 mg/ml) or of pure proteins (at 1 mg/ml) were loaded in the gel. Proteins were stained by 0.1% (w/v) Coomassie blue R250 in a mixture of 50% (v/v) ethanol and 10% (v/v) acetic acid for 30 min. The molecular mass standards (Sigma) were myosin (200.0 kDa), β-galactosidase (116.2 kDa), phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (31.0 kDa), trypsin inhibitor (21.5 kDa), lysozyme (14.4 kDa), and aprotinin (6.5 kDa). Bovine lactoperoxydase and bovine IgG (Sigma Chemical Co., St. Louis, MO, USA), bovine lactoferrin and egg's lysozyme (Serva, Heidelberg, Germany) were loaded in the gel at 1 mg/ml. Individual protein concentrations were determined by densitometry at 633 nm after Coomassie blue staining (Ultroscan XL densitometer; Amersham Pharmacia) and total protein concentration was determined by the method of Lowry *et al.* (1951) with bovine serum albumin used as the standard.

RESULTS

The whey protein concentration was largely higher in colostrum at 1 h *post-partum* than in milk at 192 h of lactation; a degree of magnitude of 3.5 was observed (Table 1).

The distinction between colostrum and milk was difficult to determine. However, by taken the protein variation into account, it might be located close to 96 h *post-partum*, at the time for which the protein concentration became almost constant. The whey protein concentration strongly decreased during the first day *post-partum* (from 198 to 118 g/l in 24 h) to reach a mean value of approximately 61 g/l between 96 and 192 h of lactation.

Table 1 Evaluation of total whey protein concentration in camel's colostrum and milk from 1 h *post-partum* to 192 h of lactation (determined by the method of Lowry *et al.*, 1951).

Time of milking *post-partum* (h)	Total protein (g/l)
1	198
2	186
4	182
6	174
12	142
24	118
48	65
72	82
96	59
120	67
144	58
168	63
192	57

Lactoferrin (identified in gel according to Abd El-Gawad *et al.*, 1996), CSA, the heavy (H55) and low chains (L30) of IgG1, the heavy chains (H45) of IgG2, the heavy chains (H42) of IgG3 (Lauwereys *et al.*, 1998), CWBP (Ochirkhuyag *et al.*, 1998), PP3 (Girardet *et al.*, 2000), and α-LA have been located on SDS-PAGE profile of camel's whey (Fig. 1).

IgG were the electrophoretic bands having the highest intensity for the first 48 h. α-Lactoglobulin was not expressed in camel's colostrum or milk. Significant amount of caseins, which did not precipitate at pH 4.6, had also been found. Figure 2 shows the FPLC profiles of whey proteins fractionated onto a cation-exchange column. Four FPLC fractions (noted F1 to F4; Fig. 2) of the camel's colostrum whey at 48 h *post-partum* were collected and characterized by SDS-PAGE (Fig. 3). In the absence of dissociating agent, caseins did not appear on the FPLC profiles, as they were discarded by filtration at 0.45 µm prior to chromatographic fractionation; thus, no casein band has been observed on the gel profile of the four FPLC fractions (Fig. 3).

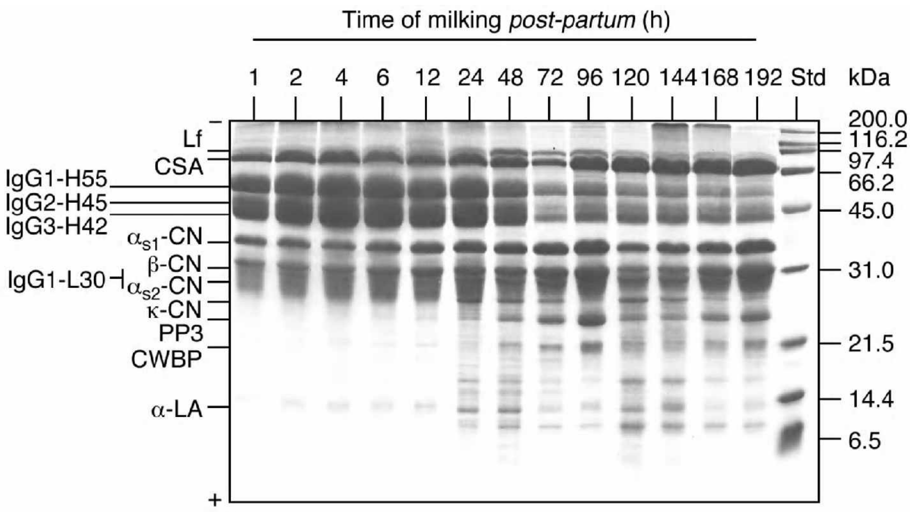

Figure 1 Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis of proteins from camel's colostrum whey and camel's milk whey. Volumes of 20 µL of each sample (at 2 mg/ml) were loaded in the gel. Revelation of proteins was performed with Coomassie blue R250. Legend: Lf, lactoferrin; CSA, camel serum albumin; IgG: immunoglobulins G; H, heavy chain; L, low chain; CN: casein; PP3, component-3 of proteose peptone; CWBP, camel whey basic protein; α-LA: α-lactalbumin; Std, molecular mass standard proteins.

The fraction F1, corresponding to the void volume of the column, contained only small amount of protein material; it was mainly constituted of CSA and α-LA. PP3 was not recovered by FPLC; it might interact with caseins that were retained by filtration prior to injection onto the chromatographic column. CSA, α-LA, and the high chains H45 of IgG2 were weakly adsorbed onto the column and were mainly recovered in F2, whereas IgG1 (composed of heavy chains H55 and light chains L30) and IgG3 (only containing heavy chains H42) were present in F3. The fraction F4 would contain lactoferrin rather than lactoperoxydase, as the retention time of F4 determined by FPLC (23.6 min; Fig. 1) corresponds to materials highly retained onto the cation-exchange column. Lactoperoxydase weakly interacts with the chromatographic support used on the contrary of lactoferrin (Ekstrand & Björck, 1986). The SDS-PAGE analysis of the whey proteins (Fig. 1) allowed one to determine the different concentrations of each main protein present in the camel's whey (the content of individual caseins soluble at pH 4.6 was not taken into account).

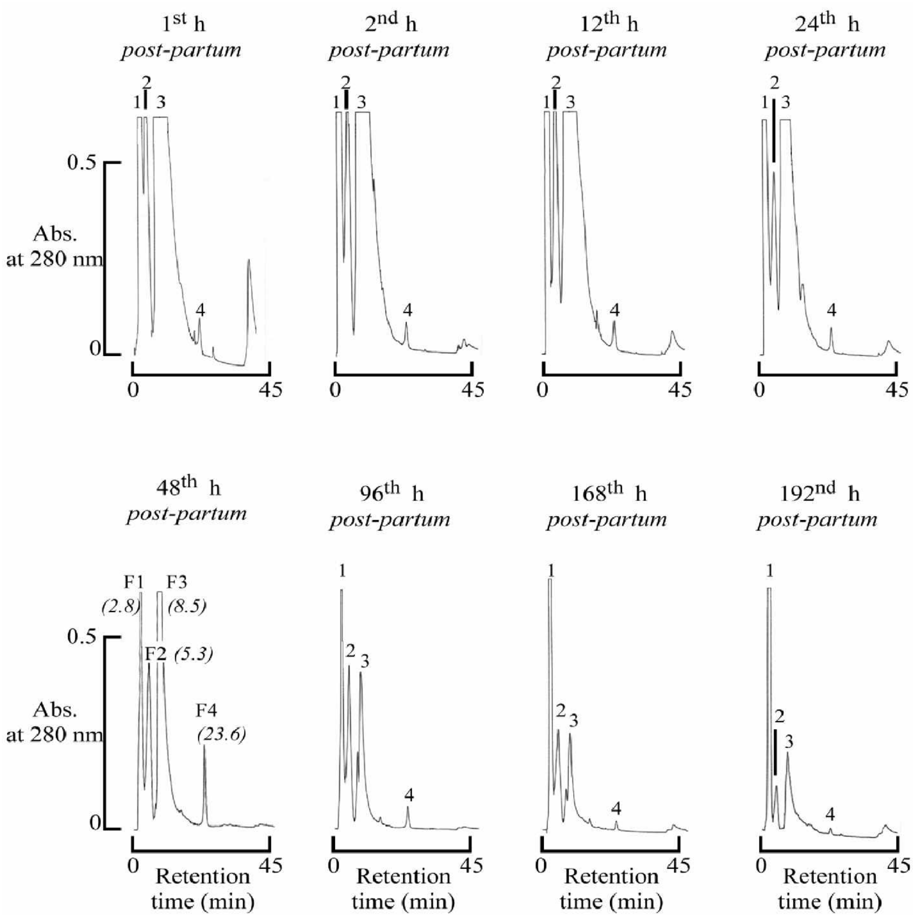

Figure 2 Cation-exchange Fast Protein Liquid Chromatography of proteins from camel's colostrum whey and camel's milk whey. Volumes of 500 µL of proteins (5 mg/ml) were loaded on the Protein Pak SP-5 PW column. A linear gradient of 0 to 1 M NaCl was performed from 5 to 30 min in 20 mM imidazole-HCl buffer, pH 7.0. Peaks of interest are numbered from 1 to 4; F1 to F4 refer to protein fractions of colostruobtained at 48 h *post-partum* and the corresponding retention times are indicated on the profile in italic.

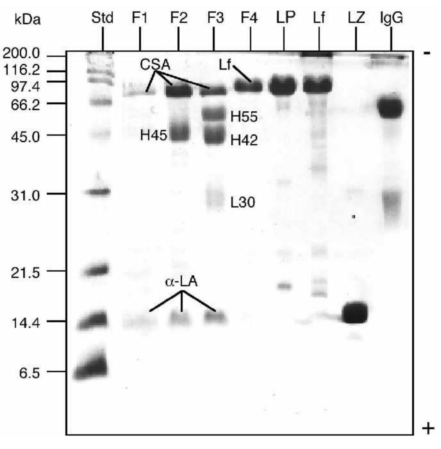

Figure 3 Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis of fractions (F1 to F4) prepared by cation-exchange Fast Protein Liquid Chromatography of proteins from camel's colostrum whey obtained at 48 h *post-partum*. Reference proteins are lactoperoxydase (LP), lactoferrin (Lf), and immmunoglobulins G (IgG) from cow, and lysozyme (LZ) from egg. Volumes of 20 µl of each sample (at 1 mg/ml) were loaded in the gel. Revelation of proteins was performed with Coomassie blue R250. Legend: Std, molecular mass standard proteins; CSA, camel serum albumin; α-LA, α-lactalbumin; H, heavy chain; L, low chain.

Only protein concentrations determined by densitometry are presented on Table 2. The lactoferrin concentration increased from 1.5 to 2.3 g/l (from 24 to 48 h) and then, dropped to 0.7 g/l (at 120 and 144 h). This result was in accordance with the evolution of the peak area of F4 which was maximal for colostrum obtained at 48 h *post-partum* (Fig. 2). For the colostrum samples collected during the first day *post-partum*, lactoferrin was present in an electrophoretically detectable amount but its concentration was difficult to determine, as the band of lactoferrin was very close to that of CSA (Fig. 1).

It was also the same for milks at 168 and 192 h. The area of the peak of F2 decreased in time (Fig. 2); then, IgG2 probably decreased in a same way. In a similar manner, the peak area of F3 was very high for the 24 first hours and decreased dramatically from 48 h *post-partum*. This was in accordance with the variation in time of the CSA and IgG amounts (Table 2). The CSA concentration decreased from 20.8 to 12.5 g/l during the first day and was stabilised to an average value of 10.8 g/l (for the four last days). The value of 20.8 g/l was, however, slightly high as it included lactoferrin which band was not well-resolved. The main proteins found in colostrum were IgG1 and enzyme inhibitory antibodies IgG2+IgG3. Their respective concentrations were 43.4 and 58.4 g/l at 1 h after parturition. The total IgG concentration (101.8 g/l at 1 h) fell sharply at 48 h (19.6 g/l). The IgG1 concentration decreased twice more rapidly than that of the enzyme inhibitory antibodies IgG2+IgG3 between 1 h and 192 h. The PP3 concentration was almost doubled between the 48 h *post-partum* (2.7 g/l) and the 168 h of lactation (4.7 g/l), and then remained almost constant at 192 h (4.9 g/l). As PP3, the basic CWBP was detected in gel only after 48 h *post-partum* and increased to reach 3.1 g/l in whey. α-Lactalbumin displayed a mean concentration of 2.2 g/l from 1 h after parturition to 192 h of lactation.

Table 2 : Evaluation of whey protein concentration (g/l) in camel's colostrum and milk determined by densitometry measurement at 633 nm of electrophorectically separated protein bands.

	Immunoglobulins subclasses IgG[a]		Whey proteins				
Time of milking *post-partum* (h)	***IgG1***	**IgG_2+IgG_3**	***Lactoferrin***	**CSA[a]**	**PP3[a]**	**CWBP[a]**	**α-LA[a]**
1	43.4	58.4	n.d.[b]	*20.8*	*n.d.*	*n.d.*	*2.2*
2	38.7	56.5	*n.d.*	24.2	n.d.	n.d.	2.4
4	36.2	53.0	n.d.	23.6	n.d.	n.d.	4.5
6	35.7	47.8	n.d.	19.3	n.d.	n.d.	2.3
12	27.5	36.9	n.d.	15.2	n.d.	n.d.	1.8
24	19.7	27.5	1.5	12.5	n.d.	n.d.	2.7
48	7.7	11.9	2.3	7.0	2.7	1.7	2.1
72	6.5	7.4	2.3	6.4	2.9	1.2	1.9
96	4.2	6.3	1.1	8.1	6.8	2.5	0.8
120	3.9	8.0	0.7	11.3	3.1	2.7	2.1
144	3.7	7.1	0.7	10.8	2.3	1.1	3.0
168	3.5	8.0	n.d.	11.9	4.7	3.5	1.5
192	2.2	5.7	n.d.	9.2	4.9	3.1	1.3

[a]CSA, camel serum albumin; IgG, immunoglobulins G; PP3, component-3 of proteose peptone; CWBP, camel whey basic protein; α-LA, α-lactalbumin. [b]n.d., not detected

DISCUSSION

Camel's colostrum was rich in IgG and CSA, which amounts were drastically reduced after 48 h *post-partum*. These results were in agreement with those mentioned by Elagamy *et al.* (1996). The mean concentration of -LA in camel's colostrum (mean value of 2.2 g/l for the thirteen samples studied) was close to that of α-LA in bovine colostrum (2.04 g/l; Levieux & Ollier, 1999). However, the colostrum as well as the milk did not contain α-lactoglobulin, which was in agreement with data previously reported (Ochirkhuyag *et al.*, 1998). The lack of -lactoglobulin has also been reported for human colostrum and milk (Jenness, 1985). Camel's colostrum is reported to have a high lactoferrin content of 5.1 g/l at the second day after parturition comparatively to bovine colostral milk (0.5 g/l; Abd El-Gawad *et al.*, 1996). In the present study, the lactoferrin concentrations in the different colostrum and milk samples were found lower, with a maximum of 2.3 g/l at 48 h. The concentration of lactoferrin in milk collected after 144 h decreased to values lower than 0.7 g/l, close to the value determined by Kappeler *et al.* (1999) in a camel's milk sample (0.22 g/l).

IgG1, in high concentration in camel's colostrum (mean value of 36.3 g/l for the first 12 h *post-partum*), decreased in time when milk replaced colostrum, as that is observed in the other mammals. But, it was worthy to note that IgG2 and IgG3 were found in very high quantity in camel's colostrum (mean value of 50.5 g/l for the first 12 h *post-partum*), whereas colostrums of other species do not seem to contain such antibodies. Camel (*Camelus dromedarius)* has unique immunological characteristics, as it contains considerable amount of enzyme inhibitory antibodies circulating in bloodstream (Lauwereys *et al.*, 1998). According to Azwai *et al.* (1996), camel's colostral IgG are not restricted to one major subclass IgG1 as in bovine colostrum, but includes three main subclasses (IgG1, IgG2 and IgG3). Hamers-Casterman *et al.* (1993) have demonstrated that the two IgG2 and IgG3 subclasses are devoid of light chains (IgG2 and IgG3 have heavy chains of 45 and 42 kDa, respectively). It has been suggested that the functional domain (the N-terminal variable region of the heavy-chain antibodies referred as VH is the minimal intact antigen-binding fragment that can be generated) of the heavy-chain antibodies interfere with several biological processes and might make good candidates for human therapy (Holt *et al*., 2003). IgG2 and IgG3 act as true competitive inhibitors by penetrating into active sites of some enzymes (Lauwereys *et al*., 1998). They might have inhibitory activity on human immunodeficiency viruses type 1 (HIV 1) reverse transcriptase, protease, and integrase, enzymes that are crucial to the HIV-1 life cycle (Ng *et al.*, 2001; Ng and Ye, 2004). The mecanism by which lysozyme, one of the main antibacterial enzymes contained in camel's milk (Elagamy *et al*., 1996), can be inhibited by heavy-chain antibodies (IgG2 and IgG3) is well-known (Desmyter *et al*., 1996). The amino-terminal part of the complementary determining region 3 (CDR3) loop located in VH protrudes from the antigen-binding surface and penetrates deeply into the active site of lysozyme (Desmyter *et al*., 1996). It could be thought that lysozyme might be inhibited in camel's colostrum and then loose a part of its activity in the presence of the high quantities of heavy-chain antibodies.

The PP3 component of camel's whey, also named lactophorin (Sørensen & Petersen, 1993) has been first described by Beg *et al.* (1987). It was only detected after 48 h *post-partum*. The PP3 concentration fluctuated between 2.7 and 6.8 g/l during the first 192 h *post-partum* for the present Tunisian camel, whereas concentrations greater than 0.9 g/l and of 1.1 g/l have been found in Arabian and Mauritanian camel's milk, respectively (Kappeler *et al.*, 1999; Girardet *et al.* 2000). Anyway, the PP3 content was higher than that of cow's milk (0.3 g/l reported by Sørensen & Petersen, 1993). PP3 of camel, cow, or of other species such as llama, goat, and ewe belongs to the glycosylation-dependent cell adhesion molecule 1 (GlyCAM-1) family (Groenen *et al*., 1995) and therefore, might prevent the occurrence of infections of the mastitis type in the lactating animal or might play a role in inhibiting the replication of pathogens in the respiratory and gastrointestinal tracts of the suckling young, as suggested by Girardet *et al*. (2000). Campagna *et al.* (2004) have shown that lactophoricin, a synthetic peptide of 23 amino acid residues corresponding to the amphipathic carboxy-terminal 113 to 135 region of lactophorin, display a growth inhibitory activity against both Gram-positive and Gram-negative bacteria. So, it could be thought that the high quantity of PP3 in camel's colostrum and milk might complete the antibacterial function of IgG when milk instead of colostrum was excreted. We can conclude that the camel colostrum has potential to treat as well to prevent certain diseases in the body. In future this will prove to be a very useful product to treat and control diseases in a natural way.

ACKNOWLEDGEMENTS

This research was conducted in the framework of the program "Livestock and widelife laboratory" funded by the Ministery of Scientific Research and Technology and capacity building of Tunisia. The encouragements of Prof. Houcine KHATTELI Director General of IRA (Institut des Regions Arides), are gratefully acknowledged. The assistance and the scientific collaboration of all the members of 'Laboratoire des Biosciences de L'Aliment, Unité Associée INRA, Université de Nancy' has been highly appreciated.

REFERENCES

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

Azwai S M, Carter S D and Woldehiwet Z (1996) Immunoglobulins of camel (*camelus dromedarius*) colostrum. *Journal Comparative Pathology* **114** 273–282.

Beg O U, von Bahr-Lindström H, Zaidi Z H and Jörnvall H (1986) A camel milk whey protein rich in half-cystine: Primary structure, assessment of variations, internal repeat patterns, and relationships with neurophysin and other active polypeptides. *European Journal of Biochemistry* **159** 195–201.

Beg O U, von Bahr-Lindström H, Zaidi Z H and Jörnvall H (1987) Characterization of a heterogeneous camel milk whey non-casein protein. *FEBS Letter* **216** 270–274.

Campagna S, Mahlot A G, Fleury Y, Girardet J M and Gaillard J L (2004) Antibacterial activity of lactophoricin, a synthetic 23-residues peptide derived from the sequence of bovine milk component-3 of proteose peptone. *Journal of Dairy Science* **87** 1621–626.

Desmyter A, Transue T R, Ghahroudi M A, Thi M H, Poortmans F, Hamers R, Muyldermans S and Wyns L (1996) Crystal structure of a camel single-domain VH antibody fragment in complex with lysozyme. *Nature Structural Biolology* **3** 803–811.

Ekstrand B and Björck L (1986) Fast protein liquid chromatography of antibacterial components in milk. *Journal of Chromatography* **358** 429–433.

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

Girardet J M, Saulnier F, Gaillard J L, Rame J P and Humbert G (2000) Camel *(Camelus dromedarius)* milk PP3: Evidence for an insertion in the amino-terminal sequence of the camel milk whey protein. *Biochemistry Cell Biolology* **78** 19–26.

Groenen M A M, Dijkhof R J M and Van der Poel J J (1995) Characterization of a GlyCAM1-like gene (glycosylation dependent cell adhesion molecule 1) which is highly and specifically expressed in the lactating bovine mammary gland. *Gene* **158** 189–195.

Hamers-Casterman C, Atarhouch T, Muyldermans S, Robinson G, Hamers C, Songa EB, Bendahman B and Hamers R (1993) Naturally occurring antibodies devoid of light chains. *Nature* **363** 446–448.

Holt L J, Herring C, Jespers L S, Woolven B P and Tomlinson I M (2003) Domain antibodies: proteins for therapy. *TRENDS Biotechnology* **21** 484–490.

Jenness R (1985) Biochemical and nutritional aspects of milk and colostrum. *In* Lactation, BL Larson (Ed.) Iowa State Univ. Press, Ames, IA, pp. 164–197, Ch. 5.

Kappeler S R, Farah Z and Puhan Z (1999) Alternative splicing of lactophorin mRNA from lactating mammary gland of the camel (*Camelus dromedarius*). *Journal of Dairy Science* **82** 2084–2093.

Kappeler S R, Farah Z and Puhan Z (2003) 5'-Flanking regions of camel milk genes are highly similar to homologue regions of other species and can be divided into two distinct groups. *Journal of Dairy Science* **86** 498–508.

Laemmli U K and Favre M (1973) Maturation of the head of bacteriophage T4. I. DNA packaging events. *Journal of Molecular Biology* **80** 575–579.

Lauwereys M, Ghahroudi M A, Desmyter A, Kinne J, Hölzer W, De Genst E, Wyns L and Muyldermans S (1998) Potent enzyme inhibitors derived from dromedary heavy-chain antibodies. *EMBO Journal* **17** 3512–3520.

Levieux D and Ollier A (1999) Bovine immunoglobulin G, -lactoglobulin, -lactalbumin and serum albumin in colostrum and milk during the early *post partum* period. *Journal of Dairy Research* **66** 421–430.

Lowry OH, Rosebrough NJ, Farr AL, **&** Randall RJ 1951 Protein measurement with the Folin phenol reagent. *Journal Biological Chemistry* **193** 265–275

Merin U, Bernstein S, Van Creveld C, Yagil R and Gollop N (2001) Camel *(Camelus dromedarius)* colostrums and milk composition during the lactation. *Milchwissenschaft* **50 (2)** 70–73.

T B, Lam T L, Au T K, Ye X Y and Wan CC (2001) Inhibition of human immunodeficiency virus type 1 reverse transcriptase, protease and integrase by bovine milk proteins. *Life Science*. **69** 2217–2223.

Ng T B and Ye X Y (2004) A polymeric immunoglobulin receptor-like milk protein with inhibitory activity on human immunodeficiency virus type 1 reverse transcriptase. Int. J. Biochem. *Cell Biology* **36** 2242–2249.

Ochirkhuyag B, Dalgalarrondo M, Chobert J M, Choiset Y and Haertle T (1998) Characterization of whey proteins from mongolian yak, khainak, and bacterian camel. *Journal Food Biochemistry* **22** 105–124.

Sørensen E S and Petersen T E (1993) Phosphorylation, glycosylation and amino acid sequence of component PP3 from the proteose peptone fraction of bovine milk. *Journal Dairy Research* **60** 535–542.