

## Camel (*Camelus dromedarius*) immunoglobulin G, $\alpha$ -lactalbumin, serum albumin and lactoferrin in colostrum and milk during the early *post partum* period

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Received 24 May 2005 and accepted for publication 19 October 2005

Colostrum and milk samples from twelve Tunisian camels were analysed for concentration of immunoglobulin G (IgG),  $\alpha$ -lactalbumin ( $\alpha$ -la), serum albumin (CSA) and lactoferrin throughout the first 14 milkings *post partum* (7 days of lactation) using single radial immunodiffusion assay. Concentrations (mg/ml, means $\pm$ SD) at first milking were IgG, 100.7 $\pm$ 60.4;  $\alpha$ -la, 2.2 $\pm$ 0.7; CSA, 8.5 $\pm$ 3.6 and lactoferrin, 1.2 $\pm$ 0.3. Large variations were recorded for IgG and CSA concentrations (11.8–211.1 mg/ml and 2.9–13.8 mg/ml respectively) Concentrations of IgG and CSA dropped abruptly in the subsequent milkings while  $\alpha$ -la concentration increased until milking 5 and then decreased slowly. Lactoferrin dropped only from milking 7. Mean IgG concentrations were 3.6 and 2.5 mg/ml at milking 9 and 13 respectively. However, IgG concentration did not differ significantly, at the 1% level, from milkings 11 to 14. The contribution of CSA to the increase in whey proteins in early milks was greater than that described in the bovine and caprine species.

**Keywords:** IgG, Albumin,  $\alpha$ -lactalbumin, lactoferrin, camel, colostrum, milk.

Similar to cattle, sheep and horses, camelids have a thick layered epitheliochorial placenta, which prevents transplacental transfer of IgG. The newborns rely on ingestion and absorption of immunoglobulins from colostrum to provide the antibodies necessary to protect them from microbial infection (reviewed by Wernery, 2001). Colostrum is generally referred to as the first milk produced after parturition. It is mostly characterized by its very high level of immunoglobulin G (IgG) which accounts for  $\geq$ 85% of the passively transferred proteins in serum of 24 h-old camelids (Garmendia & McGuire, 1987). However, proteins secreted by the mammary gland of the camel such as  $\alpha$ -lactalbumin ( $\alpha$ -la), albumin (CSA) and lactoferrin are present in higher concentrations in colostrum than in definitive milk (Garmendia & McGuire, 1987; Ungar-Waron et al. 1987; Fernandez & Oliver, 1988; Bravo et al. 1997; Kamber et al. 2001; Merin et al. 2001a, b).

The high content of IgG and other soluble proteins modify the technological properties of milk. For the bovine milk, several problems have been described such as reduced heat stability, low cheese yield, weak curd formation and poor curd characteristics (Feagan, 1979).

Cheese and pasteurized or ultra-high temperature milk contaminated with colostrum have shorter shelf life and may exhibit off-flavours. Owing to the temperature sensitivity of IgG, the use of milk containing colostrum necessitates more frequent and comprehensive cleaning of heat transfer surfaces and other equipment in dairy plants (Zawitowski & MacKinnon, 1993). For the camel milk, a similar increase in the heat sensitivity of whey proteins has been recently demonstrated (Levieux et al. 2005).

Because of these undesirable properties, commercial milk should be free from colostrum. However, regulations controlling the period for withholding post-parturition milk vary between countries. This situation arises largely because there is no clear delineation between colostrum and milk: there are gradual changes in protein composition from colostrum to definitive milk during the first week *post partum* and from milk to colostrum during the last weeks *ante-partum*. To our knowledge there is no published information describing in detail the variability of IgG and of the other major whey proteins in camel colostrum and milk during the first week of lactation.

The purpose of the present work was to study the concomitant variation in concentration of IgG,  $\alpha$ -la, CSA and lactoferrin during the first 14 milkings *post partum* (7 days of lactation) in the camel.

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## Materials and Methods

### Materials and samples

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

Individual colostrum and milk samples were obtained from the first 14 milkings *post partum* (7 d of lactation) of 12 multiparous lactating camels (*Camelus dromedarius*) at the Experimental Station of the Arid Land Institute of Medenine. Milk samples were also obtained at d 90. Samples were obtained twice a day by manual milking, after discarding the first jets ejected, and kept frozen at  $-20^{\circ}\text{C}$  until analysis.

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  $4^{\circ}\text{C}$  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  $4^{\circ}\text{C}$  for 30 min.

### Purified proteins

IgG, CSA and  $\alpha$ -la 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). CSA was purified by the same chromatographic combination but starting from camel serum.  $\alpha$ -La was purified from camel whey by gel permeation chromatography on Sephadex G100 and ion exchange chromatography on Q-Sepharose Fast Flow followed by Mono-Q HR 10/10 (Amersham-Biosciences).

For the lactoferrin purification, the third peak obtained by gel permeation chromatography of colostrum 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) 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).

### 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. 1965). Immunogens used were purified IgG,  $\alpha$ -la, CSA and lactoferrin.

### Immunochemical assay of proteins

Concentrations of IgG,  $\alpha$ -la, CSA 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  $\mu\text{l}$  aliquots of the adequately diluted samples or 3  $\mu\text{l}$  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  $37^{\circ}\text{C}$  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%.

### Statistical analysis

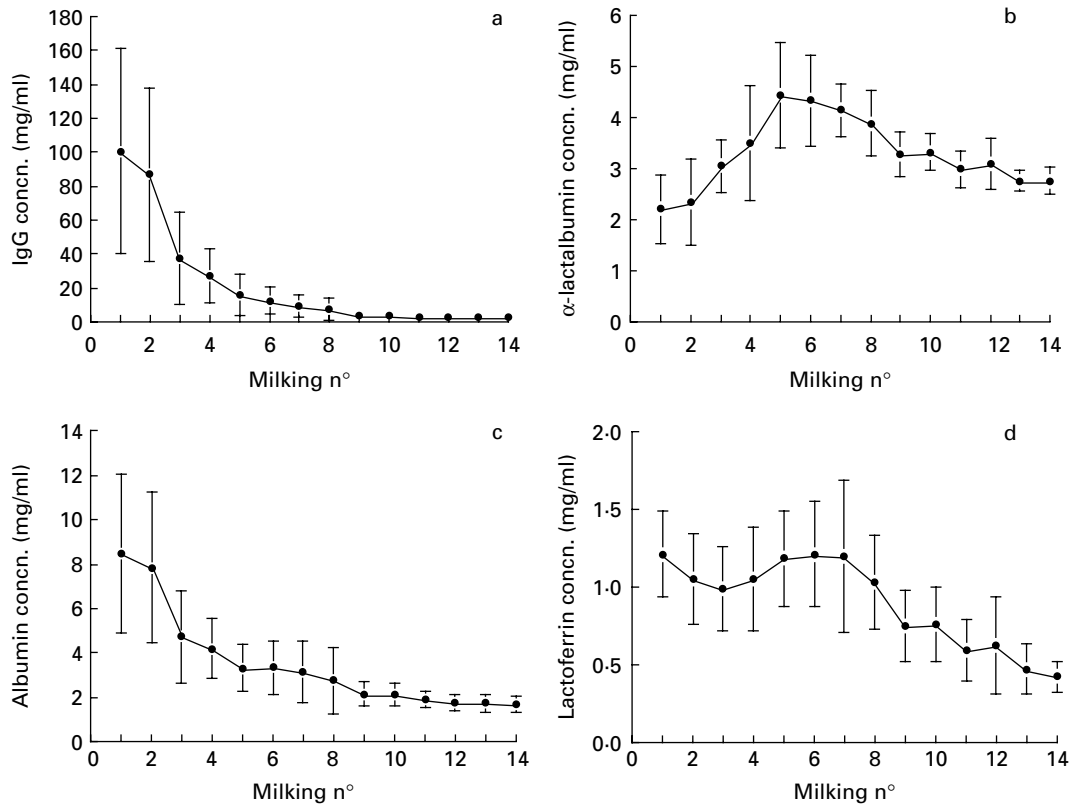
Probability and significance were calculated by Student's *t* test.

## Results

Values are given as means  $\pm$  SD. IgG concentration at the first milking were 11.8–211.1 (mean 100.7  $\pm$  60.4) mg/ml. Thereafter, they fell sharply to 2.3 mg/ml at the 14th milking (Fig. 1a). Best fitting was obtained with the equation  $y=191.1x^{-1.64}$  ( $r=0.976$ ) for milkings 1–14 and  $y=351.3x^{-1.933}$  ( $r=0.992$ ) for milkings 2–14. IgG concentration did not differ significantly, at the 1% level, from milkings 11 to 14.

$\alpha$ -La concentrations (2.19  $\pm$  0.67 mg/ml at the first milking) increased until milking 5 ( $P<0.001$ ) and then decreased slowly (Fig. 1b). Concentrations at the 14th milking (2.43–3.19 mg/ml) were still significantly ( $P<0.03$ ) higher than that for the first milking.

CSA concentration at the first milking were 2.9–13.6 (mean 8.5  $\pm$  3.58) mg/ml, falling sharply between milking 1 and 5 and then decreasing more slowly (Fig. 1c). Concentrations in milking 1–7 were significantly higher ( $P<0.01$ ) than that for milking 14.



**Fig. 1a–d.** Changes in the concentrations of (a) immunoglobulin G, (b)  $\alpha$ -lactalbumin, (c) albumin, (d) lactoferrin in the milk of 12 Tunisian multiparous camels during early lactation. Values are means with SD indicated by vertical bars.

Lactoferrin concentration in colostrums ranged from 0.74 to 1.67 (mean  $1.21 \pm 0.28$ ) mg/ml. The slow decrease between milkings 1 and 3 (Fig. 1d) was not significant ( $P > 0.05$ ) and values at the 5–7 milkings were similar to that obtained at the first milking (0.78–1.41, mean 1.20 mg/ml). Thereafter, the lactoferrin concentration dropped to a mean concentration of 0.42 mg/ml for milking 14.

Concentration (mg/ml) of the four proteins in milk samples obtained at 90 d *post partum* were IgG  $0.65 \pm 0.01$ ,  $\alpha$ -la  $1.84 \pm 0.06$ , CSA  $2.41 \pm 0.07$  and lactoferrin  $0.14 \pm 0.002$ .

The excess of whey proteins in early milks relative to their mean concentrations obtained at d 90 are presented in Table 1. IgG was the predominant whey protein in excess in milkings 1–14. Thereafter, albumin and  $\alpha$ -la were the most important proteins in excess in milkings 1–4 and 5–14 respectively.

## Discussion

The main objective of the present investigation was to describe the variation of the major soluble proteins in colostrum and milk throughout the first 14 milkings *post partum*. SRID was used to quantify these proteins because this technique makes it possible to analyse whole colostrum

or milk directly, as recommended by Flenor & Stott (1981). SRID has been previously used for the quantification of camelids IgG (Ungar-Waron et al. 1987, Hutchison et al. 1995; Fernandez & Oliver, 1988; Bravo et al. 1997; Kamber et al. 2001). However, Hutchison et al. (1995) have pointed out that 2 commercial SRID kits used for the quantification of camelids (llama) IgG provided different IgG values for the same sample. These authors reported that not only were the 2 sets of reference solutions calibrated differently, but the 'purified' IgG was not identical between manufacturers. The important effect of using different reference antigens or standards as well as the source of antibody in the immunochemical quantification of bovine IgG has been thoroughly studied (Bockhout, 1975; Li-Chan & Kummer, 1997).

In the camelids, three IgG subclasses have been identified (IgG1, IgG2 and IgG3), of which IgG2 and IgG3 are lacking the light chain (Hamers-Casterman et al. 1993) while exhibiting antibody activity (Lange et al. 2001; Cortez-Retamozo et al. 2002) and neutralizing capacity against enzymes (Conrath et al. 2001) and toxins (Meddeb-Mouehi et al. 2003). Colostral IgG in camel has been found not to be restricted to one subclass as in bovine, but to include the three IgG subclasses (Azwai et al. 1996). However, their respective concentrations have not been established and thus it is imperative to use IgG purified

**Table 1.** Excess concentration of the four major whey proteins (immunoglobulins G,  $\alpha$ -lactalbumin, albumin and lactoferrin) in the milk of camels during early lactation ( $n=12$ )

Milking no.	Excess whey protein* mg/ml				
	IgG	$\alpha$ -lactalbumin	Albumin	Lactoferrin	Total
1	100.0	0.35	6.06	1.07	107.5
2	86.1	0.49	5.44	0.91	93.0
3	37.0	1.20	2.32	0.85	41.3
4	26.8	1.64	1.80	0.91	31.2
5	15.2	2.58	0.91	1.04	19.7
6	12.0	2.48	0.95	1.07	16.5
7	8.6	2.29	0.77	1.06	12.7
8	7.1	2.03	0.37	0.89	10.3
9	3.0	1.43	-0.25	0.52	4.7
10	3.0	1.47	-0.26	0.62	4.9
11	2.5	1.13	-0.47	0.45	3.6
12	2.2	1.24	-0.62	0.48	3.3
13	1.8	0.91	-0.66	0.33	2.4
14	1.7	0.91	-0.72	0.28	2.2

\*The excess value is calculated as the difference between the protein concentration in each milking 1 to 14 and that obtained for the reference mature milk sampled at 90 d *post partum*.

from colostrum when producing antisera and standards for their immunoquantitation in colostrum or milk. Purification of IgG using affinity chromatography on protein G and protein A allows full separation of IgG subclasses (Hamers-Casterman et al. 1993). In contrast, the combination of size exclusion and ion-exchange chromatography we used does not really separate the IgG subclasses and the IgG mixture obtained can be considered as representative of the IgG composition in colostrum.

The IgG concentrations we found in the camel colostrums of the Tunisian bred were higher on average than the values found in the literature for other domestic animals such as horses (Perryman & Crawford, 1979), cattle (Butler, 1981; Levieux & Ollier, 1999), sheep (Halliday, 1978) and goat (Levieux et al. 2002). Our results are consistent with those reported for camels by Ungar-Waron et al. (1987) and those reported for llamas and alpacas by Bravo et al. (1997) and Garmendia & McGuire (1987). However, lower IgG values (mean 58 mg/ml) were found for camels in Kenya by Kamber et al. (2001).

The high variability of IgG concentration observed at the first sampling (11.8 to 211.1 mg/ml) is similar to that reported by Garmendia & McGuire (1987) for alpacas and is characteristic of non-dairy breeds. As a consequence, there is a risk for the newborns to receive colostrums with amounts of IgG insufficient to guaranty a proper transmission of immunity.

After calving, IgG concentrations fell by around 35% at each milking ( $r=0.984$ ), similarly to that reported for dairy cows and goats (Levieux & Ollier, 1999; Levieux et al. 2002). However, this decrease is higher than the 25% we calculated from the data published for the camel by

Kamber et al. (2001). IgG concentration did not differ significantly, at the 1% level, from milkings 11 to 14. Thus, on the IgG basis, time for withholding post parturition milk could be limited to the first 5 days lactation.

The increase of  $\alpha$ -la concentration during the first 5 milkings has not been observed in dairy cows or goats (Levieux & Ollier, 1999; Levieux et al. 2002). The higher level at milking 14 than at the first milking is consistent with the increase described for the camel by Merin et al. (2001a).

CSA concentration in the first milking was higher than that reported for dairy cows and goats (Perez et al. 1989; Levieux & Ollier, 1999; Levieux et al. 2002). However they were considerably lower than the 60 mg/ml reported by Merin et al. (2001b) who conclude that CSA is the major component of colostrum. This discrepancy could be explained by a misinterpretation of the gel permeation chromatogram obtained by these authors since the CSA peak is heavily contaminated by lactoferrin, lactoperoxidase and IgG isotypes devoided of light chains (IgG2, IgG3).

Colostrum and milk from camel are generally considered as particularly rich in lactoferrin from the data published by Abd El-Gawad et al. (1996). These authors, using HPLC analysis, reported a lactoferrin content of 5.1 mg/ml in one camel colostrum sampled 2 d after parturition and 0.5 mg/ml in bovine colostrum. This lactoferrin concentration in camel colostrum is considerably higher than the  $1.21 \pm 0.28$  and  $0.99 \pm 0.27$  mg/ml we found for the first and third milkings respectively and the lactoferrin concentration of bovine colostrum was generally reported between 1.0 and 4.0 mg/ml (Senft & Klobasa, 1973; Sanchez et al. 1988; Tu et al. 2002; Turner et al. 2003). At milking 14, the 0.42 mg/ml we obtained for the camel milk is compatible with the 0.34 mg/ml reported by Abd El-Gawad (1996) at 30 d after parturition and the 0.22 mg/ml reported by Kappeler et al. (1999) in late-lactation milk. These concentrations are of the same order as those reported for bovine milk: 0.25–0.30 mg/ml at 15–30 d *post-partum*, 0.05–0.15 mg/ml for mid-lactation milk and 0.15 mg/ml for milk at 270 d lactation (Welty et al. 1976; Rainard et al. 1982; Xiuyun & Yoshida, 1995; Indyk & Filonzi, 2005).

Early milk from camel presents a reduced heat stability (Levieux et al. 2005). In the bovine, several other technological problems have been described for early milks or milk contaminated with colostrum such as low cheese yield, weak curd formation and poor curd characteristics (Feagan 1979; Zawitowski & MacKinnon, 1993). IgG, a very heat sensitive protein, is the major component responsible of these changes in the technological properties of early milks. However, the other major whey proteins are also in higher concentrations in colostrum and early milk than in mature milk and contribute to their poor technological properties. We have thus tabulated values for the total concentrations of the four major whey proteins throughout early lactation and calculated the excess of proteins

relative to mature milk obtained three months *post partum* (Table 1). From milking 11 to 14 the total concentration of soluble proteins is respectively only 3.6 to 2.2 mg/ml higher than in mature milk and should not represent a technological risk.

The major concern with colostrum in camelids is the inadequate feeding of the neonate with insufficient amount of colostrum. However, camel milk industrialisation and its pasteurization may be considered in the future and technological problems linked to excess soluble proteins in milk if colostrum or early milk are added to the milk supplies will have to be taken into account.

The authors wish to thank Dr Khorchani Touhami (Head of the Livestock & Wildlife Laboratory, Arid Land Institute of Medenine; Tunisia) for his encouragements and for providing the camel milk samples.

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