Vol 13 No 1, p 53-57

SELECTED VITAMINS AND FATTY ACID PATTERNS IN DROMEDARY MILK AND COLOSTRUM

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ABSTRACT

The aim of the present study was to determine concentrations of the vitamins A, E, B_1, C and β-carotene as well as to evaluate the fatty acid patterns of dromedary milk (*Camelus dromedarius*). Therefore, camel milk from different herds in the United Arab Emirates was analysed by HPLC as well as gas chromatographic methods and compared with milk from Holstein Frisian cows of the same area. Besides fresh camel milk, pasteurised and lyophilised milk was also analysed to evaluate the influence of these preservation methods on the determined parameters. Colostrum was directly tested after birth and in 5 individuals also during the first week after parturition. Blood samples were tested from the same herds for their vitamin content in order to find an eventual relation between milk and blood levels. Vitamins A, E, B_1 and β-carotene were significantly lower in dromedary milk while vitamin C was significantly (fivefold) higher compared to bovine milk. In camel colostrum fat soluble vitamins and vitamin B_1 were higher than in mature camel milk, but vitamin C was lower in colostrum. Pasteurisation and lyophilisation caused only small but significant vitamin losses. The total content of saturated and unsaturated fatty acids was similar in camel and cow milk. The differences in the fatty acid patterns were most obvious only in omega-6 and omega-7 fatty acids. In dromedary serum vitamins A, B_1 and C were significantly higher than in cow serum; vitamin E was significantly higher in bovine serum. Regarding the vitamin content and the fatty acid composition, it was concluded that camel milk is a good alternative to cow milk for human nutrition.

Key words: Camel milk, colostrum, fatty acid pattern, vitamins

Camel milk is still the most important nutritional source for pastoralists in many Asian and African countries. However, in some oil producing countries like the United Arab Emirates, it has lost its importance for human nutrition. During the previous years the interest in camel milk has increased due to reports on its positive influence towards human health (Wernery *et al*, 2006). Discussions in the literature of possible influences of cow milk proteins on immune mediated type-1 diabetes and on other autoimmune diseases are manifold (Schrezenmeir *et al*, 2000; Wasmuth and Kolb, 2000). In this connection camel milk could be an alternative to cow milk especially in young children's diet. In addition some authors report a therapeutic effect of camel milk for type-1 diabetic patients (Agarwal *et al*, 2005; Breitling, 2002).

Efforts have been undertaken by some scientists to determine to some extent the composition of camel milk. Studies on the content of some vitamins in camel milk were carried out by Wernery *et al* (2003), Farah *et al* (1992) and Sawaya *et al* (1984) but studies with modern analytical and well-adapted procedures for the determination of various vitamins and fatty acids are missing. Furthermore, detailed information on the effects of diets and other factors on camel milk compositions are also lacking.

The aim of this study was to establish vitamin analyses taking in consideration the chemical and physical specifics of dromedary milk. Tests were performed directly on fresh milk samples and in some cases on lyophilised milk. The latter procedure was preferred for samples, which could not be analysed directly after extraction for technical and methodological reasons. This procedure avoided vitamin losses as much as possible. In addition, 10 cow milk samples and 10 camel sera and 6 cow sera were also analysed for the same vitamins.

Materials and Methods

Milk samples were drawn from 46 dromedaries from 2 herds in Dubai and one herd in Abu Dhabi. Equal amounts of milk were taken from all 4 quarters at the beginning of the milking process and mixed

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to a volume of 500 ml. Colostrum was sampled from 10 dromedaries of the 3 herds immediately after parturition. Ten milk samples were also collected from Holstein Frisian cows living in the vicinity of the dromedaries under the same climatic conditions.

Furthermore, 10 dromedary and 6 bovine blood samples were taken from the same herds for vitamin analysis. A follow up study of the milk vitamin content during the first week after birth was carried out with 5 camels. For the fatty acid analysis the following samples were collected: 7 colostrum, 4 bovine milk and 18 mature dromedary milk samples.

All camels were kept in open pens in the desert with unrestricted access to water. Good quality timothy hay was fed *ad libitum* and each dromedary also received 4 kg of pelleted concentrate daily. The bovine diet consisted of 2 kg fresh alfalfa, 4 kg of concentrate and hay *ad libitum*.

The analytical methods used in our study followed good laboratory practice (GLP). For the vitamin analysis we used 3 different RP-HPLC instruments (in Dubai: 2695 Alliance Separations Module from Waters, Milford, MA, USA; in Hannover: Knauer, Berlin and Shimadzu, Kyoto, Japan).

Milk samples were pasteurised with a ´Safgrad-Pres Vac Home´ pasteuriser (MB-47). At a temperature of 72°C the heating process was stopped and the milk was kept in the unit for 5 minutes.

For lyophilisation a ´Heto-sicc´ lyophilisator (Heto FD 2.5) was used. After freezing 200 ml milk samples at -80°C, we freeze-dried the samples for 24 hours in the lyophilisator.

For the vitamins A, E, B_1 and β-carotene determination a Supelco Discovery C18 (15cm x 4.6mm) column and for vitamin C determination a Chromsystems column (Art. 651000) were used. The fatty acids were detected by Gas Chromatography (GC). The applied double column device was from Varian, Darmstadt (Varian 3400), and the column, a Supelcowax-10 column from Supelco, Bad Homburg.

Vitamins A and E as well as β-Carotene were measured with the same analytical procedure. After alkaline hydrolysis the vitamins and ß-carotene were extracted with hexane. The solvent was evaporated, the residue resolved in mobile phase and the sample subsequently purified by HPLC. The detection of vitamin A and E was determined by fluorescence stimulation (A: excitation ë = 325nm, emission ë = 480nm, E: excitation ë = 296nm, emission ë = 330nm). Beta-carotene was determined by UV detection (ë = 450nm) (Stahl, 2005).

Vitamin B_1 was measured according to the European norm EN 14122:2003. Thiamine was released by acid hydrolysis and the phosphate bonds were split by the enzyme Takadiastase. Then thiamine was oxidised to the fluorescent thiochrome. The RP-HPLC system measured samples by fluorescence detection (excitation ë = 366nm, emission ë = 435nm) (Anonymous, 2003).

For .vitamin C determination fat was extracted with metaphosphoric acid. Sample preparation was performed according to the description in "reagent kit for HPLC analysis of vitamin C in plasma/ serum" from Chromsystems GmbH, Munich. After chromatographic separation vitamin C was measured by UV detection (ë = 245nm).

Transesterification of the fatty acids to fatty acid methyl-esters was done with acetyl chloride before determination of the fatty acid composition by gas chromatographic separation (Sallmann *et al*, 1992).

All vitamin determination of fresh milk samples were carried out at the Central Veterinary Research Laboratory in Dubai. Beta-carotene contents and fatty acid compositions were measured from lyophilised milk. These samples as well as serum samples were analysed at the Department of Physiological Chemistry, University of Veterinary Medicine, Hannover.

Results

As shown in Table 1, fresh dromedary milk contains less vitamin A, E and B_1 than cow milk. The beta-carotene level of camel milk was below the detection limit. However, the vitamin C content of

Table 1. Vitamin contents of dromedary milk, colostrum and cow milk. Statistically significant differences are shown between the groups.

	Dromedary colostrum n=10		**Fresh dromedary milk** n=46		**Fresh cow milk** n=10
Vitamin A (µg/100ml)	30.7±13.2	*P=0.006*	20.1±10	*P=0.001*	60.9±25.6
Vitamin E (µg/100ml)	136.9±98.4	*P=0.001*	32.7±12.8	*P=0.001*	171.0±114.4
Vitamin B1 (mg/100ml)	72.7±32.7	*P=0.001*	19.6±6.4	*P=0.001*	34.7±8.1
Vitamin C (mg/l)	35.6±10.6	*P=0.001*	52.5±15.8	*P=0.001*	10.5 1
β-Carotene (µg/100ml)	0.32±0.31	-	b.d.	-	99.6±62

- 1 Reference: Timmons *et al* (2001)
- b.d.: below the detection limit of 0.32 µg/100 ml in dromedary milk and serumv

camel milk was approximately 5 times higher than vitamin C content of cow milk specified by Timmons *et al* (2001).

The differences between the three camel herds regarding vitamin contents and fatty acid levels were not significant (not shown).

Vitamin A, E and B_1 levels of camel colostrum was higher than that in mature camel milk. The vitamin C content of mature camel milk was higher than the vitamin C content of colostrum.

The results of serum vitamin determination are shown in Table 2. Serum vitamin A, B_1 and C levels were higher in camels than in cows, whereas vitamin E was higher in cow serum than in serum of camels. In camel serum no β-carotene was detected while cow serum contained 496±88.8 µg/100ml.

Discussion

Regarding the main components, composition of camel milk in general is similar to that of cows and small ruminants (Zhang *et al*, 2005). However, variations of especially minor camel milk constituents

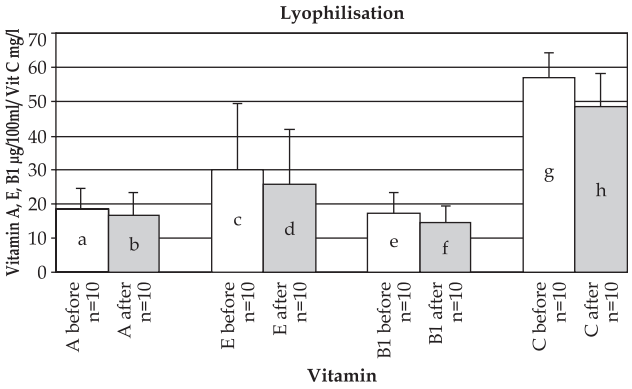

Fig 2. Vitamin regression after lyophilisation. Significant vitamin loss is shown by different letters before and after pasteurisation.

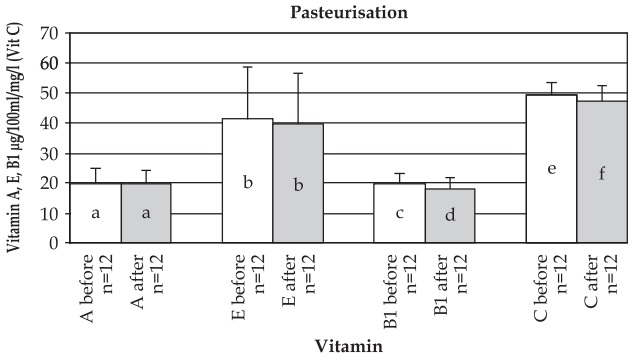

Fig 1. Vitamin regression following pasteurisation. Significant vitamin loss is shown by different letters before and after pasteurisation.

Table 2. Vitamin content of dromedary and cow serum. Statistically significant differences are shown between the groups.

	Dromedary serum n=10		**Cow serum** n=6
Vitamin A (µg/100ml)	39.2±6.6	P=0.004	27.2±7
Vitamin E (µg/100ml)	161.1±67.7	P ≤ 0.001	1168±247.2
Vitamin B1 (mg/100ml)	7.6±0.9	P=0.028	5.9±0.9
Vitamin C (mg/l)	5.4±1.1	P=0.002	3.3±0.3
β-Carotene (µg/100ml)	b.d.	-	496±88.8

- b.d.: below the detection limit of 0.32 µg/100ml in dromedary milk and serum

Table 3. Fatty acid pattern of dromedary milk, colostrum and cow milk. Statistically significant differences are shown between the groups. n.s. means ´not significant´.

Weight %	**Dromedary colostrum 1. day p.p.**		**Dromedary milk**		**Cow milk**
	n=7 n=7		n=18		n=4
C12:0	0.4	P=0.004	0.7	P=0.003	2.5
C14:0	6.8	P≤0.001	11.4	P=0.029	8.7
C16:0	30	n.s	30.4	P=0.030	33
C18:0	12.5	n.s	13.9	n.s	13.3
C20:0	0.3	n.s	0.3	n.s	0.2
C22:0	0.1	n.s	0.1	n.s	0.1
C24:0	0.3	n.s	0.1	n.s	0.1
saturated FA	**50.4±3.3**	**P≤0.001**	**56.9±3.4**	**n.s**	**57.9±4.2**
C18:3n3	0.8	n.s	0.7	P=0.012	0.3
C20:5n3	0.3	n.s	0.2	n.s	0.1
C22:5n3	0.3	P=0.036	0.2	P=0.009	0.1
n3-FA	**1.4±0.6**	**n.s**	**1.0±0.5**	**n.s**	**0.5±0.1**
C18:2n6	5.5	P=0.006	3	P=0.001	4.7
C18:3n6	0.1	n.s	0.1	n.s	0.1
C20:2n6	0.1	n.s	0	n.s	0
C20:3n6	0.1	P=0.029	0.1	P≤0.001	0.2
C20:4n6	0.4	P=0.015	0.3	n.s	0.3
n6-FA	**6.2±3**	**P=0.006**	**3.5±0.8**	**P≤0.001**	**5.2±0.8**
C16:1n9	0.9	n.s	0.8	P=0.015	0.3
C18:1n9	30.5	P=0.001	25.8	P≤0.001	32.8
C20:1n9	0.4	P=0.007	0.2	P≤0.001	0.8
C20:3n9	0.1	n.s	0.1	P=0.003	0
C22:1n9	0.2	P=0.006	0	P=0.007	0.1
n9-FA	**32.1±3.2**	**P≤0.001**	**27.0±2.6**	**P≤0.001**	**34.1±3.6**
C16:1n7	7.4	P=0.009	10.2	P=0.003	1.7
C18:1n7	2.4	P=0.001	1.4	P≤0.001	0.6
n7-FA	**9.8±2.2**	**n.s**	**11.6±1.3**	**P=0.003**	**2.3±0.2**
unsaturated FA	**49.5±3.2**	**P≤0.001**	**43.1±3.4**	**n.s**	**42.1±4.1**
total:	**100**		**100**		**100**

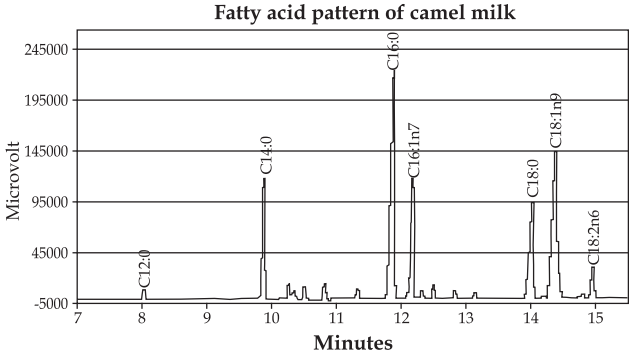

Fig 3a. Fatty acid pattern of camel milk.

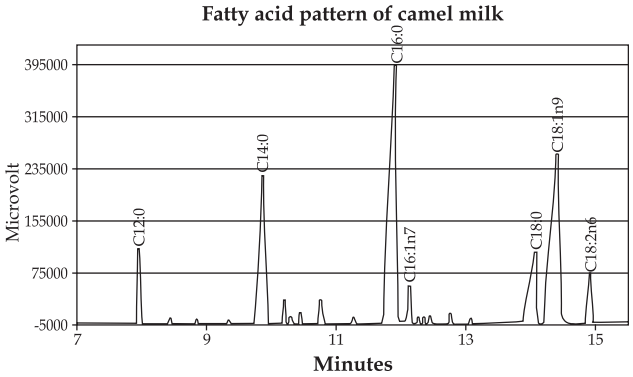

Fig 3b. Fatty acid pattern of cow milk.

(Gnan and Sheriha, 1986) are attributed to different breeds, feeding, stage of lactation or sampling techniques (Abu-Lehia, 1987; Alshaikh and Salah, 1994).

Our results regarding the contents of vitamin A, E, B_1, C and β-carotene in dromedary mature fresh milk are significantly different compared to fresh bovine milk. Dromedary milk generally contains less vitamin A, E, B_1, and β-carotene. The β-carotene content of camel milk was not even detectable in our study. In contrast, data on vitamin C were nearly 5 times higher in dromedary milk than those in cows milk (Table 1). These findings in the main are in agreement with data published by Farah *et al* (1992) and Farah (1993). The result for vitamin C makes sense in a teleological way of thinking because fruits and vegetables are rarely a compound of human food in arid zones and ascorbic acid intake via camel milk may help to avoid vitamin undersupply in humans.

Surprisingly the vitamin C content of dromedary colostrum is lower than in mature milk. The colostral content is still threefold higher than that of mature cow milk. This means good supply for the neonate calves which cannot produce endogenous vitamin C until 4 months of age (Hidiroglou *et al*, 1995) and must rely on the intake via colostrum.

When vitamin results in sera of dromedaries and cows were compared, the picture looks different (Table 2). While serum vitamins A, B_1 and C are higher in dromedaries compared to cows, the amount of vitamin E is significantly lower (by nearly one order of magnitude). In respect to the antioxidative action of the two micronutrients vitamin C and E, in dromedaries vitamin C seems to be the main active milk agent, whereas vitamin E plays an important role in blood. In cows the most important antioxidative active agent in both organs is vitamin E. At present there is good evidence for the *in vivo* efficacy of vitamin C in aqueous systems and for vitamin E in lipophilic areas to protect cellular and liquid biological systems against oxidative damages. In addition, many studies showed effective interaction between both vitamins in a way that ascorbic acid acts as a competent regenerator for vitamin E (Niki, 1987; Guo and Parker, 2000).

Therefore, it seems reasonable to hypothesise a compensatory function of vitamin C in mature dromedary milk. A lack of vitamin E in the camel's diet may cause a higher vitamin C production in the liver and a high transfer to the milk in order to compensate an otherwise low antioxidative protection of the animals and their offspring. In order to better understand the relationship between both micronutrients in camels, feeding studies are needed.

At present there is no research work published on the molecular mechanisms responsible for the extraordinary high vitamin C accumulation in the dromedary milk. For other species simple diffusion, facilitated diffusion and active mechanisms are reported as transport procedures over the plasma cell membranes (Wilson, 2005). In cattle active transport and eventually facilitated diffusion seem to be mainly involved in vitamin C transport which follows Michaelis-Menten kinetics and is not influenced by the diet (Weiss, 2001).

Pasteurisation is a good conservation method for camel milk. As Wernery *et al* (2003) already ascertained, we also found that vitamin losses in pasteurised camel milk occur.

Our lyophilisation results should be carefully evaluated, because a full homogenisation of milk powder was difficult to achieve. The reason for this was a sometimes insufficient freeze drying process with remaining water residues in the samples and clumped milk powder.

The strong relation between fatty acid pattern in tissues and body fluids and the diet, generally makes it difficult to compare own results with results from

other authors. The fraction of unsaturated fatty acids in the fatty acid pattern of camel milk was higher (43.1%) in our study than findings by Gorban and Izzeldin (2001) (30.2%) and Farah *et al* (1989) (35%). The milking camels in our study received a rich diet compared to camels that browse in the desert. This may explain the lower grade of saturation in fatty acid pattern in the milk of camels in our study. Other factors like lactation status and other milking conditions also may influence the fatty acid pattern of milk and should be kept in mind while comparing results from different authors.

Regarding the macro- and micronutrients of camel milk, it can be concluded, that the nutritional value of camel milk is equivalent to cow milk, and no nutritional disadvantages concerning the investigated vitamins and fatty acid composition should be expected by consuming camel milk. The high vitamin C content of dromedary milk is of great advantage for desert dwellers.

In addition, camel milk does not seem to induce allergies or *Diabetes mellitus* as reported for cow milk (Scott, 1990; Dahl-Jorgensen *et al*, 1991). On the contrary, Agrawal *et al* (2005) have shown a positive effect on diabetes patients when camel milk was consumed daily. However, more research needs to be carried out to elucidate these claims.

Acknowledgement

We are thankful to Andrea Widdel-Bigdely and Uwe Glockenthoer (Hannover) as well as Asha Annie Varghese (CVRL Dubai) for their support and technical assistance.

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