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Lipid Fractions and Fatty Acid Composition of Colostrums, Transitional and Mature She-Camel Milk During the First Month of Lactation

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Abstract: Fatty acid composition, triglycerides, cholesterol and tocopherols were determined in colostrums, transitional and mature milk. With progress of lactation, triglycerides and percentage medium chain fatty acids increased whereas tocopherols, cholesterol and percentage long chain polyunsaturated fatty acids decreased. These changes reflect augmented *de novo* synthesis of fatty acids (12:0, 14:0, 16:0 and 18:0) in the mammary gland and a tendency of increasing fat globule size as milk matures. Transitional and mature milks but particularly colostrums, contained higher concentrations of components considered to be derived from the fat-globule membrane (cholesterol, tocopherols, percentage long-chain polyunsaturated fatty acids). On the same time, serum concentration of cholesterol, triglycerides, total lipids, high density lipoprotein-cholesterol, low density lipoprotein and very low density lipoprotein cholesterol were estimated and revealed a higher level in older camel. Differences from data are discussed in relation to analytical methods and possible consequences for lipid digestion, lipid absorption, growth and brain development.

Key words: Camel milk, colostrum, transitional and mature milk, lipid fractions, α -tocopherols, fatty acid composition, serum lipid profile

INTRODUCTION

Studies on lipids and lipoproteins in domestic animals have made it clear that species variations exist and that even within species significant differences occur (Nazifi *et al.*, 2000). Physical and chemical properties of lipid dromedary camel milk were subject of many studies (Ali and Omar, 2001; Farah, 1993; Abu-Leihia, 1989; Sawaya *et al.*, 1984). The results showed that camel milk lipids have a lower proportion of short chain fatty acids and saturated fatty acids than cow milk.

With regard to macronutrients, the lipid fraction of milk seems to be of crucial importance. Under normal conditions it does not only provide the main source of energy but in addition contain fatty acids and fat-soluble vitamins that are essential to sustain normal growth and brain development (Walker, 1967).

With progress of lactation, triglycerides and percentage medium-chain fatty acids increased whereas α -tocopherols, cholesterol and percentage long-chain polyunsaturated fatty acids decreased (Rudy *et al.*, 1991). These changes reflect augmented *de novo* synthesis of fatty acids (8:0, 10:0, 12:0 and 14:0) in the mammary gland and a tendency of increasing fat-globule size as milk matures (Rudy *et al.*, 1991). Transitional and mature milks, but particularly colostrums, contained higher concentrations of components considered to be derived from the fat-globule membrane (cholesterol, tocopherols, percentage long-chain polyunsaturated fatty acids) compared with those reported for Western countries (Rudy *et al.*, 1991).

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In this study we present the composition of the lipid fractions in colostrums, transitional and mature camel milk. We measured the concentrations of milk triglycerides, cholesterol and tocopherols and determined the fatty acid concentration and composition of the total lipid fractions of milk. Also the concentration of serum lipids and lipoproteins during the first month of lactation were investigated and search to justify such dynamics of changes in relation to the specific needs of growing neonates.

MATERIALS AND METHODS

Milk Samples

Milk samples were collected from camels in Marsa Matrouh Farm (Animal Production Institute, Dokki, Egypt) at varying stages of lactation, samples were collected in the morning during winter. Samples (350 mL from each camel) were collected in polyethylene bottles and kept on ice during transportation to the laboratory where they were stored at 30°C until analyzed. All camels milk are taken during the 1st month of lactation at interval period, we had delivered at term between 0 day and 5 day (colostrums), 6 day and 10 day (transitional) and the rest milk till 30 day (mature) milk.

All samples were taken with supervision by manual expression and were collected in sterile containers without preservatives.

Fatty acid standards (as methyl esters) was obtained from Merck (Darmstadt, Germany) and triacylglycerols standard from Reagent Laboratories (London, England).

Extraction of Camel Milk Lipids

Lipids were extracted four times from milk using the method of Rose-Gottlieb as reported by (Pearson, 1976), using ammonia, methanol and diethyl ether. Lipids were dried in a vacuum oven for 1 h at 45°C. Total lipids were weighed after drying.

For the Stabilization of Tocopherols

Two hundred microliter sample was pipetted into a tube containing 0.5 mL antioxidant solution A (25 mmol potassium EDTA and 910 mmol vitamin C L⁻¹ water) and 1.3 mL antioxidant solution B (110 mmol pyrogallol and 250 mmol butylated hydroxyl toluene L⁻¹ methanol). The remaining milk was put into plastic tubes. All tubes were capped and frozen at -20°C until analyzed. When thawed, the contents of the tubes were carefully mixed to ensure representative sampling.

Fatty Acid Methyl Esters

The fatty acid methyl esters of milk of samples were prepared by the procedure of (Sheppard and Iverson, 1975). They were identified by their retention time in comparison with standards and were expressed as percent of total.

Gas-Chromatography

A Pye Unicam series 304 chromatography (Pye Unicam Ltd., UK) with flame ionization detector was used to analyse the fatty acid methyl esters in using 10% polyethylene glycol adipate column (PEGA, 2.5 m length, 4 mm diameter). The temperature of injector, column and detector were 190, 195 and 220°C, respectively. Nitrogen served as a carrier gas at a flow rate of 60 mL min⁻¹ while the flow rates of hydrogen and air were 60 and 480 mL min⁻¹, respectively. This condition was achieved for separation of fatty acid of triacylglycerols of camel milk and colostrums. The same results were obtained when we used temperature programming. Starting column temperature was 140°C and final temperature was 200°C with increase rate of 5°C per min (Steege *et al.*, 1987).

Cholesterol was determined by a separate capillary gas-chromatographic program as previously described by (Muskiet *et al.*, 1983). The internal standard for quantification was 5β-cholestan-3α-ol.

Tocopherol was quantified by high-performance liquid chromatography with fluorescence detection according to Lehman and Martin (1983) by using tocol as an internal standard.

Triglycerides was quantified by gas liquid chromatography as previously described by Stuart *et al.* (1968).

Blood Samples

Blood samples were collected from the jugular vein at the same time of milk collection. The serum was separated by centrifugation and stored at 20°C until used.

Biochemical Analysis of Serum

- Cholesterol, triglycerides, total lipids were estimated using commercial chemical reagent kits.
- HDL-cholesterol was measured by a precipitation method. In the first step, the precipitation reagent (sodium phosphotungstate with magnesium chloride) was added to the serum to aggregate non-HDL lipoproteins, which were sedimented by centrifugation, then the residual cholesterol was measured by an enzymatic method as described by Burtis and Ashwood (1994).
- LDL cholesterol was calculated as the difference between cholesterol in the precipitate and that in the HDL cholesterol.
- VLDL-cholesterol was estimated as one-fifth of concentration of triglycerides (Friedewald *et al.*, 1972).

Statistical Analysis

Data were analysed by one-way ANOVA and regression analysis, using Spss/Pc soft ware and Duncan's multiple range test was used to detect significance differences among the means (Norusis, 1993).

All values were expressed as mean±standard deviation with p≤0.05 being regarded as a significant result.

RESULTS

Relative saturated fatty acids content (Lauric acid (C12:0); Myristic acid (C14:0) and Stearic acid (C18:0)), increased with duration of lactation while Palmitic acid (C16:0) was decreased (Table 1).

The relative amount of the monounsaturated fatty acids (oleic acid (C18:1)-ω-9) was lower in mature than in transitional milk and colostrums. Among the two parent essential Polyunsaturated fatty acids Linoleic acid (18:2)-ω-6, was greater in transitional than in colostrums and in mature milk. While, α-Linolenic (18:3)-ω-3 was low in transitional than in colostrums and mature milk (Table 2).

Changes in percentages of fatty acids subgroups, As lactation progressed, there is changes in the relative amounts of individual fatty acids, relative saturated fatty acids content increased from colostrums till mature milk and there is a rise in medium chain fatty acids, at the same time there was a relative decline in long chain saturated fatty acid while, the relative amount of the major

Table 1: Changes in saturated fatty acid concentrations of camel's milk fat during the 1st month of lactation

Duration of lactation	C12:0 (Lauric)	C14:0 (Myristic)	C16:0 (Palmitic)	C18:0 (Stearic)
1st day	1.10	8.00	27.30	6.50
3rd day	0.90	7.85	27.00	6.50
5th day	0.95	6.75	26.50	6.20
7th day	1.35	8.30	26.00	8.00
10th day	2.05*	11.50**	22.50***	11.00****
15th day	1.55	10.00	22.00	7.70
21 day	1.60	10.00	20.90	7.00
30 day	4.50*	16.00**	22.50***	8.50

*Lauric acid show a significant increase in transitional milk then continuous increase till reach mature milk. LSD at 0.05 between colostrums and mature milk = 0.95; **Myristic acid show a significant increase in transitional milk and continuous increase till reach mature milk. LSD at 0.05 between colostrums and mature milk = 2.15; ***Palmitic acid show a significant decrease from colostrums till reach mature milk. LSD at 0.05 between colostrums and mature milk = 3.10; ****In Stearic acid, there is an increase till reach transitional milk then decrease again in mature milk. LSD between colostrums and transitional milk = 1.52

Table 2: Changes in polyunsaturated fatty acid concentrations of camel's milk fat during the 1st month of lactation

Duration of lactation	C18:1 Olic acid	C18:2 Linoleic	C18:3 α -Linolenic
1st day	28.50	5.20	4.50
3rd day	26.70	7.50	4.60
5th day	24.50	7.50	4.30
7th day	20.00***	7.00**	4.00
10th day	19.50***	5.10	3.90*
15th day	17.50	4.80	3.91
21 day	15.90	4.90	3.95
30 day	13.70***	4.00	4.75ns

***Olic acid there is a significant decrease from colostrums till transitional milk and then continuous decrease till mature milk. LSD at 0.05 between colostrums and mature milk = 7.50; **In Linoleic acid show a significant increase from colostrums till reach transitional milk and return decrease till reach mature milk. LSD at 0.05 between colostrums and transitional milk = 1.12; * α -Linolenic there is a slightly decrease from colostrums till transitional milk then increased again in case of mature milk

Table 3: Composition (g/100 g) of main fatty acid subgroups in colostrums, transitional and mature milk samples of camel's milk

Fatty acids	Colostrums at day 5	Transitional milk at day 10	Mature milk at day 30	LSD at 0.05
SAFAs	42.90±4.70	47.05±5.10	51.50±6.20***	2.75
MCASFAs(12:0-14:0)	9.10±6.50	13.55±1.40	20.50±1.50***	3.23
LCASFAs(16:0-18:0)	33.80±3.50	33.50±2.90	31.00±2.60**	1.50
PUFAs(18:1-18:2-18:3)	38.20±3.40	28.50±2.20	22.45±1.00***	4.15
Omega 6(18:2) ω -6	7.50±0.65	5.10±0.49	4.50±0.43**	1.42
Omega 3(18:3) ω -3	4.00±0.36	3.90±0.32	4.75±0.45**	0.34

***: Highly significant increase in mature milk in case of SAFAs (saturated fatty acids) and MCFAs (medium chain fatty acids) but decrease in LCFAs (long chain fatty acids) and PUFAs (polyunsaturated fatty acids) than in colostrums. Omega 6 show: Significant decrease but omega 3 show: Significant increase in mature milk, **: Moderate significant increase

Table 4: Concentrations of triglycerides, total cholesterol and α -tocopherol in camel's milk during the 1st month of lactation

Duration of lactation	Triglycerides (TG) (mmol L ⁻¹)	Cholesterol (mmol L ⁻¹)	α -Tocopherol (μ mol L ⁻¹)
1st day	15.5±1.4	1.15±0.900	60.5±5.24
3rd day	15.7±1.5	1.09±0.700	60.1±5.70
5th day	16.1±1.6	1.09±0.920	56.5±4.52
7th day	16.0±1.4	1.00±0.750	40.3±3.95
10th day	24.5±2.2	0.75±0.060	37.5±3.22
15th day	27.3±2.5	0.63±0.050	35.5±3.10
21 day	30.5±2.9	0.56±0.045	30.5±2.94
30 day	35.7±3.2***	0.49±0.043***	26.2±2.23***

***: Highly significant increase in mature milk in case of triglycerides but decrease in cholesterol and α -tocopherol in mature milk than in colostrums; The ratio of cholesterol to triglycerides = 7.42 in colostrums and 1.37 in mature milk

Table 5: Concentrations of total cholesterol, triglycerides, total lipid, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL) cholesterol in camels serum during the 1st month of lactation

Duration of lactation	Cholesterol (mmol L ⁻¹)	Triglycerides (mmol L ⁻¹)	Total lipids (g L ⁻¹)	HDL (mmol L ⁻¹)	LDL (mmol L ⁻¹)	VLDL (mmol L ⁻¹)
1st day	0.90±0.050	0.35±0.030	2.29±0.21	0.32±0.029	0.26±0.021	0.15±0.012
3rd day	0.95±0.060	0.38±0.035	2.60±0.25	0.35±0.030	0.26±0.024	0.14±0.013
5th day	0.97±0.070	0.39±0.037	2.75±0.29	0.33±0.031	0.24±0.022	0.14±0.011
7th day	0.98±0.080	0.40±0.040	3.00±0.30	0.37±0.036	0.25±0.023	0.15±0.014
10th day	0.99±0.090	0.44±0.045	3.60±0.34	0.35±0.032	0.23±0.021	0.16±0.014
15th day	0.99±0.085	0.46±0.044	3.81±0.35	0.40±0.038	0.24±0.023	0.19±0.017
21 day	1.18±0.100	0.48±0.043	3.80±0.36	0.35±0.032	0.25±0.020	0.20±0.019
30 day	1.20±0.110*	0.50±0.05*	3.91±0.38*	0.39±0.037*	0.29±0.016*	0.23±0.020*
LSD at 0.05	0.10	0.08	0.90	0.02	0.01	0.05

*: Slight increase in mature milk than in colostrum

polyunsaturated fatty acids was lower. Among the two parent essential polyunsaturated fatty acids the percentage of linoleic acid (18:2 ω -6) was lower from colostrums till mature milk whereas that of linolenic acid (18:3 ω -3) was slightly higher from colostrums to mature milk (Table 3).

A significant increase in concentration of triglycerides in mature milk than in colostrums but free cholesterol and α -tocopherol show a significant decrease in mature milk than colostrums (Table 4).

On the same time, an increase on serum concentration in cholesterol, triglycerides, total lipids, high density lipoprotein-cholesterol, low density lipoprotein and very low density lipoprotein cholesterol in older animals (Table 5).

DISCUSSION

Total Fatty Acid Composition

Data presented in Table 1 and 3 show that, mature milk had higher amounts of saturated fatty acids than colostrums as a result of elevated levels of 12:0-14:0 and 16:0 fatty acids in milk, this results are similar to Ali and Omar (2001). Even numbered saturated fatty acids 14:0-16:0-18:0 in camel milk lipid consider as the major components of total fatty acids. The presence of medium-chain fatty acids in camel milk could indicate their synthesis in the mammary gland. These results are similar to those reported by Ali and Omar (2001), Abou-Lehia (1989) and Sawaya *et al.* (1984). Camels are capable of producing these saturated fatty acids by cellulose fermentation in the rumen (Ali and Omar, 2001; Kurtz, 1974).

Koiter *et al.* (1989) revealed that, increased *de novo* synthesis of MCFAs (12:0-14:0) by the lactating cell implies an enhanced flux of glucose over the basal plasma membrane, this may be induced by an augmented responsiveness of the mammary gland to insulin after gestation, which may be due to an increasing number of insulin receptors on the mammary gland. These saturated fatty acids constitute an energy source that is independent from bile acids for its absorption (Thomposon *et al.*, 1985; Bach and Babayan, 1982; Insull, 1965). Ingestion of large amount of MCFAs (by neonate) induces excessive β -oxidation and subsequently a superfluous synthesis of ketone bodies (Mortensen and Gregersen, 1980). As a consequence, MCFAs may become ω -oxidized which eventually leads to a waste of energy-rich dicarboxylic acids. Furthermore, a relatively high energy intake from MCFAs may enhance the absorption of calcium, magnesium (Tantibhedhyangkul and Hashim, 1978) and amino acids (Holtzapple *et al.*, 1972). All these factors may contribute to an increased speed of growth during the first month of life. This result are similar to those of dromedary camel milk reported by Zhang *et al.* (2005), Gorban and Izzeldin (2001), Abu-Lehia (1989) and Sawaya *et al.* (1984).

The major polyunsaturated fatty acids were 18:1, 18:2 and 18:3 (Table 2 and 3). The unsaturated fatty acids of colostrums 18:1 was higher than the corresponding milk values and this could account for the higher total unsaturated fatty acids in triacylglycerols of the colostrums when compared to that milk, this result agree with Ali and Omar (2001). 18:2 and 18:3 increase in colostrums which decrease lipogenesis and esterification, Also it increase oxidation of fatty acids in liver and increase gluconeogenesis in ruminant hepatocytes which help in growing neonates.

According to Gnan and Sheriha (1986), camel's milk fat contained high levels of polyunsaturated fatty acids and factors that affect the fatty acids composition of camel milk include breed, feeding, composition of dietary fat, dietary protein, seasonality and region and stage of lactation (Gorban and Izzeldin, 2001; Palmquist *et al.*, 1993). This result agree with Zhang *et al.* (2005) and Ali and Omar (2001).

Lipid Classes and Fat Globules

Results of present study, show notable changes in lipid fractions of milk with duration of lactation, this result agree with Gorban and Izzeldin (1999) and Ali and Omar (2001). Triglyceride

concentrations were lower in colostrums than in mature milk, which coincided with a decrease of cholesterol and the percentage of LC PUFAs ($\omega 3$ and $\omega 6$) (Table 4). As a consequence, the ratio of cholesterol to triglycerides = 7.42 in colostrums and 1.37 in mature milk. The core of the fat globule in milk is mainly comprised of triglyceride whereas its membrane contains the majority of milk cholesterol and phospholipids, the latter contain an abundance of long chain polyunsaturated fatty acids, which useful for the growth of the neonates (Ali and Omar, 2001; Muskiet *et al.*, 1988; Harzer *et al.*, 1983; Ruegg and Blanc, 1981).

While, cholesterol and tocopherol were increased in colostrums than in mature milk in present study, this result was similar to that reported by Rudy *et al.* (1991). Assuming that the vast majority of vitamin E is secreted as a constituent of the fat-globule membrane, a decline of the vitamin E in mature than in colostrums milk may further support this concept. Another explanation is a higher contribution of constituents from white cell membranes during early lactation (Rudy *et al.*, 1991).

Serum Lipid and Lipoprotein Profile

Age had a significant effect on the serum concentration of cholesterol, triglycerides, total lipids, high density lipoprotein-cholesterol and very low density lipoprotein cholesterol of camel (Table 5) the values were being higher in older animals. The concentration of serum cholesterol of present study was similar to the values reported by Nazifi *et al.* (2000), Al-Ani *et al.* (1992) and Wasfi *et al.* (1987). It was lower than the values reported for cows, sheep goats and llamas (Kaneko, 1989). The concentration of triglyceride in the serum of present study was lower than the value reported by Nazifi *et al.* (2000) but similar than the result reported by Wasfi *et al.* (1987). The concentration of lipoprotein (HDL, LDL and VLDL) in the serum of present study is similar to the result of Nazifi *et al.* (2000) in Iranian male dromedary.

Braemwald (1995) and Kleinveld (1996) reported that in human, there was a slight increase in the concentrations of serum cholesterol and triglycerides in advanced age (Noro *et al.*, 1993) found a high concentration of low density lipoprotein and a low concentration of high density lipoprotein in calves immediately after birth. By 6 days of age, low density lipoprotein concentration had decreased and the high density lipoprotein concentration had increased to the levels found in adult animals (Noguchi, 1993) reported that, in humans, the concentrations of low density lipoprotein and very low density lipoprotein increased and the concentration of high density lipoprotein decreased with increasing age (Hugi and Blum, 1997) reported that, in calves the concentration of serum cholesterol increased transiently with age, but serum triglycerides did not show a consistent change.

In this study, significant correlations were observed between the serum cholesterol and the triglycerides and various lipoproteins.

CONCLUSIONS

Present data indicate that changes occurring in camel milk during the 1st month of lactation is a useful nutritional attribute since saturated fatty acids are rapidly metabolized by camel tissue before they have a chance to be excreted in the milk and constitute an energy source and induces excessive β -oxidation and subsequently a superfluous synthesis of ketone bodies and may enhance the absorption of calcium, magnesium and amino acids. Also, it appears that biohydrogenation of polyunsaturated fatty acids is less extensive in the rumen of the camel and polyunsaturated fatty acids was high in colostrums of camel milk, All these factors may contribute to be the specific needs of growing neonates and increased speed of growth during the first month of life.

In addition, the study show that age had a significant effect on serum concentration of cholesterol, triglycerides, total lipids, high density lipoprotein-cholesterol and very low density lipoprotein cholesterol of camel, the values being higher in older animals.

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