

*Full Length Research Paper*

# Concentrations of conjugated linoleic acids in milk and tissues from single-humped Arabian camel (*Camelus dromedaries*) kept under intensive standardized management

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The present study aimed to determine the amounts of two CLA isomers, c9t11-CLA and t10c12-CLA, in products from *Camelus dromedarius* and, to compare their CLA levels with those reported in the literature for true ruminants. Weight percentages of c9t11-CLA and t10c12-CLA in different camel products were in the range between 0.37 to 0.80 and 0.04 to 0.14% of total fatty acid methyl esters, respectively, with the highest values found in liver, milk and meat, and the lowest found in perirenal and hump adipose tissue. Comparison of camel data with literature data for true ruminants indicates that no major differences exist between products from *C. dromedarius* and true ruminants with respect to their CLA content.

**Key words:** Conjugated linoleic acid, camel, milk, meat.

## INTRODUCTION

Conjugated linoleic acid (CLA) isomers are a naturally occurring group of positional and geometrical isomers of linoleic acid (LA; c9c12-18:2) characterized by the presence of conjugated double bonds (Steinhart et al., 2003). CLA are biologically highly active compounds which have attracted great scientific interest due to several beneficial properties. Such properties include inhibitory effects on atherogenesis, carcinogenesis, inflammation, allergic sensitization, obesity, and diabetes (Eder and Ringseis, 2010). The most important sources of CLA in the human diet are products from ruminants (milk, dairy products, meat) (Chin et al., 1992; Steinhart et al., 2003). This is due to the fact that CLA isomers are produced in the rumen during microbial biohydrogenation of dietary polyunsaturated fatty acids and in tissues through  $\Delta 9$ -desaturation of the rumen-derived trans-vaccenic acid (t11-18:1) (Griinari et al., 2000; Corl et al., 2001). The predominant CLA isomer in ruminant-derived products is c9t11-CLA, contributing to more than 90% of

total CLA (Steinhart et al., 2003), and it is now accepted that endogenous synthesis by  $\Delta 9$ -desaturation contributes most to this CLA isomer in ruminant-derived products (Palmquist et al., 2004). Since  $\Delta 9$ -desaturation of fatty acids also occurs in tissues of single-stomached species, non-ruminant-derived meat also contains CLA, but at a much lower content (Fritsche and Steinhart, 1998; Kuhnt et al., 2006).

In addition to natural foodstuffs, dietary CLA supplements can also contribute to CLA intake in humans. Such products differ from natural foodstuff in that they contain high percentages of t10c12-CLA (up to 50% of total CLA) (Steinhart et al., 2003), whereas this CLA isomer is only a minor component in dairy products or meat. According to recent studies, average daily intakes of CLA in Europe and the US are estimated to be in the range of 100 to 400 mg (Fritsche and Steinhart, 1998; Wolff and Precht, 2002; Ritzenthaler et al., 2001). In contrast to Europe and the US, where products from cattle, and, to a lesser extent, from sheep and goat are the most important ruminant-derived products in the human diet, products from other ruminants like camels also contribute to human diet in arid and semi-arid areas

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of the world, in particular Africa and the Middle East. Camel milk is a very important part of the diet of nomadic people in these areas because other milk-producing species would either give not sufficient milk or would not survive in this arid climate (Schwartz, 1992). However, camel milk is also produced in dairy farms in North African countries and can therefore be provided to the general public on a larger scale (Schwartz and Walsh, 1992). Other camel products like meat and hump adipose tissue are usually rarely consumed among nomadic people, and only consumed occasionally in critical periods of food shortage or for ritual and sacrificial purposes (Dahl and Hjort, 1976). However, a considerable number of camels are bred specifically for slaughter in East African countries, from which camels are exported to North African countries including Libya, Egypt, Saudi Arabia and Gulf States (Schwartz and Walsh, 1992) for the production of meat and meat products.

Although camel is not a true ruminant (the omasum is lacking in Tylopoda with the single family Camelidae), it has been shown that biohydrogenation by its rumen microflora leads to the same trans-18:1 isomers including the precursor of c9t11-CLA, vaccenic acid, in approximately the same proportions as in true ruminants (Ruminantia species), like cattle (Wolff et al., 2001). This suggests that concentrations of CLA isomers in products originating from Tylopoda species are similar to those from Ruminantia species like *Bos*, *Ovis* and *Capra*. The available literature, however, shows that products from Tylopoda species have not yet been examined with regard to the presence of CLA isomers. Therefore, the aims of the present study were, first, to determine the amounts of two CLA isomers, c9t11-CLA and t10c12-CLA, in different products (milk, hump, meat, liver, perirenal adipose tissue) from the single-humped Arabian camel (*Camelus dromedarius*), and second, to compare the CLA levels found in camel products with those reported in the literature for true ruminants.

## MATERIALS AND METHODS

### Sample collection

Ten individual samples each of different camel products including 0.5 L milk and 0.5 kg each of hump, meat and liver, and 0.25 kg perirenal adipose tissue were purchased on five different market places in Tripoli (Libya). According to the information from the salespersons, the camel products were only obtained from camels kept in local dairy farms under intensive standardized management in the near of Tripoli. The samples were immediately placed on ice and transported to the Reference Laboratories for Food Analysis, Biotechnology Research Center in Tripoli (Libya). Samples were stored at -20°C pending analysis.

### Lipid extraction

Within 1 week post sampling total lipids of milk, hump, meat, liver, and perirenal fat tissue were extracted with a mixture of hexane and

isopropanol (3:2 v/v) (Hara and Radin, 1978). For lipid extraction, 100 mg of milk and 500 mg of each tissue sample were thoroughly minced with a scalpel on an ice cold glass plate and weighed into a test tube. Subsequently, the hexane and isopropanol-mixture was added to the test tubes, the test tubes were sealed with a Teflon-coated screw cap, and agitated using a G 25 Incubator Shaker (New Brunswick Scientific, Edison, New Jersey, USA) at 250 rpm at room temperature. After 18 h, lipid extracts were transferred into brown glass vials, sealed with a cap and stored at -20°C pending fatty acid analysis.

### Fatty acid analysis

Following total lipid extraction, lipids from 1 ml of the extracts were dried under a stream of nitrogen and methylated with trimethylsulfonium hydroxide (Butte, 1983). Fatty acid methyl esters (FAME) were separated by gas chromatography, using an Agilent 6890 N system (Santa Clara, CA, United States) equipped with an automatic split injector, a polar capillary column (HP-INNOWax 19091 N-133; 30 m, 0.25 mm internal diameter, 0.25 µm film thickness; HP-INNOWax/Agilent), and a flame ionization detector. Hydrogen was used as the carrier gas with a flow rate of 1 ml/min. FAME was identified by comparing their retention times with those of individually purified standards.

### Statistical analysis

Means and standard deviations (SD) were calculated from values of individual samples using the Minitab Statistical Software Release 13.0 (Minitab, State College, PA, USA). Differences in fatty acid concentrations between tissues were analyzed by one-way ANOVA. All data presented are means ± SD for n = 10 samples.

## RESULTS AND DISCUSSION

As demonstrated in Table 1, the most abundant fatty acids in lipids from camel products were oleic acid (1), palmitic acid (2), stearic acid (3), and, with the exception of the liver, myristic acid (4). In the liver but also in muscle, relatively high levels of linoleic acid were found which is attributable to their higher percentage of phospholipids in total lipids than in the other tissues where triglycerides are the predominant lipids. Considering literature data on milk fatty acid composition from true ruminants (Khanal and Olson, 2004), it is apparent from Table 1 that especially the milk fatty acid composition differs greatly between camel and true ruminants. Camel milk is characterized by lower proportions of short-chain and medium-chain (C4 to C14) fatty acids but greater proportions of unsaturated fatty acids like oleic and palmitoleic acid compared with sheep, goat or cow. The main finding of the present study is that CLA isomers are present in camel products. As shown in Table 1, weight percentages of c9t11-CLA, which is the most abundant CLA isomer in lipids from true ruminants, in different camel products were in the range between 0.37 and 0.80% of total FAME, with the highest values found in liver and milk (0.87 and 0.86% of total FAME, respectively), and the lowest observed in perirenal and hump adipose tissue (0.43 and 0.38% of

**Table 1.** Fatty acid composition of total lipids of different products from *Camelus dromedarius*.

Fatty acid	Milk	Perirenal fat	Hump	Muscle	Liver
	g/100 g total FAME				
8:0	0.35 ± 0.15 <sup>c</sup>	0.21 ± 0.04 <sup>c</sup>	0.26 ± 0.14 <sup>c</sup>	1.80 ± 0.46 <sup>a</sup>	0.72 ± 0.19 <sup>b</sup>
10:0	0.27 ± 0.12 <sup>c</sup>	0.13 ± 0.02 <sup>d</sup>	0.13 ± 0.08 <sup>d</sup>	0.48 ± 0.16 <sup>b</sup>	1.27 ± 0.39 <sup>a</sup>
11:0	0.14 ± 0.06 <sup>c</sup>	<0.1 <sup>d</sup>	<0.1 <sup>d</sup>	1.48 ± 0.42 <sup>a</sup>	0.58 ± 0.23 <sup>b</sup>
12:0	3.11 ± 1.09 <sup>ab</sup>	0.49 ± 0.06 <sup>d</sup>	2.10 ± 0.32 <sup>b</sup>	4.27 ± 1.39 <sup>a</sup>	1.32 ± 0.42 <sup>c</sup>
13:0	0.58 ± 0.17 <sup>a</sup>	<0.1 <sup>b</sup>	0.43 ± 0.11 <sup>a</sup>	<0.1 <sup>b</sup>	<0.1 <sup>b</sup>
14:0	12.0 ± 1.8 <sup>a</sup>	7.3 ± 0.7 <sup>b</sup>	6.7 ± 1.5 <sup>b</sup>	7.9 ± 1.6 <sup>b</sup>	4.7 ± 0.8 <sup>c</sup>
c9-14:1	1.70 ± 0.52 <sup>a</sup>	0.14 ± 0.03 <sup>c</sup>	0.32 ± 0.20 <sup>b</sup>	0.27 ± 0.12 <sup>b</sup>	0.34 ± 0.12 <sup>b</sup>
15:0	0.75 ± 0.06 <sup>b</sup>	0.81 ± 0.32 <sup>b</sup>	0.80 ± 0.23 <sup>b</sup>	0.58 ± 0.28 <sup>b</sup>	1.77 ± 0.58 <sup>a</sup>
c9-15:1	0.22 ± 0.04	0.23 ± 0.05	0.25 ± 0.05	0.39 ± 0.14	0.23 ± 0.06
16:0	22.8 ± 1.5 <sup>a</sup>	24.8 ± 2.0 <sup>a</sup>	24.1 ± 2.6 <sup>a</sup>	19.1 ± 2.6 <sup>b</sup>	24.2 ± 3.5 <sup>a</sup>
c9-16:1	8.30 ± 1.21 <sup>a</sup>	3.71 ± 0.67 <sup>b</sup>	3.11 ± 1.03 <sup>b</sup>	3.31 ± 0.63 <sup>b</sup>	2.74 ± 0.50 <sup>b</sup>
17:0	0.55 ± 0.09 <sup>b</sup>	2.52 ± 0.48 <sup>a</sup>	2.92 ± 1.16 <sup>a</sup>	0.83 ± 0.25 <sup>b</sup>	2.06 ± 0.34 <sup>a</sup>
c9-17:1	0.76 ± 0.07	0.87 ± 0.15	1.17 ± 0.30	0.93 ± 0.14	0.96 ± 0.12
18:0	13.83 ± 1.70 <sup>b</sup>	18.02 ± 3.16 <sup>a</sup>	16.24 ± 3.06 <sup>ab</sup>	13.45 ± 2.67 <sup>b</sup>	16.12 ± 1.95 <sup>ab</sup>
c9-18:1	29.4 ± 2.3 <sup>b</sup>	37.4 ± 2.5 <sup>a</sup>	37.8 ± 4.5 <sup>a</sup>	31.5 ± 3.6 <sup>b</sup>	25.1 ± 4.7 <sup>c</sup>
18:2n-6	2.83 ± 0.33 <sup>b</sup>	1.41 ± 0.29 <sup>c</sup>	1.71 ± 0.57 <sup>c</sup>	7.89 ± 1.49 <sup>a</sup>	7.15 ± 0.98 <sup>a</sup>
18:3n-6	<0.1 <sup>c</sup>	0.73 ± 0.29 <sup>a</sup>	0.68 ± 0.32 <sup>a</sup>	0.28 ± 0.10 <sup>b</sup>	0.25 ± 0.11 <sup>b</sup>
18:3n-3	0.20 ± 0.03 <sup>b</sup>	0.20 ± 0.08 <sup>b</sup>	0.14 ± 0.06 <sup>b</sup>	0.71 ± 0.28 <sup>a</sup>	0.66 ± 0.23 <sup>a</sup>
c9t11-CLA	0.80 ± 0.15 <sup>a</sup>	0.39 ± 0.14 <sup>bc</sup>	0.37 ± 0.15 <sup>bc</sup>	0.50 ± 0.16 <sup>b</sup>	0.74 ± 0.18 <sup>ab</sup>
t10c12-CLA	0.06 ± 0.02 <sup>c</sup>	0.04 ± 0.02 <sup>c</sup>	0.05 ± 0.02 <sup>c</sup>	0.11 ± 0.03 <sup>b</sup>	0.14 ± 0.05 <sup>a</sup>
20:0	0.20 ± 0.03 <sup>c</sup>	0.13 ± 0.05 <sup>d</sup>	0.12 ± 0.03 <sup>d</sup>	0.81 ± 0.29 <sup>b</sup>	1.94 ± 0.31 <sup>a</sup>
c11-20:1	0.22 ± 0.04 <sup>c</sup>	0.11 ± 0.04 <sup>d</sup>	0.10 ± 0.05 <sup>d</sup>	0.32 ± 0.12 <sup>b</sup>	0.71 ± 0.17 <sup>a</sup>
20:2n-6	<0.1 <sup>c</sup>	0.11 ± 0.05 <sup>b</sup>	0.10 ± 0.06 <sup>b</sup>	<0.1 <sup>c</sup>	4.45 ± 0.41 <sup>a</sup>
21:0	0.18 ± 0.09 <sup>b</sup>	<0.1 <sup>c</sup>	<0.1 <sup>c</sup>	2.25 ± 0.69 <sup>a</sup>	<0.1 <sup>c</sup>
20:3n-6	<0.1 <sup>b</sup>	<0.1 <sup>b</sup>	<0.1 <sup>b</sup>	0.63 ± 0.17 <sup>a</sup>	0.54 ± 0.13 <sup>a</sup>
20:4n-6	0.24 ± 0.09 <sup>a</sup>	<0.1 <sup>b</sup>	<0.1 <sup>b</sup>	<0.1 <sup>b</sup>	0.19 ± 0.11 <sup>a</sup>
20:3n-3	<0.1 <sup>c</sup>	<0.1 <sup>c</sup>	0.13 ± 0.11 <sup>b</sup>	0.32 ± 0.19 <sup>a</sup>	<0.1 <sup>c</sup>
20:5n-3	<0.1 <sup>b</sup>	<0.1 <sup>b</sup>	<0.1 <sup>b</sup>	0.43 ± 0.28 <sup>a</sup>	0.38 ± 0.14 <sup>a</sup>
22:1	0.24 ± 0.13 <sup>a</sup>	<0.1 <sup>c</sup>	0.10 ± 0.07 <sup>b</sup>	<0.1 <sup>c</sup>	<0.1 <sup>c</sup>
24:0	0.25 ± 0.12 <sup>b</sup>	0.11 ± 0.06 <sup>c</sup>	0.11 ± 0.08 <sup>c</sup>	<0.1 <sup>c</sup>	0.54 ± 0.19 <sup>a</sup>
24:1	<0.1 <sup>c</sup>	0.13 ± 0.09 <sup>b</sup>	<0.1 <sup>c</sup>	0.52 ± 0.37 <sup>a</sup>	0.43 ± 0.23 <sup>a</sup>
22:6n-3	<0.1 <sup>b</sup>	<0.1 <sup>b</sup>	<0.1 <sup>b</sup>	<0.1 <sup>b</sup>	0.38 ± 0.09 <sup>a</sup>
SFA	55.0 ± 3.0	54.5 ± 2.9	54.0 ± 5.0	52.4 ± 3.3	55.1 ± 4.9
MUFA	40.8 ± 2.8 <sup>a</sup>	42.4 ± 3.0 <sup>a</sup>	42.8 ± 4.7 <sup>a</sup>	37.1 ± 4.1 <sup>ab</sup>	30.4 ± 4.4 <sup>b</sup>
PUFA	4.26 ± 0.54 <sup>c</sup>	3.04 ± 0.56 <sup>c</sup>	3.39 ± 0.95 <sup>c</sup>	10.5 ± 1.5 <sup>b</sup>	14.4 ± 1.3 <sup>a</sup>

Data represent mean ± SD for n = 10 individual samples. Means with different superscript letters differ (P < 0.05). SFA = short-chain fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

total FAME, respectively). Weight percentages of c9t11-CLA in meat were between those in liver and muscle and those in adipose tissues. These levels of c9t11-CLA are in agreement with those reported for products from true ruminants.

For instance, the c9t11-CLA content in milk, meat and adipose tissue from cattle was found to vary in a wide range of 0.3 to 3.3% of fat (White et al., 2001; Lawless et al., 1998; Kelly et al., 1998; Kelsey et al., 2003), 0.1 to 1.5% of fat (Gillis et al., 2004; Poulson et al., 2004), and

0.3 to 1.7% of fat (Gillis et al., 2004; Poulson et al., 2004), respectively. Similar as in cows, c9t11-CLA content was reported to vary greatly in milk from goats, 0.58 to 1.1% of fat (Parodi, 1999) and milk, 0.8 to 3.0% of fat (Parodi, 1999; Luna et al., 2005), meat, 0.1 to 1.0% of fat (Santercole et al., 2007; Bolte et al., 2002; Khanal and Olson, 2004) and adipose tissue from sheep, 1.7% of fat (Khanal and Olson, 2004). The great variation in c9t11-CLA content in milk and tissues from ruminants has been primarily explained by diet related factors; e.g. c9t11-CLA

**Table 2.**  $\Delta 9$ -desaturase indices calculated from weight percentages of fatty acids in milk lipids from *Camelus dromedarius*.

$\Delta 9$ -Desaturase index	Mean <sup>1</sup>	SD <sup>1</sup>	CV <sup>1</sup>
c9-14:1/14:0	0.14	0.03	21.4
c9-16:1/16:0	0.36	0.04	11.1
c9-17:1/17:0	1.42	0.24	16.9
c9-18:1/18:0	2.16	0.40	18.5

<sup>1</sup>Data represent mean, SD, and CV for n = 10 individual milk samples.

content in milk and tissues of cows is low in high-performance cows, fed concentrate-rich rations but markedly increased as a result of grazing on pasture (Kelly et al., 1998; Kay et al., 2004), and supplementing total mixed rations with either plant oils or oil seeds (Madron et al., 2002). These feeding strategies increase c9t11-CLA content in tissues mainly through increased formation of t11-18:1 in the rumen, which is absorbed and subsequently converted to c9t11-CLA in tissues via the action of  $\Delta 9$ -desaturase. Since the camel samples used in this study originated from camels kept under North African dairy production systems with a standardized feeding management system (Schwartz and Walsh, 1992), where all animals are offered a similar ration based on wheat straw, oat hay, meadow hay and concentrates, it is not surprising that the variation in c9t11-CLA content in milk, meat, and other tissues among the individual camel samples was small.

Besides diet related factors, animal-to-animal variation is also considered to be an important factor explaining the large variation in ruminant milk and tissue c9t11-CLA content. Given that endogenous synthesis of CLA is the major source of CLA (Palmquist et al., 2004), differences in the activity of  $\Delta 9$ -desaturase, which was shown to vary over 3-fold among individual cows (Kelsey et al., 2003; White et al., 2001), has been made responsible for the great variation in milk and tissue c9t11-CLA content among individual cows. For evaluation of inter-animal variation in  $\Delta 9$ -desaturase activity in mammary gland tissue, which is known to possess a high activity of this enzyme, we calculated different  $\Delta 9$ -desaturase activity indices; that is the product/substrate ratios for  $\Delta 9$ -desaturase in milk lipids. As shown in Table 2, the variation in  $\Delta 9$ -desaturase indices between different milk samples was very small being indicative of low inter-individual differences in tissue  $\Delta 9$ -desaturase activity, which likely also contributes to the small variation in c9t11-CLA content among the camel samples investigated. The t10c12-CLA content in camel lipids, like in lipids from true ruminants (Kraft et al., 2003; Piperova et al., 2000; Peterson et al., 2003; Gillis et al., 2004; Poulson et al., 2004), was generally very low ranging between 0.04 and 0.14% of total FAME. As observed for c9t11-CLA, the lowest values were found in perirenal and

hump adipose tissue (0.04 and 0.05%, respectively), followed by milk (0.06%) and muscle (0.11%). The highest weight percentage of t10c12-CLA was observed in the liver (0.14%).

These levels of t10c12-CLA found in camel products are quite similar to those reported for true ruminants. For instance, weight percentages of t10c12-CLA in muscle (longissimus dorsi) of beef cattle and bison were reported to be in the range of 0.01 to 0.12% (Rule et al., 2002; Poulson et al., 2004). Weight percentages of t10c12-CLA in different adipose tissue depots of beef cattle were found to be in the range of 0.01 to 0.05% (Gillis et al., 2004; Poulson et al., 2004). In milk, weight percentages of t10c12-CLA were reported to be approximately 0.01% of total FAME in sheep (Luna et al., 2005), and to vary in the range of 0.01 and 0.1% of total fatty acids in cows (Piperova et al., 2000; Peterson et al., 2003). In contrast to c9t11-CLA, t10c12-CLA in milk and tissues is considered to be exclusively formed in the rumen from polyunsaturated fatty acids via the action of a specific microbial isomerase. Endogenous formation of t10c12-CLA in tissues from the trans-fatty acid t10-18:1, which is formed in the rumen and absorbed from the intestine, is not possible due to the lack of  $\Delta 12$ -desaturase in mammals. The great variation in t10c12-CLA content observed in cow products has been explained by diet related factors, e.g. the milk fat content of t10c12-CLA markedly increases as a result of feeding a milk fat depressing diet (high concentrate/low forage diet, total mixed ration supplemented with plant oils or fish oils) (Bauman and Griinari, 2003; Piperova et al., 2000; Abu-Ghazaleh et al., 2002). Since the variation in t10c12-CLA content was small in the camel products investigated, we suggest that the camels from which the samples were obtained were fed similar rations based on typical North African dairy production standards.

Collectively, the comparison of the present data with data for true ruminants indicates that no major differences exist between products from *C. dromedarius* and true ruminants with respect to their CLA content. Similar observations have been made earlier regarding the proportions and the spectrum of trans- and cis-18:1 fatty acids, which are typical rumen biohydrogenation products (Wolff et al., 2001). Although the present study has the limitation that the sample size was relatively small and there were some experimental uncertainties (e.g., age and sex of the camels were unknown), our observations are supportive of the assumption that the rumen microflora is quite similar between Tylopoda and Ruminantia species (Wolff et al., 2001), despite the fact that these two suborders have evolved profound differences in anatomy, morphology, physiology and dietary habits since the Eocene (ca. 50 million years). Regarding that camel products are part of the diet in the North African population, we suggest that consumption of camel products, particularly those which are rich in fat (hump), contributes to CLA uptake in these countries.

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