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Donkey's milk detailed lipid composition

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1. ABSTRACT

Donkey's milk (DM) has recently aroused scientific interest, above all among paediatric allergologists. A deeper knowledge of both proteins and fats in donkey's milk is necessary to evaluate the immunological, physiological and nutritional properties. By using the most refined techniques for fatty acids analysis, the paper offers a detailed comparative analysis of the lipid fractions of DM as well as of human and cow milk, also indicating the distribution of fatty-acid moieties among sn-1/3 and sn-2 positions of the glycerol backbone. In DM the position of fatty acids on glycerol backbone, above all of long chain saturated fatty acids, is very similar to that of human milk: this fact, in conjunction with the relatively high contents of medium-chain triglycerides, makes the lipids in DM, through quantitatively reduced, highly bioavailable. The high PUFA n-3 content of donkey's milk, and especially its low n-6/n-3 ratio, acquires particular interest in subjects affected by cow's milk protein allergy. Whole DM might also constitute the basis forformulas suitable for subjects in the first year of life.

2. INTRODUCTION

The need to use natural food products that safeguard biodiversity and that have an application in the clinical and nutritional fields is strongly felt by many consumers.

In recent years the interest in donkey milk (DM) has considerably increased above all among paediatric allergologists and nutritionists. DM hypoallergenic properties have recently been demonstrated in 38 of 46 highly problematic and pluriallergic children (83% tolerability) (1). A similar figure (88% tolerability) was reported by Vita *et al.* (2) in a study assessing the tolerability and clinical effect of DM compared with goat milk in a single-blind, controlled, randomised study on twenty-eight cow's milk protein (CMP) allergic children with atopic dermatitis.

Beside protein content, the essential fatty acids (EFA) composition of foods, with special emphasis on the ratio of omega-6/omega-3 EFA, has also been shown to have a role in inflammatory and autoimmune diseases (3).

The knowledge of the composition of milk lipids, in terms of triacylglycerols (TAG), is fundamental in order to explain the nutritive and organoleptic features of milk. Moreover, information concerning the distribution of fatty-acid moieties among sn-1, sn-2 and sn-3 positions of the glycerol backbone, in addition to the fatty-acid composition, is of great importance from the biochemical and nutritional standpoints (4).

Although several studies have been published on the composition and the physico-chemical and nutritional properties of DM protein components (5-7), only a few have been published on the characterisation of its lipid fraction (8). Thus, a deeper knowledge of fats in DM is necessary to better understand the immunological, physiological and nutritional properties of DM. By using the most refined techniques for fatty acids analysis, the paper offers a detailed comparative analysis of the lipid fractions of DM as well as of human (HM) and cow milk (CM), also indicating the distribution of fatty-acid moieties among sn-1/3 and sn-2 positions of the glycerol backbone.

3. MATERIALS AND METHODS

3.1. Sample preparation

Holder-pasteurized CM (commercial), HM (milk bank of OIRM - St. Anna Hospital. Turin - Italy) and pooled DM (Montebaducco farm, Reggio Emilia - Italy) were skimmed by centrifugation at 2000g for 30' at 4°C. Fat fraction and skimmed milk were stored at -20°C until use.

3.2. Sample treatment

1 ml of each milk sample was thawed, warmed to 37°C and vortexed vigorously before analysis. Prior to extraction, the samples were flushed with nitrogen to minimize oxidation. Lipids were extracted using the method described by Folch *et al.* (9). For HPLC analysis, the extract was dried under a gentle stream of N_2 and the residue was dissolved in CH_3CN/CH_2Cl_2 (95/5). For GC analysis, the extract was dried under N_2 and the residue was treated with boron trifluoride–methanol (14%, w/v) for transesterification of the total lipid fractions, and consequently the fatty acid methyl esters were extracted using hexane (10).

3.3. GC Analysis

GC-MS determination was carried out on a VARIAN 3800 gas chromatograph equipped with VARIAN 8400 autosampler and SATURN 2000 ion-trap mass selective detector. A VF-23 MS (Varian) (50% cyanopropylsilicone) fused silica capillary column (30 m long, 0.25 mm i.d. and 0.25 micrometer film) was used. Both injector and detector temperatures were set to 250 °C. The samples (1 microliter) were injected in splitless mode for 90 sec, after which the split ratio was 25:1. The oven was programmed as follows: 70°C for 1.0 min, increased at 5°C/min to 230°C, and held constant for 10 min. Helium was used as carrier gas at a flow rate of 1.0 ml/min.

The detector response factors for different fatty acids were calculated by analyzing known quantities of authentic standards (Supelco, Inc., Bellefonte, PA). The fatty-acid areas in each chromatogram were automatically integrated by computer (VARIAN WS 6.4). Results are expressed as g/100 g total fatty acid.

3.4. HPLC Analysis and Mass Conditions

HPLC-APCI-MS analysis was performed using a ThermoFinnigan Surveyor liquid chromatograph equipped with an ion-trap mass-spectrometer detector ThermoFinnigan LCQ Deca XPPlus, mounting an APCI source. The column was a RP-C18 Lichrosphere (4.5 x 250 mm, 5 µm, Merck) and the flow rate was 1 ml/min. The gradient program used for chromatographic separation was composed by mixture of acetonitrile / dichloromethane in different proportion and time: held for 5 min, (95:5), then linear from 5 to 10 min (90:10) and held for 15 min; linear from 25 to 35 min (70:30) and held for 10 min; linear from 45 to 75 min (40:60).

Due to the marked difference in response of the diverse TAG, quantification was done by correcting the signals obtained experimentally, applying a response factor determined empirically from the ratios between the response of trioleylglycerol and that of three different standards (tripalmitoylglycerol, tristearoylglycerol and trilinoleylglycerol) at equal concentrations (10 ppm). This factor was found to be adequate, enabling a good correspondence between the fatty acid composition, determined from analysis of the TAG, and that obtained with the previously-described trans-methylation method. To reduce interference, peak height intensities were considered.

The capillary and APCI vaporizer temperatures were set at 200°C and 450°C, respectively. Source voltage and capillary voltage were 4.50 kV and 16.00 V. Sheath and auxiliary gas flows were 60 and 20 ml/min.

4. RESULTS AND DISCUSSION

4.1. Fatty acid analysis

Fatty acids, determined after methylation-through gas chromatography, are shown (Table 1) both as percentage composition versus total fat content and as milligrams in 100ml of DM, HM and CM.

DM significantly differs from HM and CM for its lower total lipid content (0.94% vs 3.6% and 3.8%, respectively) which reflects in its lower caloric content (408 Kcal/l, vs. 690 Kcal/l and 660 Kcal/l, respectively). It contains few SFA (5.46 g/l) and few monounsaturated fatty acids (MUFA) (1.96 g/l); the PUFA content of DM is slightly higher than in CM and lower than in HM (1.69 vs 5.78 and 1.31 g/l, respectively). Among the SFA contained in DM, medium chain fatty acids (MCFA) predominate (Table 2); together with palmitic acid they account for almost the totality of SFA. From the nutritional standpoint, MCFA undergo preferential intestinal absorption versus long chain fatty acids (LCFA) (11), which are present in very small quantities in DM and in much higher quantities in CM

Table 1. Fatty acid composition of milk samples. (DM - Donkey's milk; HM – Human milk and CM – Cow's milk)

Name		Abb.	D.M.	H.M.	C.M.	D.M.	H.M	C.M.
			g /100 g of fat			mg/100 ml of milk		
Butyrric Acid	C4:0		0.57	0.01	3.77	5.34	0.38	137
Caproic Acid	C6:0		1.16	0.02	2.32	10.9	0.77	84.4
Caprylic Acid	C8:0		2.33	0.10	1.39	21.8	3.85	50.6
Capric Acid	C10:0		6.58	0.15	3.34	61.6	5.77	122
Undecanoic Acid	C11:0		0.67	0.01	0.13	6.27	0.38	4.73
Lauric Acid	C12:0		6.99	6.54	4.15	65.4	252	151
Tridecanoic Acid	C13:0		3.72	0.02	0.19	34.8	0.77	6.92
Myristic Acid	C14:0		6.67	5.38	11.3	62.4	207	411
Myristoleic Acid	C14:1		0.21	0.34	0.78	1.97	13.1	28.0
Pentadecanoic Acid	C15:0		0.3	0.23	0.36	2.81	8.85	13.1
Palmitic Acid	C16:0		26.3	20.0	28.8	246	770	1050
Palmitoleic Acid	C16:1		2.25	3.10	1.55	21.1	119	56.4
Margaric Acid	C17:0		0.21	0.28	0.53	1.95	10.8	19.3
Stearic Acid	C18:0		2.68	6.15	14.2	25.1	237	517
Oleic Acid	C18:1 n-9		17.0	32.6	20.7	159	1250	753
Octadecenoic Acid (isomer)	C18:1i		1.14	7.99	2.10	10.7	307	76.4
Linoleic Acid	C18:2 n-6	LA	9.50	12.2	2.44	88.9	469	88.8
a-Linolenic Acid	C18:3 n-3	ALA	7.25	1.14	0.48	67.9	43.9	17.5
c-Linolenic Acid	C18:3 n-6		0.14	0.05	0.18	1.31	1.92	6.35
Arachidic Acid	C20:0		0.11	0.23	0.18	1.03	8.85	6.55
Eicosaenoic Acid	C20:1 n-11		0.33	0.05	0.14	3.09	1.91	5.10
Eicosadienoic Acid	C20:2 n-6		0.33	0.39		3.10	15.0	
Eicosatrienoic Acid	C20:3 n-3		0.11	0.05		1.03	1.93	
Arachidonic Acid	C20:4 n-6	AA	0.07	0.59	0.22	0.66	22.7	8.01
Eicosapentaenoic Acid	C20:5 n-3	EPA	0.26	0.02	0.06	2.43	0.76	2.18
Docosanoic Acid	C22:0		0.05	0.38	0.05	0.47	14.6	1.82
Docosapentaenoic Acid	C22:5 n-3		0.07	0.18	0.18	0.66	6.93	6.42
Docosahesaenoic Acid	C22:6 n-3	DHA	0.28	0.40	0.05	2.62	15.4	1.82

Table 2. Fatty acid composition of milk samples. Summarized data.

	D.M	H.M	C.M.	D.M	H.M	C.M.
	g/ 100 g fat	g / 100 g fat	g /100 g fat	mg/100 ml milk	mg/ 100 ml milk	mg /100 ml milk
Total fat				0.94%	3.8%	3.6%
Saturated	58.3	39.5	70.8	546	1520	2580
of which MCT	27.0	12.2	20.5	252	469	746
Monounsaturated	20.9	44.0	25.2	196	1690	920
Polyunsaturated	18.0	15.0	3.62	169	578	131
of which						
Polyunsaturated n-6	10.0	13.2	2.84	94.0	509	103
Polyunsaturated n-3	7.97	1.79	0.78	74.6	68.9	28.0
Ratio n-6 / n-3	1.26	7.38	3.65			
Ratio unsat./sat.	0.31	0.38	0.05			
Ratio LA/ALA	1.31	10.7	5.08			
Ratio AA/EPA	0.27	29.5	3.67			

Among PUFA, in absolute quantities, PUFA n-3 contained in DM (746 mg/l) are slightly higher than HM (689 mg/l), and much higher than CM (280 mg/l) (Table 2); this is one of the chief points of interest in this milk. Also when the comparison is extended to include the formulas currently available to treat CMPA, DM possesses a higher n-3 content (12). Moreover, in terms of percentage fatty acid composition, PUFA content of DM is higher than that of HM and, particularly, of CM (Table 2).

In all three types of analyzed milk, PUFA-n-3 and PUFA-n-6 are mainly represented by two essential fatty acids, precursors of the two series, alpha-linolenic acid (ALA) and linoleic acid (LA), respectively. However, whereas in DM these amount to more than 90% of their respective classes, in HM and in CM the amounts are smaller, particularly with regard to ALA (63.7% and 61%, respectively of their classes).

The percentage content of ALA in DM is particularly high (7.25%) compared to that of HM (1.14%), whereas the percentage of LA is comparable in DM and HM. This reflects in a lower LC-PUFA n-6 / n-3 ratio and also in a lower LA/ALA ratio, in DM compared to HM. With regard to CM, the percentage contents of ALA and LA are much lower than those of DM and HM, whereas LC-PUFA n-6 / n-3 and LA/ALA ratios are intermediate between those of DM and HM (Table 2). The formulas available to treat CMPA have LA/ALA ratios that range from similar to that of HM to nine times higher, due both to the lower ALA content and to the higher LA content (12).

The percentage content of arachidonic acid (AA) in DM is low if compared to that of CM and HM, the difference being even more marked if absolute values are compared. The AA/EPA (eicosapentaenoic acid) ratio is also decidedly lower in DM than in CM and, particularly, in HM (Table 2), as well is the AA/DHA ratio (0.25 vs 1.4 in HM).

The compositional peculiarities of DM (i.e. higher PUFA n-3 content, lower PUFA n-6 / n-3 ratio and

lower AA/EPA ratio with respect to HM and CM) make this animal milk of particular interest for infant nutrition. Indeed, it has been reported that a higher LC-PUFA n-6 / n-3 ratio in HM is correlated with a higher risk of developing atopical diseases and/or allergic sensitization in the first few months of life. This ratio, and more specifically the AA/EPA ratio, is higher in milk from mothers of atopic as compared to non-atopic children (13, 14). Besides, low levels of n-3 LC-PUFA in human milk have been correlated to the development of symptoms of allergic disease at 18 months of age (13). In a selected sample of high-risk breastfed infants, the higher n-6 / n-3 ratio in milk was associated with the risk of eczema at 6 months (15), likewise, breast milk rich in SFA and low in n-3 fatty acids may be a risk factor for atopic dermatitis in the infant (16). Finally, low levels of n-3 LC-PUFA may be related to the development of atopic sensitization against food proteins in children during the first year of life (17).

Prostaglandin E2 and leukotriene B4, eicosanoids derived from AA, are important factors promoting atopic inflammation; in contrast, n-3 fatty acids and eicosanoids derived from them have been demonstrated to possess anti-inflammatory properties, because of their ability to interfere with AA metabolism and because they give rise to inflammation-resolving mediators termed resolvins (18). A relative lack of n-3 LC-PUFA may thus bring about an atopic state (14, 19). These fatty acids have also been shown to possess potent immunomodulatory activity, exercised on components of both natural and acquired immunity (20), and they may also exert their effects by modulating signal transduction and/or gene expression within inflammatory and immune cells, and by influencing the Th1/Th2 balance (15).

Among the LC-PUFA n-3, the principal metabolic activity is attributed to DHA. In DM, DHA accounts for 0.28% of the total lipid content, a proportion that lies within the recommended range (21, 22); it is comparable to the percentage contained in HM and higher than that in CM. Given that the synthesis of DHA from its precursor appears to be limited *in vivo*, this finding acquires considerable significance (23, 24). Furthermore, feeding LC-PUFA n-3 alone decreases AA status; this reduction occurs when DHA, and especially EPA, is given (25). Levels of n-6 and n-3 LC-PUFA should be in the same range as those found in HM to ensure the best effects on growth, cognitive development and visual acuity, and to limit the side effects due to a lack of balance between n-6 and n-3.

4.2. TAG analysis

The triacylglycerols (TAG) of the different milks were analyzed through HPLC/APCI/MS and tandem mass spectrometry, as described in the Materials and Methods section. Using these techniques, the size of the response signal varies markedly as a function of the overall number of carbon atoms in the acyl chain and of the degree of unsaturation. For example, concentrations being equal, the signal produced by trioleoylglycerol is, respectively, 20 and 5 times more intense than those produced by tristearoylglycerol and tripalmitoylglycerol. In consequence, correction factors must be used to estimate the proportion of the different TAG in the fat of each type of milk. To determine these factors, a standard mixture was analyzed containing four TAG: trioleoylglycerol, tristearoylglycerol, tripalmitoylglycerol and trilinoleoylglycerol, at 10 ppm each. Optimal linear correction (r=0.999) was obtained by graphing the ratio between the peak area corresponding to each standard and the area corresponding to the peak generated by trioleoylglycerol vs. the ratio between the overall number of the carbon atoms in the acyl chain and the number of unsaturations +1. The parameters of the straight line thus obtained were used to calculate, for each TAG identified, the corresponding correction factor. The congruence of the correction factors applied was shown by the generally high correspondence between the percentage composition of each individual FA calculated with this method and that obtained through transmethylation and subsequent gas chromatographic analysis.

Not considering regioisomers, 160 TAG were identified overall, and are reported in Table 4 (abbreviations are in Table 3). Table 4 also shows the percentage of the lipid fraction that each TAG represents in each type of milk. Numerous TAG containing short chain fatty acids (butyrric and caproic acids) are present in CM, but only small amounts in the TAG identified in the other two milks. For its part, DM differs from the others for the high number of TAG containing linolenic acid and for the high concentration of MCFA.

The characteristics of the three milks are summarized in the histograms in Figure 1 and Figure 2. In Figure 1 the TAG of each type of milk, expressed as percentage of total lipids, are grouped by the overall number of carbon atoms of the acyl chain (CN), whereas in Figure 2 the same TAG are grouped by the partition number (PN), defined as the difference between CN and double the number of unsaturations (DB), i.e. PN = CN-2*DB. Figure 1 shows that, in the case of HM, the percentage of TAG increases almost exponentially as CN increases, reaching its maximum at 52 carbon atoms (35.2% of the total triglycerides). This band is chiefly represented by triglycerides containing palmitic acid and two FA at 18 carbon atoms. DM also presents a maximum at 52 carbon atoms (22.5%), although the upward trend is less regular, and a marked proportion, above 10% of the total TAGs, lie at 44 and 46 carbon atoms. In CM on the contrary over half (53%) of the TAGs are triglycerides with fewer carbon atoms (from 36 to 40) with the maximum (22%) at 38 atoms. This preponderance of lower CN values is essentially due to the marked presence of triglycerides containing SFA; indeed, the peak at 38 CN chiefly consists of a combination of the fatty acids Bu-S-P, Bu-P-O and Co-P-P. For HM, a comparison of Figure 1 with Figure 2 shows they both have very similar profiles, though that in Figure 2 is displaced to lower PN values, with the maximum at PN 48, indicating that the most abundant triglycerides in HM have at least two unsaturations. On the contrary, in DM the Figure 2 profile is markedly different from that in Figure 1, presenting a roughly bell-shaped distribution, with a maximum centered around 42 PN. This

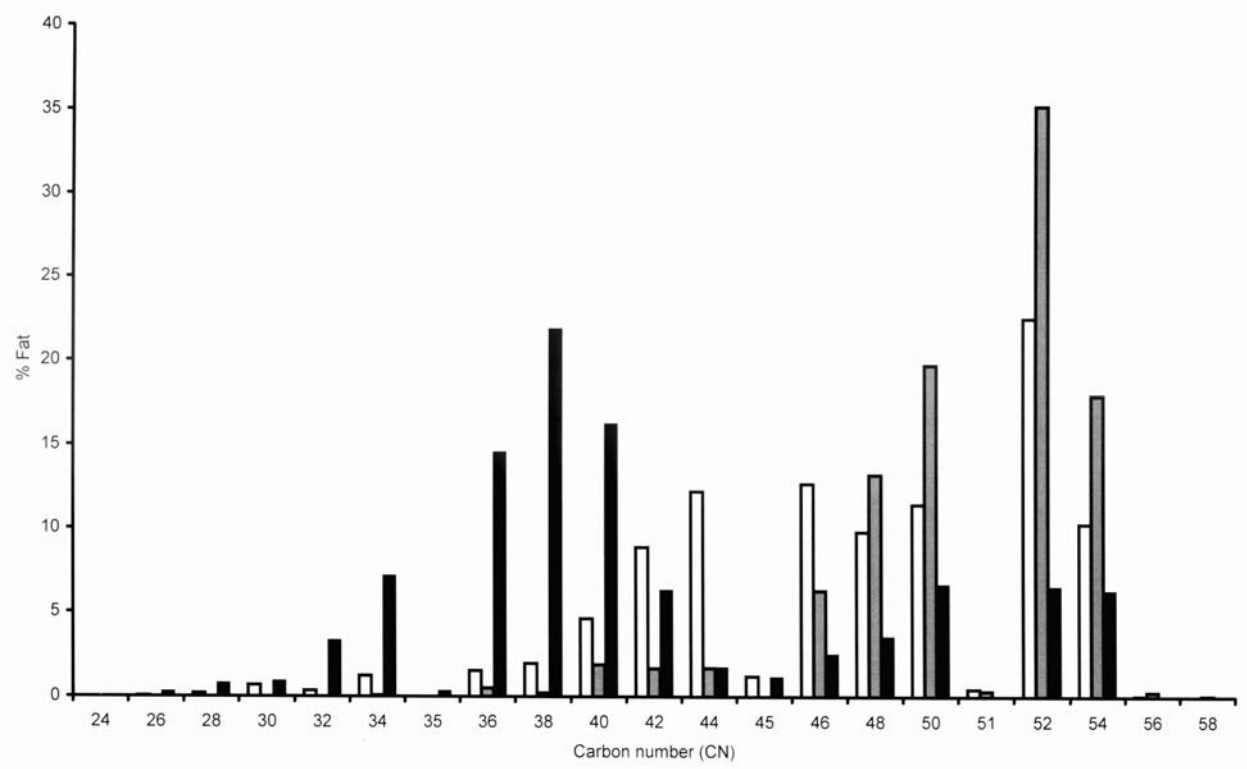

Figure 1. Distribution of TAGs within different carbon number (CN) groups in donkey, human and cow's milk. DM: white, HM: grey, CM: black.

means that in DM the predominant TAG have generally a higher number of unsaturations than they do in HM, together with greater variability. In marked contrast with these two milks, the CM profile in Figure 2 is very similar to that in Figure 1, in both cases maintaining a maximum at 38 PN, while slightly modifying the relative abundances of the vicinal bars at 36 and 40 PN, indicating that the most abundant TAG in this type of milk are prevalently saturated.

This is further confirmed by the fact that the saturated TAG in CM amount to 59.7% of the total fat content, whereas in DM and HM the saturated triglycerides account respectively for 12.4% and for 12.7%. In particular, in DM 2.34% of saturated TAG comprise medium-chain triglycerides (MCT), while in the other two milks MCT accounts for approximately 0.6% (0.55% in HM and 0.56% in CM). In HM the predominant part is represented by TAG whose degree of unsaturation is exclusively due to the presence of MUFA (56.5%, versus 38.8% in CM and 23.7% in DM). On the contrary, in DM, TAG containing at least one LC-PUFA predominate (62.7%), while in HM these account for 30.8% of total fats and in CM only for 1.48%.

One of the advantages offered by the use of APCI-MS to analyze the triglycerides is that it enables the fatty acid that occupies position sn-2 in the glycerol backbone to be determined (26). In the case of complex samples, such as those examined here, it is also important to have tandem-mass spectrometry available to resolve interferences due to co-eluting or partially co-eluting TAG (27). In this study both techniques were used and, for most TAG, it was possible to identify 2-acyloylglycerol. In several cases the contemporary presence of more than one regioisomer was detected, but since one was always markedly predominant over the others the most abundant isomer is presented in the results.

The results of this analysis are given in Table 5, which lists, in order of decreasing abundance, the TAG that account for up to 80% of the total lipid content (in the case of DM) or that are present in concentrations above 1% (HM and CM). From the table it is clear that the number of TAG constituting 80% of the lipid fraction in DM is larger (38 TAG) than it is in the other two types of milk (16 for HM and 24 for CM); in the case of HM and CM few (1 or 2) TAG are markedly preponderant over the others, in DM this occurs to a much smaller extent.

Among the most abundant TAG listed in Table 5, seven regioisomers (OPO, PPO, OPLa, OPL, LPL, LOL and CPO) are present both in HM and in DM, and four (PPO, OOO, SPM and OSO) appear in both HM and CM; another three TAG, constituted of the same FA, are present in the latter two milks, and one in DM and in HM, but as differentiated isomers (OPO/POO, OPS/OSP and PPS/PSP in HM/CM and SPM/MSP in HM/DM): in all these cases the sn-2 position is occupied by palmitic acid in HM and by another FA in the other two milks.

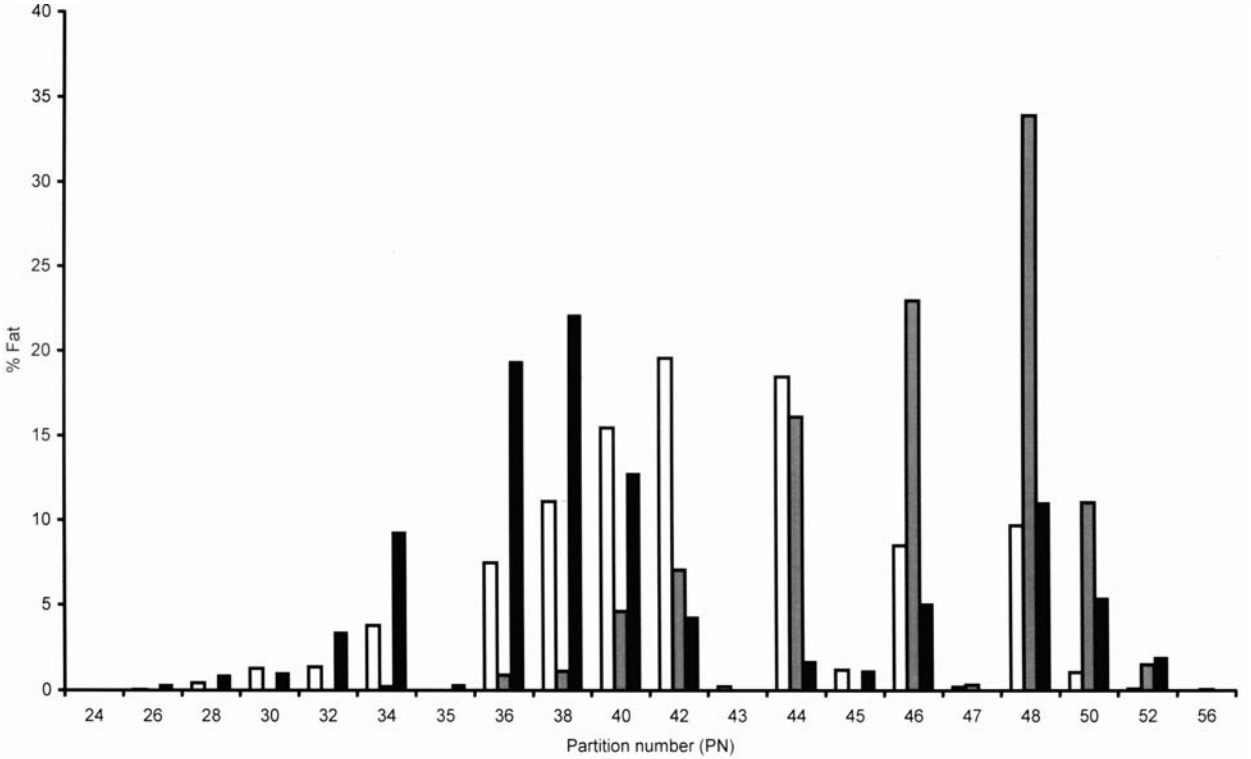

Figure 2. Distribution of TAGs within different PN groups in donkey, human and cow's milk. (PN = CN – 2 x number of double bonds). DM: white, HM: grey, CM: black.

Table 3. – Fatty acid names and symbols used in the text and tables

PM	Symbol	Name	Notation
88	Bu	Butyrric Acid	C4:0
116	Co	Caproic Acid	C6:0
144	Cy	Caprylic Acid	C8:0
170	Cl	Decenoic Acid	C10:1
172	C	Capric Acid	C10:0
200	La	Lauric Acid	C12:0
214	Td	Tridecanoic Acid	C13:0
226	Mo	Myristoleic Acid	C14:1
228	M	Myristic Acid	C14:0
242	Pd	Pentadecanoic	C15:0
254	Po	Palmitoleic Acid	C16:1
256	P	Palmitic Acid	C16:0
268	Va	Vaccenic Acid	C17:1
270	Ma	Margaric Acid	C17:0
278	Ln	Linolenic Acid	C18:3
280	L	Linoleic Acid	C18:2
282	O	Oleic Acid	C18:1
284	S	Stearic Acid	C18:0
306	Me	Eicosatrienoic Acid	C20:3
310	Ga	Eicosaenoic Acid	C20:1
312	Ar	Arachidic Acid	C20:0
328	Dh	Docosahesaenoic Acid	C22:6
338	Er	Docosaenoic Acid	C22:1
340	Be	Docosanoic Acid	C22:0
366	Ne	Tetracosaenoic Acid	C24:1
368	Li	Lignoceric Acid	C24:0

Table 6 reports the percentage fractions in sn-2 and sn-1/3 positions of the glycerol backbone of fatty-acid moieties exceeding 1% of the total FA content. Palmitic acid is present in DM mainly at sn-2 (72.9% of the total), as also observed in HM (74.8%) and, to a lesser extent, in CM (47.1%). In contrast, the palmitic acid present in vegetable oils, which are commonly used in the manufacture of infant formulas, is esterified at the sn-1 and sn-3 positions (28), while the sn-2 position is usually occupied by an unsaturated fatty acid.

The effect of the distribution of the other fatty acids on the glycerol backbone, above all of palmitic acid, has been widely investigated. It has been reported

Table 4. Triacylglycerol composition of the fat milk samples (% of fat).

TAG	DM	HM	CM	TAG	DM	HM	CM	TAG	DM	HM	CM
Bu-Bu-P			0.015	Bu-O -O			1.71	M -Po-O	0.87		
Bu-Bu-O			0.011	C -La-L		0.072		M -P -O	1.639	2.344	0.591
Bu-C -La			0.078	Co-P -O		0.135	2.79	M -P -S	1.409	5.216	2.306
Bu-Cy-M			0.085	Bu-O -S			1.87	P -P -P	0.936		
Bu-Co-P			0.108	C -La-O	1.25	0.438		C -L -Dh		0.013	
Cy-Cy-C	0.04			C -M -P	0.643		2.23	C -O -Dh		0.077	
Bu-Co-O			0.04	Cy-P -P	0.865		3.5	M -Ln-Ln	0.805		
Co-C -La			0.045	Co-P -S			3.88	Po-P -Ln	2.561		
Bu-C -M			0.341	La-La-P		1.187		M -L -O		2.544	0.324
Bu-Cy-P			0.285	Co-L -O			0.08	P -P -Ln	2.504		
Bu-Co-S			0.052	Cy-P -Ln	1.37			M -O -O		5.277	2.418
Cy-Cy-La	0.186			C -M -Ln	2.384			P -P -L	0.794	1.456	
Bu-Cy-O			0.15	La-La-Ln	1.947			Po-P -O	1.778		
Co-C -M			0.197	Co-O -O			0.787	P -P -O	2.648	6.35	2.793
Bu-C -P			0.542	Cy-P -O	0.823		1.728	P -P -S	0.348	4.009	1.108
Cy-C -La	0.673			Co-O -S			0.788	Pd-Ln-O	0.231		
Co-Cy-O			0.039	C -M -O			0.145	Pd-O -O		0.324	
Bu-C -O			0.233	La-M -Po			0.024	P -Va-O	0.209		
Co-C -P			0.24	La-La-O		1.059		La-L -Dh		0.034	
Co-La-M			0.318	C -Po-P	0.449			La-O -Dh		0.152	
Bu-La-P			1.7	La-M -P		0.601	1.71	P -Ln-Ln	2.819		
Bu-M -M			0.769	C -P -P	1.896		0.84	P -Ln-L	3.6		
C -C -La	0.339			Cy-P -S			0.191	Po-Ln-O	0.932		
Cy-Cy-Ln	0.173			Cy-Ln-L	0.258			P -L -L	1.259	3.533	0.106
Co-C -O			0.071	Cy-L -L		0.029		P -Ln-O	6.382		
Bu-La-O			0.246	Cy-Ln-O	0.579			Po-L -O	0.415		
Bu-M -P			6.82	Cy-L -O		0.145		P -L -O	2.118	8.579	0.423
C -C -M	0.446	0.074		C -P -Ln	5.357			Po-O -O	0.454	1.614	
Cy-C -P	0.275			Cy-O -O	0.671	0.097	0.483	P -O -O	4.061	15.407	3.753
C -La-La	0.325			La-M -O			0.49	P -O -S	0.468	4.748	2.215
Bu-Pd-P			0.325	C -P -O	3.775	1.414	0.673	P -S -S		1.174	
Cy-Cl-Ln	0.071			Mo-M -P			0.078	Ln-Ln-L	0.835		
Cy-C -Ln	0.601			La-P -P	1.58			Ln-L -L	0.703	0.095	
Bu-Mo-O			0.026	Td-Pd-Ma	1.197		1.146	Ln-Ln-O	1.935		
Bu-M -O		0.016	1.91	La-La-Dh		0.016		L -L -L		0.542	
Co-La-O			0.05	C -Ln-Ln	0.405			Ln-L -O	2.474	0.527	
Cy-C -O	0.217			C -Ln-L	0.44			L -L -O	0.918	3.501	0.056
Bu-P -P			12.6	C -L -L		0.196		Ln-O -O	1.758		
C -La-M		0.482		C -Ln-O	2.055			L -O -O	0.721	3.713	
Cy-La-P	0.63			C -L -O	0.931	0.726		O -O -O	0.63	6.982	2.195
Bu-P -Ln	0.101			La-P -Ln	3.607			O -O -S	0.226	2.298	2.085
Cy-C -Me	0.918			C -O -O	0.995	0.722	0.295	P -O -Ga	0.018		
Bu-P -L		0.013	0.206	La-P -L	0.972	0.787		O -S -S	0.054	0.282	1.93
Bu-P -O		0.124	5	La-P -O	2.286	3.831	0.282	P -L -Dh		0.162	
Cy-La-O		0.075		M -M -O			0.188	O -O -Ar		0.06	
C -La-Po	0.122			M -Po-P			0.094	P -O -Er	0.048		
Co-P -P	0.078		6.66	M -P -P	0.997		1.63	P -O -Be		0.049	
Bu-P -S			10	Cy-O -Dh		0.015		L -L -Dh		0.05	
C -La-P	0.435			La-L -L		0.683		L -O -Dh		0.312	
La-La-M	0.296			La-Ln-O	2.037	0.3		O -O -Dh		0.513	
Bu-L -O			0.287	La-L -O	0.684	1.952		P -O -Ne		0.041	
C -La-Ln	0.84	0.062		Mo-P -L	1.694			P -O -Li		0.058	
Co-P -Ln	0.128			La-O -O		2.713	0.633				
Cy-M -Ln	0.89			M -P -L	0.515						

that the 2-monoacylglycerols of the SFA are much more easily absorbed than free fatty acids and that, in particular, in a study on rats, a high 2-palmitoyl TAG content in the lipid fraction increases fat absorption (29). Reports confirming that palmitic acid is also absorbed as 2-monoacylglycerol in infants have been published (30, 31). The absorption of free fatty acids varies greatly, depending on their chemical structure. MUFA and PUFA are well absorbed, as are SFA of chain length 12 carbons or less. The coefficient of absorption of free long chain SFA, i.e. palmitic acid, is relatively low (32) due in part to a melting point above body temperature, and also to the tendency of these fatty acids to form hydrated fatty acid soaps with minerals such as calcium or magnesium at the pH of the intestine, after which they are partially excreted with the feces (33). In parallel with efficient absorption of 2-palmitoylglycerol, the absorption of stearic acid was found to be increased, which could not be explained by its stereospecific position in TAG. It was suggested that the more efficient micellization in the presence of 2-palmitoylglycerol may also enhance the absorption of stearic acid (34). In addition, positional isomerism in infant formula TAG has been reported to affect the mineral balance and plasma lipid composition in premature infants (35, 36).

Table 5. Proportion of the major sn2- TAG in the different samples of milk, ordered by decreasing concentration (% fat).

DM			HM			CM		
TAG	%fat	Sum	TAG	%fat	Sum	TAG	%fat	Sum
O -P -Ln	6.38	6.382	O -P -O	15.4	15.4	Bu-P -P	12.6	12.6
C -P -Ln	5.36	11.74	L -P -O	8.58	24.0	Bu-S -P	10.0	22.6
O -P -O	4.06	15.80	O -O -O	6.98	31.0	Bu-P -M	6.82	29.4
C -P -O	3.77	19.58	P -P -O	6.35	37.3	Co-P -P	6.66	36.1
Ln-P -La	3.61	23.18	O -M -O	5.28	42.6	Bu-P -O	5.00	41.1
L -P -Ln	3.60	26.78	S -P -M	5.22	47.8	Co-S -P	3.88	44.9
P -Ln-Ln	2.82	29.60	O -P -S	4.75	52.6	O -O -P	3.75	48.7
P -P -O	2.65	32.25	P -P -S	4.01	56.6	Cy-P -P	3.50	52.2
Po-P -Ln	2.56	34.81	La-P -O	3.83	60.4	P -P -O	2.79	55.0
P -P -Ln	2.50	37.31	O -O -L	3.71	64.1	Co-P -O	2.79	57.8
O -Ln-L	2.47	39.79	L -P -L	3.53	67.6	O -O -M	2.42	60.2
C -M -Ln	2.38	42.17	L -O -L	3.50	71.1	S -P -M	2.31	62.5
O -P -La	2.29	44.46	O -O -La	2.71	73.9	C -P -M	2.23	64.7
O -P -L	2.12	46.58	O -M -L	2.54	76.4	O -S -P	2.22	66.9
C -O -Ln	2.06	48.63	O -P -M	2.34	78.7	O -O -O	2.20	69.1
Ln-O -La	2.04	50.67	O -S -O	2.30	81.0	O -S -O	2.09	71.2
La-La-Ln	1.95	52.61	L -O -La	1.95	83.0	S -S -O	1.93	73.2
O -Ln-Ln	1.94	54.55	O -O -Po	1.61	84.6	Bu-O -M	1.91	75.1
C -P -P	1.90	56.45	P -P -L	1.46	86.1	Bu-S -O	1.87	76.9
O -P -Po	1.78	58.22	O -P -C	1.41	87.5	Cy-O -P	1.73	78.7
O -Ln-O	1.76	59.98	La-P -La	1.19	88.7	Bu-O -O	1.71	80.4
L -P -Mo	1.69	61.68	S -S -P	1.17	89.8	La-P -M	1.71	82.1
O -P -M	1.64	63.31	La-La-O	1.06	90.9	Bu-La-P	1.70	83.8
P -La-P	1.58	64.89				P -P -M	1.63	85.4
S -M -P	1.41	66.30				Td-Ma-Pd	1.15	86.6
Cy-P -Ln	1.37	67.67				P -S -P	1.11	87.7
L -P -L	1.26	68.93						
C -O -La	1.25	70.18						
Ma-Pd-Td	1.20	71.37						
P -P -M	1.00	72.37						
C -O -O	0.99	73.37						
La-P -L	0.972	74.34						
P -P -P	0.936	75.27						
Ln-O -Po	0.932	76.21						
O -L -C	0.931	77.14						
C -Me-Cy	0.918	78.06						
L -O -L	0.918	78.97						
Cy-M -Ln	0.890	79.86						
O -Po-M	0.870	80.73						

Table 6. Distribution of the major fatty acids of Donkey, Human and cow's milk between sn-2 and sn-1,3 positions of triacylglycerols

		DM		HM		CM	
		%sn-1/3	**% sn-2**	**%sn-1/3**	**% sn-2**	**%sn-1/3**	**% sn-2**
Butyrric Acid	C4:0	ns [1]	ns [1]	ns [1]	ns [1]	100	0
Caproic Acid	C6:0	ns [1]	ns [1]	ns [1]	ns [1]	100	0
Caprylic Acid	C8:0	98.0	1.98	ns [1]	ns [1]	100	0
Capric Acid	C10:0	98.2	1.79	97.9	2.05	99.0	0.97
Lauric Acid	C12:0	69.4	30.6	91.7	8.32	55.5	44.5
Myristic Acid	C14:0	50.9	49.1	50.5	49.5	91.1	8.94
Palmitoleic Acid	C16:1	75.7	24.3	ns [1]	ns [1]	ns [1]	ns [1]
Palmitic Acid	C16:0	27.1	72.9	25.2	74.8	52.9	47.1
Linolenic Acid	C18:3	78.5	21.5	ns [1]	ns [1]	Not Found	
Linoleic Acid	C18:2	83.5	16.5	95.7	4.27	91.1	8.90
Oleic Acid	C18:1	80.0	20.0	80.4	19.6	64.5	35.5
Stearic Acid	C18:0	76.4	23.6	79.9	20.1	13.0	87.0
DHA	C22:6	Not Found	100	0	Not Found		

[1]The number of TAGs containing the reported fatty acid was less than 5.

In DM the position of fatty acids on glycerol backbone, above all of LC-SFA, is very similar to that of HM: this fact, in conjunction with the relatively high contents of MCT, makes the lipids in DM, through quantitatively modest, highly bioavailable.

However, the low overall lipid content, together with the lower percentages of EPA and DHA, whose synthesis *in vivo* is limited (23, 24), and with the low LC-PUFA n-6 content (with particular reference to AA, which is recognized to play an important role in cognitive development) make DM inadequate as an exclusive food in infants for the first year of life; nevertheless, the better quality of the lipid pattern in DM versus CM might make this milk a better substrate to produce formulas (starting or follow on) to be used for feeding of newborns and infants in the early months of life, both for prevention and therapy of

atopy. In this case, the defattening process usually applied for cow milk, would not be necessary and a formulation would just require a slight increases of DHA EPA and AA.

Within a well balanced and integrated diet, DM is a precious source of essential fatty acids. These fatty acids are of particular importance in the diet of subjects with CMPA, especially if affected by multiple food allergy. These subjects are at risk of developing a deficiency in EFA and particularly in n-3 LC-PUFA, which are especially necessary for adequate growth, neurological development and cardiovascular health. (37).

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Abbreviations: CM, cow milk; DM, donkey milk; HM, human milk; DHA, 4,7,10,13,16,19-docosahexaenoic acid; EPA, 5,8,11,14,17-eicosapentaenoic acid; AA, arachidonic acid; ALA, a-linolenic acid; LA, linoleic acid; FA, fatty acid; SFA, saturated FA; MCFA, medium chain FA; LCFA, long chain FA; EFA, essential FA; PUFA, polyunsaturated FA; MUFA, monounsaturated FA; LC-PUFA, long chain PUFA; TAG, triacylglycerol; MCT, medium chain triacylglycerol; CMP, cow's milk protein; CMPA, CMP allergy; PN, partition number; CN, carbon number; GC, gaschromatography; HPLC, high performance liquid chromatography; MS, mass spectrometry; APCI, atmospheric pressure chemical ionization

Key Words: Cow's Milk Protein Allergy; Fatty Acids; Mass Spectrometry; Nutritional Value

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