Note

Comparative composition of total and *sn*-2 fatty acids in bovine and ovine milk fat

Deven H. Mistry, Edward J. DePeters, Juan F. Medrano*

Department of Animal Science, University of California, Davis,
One Shields Avenue, Davis, CA 95616-8521, USA

Abstract – Similar diets were fed to cattle and sheep to compare the total and the *sn*-2 fatty acid (FA) composition of milk fat. The study confirmed that substrate availability in the bovine and ovine mammary gland is a determining factor in the *sn*-2 FA composition. The acyltransferase that esterifies the FA at the *sn*-2 position is specific for FA of chain length C14 to C18 and has a reduced affinity for short chain FA in cattle and sheep.

fatty acid / *sn*-2 / triglyceride composition / lysophosphatidic acid acyltransferase

Résumé – Comparaison de la composition du lait en acides gras totaux et *sn*-2 chez les bovins et les ovins. L'étude a confirmé que la disponibilité du substrat dans la glande mammaire des bovins et des ovins est un facteur déterminant de la composition en acide gras en position *sn*-2 des matières grasses du lait. L'acyltransférase qui estérifie les acides gras à la position *sn*-2 agit surtout sur les acides gras de type C14 à C18, et n'a qu'une action réduite sur les acides gras à chaîne courte, chez les bovins et les ovins.

acide gras / *sn*-2 / composition en triglycéride / lysophosphatidique acide acyltransférase

1. INTRODUCTION

The distribution of fatty acids (FA) on the backbone of the triglyceride molecule is non-random, affecting the melting point and hardness, as well as digestibility of lipids [4]. The nutritional characteristics of ruminant milk are determined, in part, by the triglyceride structure. In humans, due to the specificity of the pancreatic lipase for the *sn*-1 and *sn*-3 positions of the triglyceride molecule, the FA present at the *sn*-2 position is preferentially absorbed because it remains in the monoacyl glycerol

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* Correspondence and reprints
Tel.: (530) 752 6786; fax: (530) 752 0175; e-mail: jfmedrano@ucdavis.edu

form [12]. Ruminant milk fat is unique compared to plant oils because it has a high proportion of medium and long chain-saturated FA (C12:0 to C18:0) at positions *sn*-1 and *sn*-2 and, short chain (C4:0 to C10:0) and unsaturated fatty acids at position *sn*-3. Medium and long chain saturated FA are hypercholesterolemic because they raise low-density lipoprotein (LDL) cholesterol levels, resulting in an increased risk of coronary heart disease. Triglycerides that are high in total saturated FA content but contain only a small percentage of saturated FA at the *sn*-2 position have been shown to be hypocholesterolemic [7]. The metabolic effect of dietary FA in humans, especially from consumption of milk products, is determined in part by their positional distribution on the triglyceride molecule [5, 8, 12].

Bovine and ovine milks have been reported to differ in the proportions of FA at the *sn*-2 position [1, 9], with bovine milk fat having a higher percentage of C16:0 and a lower percentage of unsaturated FA, such as C18:2 and C18:3, than ovine milk fat. To evaluate differences in stereochemistry of milk fat, nine Holstein cows and eight Suffolk ewes were fed a similar diet for a period of ten days, and milk samples were collected and analyzed for total FA and *sn*-2 FA composition.

2. MATERIALS AND METHODS

Ewes and cows were on standard flock and herd diets containing concentrate and forage ingredients prior to being fed the experimental diet. Composition of the experimental diet as a percentage of dry matter was 5.21% dried beet pulp, 13.1% cotton seed with lint, 0.67% sugar cane molasses, 2.7% soybean meal, 34.5% barley/corn (50/50), 1.9% mineral buffer (not included in sheep diet), and 41.8% chopped alfalfa hay. The %FA composition of the diet was 0.19 (C12), 0.72 (C14), 26.9 (C16), 0.43 (C16:1 c), 0.23 (C17), 3.2 (C18), 15.3

(C18:1 c9 and 10), 48.7 (C18:2) and 4.4 (C18:3). Approximately 100 mL of milk were collected manually from all animals on days eight and ten in a vial containing a preservative (Broad Spectrum Microtabs® II D&F Control Systems Inc., San Ramon, CA, USA). Milk samples were placed in ice in tubes completely full, and stored for 12 d without any headspace at –20 °C until assayed for fatty acids.

Fat was extracted [3] and total FA composition of the milk fat was determined by gas chromatography of methyl esters [2]. The *sn*-2 FA composition of the triglyceride was done according to [3, 6].

The mean FA content of the two samples collected on days eight and ten, and the mean bovine and ovine FA compositions were calculated and compared using one way analysis of variance [11]. The standard deviations of the measurements were calculated as the square root of the mean square error. A one-tailed *t*-test was calculated to compare if the ratio of the *sn*-2 FA to total FA was equal to one [11].

3. RESULTS

Significant ($P < 0.05$) differences in total FA content and in the *sn*-2 FA content of the milk fat between the two sampling days were detected only in ovine milk fat. The mean total FA content was higher on the first day than the second day for C16:0 (26.4% vs. 24.7%), C18:1 trans 11 (2.6% vs. 1.4%) and C18:2 cis 9 trans 11 (4.2% vs. 1.4%). For the *sn*-2 FA of ovine milk fat, C14:0 was lower on day eight (15.7%) than day ten (19.7%), and C18:1 trans 11 was higher on day eight (4.2%) than day ten (1.4%). The reason for the differences is not known but may be due to lack of buffering and minerals in the sheep diet. Differences between the two sampling days were considered small, and the data were averaged.

4. DISCUSSION

In general, the total FA compositions of milk fat from bovine and ovine agree with previous reports [1, 9]. However, these reports did not contrast animals fed the same diet and therefore it is difficult to make comparisons on the nature of the differences between species.

An important observation was that the *sn*-2 FA composition reflected the total FA composition. The higher the concentration of an individual FA in milk fat, the higher the concentration of that FA at the *sn*-2 position (Tab. I). The *sn*-2 C12:0 was significantly different between the two species, higher in ovine (2.9%) and lower in bovine (0.8%), similarly, in total FA composition C12 was higher in ovine (3.8%) and lower in bovine (2.7%). The same trend was observed for C16:0, which was higher in bovine (31.3 vs. 25.6%). This suggests that the substrate availability in the ruminant

Table I. Comparison of mean adjusted milk fat fatty acid (FA^{AD})^a composition and the proportion of fatty acids in the *sn-2* position (FA^{sn-2}) in cow and sheep milk fat. Standard deviation of the measurements are shown in parenthesis.

Fatty Acid	Bovine (n = 9)			Ovine (n = 8)		
	FA^{AD}	FA^{sn-2}	(FA^{sn-2})/(FA^{AD})	FA^{AD}	FA^{sn-2}	(FA^{sn-2})/(FA^{AD})^c
C12:0	4.56 (0.845)	0.78 (1.034)	0.17**	9.93 (1.277)	2.86 (3.016)	0.29**
C14:0	13.25 (1.097)	11.60 (4.817)	0.88	15.46 (1.636)	17.68 (2.658)	1.14**
C16:0	36.18 (2.200)	40.16 (3.182)	1.11**	34.51 (1.711)	34.60 (3.114)	1.00
C16:1 cis	2.44 (0.529)	3.42 (1.362)	1.40**	1.05 (0.191)	1.49 (0.291)	1.42**
C18:0	11.91 (2.047)	10.60 (2.908)	0.89	12.88 (2.062)	11.16 (2.848)	0.87**
C18:1 trans 9	0.31 (0.125)	0.32 (0.121)	1.02	0.37 (0.075)	0.47 (0.382)	1.27
C18:1 trans 11	1.56 (1.395)	1.26 (1.280)	0.81	2.71 (1.359)	2.81 (1.307)	1.04
C18:1 cis 9 and 10	24.14 (3.091)	24.67 (4.170)	1.02	17.30 (2.461)	21.23 (2.671)	1.23**
C18:2	4.13 (0.689)	5.41 (1.738)	1.31**	4.27 (0.808)	6.05 (1.134)	1.42**
C18:3	0.63 (0.083)	0.65 (0.269)	1.03	0.44 (0.081)	0.40 (0.100)	0.91
C18:2 cis 9 trans 11	0.89 (0.590)	1.14 (1.074)	1.28	1.05 (0.402)	1.26 (0.445)	1.20**

^{a} FA^{AD} adjusted FA composition of milk fat calculated as the % of each FA in relation to the total sum of C12:0 + C14:0 + C16:0 + C16:1 c + C18:0 + C18:1 t9 + C18:1 t11 + C18:1 c9 and 10 + C18:2 + C18:3 + C18:2 c9 t11.
** The ratios are different from 1 ($P < 0.01$).

mammary gland may be the determining factor for which FA is esterified at the *sn*-2 position of the triglyceride molecule by the lysophosphatidic acid acyltransferase (LPAAT). To examine this relationship a ratio of the *sn*-2 FA concentration to an adjusted value of the total milk fat FA (Tab. I) was calculated. The adjustment consisted of calculating the percentage of each FA in relation to the total of C12:0 + C14:0 + C16:0 + C16:1 cis + C18:0 + C18:1 trans 9 + C18:1 trans 11 + C18:1 cis 9 and 10 + C18:2 + C18:3 + C18:2 cis 9 trans 11 so that the same FA were considered. A ratio of *sn*-2 FA to the total FA for each individual FA was determined, and the ratio was compared to determine if it was significantly different from one using a one-tail *t*-test. For the bovine, only C12:0, C16:0, C16:1 cis, and C18:2 differed significantly from one for total FA composition. For the ovine, C12:0, C14:0, C16:1 cis, C18:0, C18:1 cis 9 and 10, C18:2, and C18:2 cis 9 trans 11 differed significantly from one. The amount of C12:0 was much lower at the *sn*-2 position than the total FA for both species, which agrees with the specificity of the LPAAT enzyme [6]. In the bovine, the proportion of C16:0, C16:1 cis, and C18:2 was higher at the *sn*-2 position than in the total FA content, with C16:1 cis and C18:2 being the highest. This conforms to previous observations [1], where the affinity of the human LPAAT alpha was C16:0 ~ C16:1 ~ C18:2 > C18:3 > C20:4. However, the affinity changed with the substrate availability. Specificity of the LPAAT for chain length was also observed for the bovine, C16 > C14 > C12 > C10 > C8 [10].

Based on the current findings, in the ruminant mammary gland the fatty-acyl molecule attached to the *sn*-2 of the triglyceride is influenced by substrate availability. Earlier Marshall and Knudsen [10] proposed that in vivo concentration of the fatty acyl-CoA influenced the *sn*-2 position. The LPAAT appears to be specific for FA of chain length C14 to C18 and has a reduced affinity for short chain FA. This is consistent with observations in plants where mass action of the available acyl groups drove triglyceride biosynthesis [10].

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