

## Differences on the Fatty Acids and Triglycerides Profile of Conventional VS Ecological Milk from Asturias, Spain

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### ABSTRACT

The effect on the fatty acids (FA) and triglycerides (TG) profile of ecological vs conventional cow's milk from Asturias (northern of Spain) were studied. From all fatty acids analyzed short, medium and long chain fatty acids, only C16:0 (medium chain) and C18:1, C18:2, C18:3 and C18:2 c9t11 (long chain) presented significant differences ( $p < 0.05$ ). The sum of saturated fatty acids (SFA), unsaturated fatty acids (UFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and the atherogenicity index (AI) showed differences ( $p < 0.05$ ). Triglycerides CN40 (medium atom carbon length) and C44, CN46, CN48, CN50, CN52 and CN54 (long atom carbon length) showed differences ( $p < 0.05$ ) between ecological vs conventional milk.

**Keywords:** ecological cow's farm; conventional cow's farm; milk fat; fatty acid; triglycerides; gas chromatography.

### INTRODUCTION

Bovine milk fat contains a large number of FA, some of which may be of potential benefit to human health, including polyunsaturated fatty acids (C18:1, C18:2 and C18:3) and conjugated linoleic acids (c9t11). Milk fat is formed basically by triglycerides (TG) that contain short (C4-C10), medium (C12-C16) and long (C18-C20) chain FA (German *et al.* 2009; Parodi, 2009; Mayer and Fiechter, 2012). The long chain FA are originated directly from the FA of the blood plasma, the short chain FA are biosynthesized in the mammary glands and the medium chains are biosynthesized by both ways (Alonso *et al.* 1999; Markiewicz-Kęszycka *et al.* 2013). Several factors exist that influence the composition of FA in milk such as the feeding (Carroll *et al.* 2006; Morales-Almaráz *et al.* 2011; White *et al.* 2001), genetics (Gaunt, 1981; Soyeurt & Gengler, 2008) and seasonal factors (Hinrichs *et al.* 1992).

The aim of this study was to analyze the conventional and ecological feeding on the evolution of the FA and TG composition of the milk fat from Asturias (Spain). In order to monitor and improving the quality of milk fat

composition in the milk from Asturias, which is the second leading region in milk production from Spain. The FA by grouping them according to unsaturation and the length of the carbon chain was also studied.

### MATERIALS AND METHODS

#### Chemical and Reagents

Standards of FA and TG and all reagents grade were supplied by Sigma (St. Louis MO, USA). Deionized water was prepared by a water purification system (Millipore Co., Burlington, MA, USA).

#### Milk Collection

Homogeneous samples from Friesian cow's milk feeding on conventional and ecological pastures were collected in flasks and transported at 4°C from twelve farmers during spring and summer from Asturias, Spain. Samples were collected from all the cows and mixed according to the milk production of each individual cow to get one representative sample per farm.

#### Extraction of Fat

The milk was centrifugated at 6000 r.p.m and the separated creams were extracted with

petroleum ether and anhydrous sodium sulphate (Alonso, Fontecha, Lozada et al. 1999) and concentrate on a rotatory evaporator at 40-50°C. 0.2 µl of a solution 0.05% in hexane (w/v) was injected for gas chromatographic analysis.

### Fatty Acids Analysis

The preparation and analyses of the fatty acids methyl esters (FAME) were based on the method proposed by Alonso *et al.* (2000) by gas chromatography (GC). About 100 mg of milk fat was weighed and dissolved in 1 ml of hexane. Then, 0.1 ml of methanolic potassium hydroxide (2 M) was added and the mixture was stirred for 1 min and left to rest for 15 min. Next, the hexane layer was separated, and 0.1 µL of the hexane fraction was injected into the GC. The GC analysis of FAME was performed on an Agilent Technologies GC Agilent Technology 5975 B (Palo Alto, CA, USA) equipped with a flame ionization detector (FID). Analysis were performed with a CP Sil 88 column (100 m x 0.25 mm i.d.) containing 100% cyanopropyl siloxane, stationary phase, with 0.20 µm film thickness (Chrompack, Middelburg, The Netherlands). The initial temperature of 70°C was maintained for 3 min, then raised to 175°C at a rate of 1.3°C/min for 10 min. The split ratio was 1:50, and the carrier gas was helium with a flow rate of 1 ml/min. The injector and detector temperatures were 250°C.

### Triglyceride Analysis

The GC analysis of TG was on Agilent Technologies GC Agilent Technology 5975 B (Palo Alto, CA, USA) equipped with a FID using a WCOT, fused silica capillary column (25m x 0.25 mm) coated with TAP-CB (df: 0.10 µm) Chrompack, Middelburg, The Netherlands) as proposed (Alonso, 1993). Experimental chromatographic conditions were: He carrier 17.0 psi head pressure. Initial column temperature 280°C, hold for 1 min., rising to 350°C at 3°C/min with a split ratio was 1:50.

### Statistical Analysis

Statistical analysis was performed using SPSS-PC + 4.0 Software (SPSS Inc., Chicago, IL, USA). Milk was specified as a random effect. The data were subjected to ANOVA for the interaction of two factors in which one is the conventional feeding and the other is the ecological feeding.

## RESULTS AND DISCUSSION

Table 1, gathered the FA composition between conventional vs ecological milk. Differences were found ( $p < 0.05$ ) for the FA C16:0 ( $34.04 \pm 2.16\%$  to  $28.06 \pm 2.04\%$ ), C18:1 ( $19.91 \pm 1.85\%$  to  $23.87 \pm 2.18\%$ ), C18:2 ( $2.24 \pm 0.27\%$  to  $2.70 \pm 0.25\%$ ), C18:3 ( $0.47 \pm 0.04\%$  to  $0.93 \pm 0.07\%$ ) and C18:2 c9t11 ( $0.44 \pm 0.05\%$  to  $1.71 \pm 0.09\%$ ). These results were in accordance to those reported by Jahreis *et al.* (1996), although the polyunsaturated fatty acids were lower than that of the present study. The Table 2 summarises the average values of the different FA classified by nutritional groups according to the carbon chain, saturated ( $73.85 \pm 2.85\%$  to  $67.62 \pm 2.78\%$ ), unsaturated ( $26.05 \pm 1.98\%$  to  $32.88 \pm 1.72\%$ ), monounsaturated ( $23.40 \pm 1.52\%$  to  $27.14 \pm 1.15\%$ ), polyunsaturated ( $3.15 \pm 0.25\%$  to  $5.24 \pm 0.31\%$ ) shows differences ( $p < 0.05$ ), along with the degree of unsaturation (saturated and unsaturated FA) between conventional vs ecological milk. Atherogenicity index (AI) are commonly used to assess the nutritional value and consumer health and is required in the diet to combat the lifestyle (Jahreis&Fritsch,1996). The AI was significant ( $p < 0.05$ ) between conventional and ecological milk ( $3.27 \pm 0.24\%$  to  $2.46 \pm 0.19\%$ ). The average TG profiles of conventional vs ecological milk fat are shown in Table 3. The TG C40 ( $9.61 \pm 0.18\%$  to  $9.18 \pm 0.21\%$ ), C44 ( $7.36 \pm 0.27\%$  to  $6.95 \pm 0.08\%$ ), C46 ( $8.12 \pm 0.41\%$  to  $7.52 \pm 0.24\%$ ), C48 ( $9.67 \pm 0.52\%$  to  $9.35 \pm 0.55\%$ ), C50 ( $10.93 \pm 0.35\%$  to  $11.64 \pm 0.38\%$ ), C52 ( $8.06 \pm 0.25\%$  to  $8.66 \pm 0.29\%$ ) and C54 ( $2.81 \pm 0.25\%$  to  $3.55 \pm 0.31\%$ ) were found to be significantly different ( $p < 0.05$ ) in conventional compared to ecological milk. These results are in accordance to those found by (Pilarczyk *et al.* 2015; Pustjens *et al.* 2015) in a study in conventional and organic butter. This may be due to the fact that these groups of TG have a higher proportion of unsaturated and polyunsaturated fatty acids esterified and make them healthier. Taking into account the different effects that single FA might have on the human health on the probability of increasing the incidence of pathogenic phenomena, such as atheroma or thrombosis formation, the milk fat studied in those ecological farmers were healthier than conventional farmer.

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In conclusion, milk FA and TG composition are affected by feeding system. All FA analyzed short, medium and long chain FA, only C16:0 (medium chain) and C18:1, C18:2, C18:3 and C18:2 c9t11 (long chain) presented significant differences ( $p < 0.05$ ). The sum of saturated FA, unsaturated FA, monounsaturated FA, polyunsaturated FA and the

atherogenicity index (AI) showed differences ( $p < 0.05$ ). TG CN40 (medium chain) and C44, CN46, CN48, CN50, CN52 and CN54 (long chain) showed differences ( $p < 0.05$ ) between conventional and ecological milk. The milk fat studied in those ecological farmers were healthier than conventional farmer.

**Table1.** Fatty acids composition (% of total fatty acids) of conventional and ecological milk fat from Asturias, Spain.

Fatty acids	Conventional milk	Ecological milk
C4:0	3.22 ± 0.24 <sup>a</sup>	3.19 ± 0.28 <sup>a</sup>
C6:0	1.95 ± 0.17 <sup>a</sup>	1.94 ± 0.19 <sup>a</sup>
C8:0	1.21 ± 0.11 <sup>a</sup>	1.32 ± 0.16 <sup>a</sup>
C10:0	2.83 ± 0.26 <sup>a</sup>	2.56 ± 0.25 <sup>a</sup>
C10:1	0.35 ± 0.05 <sup>a</sup>	0.39 ± 0.05 <sup>a</sup>
C12:0	3.33 ± 0.28 <sup>a</sup>	3.60 ± 0.35 <sup>a</sup>
C14:0	11.94 ± 1.54 <sup>a</sup>	12.31 ± 1.39 <sup>a</sup>
C14:1	0.97 ± 0.06 <sup>a</sup>	1.04 ± 0.07 <sup>a</sup>
iC15:0	0.24 ± 0.03 <sup>a</sup>	0.20 ± 0.04 <sup>a</sup>
aiC15:0	0.54 ± 0.05 <sup>a</sup>	0.59 ± 0.06 <sup>a</sup>
C15:0	1.36 ± 0.07 <sup>a</sup>	1.34 ± 0.08 <sup>a</sup>
C16:0	34.04 ± 2.16 <sup>a</sup>	28.06 ± 2.04 <sup>b</sup>
iC17:0	0.55 ± 0.05 <sup>a</sup>	0.56 ± 0.06 <sup>a</sup>
C17:0	0.53 ± 0.06 <sup>a</sup>	0.58 ± 0.06 <sup>a</sup>
C16:1	2.17 ± 0.25 <sup>a</sup>	1.84 ± 0.19 <sup>a</sup>
C18:0	11.71 ± 1.25 <sup>a</sup>	11.27 ± 1.35 <sup>a</sup>
C18:1	19.91 ± 1.85 <sup>a</sup>	23.87 ± 2.18 <sup>b</sup>
C18:2	2.24 ± 0.27 <sup>a</sup>	2.70 ± 0.25 <sup>b</sup>
C18:3	0.47 ± 0.04 <sup>a</sup>	0.93 ± 0.07 <sup>b</sup>
C18:2 (c9t11)	0.44 ± 0.05 <sup>a</sup>	1.71 ± 0.09 <sup>b</sup>

<sup>a,b</sup>Different letters in the same row mean significant differences ( $p \leq 0.05$ ).

( $n=12$ )

**Table2.** Total fatty acids composition of saturated, unsaturated, monounsaturated, polyunsaturated and atherogenicity index (AI) of conventional and ecological milk fat from Asturias, Spain.

Fatty Acids	Conventional Milk	Ecological Milk
Saturated	73.85 ± 2.85 <sup>a</sup>	67.62 ± 2.78 <sup>b</sup>
Unsaturated	26.05 ± 1.98 <sup>a</sup>	32.88 ± 1.72 <sup>b</sup>
Monounsaturated	23.40 ± 1.52 <sup>a</sup>	27.14 ± 1.15 <sup>b</sup>
Polyunsaturated	3.15 ± 0.25 <sup>a</sup>	5.24 ± 0.31 <sup>b</sup>
Atherogenicity index (AI)	3.27 ± 0.24 <sup>a</sup>	2.46 ± 0.19 <sup>b</sup>

<sup>a,b</sup>Different letters in the same row mean significant differences ( $p \leq 0.05$ ). ( $n=12$ )

AI: (C12:0 + 4 x C14:0 + C16:0/MUFA + PUFA).

**Table3.** Triglycerides composition (% of total triglycerides) of conventional and ecological milk fat from Asturias, Spain.

Triglyceride	Conventional milk	Ecological milk
CN24	0.16 ± 0.02 <sup>a</sup>	0.13 ± 0.01 <sup>a</sup>
CN26	0.28 ± 0.01 <sup>a</sup>	0.21 ± 0.02 <sup>a</sup>
CN28	0.56 ± 0.03 <sup>a</sup>	0.51 ± 0.02 <sup>a</sup>
CN30	1.28 ± 0.11 <sup>a</sup>	1.22 ± 0.12 <sup>a</sup>
CN32	2.65 ± 0.15 <sup>a</sup>	2.58 ± 0.14 <sup>a</sup>
CN34	6.51 ± 0.19 <sup>a</sup>	6.39 ± 0.21 <sup>a</sup>
CN36	11.74 ± 0.24 <sup>a</sup>	11.53 ± 0.28 <sup>a</sup>
CN38	12.36 ± 0.26 <sup>a</sup>	12.09 ± 0.32 <sup>a</sup>
CN40	9.61 ± 0.18 <sup>a</sup>	9.18 ± 0.21 <sup>b</sup>

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CN42	7.19 ± 0.21 <sup>a</sup>	6.86 ± 0.22 <sup>a</sup>
CN44	7.36 ± 0.27 <sup>a</sup>	6.95 ± 0.08 <sup>b</sup>
CN46	8.12 ± 0.41 <sup>a</sup>	7.52 ± 0.24 <sup>b</sup>
CN48	9.67 ± 0.52 <sup>a</sup>	9.35 ± 0.55 <sup>b</sup>
CN50	10.93 ± 0.35 <sup>a</sup>	11.64 ± 0.38 <sup>b</sup>
CN52	8.06 ± 0.25 <sup>a</sup>	8.66 ± 0.29 <sup>b</sup>
CN54	2.81 ± 0.25 <sup>a</sup>	3.55 ± 0.31 <sup>b</sup>

<sup>a,b</sup>Different letters in the same row mean significant differences ( $p \leq 0.05$ ). ( $n=12$ )

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