The Contents of *Trans* Fatty Acids and CLA in Cow Colostrum and Milk Fat in the Early Lactation Period

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Abstract

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The *trans* fatty acids as well as c*is*-9 *trans*-11 C18:2 (CLA) were determined in the colostrum and milk fat of individual cows during up to 82 days of lactation. The analyses were performed using gas chromatography (GLC) on a capillary column (CP Sil 88) combined with the method of argentation thin-layer chromatography (Ag-TLC). The results obtained in the study indicated that the milk fat of individual cows, kept under identical living and feeding conditions, was characterised by an extremely high variability of *trans* isomers concentration, especially of those of C18:1 acid. The differences referred not only to the total content of *trans* C18:1 isomers but also to the proportion of *trans*-10 and *trans*-11 isomers of C18:1. In some fat samples, a high concentration of *trans* C18:1 isomers was observed and *trans*-10 isomer of C18:1 was found to dominate. In the case of all the cows, the colostrum fat was characterised by a lower average content of the examined acids as compared to the milk fat. The average value of CLA in the colostrum fat of all the cows under study was 0.34%, and in the milk fat 0.42% of total fatty acids.

Keywords: milk fat; *trans* C18:1; *trans* C18:2; *cis*-9 *trans*-11 C18:2 (CLA); lactation period; GLC/TLC-$AgNO_3$ analysis

Milk fat is characterised by a high variability in the fatty acid composition caused by multiple factors, among which belongs the lactation period. The research by Jaworski and Jaworska (1973) indicated that substantial differences in the quantitative fatty acid composition of milk fat linked to the lactation period occurred mainly in the early and late periods of lactation. According to the authors' results, milk fat of cows in the early period of lactation was characterised by higher concentrations of unsaturated fatty acids (especially in the second month) in comparison to the later lactation phases. In the third month of lactation, the concentration of higher saturated fatty acids increased, whereas that of unsaturated acids (mainly oleic and linoleic) decreased. The results of a study by Palmquist *et al.* (1993) indicated that the milk fat from cows in the first week of lactation had a low content of short-chain fatty acids (except for C4:0 acid) and a high content of C18:0 and C18:1 acids. The concentration of short-chain fatty acids in the milk fat increased until the 8th week of lactation, which was accompanied by a decrease in the fatty acids from the C18 group.

So far, the studies performed concerned the determination of the total fatty acid composition of milk fat of individual lactating cows. Little is known about the variation of *trans* fatty acid isomers among individual samples of milk in the lactation period. The objective of the present investigation was to evaluate the content of *trans* unsaturated fatty acids and the concentration of *cis*-9 *trans*-11 C18:2 acid (CLA) in the colostrum and milk fat from individual cows in the early period of lactation.

MATERIAL AND METHODS

Experimental material. The experimental material comprised the fat extracted from the milk of Holstin-Friesian cows originating from a multi-herd barn of the Research and Production Station in Bałcyny. All cows involved in the study were in the second lactation and calved in the period of 19–29 January 2002. Throughout the experimental period, the cows were fed indoors with the same mixed feed consisting of: 27 kg of corn silage, 2 kg of lucerne hay silage, 10 kg of grass hay silage and 10 kg of concentrate mix. From day 1 to day 8 of lactation, the cows were additionally given hay.

The samples of colostrum and milk were taken separately from each of the six experimental cows, beginning from the first milking performed on the day of calving (samples marked as No. 1). Subsequent samples originated from morning milking on days 2, 4, 6, 8, 10, 17, 24, 38, 52, and 82 after parturition.

Analytical methods. The fat contents of colostrum and milk samples were determined by the Gerber method (Anonym 2002). The extraction of fat from the colostrum and samples was based on the method of Röese-Gottlieb (Anonym 1996).

Methyl esters of fatty acids were prepared according to the IDF method (Anonym 1999).

The total fatty acid composition of the extracted fat was determined by direct gas chromatography (GLC) on a Hewlett-Packard 6890 chromatograph with a flame ionisation detector under the following conditions: a CP Sil 88 (100 m × 0.25 mm) with 0.20 μm film capillary column; column temperature of 60°C (1 min) – 180°C, Δt = 5°C/min; sample injector: split 100:1; temperature of the detector and sample injector reaching 250°C and 225°C, respectively; and helium used as a carrier gas at a flow rate of 0.8 ml/min.

To determine all positional *trans* isomers of C18:1 acid, a combination of gas chromatography (GLC) and thin-layer chromatography (Ag-TLC) was applied. The methyl esters of fatty acids were separated into four fractions by thin-layer chromatography using silver nitrate: saturated, *trans*-monoenoic, *cis*-monoenoic, and polyenoic (Żegarska *et al*. 1996). The *trans*-monoenoic fraction was eluted from the plates together with the saturated fraction with ethyl ether. After the evaporation of ether, the methyl esters were dissolved in hexane and used for GLC analyses under the same chromatographic conditions as the total fatty acid methyl esters, but in a splitless system.

The identification of *trans* fatty acids was based on the standards (Sigma and Supelco) and literature data (Henninger & Ulberth 1994; Wolff 1994; Precht & Molkentin 1997).

The contents of positional *trans* isomers of C18:1 acid were calculated on the base of C18:0 acid content as obtained in the determination of the total fatty acid composition.

All samples were analysed in duplicates and the mean values were reported. The content of fatty acids was expressed as the percentage of the total fatty acids (wt%).

RESULTS AND DISCUSSION

Figure 1 presents the total content of *trans* C18:1 isomers in colostrum and milk fat of individual cows during up to 82 days of lactation. The lowest content of *trans* C18:1 isomers, ranging from 1.32% to 2.45%, was noted in the colostrum samples taken on the day of calving. On subsequent days of lactation, the content of these isomers in the colostrum and milk increased, however, to a different extent with each cow examined. In the colostrum and milk of cows No. 182, 144, and 142, the total content of *trans* C18:1 isomers fluctuated negligibly. The level of *trans* C18:1 isomers in the colostrum and milk fat of the other cows was found to be highly diversified. On days 17, 24, and 52 of lactation, a remarkably high content of those isomers was observed in the milk fat of cows No. 208 and 109 (Figure 1). In the samples collected from cow No. 232, a high concentration of *trans* C18:1 isomers was observed as early as on the 8th day of lactation (7.80%) and it fluctuated on the subsequent days of lactation.

The results point to a high differentiation, not only in the total content of *trans* C18:1 isomers, but also in the contents of individual positional *trans* isomers of this acid. These differences referred mainly to *trans*-10 and *trans*-11 isomers of C18:1 acid. The contents of the remaining positional *trans* isomers of C18:1, i.e. *trans*-4, *trans*-5, *trans*-6–8, *trans*-9, *trans*-12, *trans*-13, *trans*-14, *trans*-15, and *trans*-16, did not demonstrate high variations either in colostrum fat or milk fat.

The bulk milk fat is characterised by a high level of *trans*-11 isomer which constitutes ca. 50% of the total C18:1 isomers (Wolff 1994; Precht & Molkentin 1997). The *trans*-10 isomer occurs

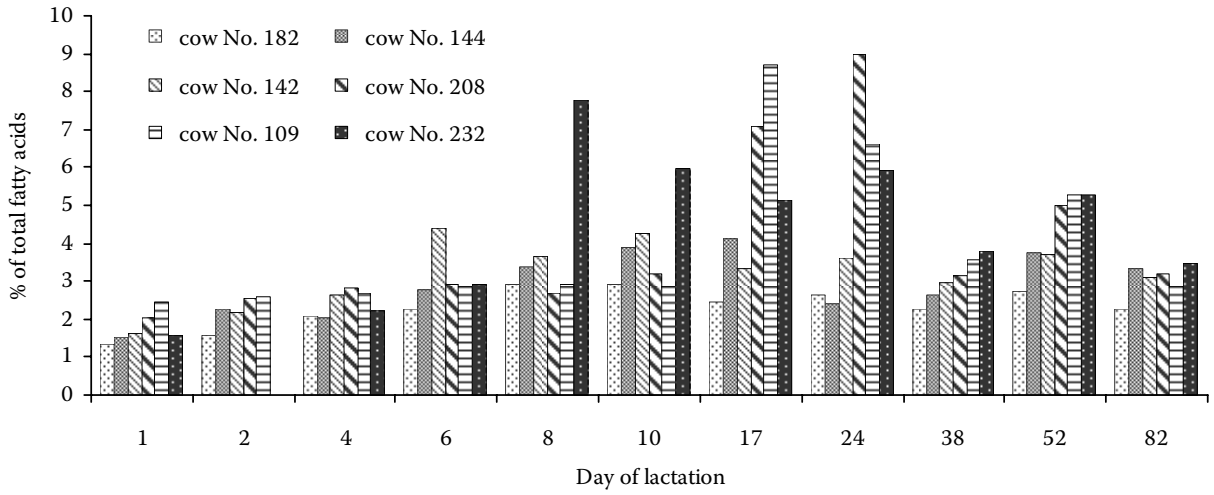

Figure 1. Sum of *trans* C18:1 isomers in colostrum and milk fat from individual cows

in low amounts (ca. 5%) (Parodi 1976; Precht & Molkentin 1994, 1996).

As shown in Figure 2, on some days of lactation, the dominant isomer in the fat of some samples was not vaccenic acid (*trans*-11) but *trans*-10 C18:1 isomer. The level of t*rans*-10 isomer in the milk fat of the cows No. 208 and 109 on day 17 and 24 of lactation was substantially higher than that of *trans*-11 isomer. In the samples taken from cow No. 232, the percentage of that isomer was found to be very high (about 58% of total *trans* C18:1 isomers) on day 8 of lactation. A high content of *trans*-10 isomer was found also in the milk fat of that cow on day 10, 17, 24, and 52 of lactation. The high concentration of *trans*-10 isomer in the milk fat of cows No. 208, 109, and 232 corresponded to a high total concentration of *trans* C18:1 isomers (Figure 1). The data in Figure 2 indicate that the proportions of *trans*-10 and *trans*-11 isomers of C18:1 acid were not similar even in the colostrum and milk fat of the cows No. 142 and 144 characterised by small variations in the total content of *trans* C18:1 isomers.

The investigations by Griinari *et al.* (1998) and Piperova *et al.* (2000) indicate that a high concentration of *trans*-10 C18:1 isomer is observed in the case of feeding cows a concentrated mixture with a low content of fibre. Griinari *et al.* (1998) obtained a high concentration of *trans*-10 C18:1 isomer in the milk fat of cows fed a low-fibre mixture supplemented with corn oil. The study of Piperova *et al.* (2000) indicated that the cows fed a diet with a high proportion of concentrate and 5% addition of soybean oil were characterised by an increasing concentration of *trans* C18:1 isomers, with *trans*-10 isomer dominating. The latter constituted almost 60% of the total content of *trans* C18:1 isomers, whereas *trans*-11 isomer – as little as 11%. In addition, the milk of these cows contained 43% less fat than those of the cows fed a control diet.

The samples of colostrum and milk were also analysed for the fat content. The highest fat content was found in the colostrum on the day of calving. The fat content fluctuated in the samples collected on subsequent days of lactation. In some milk samples it was found to be low (on day 24 of lactation, the milk of cow No. 208 contained as little as 2% of fat, and that of cow No. 109 – as little as 2.2% of fat). The average contents of fat in the colostrum and milk of all cows amounted to 5.5% and 3.7%.

The fat content in the samples tested was negatively correlated with the total content of *trans* C18:1 isomers ($r = -0.561$, $\alpha = 0.01$). A very similar correlation coefficient ($r = -0.55$) between those characteristics of milk fat of the control group of cows after 13–14 weeks of lactation was reported by Precht *et al.* (2002). A higher negative correlation coefficient ($r = -0.850$) for milk fat of individual cows was found by Wonsil *et al.* (1994).

The total content of *trans* isomers of C18:2 acid in the samples of colostrum and milk of individual cows is presented in Figure 3. The lowest content of these isomers was observed in the colostrum on the first day of lactation. In the colostrum and milk samples from cows No. 208, 109, and 232, the content of *trans* C18:2 isomers was subject

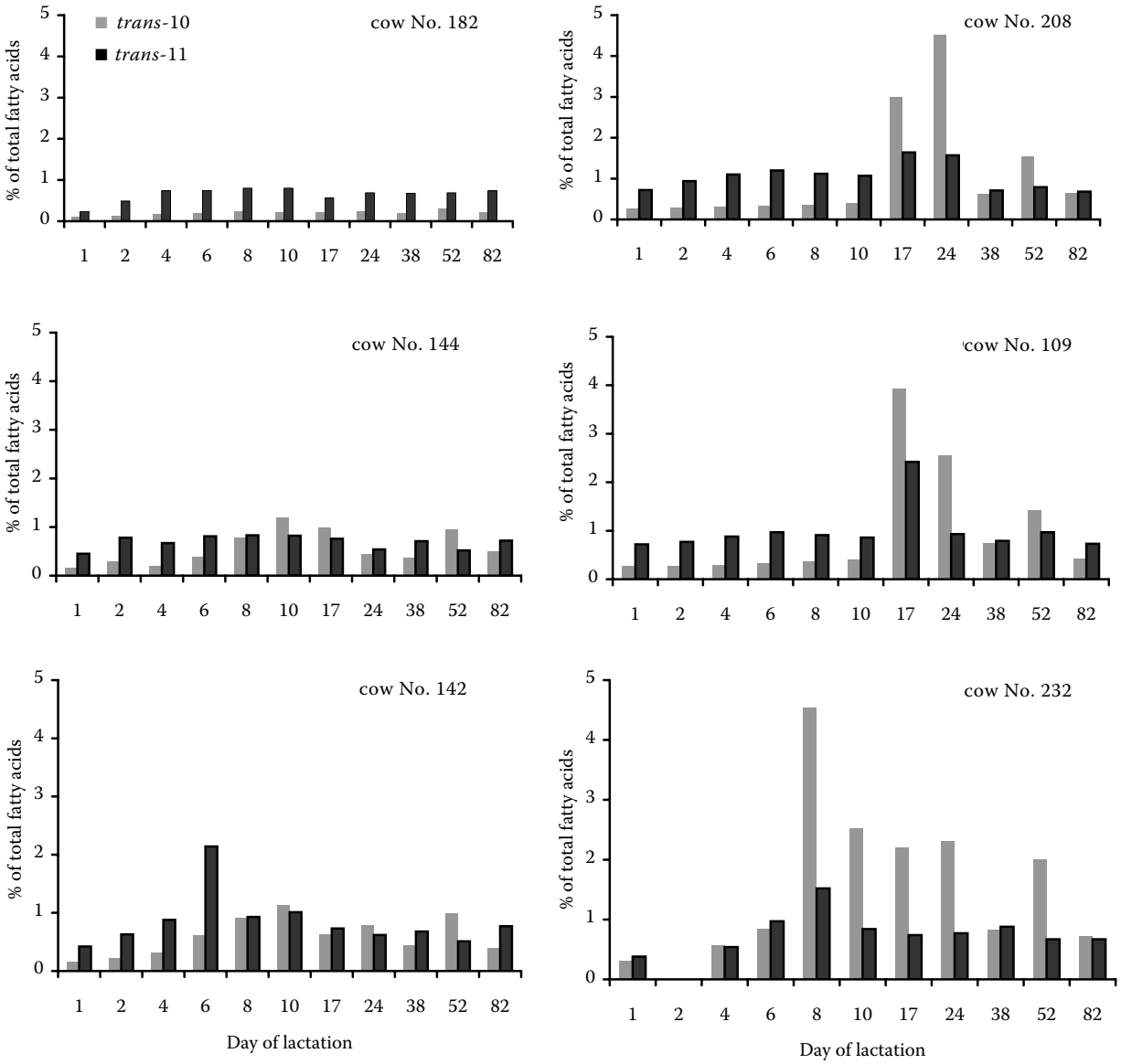

Figure 2. Content of *trans*-10 and *trans*-11 isomers of C18:1 in colostrum and milk fat from individual cows

to negligible changes up to day 10 of lactation. On days 17 and 24 of lactation, it was found to increase considerably. On the subsequent days of lactation, the content of *trans* C18:2 isomers was observed to fluctuate. In the colostrum and milk fat of cow No. 182, no apparent changes occurred in the content of *trans* C18:2 isomers, contrary to the samples taken from cows No. 144 and 142 demonstrating a substantial increase in the contents of those isomers on day 52 of lactation.

The content of *cis*-9 *trans*-11 C18:2 acid (CLA) in the colostrum fat of the cows on the first day of lactation ranged between 0.22% (cow No. 232) and 0.28% (cow No. 109) (Figure 4), with the exception of cow No. 208 (0.41%). In the samples taken from that cow, CLA content was observed to fluctuate, demonstrating the highest content (0.77%) on day 17 of lactation. On the same day, a considerable increase was also observed in the content of *cis*-9 *trans*-11 C18:2 acid in the milk fat of cow No. 109. In the colostrum and milk samples taken from cow No. 142, an increase in CLA content was observed as early as on day 6 of lactation. The subsequent days of lactation revealed fluctuations in CLA content. In the colostrum and milk fat of cows No. 182 and 144, no remarkable changes were observed in the content of this acid.

The results presented in Table 1 indicate that *trans* C18:1, *trans* C18:2 and CLA contents in the milk fat were higher compared to the colostrum

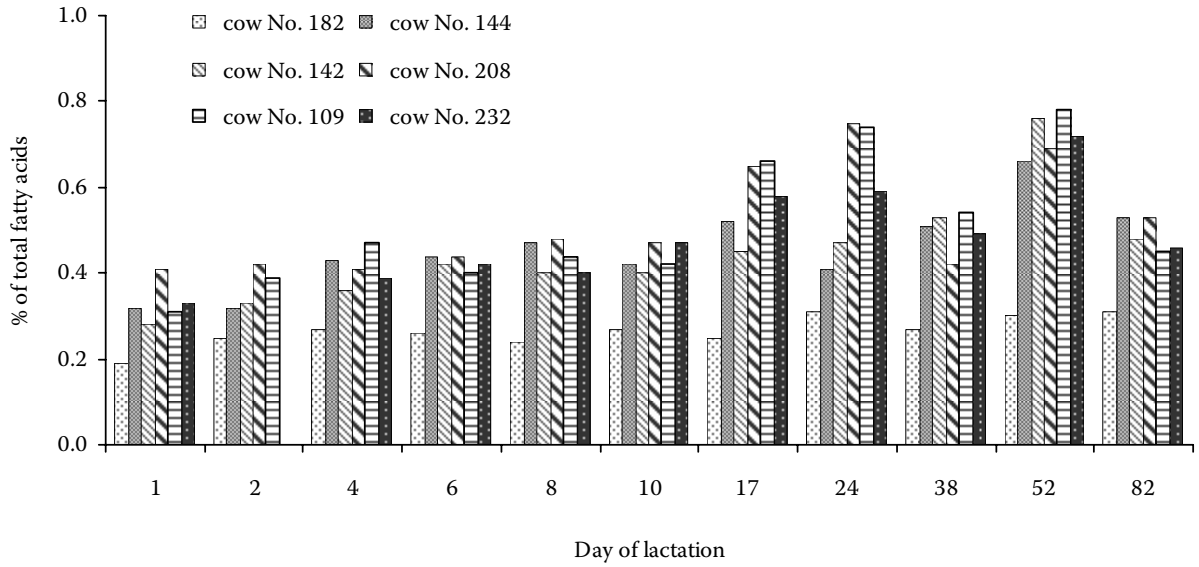

Figure 3. Content of *trans* C18:2 isomers in colostrum and milk fat from individual cows

fat. However, due to the very high standard deviations of the results, only in some cases were the differences significant. However, taking into account the colostrum and milk fat from all cows, significant differences in the average values of these acids were found.

The sum of *trans* C18:1 isomers in the samples tested was significantly positively correlated with the sum of *trans* C18:2 isomers (r = 0.759) and CLA content (r = 0.673). The coefficient of correlation between the CLA content and the proportion of *trans*-11 C18:1 isomer (r = 0.731) was similar to that reported by Jiang *et al.* (1996) for the milk fat obtained from individual cows (r = 0.78).

The total content of *trans* C18:1 isomers in bulk milk ranges from 1.29 to 6.75% (Precht & Molkentin 1996). The results of this work show that in the case of individual cows, the concentrations of *trans* C18:1 isomers in the milk fat may substantially differ from their contents in the bulk milk fat. As reported by Griinari *et al.* (1998), the synthesis of high amounts of C18:1 acid *trans* isomers requires the presence of a substrate (unsaturated fatty acids) and an altered rumen

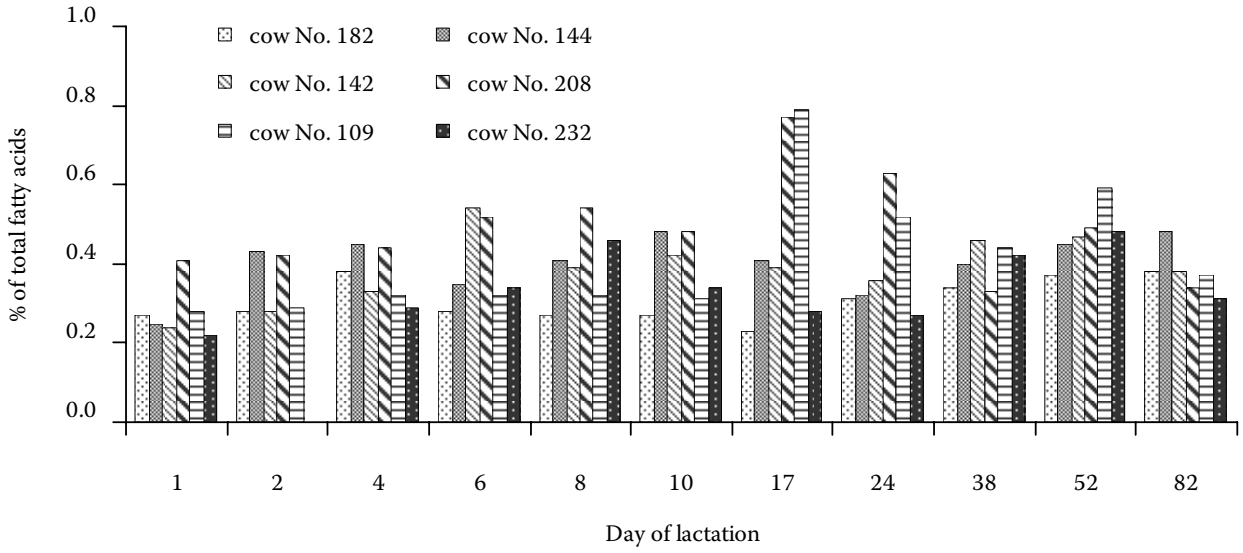

Figure 4. Content of *cis*-9 *trans*-11 C18:2 (CLA) isomers in colostrum and milk fat from individual cows

Table 1. Contents of *trans* C18:1, *trans* C18:2 and *cis*-9 *trans*-11 18:2 (CLA) in colostrum and milk fat from individual cows

Cow No.	Fatty acids	Colostrum fat (1–6 day of lactation)	*Milk fat (8–82 day of lactation)*	Sig. level
		mean ± SD		
182	Σ *trans* 18:1	1.82 ± 0.44	*2.60 ± 0.27*	**
	Σ *trans* 18:2	0.24 ± 0.04	*0.28 ± 0.03*	
	cis-9 *trans*-11 18:2 (CLA)	0.30 ± 0.05	*0.31 ± 0.06*	
144	Σ *trans* 18:1	2.15 ± 0.52	*3.31 ± 0.73*	*
	Σ *trans* 18:2	0.38 ± 0.07	*0.50 ± 0.08*	*
	cis-9 *trans*-11 18:2 (CLA)	0.37 ± 0.09	*0.42 ± 0.06*	
142	Σ *trans* 18:1	2.71 ± 1.20	*3.52 ± 0.43*	
	Σ *trans* 18:2	0.35 ± 0.06	*0.50 ± 0.12*	
	cis-9 *trans*-11 18:2 (CLA)	0.35 ± 0.13	*0.41 ± 0.04*	
208	Σ *trans* 18:1	2.59 ± 0.40	*4.75 ± 2.41*	
	Σ *trans* 18:2	0.42 ± 0.01	*0.57 ± 0.12*	**
	cis-9 *trans*-11 18:2 (CLA)	0.45 ± 0.05	*0.51 ± 0.16*	
109	Σ *trans* 18:1	2.65 ± 0.18	*4.69 ± 2.28*	
	Σ *trans* 18:2	0.39 ± 0.07	*0.58 ± 0.15*	*
	cis-9 *trans*-11 18:2 (CLA)	0.30 ± 0.02	*0.48 ± 0.17*	
232	Σ *trans* 18:1	2.23 ± 0.69	*5.35 ± 1.45*	**
	Σ *trans* 18:2	0.38 ± 0.05	*0.53 ± 0.11*	
	cis-9 *trans*-11 18:2 (CLA)	0.28 ± 0.06	*0.37 ± 0.09*	
All cows	Σ *trans* 18:1	2.36 ± 0.35	*4.04 ± 1.05*	**
	Σ *trans* 18:2	0.36 ± 0.06	*0.49 ± 0.11*	**
	cis-9 *trans*-11 18:2 (CLA)	0.34 ± 0.06	*0.42 ± 0.07*	**

values are significantly different; $^{**}P \leq 0.01$, $^{*}P \leq 0.05$

environment, which in turn leads to an incomplete biohydrogenation. The high level of *trans* C18:1 isomers in the milk fat is linked to feeding cows with a highly-concentrated mixture with a low content of fibre (Griinari *et al*. 1998; Piperova *et al*. 2000). The cows under study originated from a multi-herd barn and were fed high concentrate diets. The fibre contained in the feed ration was administered in a disintegrated form. The feeding regime applied could, therefore, cause a decrease in the pH of the rumen contents, which may have altered its bacterial ecosystem. At high rations of concentrate feed, as reported by Chilliard *et al*. (2000), the rate of hydrogenation decreases and the milk contains more intermediate products of the biohydrogenation process. Such a high variability of the contents of these isomers, apart from physiological factors, was determined to a high degree by the individual traits of cows and probably by the differences in their feed intake on some days of lactation, as shown by the differences in the contents and composition of *trans* isomers of C18:1 acid.

CONCLUSIONS

The results of this work indicate that the colostrum and milk fat of individual cows in the early period of lactation and kept under the same living and feeding conditions, were characterised by an extremely high variability in the *trans* isomers concentration, especially in those of C18:1 acid.

The changes in the total concentration of *trans* C18:1 isomers resulted mostly from the changes in the proportion of the positional isomers *trans*-10 and *trans*-11. In the case of all the cows examined, the colostrum fat was characterised by lower average contents of *trans* C18:1, *trans* C18:2 and *cis*-9 *trans*-11 C18:2 in comparison to the milk fat.

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