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ANNUAL CHANGES IN THE CONTENT OF UNSATURATED FATTY ACIDS WITH 18 CARBON ATOMS, INCLUDING *CIS*9*TRANS*11 C18:2 (CLA) ACID, IN MILK FAT

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The fatty acid composition of milk fat during a year was determined by gas chromatography with the use of a 100-m capillary column with high-polar liquid phase. The experimental material were samples of fat isolated with the extraction method from 30% non-pasteurised cream.

The study demonstrated that the range of changes in the content of *trans* isomers of milk fat over a year was significantly higher than that of *cis* isomers of those acids. The content of C18:1 *trans* isomers and *cis*9*trans*11 C18:2 (CLA) acid was *ca*. 3-fold higher, whereas that of *trans* isomers of C18:2 acid – about 2.5-higher in milk fat from summer feeding of the cows as compared to their concentrations in milk fat from winter feeding. The content of *cis*9*trans*11 C18:2 acid in milk fat during a year ranged from 0.39 to 1.76% of the total fatty acid composition. The average content of oleic acid in milk fat from the period of summer feeding accounted for 20.63% and was higher by 2.6 percentage units as compared to fat content in the winter feeding. The concentration of linoleic acid in fatty acid composition was almost constant during a year. The contribution of linolenic acid was reported to range from 0.38 to 1.46% and its content of milk fat from the period of summer feeding was twice as much as that of milk fat from the winter feeding period.

INTRODUCTION

Milk fat is characterised by a highly complex composition of fatty acids. Up to date over 400 fatty acids were identified in milk fat [Jensen, 2002]. Unsaturated fatty acids with 18 carbon atoms constitute *ca*. 25% of the total fatty acids of milk fat. That group includes acids with a high biological activity such as: oleic, linoleic, linolenic, vaccenic, linoleic with a conjugated system of bonds. Milk fat is the main source of linoleic acid with the conjugated system of bonds, *i.e*. *cis*9*trans*11 C18:2 acid, that possesses anticarcinogenic, antiatherogenic and immunomodulating properties [Cook & Pariza, 1998; Kritchevsky, 2000]. Whereas, *trans* vaccenic acid (*trans* 11 C18:1), the major *trans* isomer of fatty acids of milk fat, is a substrate in endogenous synthesis of linoleic acid with conjugated bonds (CLA) [Turpeinen *et al*., 2002].

Previous extended research on milk fat carried out in Poland [Jaworski, 1978; Żegarska, 1988; Staniewski, 2000] have demonstrated a high variability of fatty acid composition. However, chromatographic columns with the packing used in those studies did not allow the *cis* fatty acids to be separated from the *trans* ones of the same length of the carbon chain. The content of linoleic acid with the conjugated bonds was not determined either, since the acid was discharged from the chromatographic column together with linolenic acid, which resulted in its increased content.

The studies were aimed at evaluating the content of unsaturated fatty acids from the C18 group, including CLA, in fat of bulk milk during a year, using the capillary column chromatography.

MATERIALS AND METHODS

Material

The material analyzed was milk fat extracted from 30% non-pasteurized cream. The cream, obtained directly after centrifugation of the bulk milk, was collected in the Olsztyn Dairy Cooperative 8 times per month during a year.

Methods

Fat was extracted from the cream according to the method of Roese-Gottlieb [IDF Standard 1D:1996]. After two-fold extraction, ether solution was filtered through a filter with anhydrous sodium sulfate. The solvent was distilled using a vacuum evaporator and its residues were removed by blowing the fat with nitrogen.

Methyl esters of fatty acids of milk fat were prepared according to the IDF method [IDF Standard 182:1999].

Separation of methyl esters of fatty acids of milk fat was carried by the gas chromatography method using a Hewlett Packard 6890 chromatograph with flame-ionization detector. The separation was carried out under the following conditions: CP Sil 88 capillary column (100m × 0.25 mm i.d.), film thickness of liquid phase – 0.20 μm, column temperature: 60°C (1 min) – 180°C, $\Delta t = 5°C$/min; sample injector's temperature – 225°C; detector's temperature – 250°C; carrier

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gas – helium (flow rate: 0.8 mL/min), injector – split 1:100. Identification of methyl esters of the *trans* fatty acids and linoleic acid with conjugated bonds was carried out by comparing their retention times with those of standards (Sigma). The percent content of fatty acids was calculated in reference to the total fatty acid composition (weight percentage).

RESULTS AND DISCUSSION

Annual changes in the content of *trans* and *cis* isomers of C18:1 acid determined were presented in Figure 1. Changes in the contents of *trans* isomers of C18:2 acid, C18:2 acid with the conjugated bonds as well as linoleic and linolenic acids were shown in Figure 2. The average content and fluctuations in the concentration of unsaturated fatty acids with 18 carbon atoms in milk fat during summer and winter feeding as well as during transition period (May, October and November) were compiled in Table 1.

As shown in Figure 1A, the total content of C18:1 *trans* isomers in milk fat was subject to considerable fluctuations during a year. During winter feeding of the cows (January––April), the content of those acids remained at a similar level (*ca.* 1.4% of the total fatty acids). In the fat from May, an increase was observed in the concentration of C18:1 *trans* isomers up to a level of 3.97%. This was caused by the transition into summer feeding characterized by a high concentration of polyenolic acids (*ca.* 50% of linolenic acid and *ca.* 15–25% of linoleic acid [Precht *et al*., 1985]). In the fat from the summertime (June-September), the content of C18:1 *trans* isomers was similar, yet the highest content of those isomers was determined in the fat from August. In the fat of milk from consecutive months, the concentration of C18:1 *trans* isomers was observed to decrease significantly

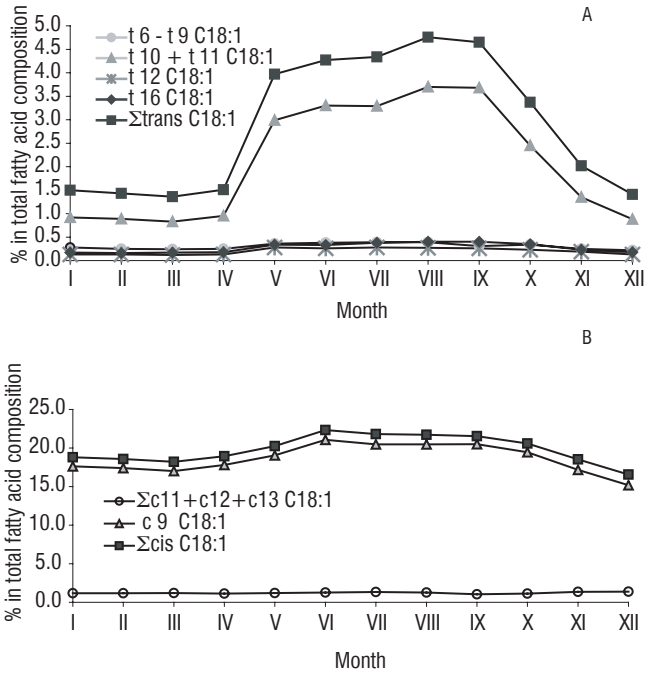

FIGURE 1. Annual changes in the content of (A) *trans* isomers of C18:1 acid and (B) *cis* isomers of C18:1 acid in milk fat.

TABLE 1. Contents of unsaturated fatty acids with 18 carbon atoms in milk fat from the periods of summer and winter feeding and transition period.

Fatty acids	Summer feeding (June – September)			Winter feeding (January – April and December)			Transition period (May, October, November)		
	Min	Max	$\bar{X}$ ± SD	Min	Max	$\bar{X}$ ± SD	Min	Max	$\bar{X}$ ± SD
Trans isomers of C18:1									
t 6 – *t* 9	0.28	0.47	0.37^{A} ± 0.04	0.21	0.30	0.25^{C} ± 0.03	0.20	0.40	0.32^{B} ± 0.06
t 10 + *t* 11	2.74	4.53	3.49^{A} ± 0.37	0.77	1.24	0.89^{C} ± 0.09	0.88	3.86	2.26^{B} ± 0.88
t 12	0.00	0.31	0.26^{A} ± 0.05	0.00	0.16	0.12^{C} ± 0.02	0.15	0.33	0.23^{B} ± 0.05
t 16	0.28	0.44	0.38^{A} ± 0.04	0.14	0.27	0.17^{C} ± 0.02	0.17	0.38	0.31^{B} ± 0.07
Σ *trans* C18:1	3.57	5.37	4.50^{A} ± 0.38	1.26	1.84	1.43^{C} ± 0.11	1.43	4.92	3.12^{B} ± 1.04
c 9	19.56	22.41	20.63^{A} ± 0.61	14.43	20.05	17.01^{C} ± 1.25	15.29	20.62	18.55^{B} ± 1.42
c 11	0.56	1.13	0.91^{a} ± 0.15	0.71	1.06	0.93^{a} ± 0.08	0.74	1.30	0.91^{a} ± 0.14
c 12	0.15	0.28	0.21^{a} ± 0.04	0.12	0.27	$0.19^{a.b}$ ± 0.04	0.17	0.35	0.22^{b} ± 0.05
c 13	0.06	0.15	0.10^{a} ± 0.02	0.06	0.12	0.09^{b} ± 0.02	0.08	0.15	0.11^{a} ± 0.02
Σ *cis* C18:1	20.57	23.72	21.85^{A} ± 0.34	15.79	21.34	18.22^{C} ± 0.97	16.40	21.82	19.78^{B} ± 1.11
Trans isomers of C18:2									
c 9 *t* 13	0.18	0.45	0.30^{A} ± 0.07	0.09	026	0.14^{B} ± 0.04	0.18	0.46	0.30^{A} ± 0.08
c 9 *t* 12	0.07	0.14	0.11^{B} ± 0.02	0.00	0.18	0.11^{B} ± 0.05	0.08	0.35	0.15^{A} ± 0.07
t 9 *c* 12	0.00	0.09	0.06^{a} ± 0.02	0.00	0.11	0.04^{a} ± 0.04	0.00	0.08	0.05^{a} ± 0.02
t 11 *c* 15	0.33	0.67	0.49^{A} ± 0.09	0.08	0.21	0.12^{C} ± 0.03	0.16	0.54	0.36^{C} ± 0.12
Σ *trans* C18:2	0.65	1.19	0.96^{A} ± 0.16	0.29	0.61	0.41^{C} ± 0.08	0.51	1.07	0.86^{B} ± 0.16
*c*9 *t* 11 C18:2 (CLA)	1.06	1.76	1.40^{A} ± 0.16	0.32	0.52	0.40^{C} ± 0.05	0.46	1.49	1.02^{B} ± 0.33
c9 c 12 C18:2	1.24	1.58	1.37^{a} ± 0.08	1.22	1.62	1.37^{a} ± 0.09	1.04	1.46	1.31^{a} ± 0.11
c9 c12 c15 C18:3	0.70	1.46	1.05^{A} ± 0.22	0.38	0.69	0.50^{B} ± 0.07	0.60	1.02	$0.77^{A.B}$ ± 0.13

Values denoted in lines with the same letters are not statistically different. a, b – statistically significant at p≤0.05; A, B – statistically significant at p≤0.01)

in October and November, and down to 1.41% in December. The range of changes in the sum of C18:1 *trans* isomers in milk fat during a year resulted from changes in the content of *trans* 10+*trans* 11 isomers. The content of those isomers accounted for 0.83% in the fat from March, and for 3.70% in that from August. The average content of *trans* 10+*trans* 11 isomers in milk fat from the summer feeding (3.49%) was over three-fold higher as compared to their content in fat from the winter feeding period (0.89%) (Table 1). On average, the content of *trans* 10 + *trans* 11 isomers in fat reached 77.6% during summer feeding, 62.2% during winter feeding, whereas during transition period 72.4% of the total C18:1 *trans* isomers.

The separation carried out under the applied conditions did not allow separating *trans* 10 and *trans* 11 isomers of C18:1. In the total content of both these isomers, *ca.* 90% is constituted by *trans* 11 isomer (vaccenic acid) [Precht & Molkentin, 1994,1996], the major *trans* isomer of the fatty acids of milk fat. The *trans* 10 isomer constitutes as little as 4.7% of the total *trans* isomers of C18:1 acid [Precht & Molkentin, 1994]. According to Parodi [1976], the content of *trans* 10 isomer in milk fat reaches 5.5% of the total *trans* isomers of C18:1 acid. Investigations of Precht & Molkentin [1997a] showed that the content of C18:1 *trans* 10 isomer in the fat of bulk milk did not change during a year. The average content of that isomer in milk fat in the period of winter feeding accounted for 0.16%, whereas in fat from the summer feeding – for 0.18% of the total fatty acids. For this reason, changes in the content of vaccenic acid have been found to determine changes in the sum of those two *trans* isomers of C18:1.

The other determined C18:1 *trans* isomers, *i.e.* the total content of *trans* 6 to *trans* 9, *trans* 12 and *trans* 16, were found to occur in milk fat in very low concentrations (Table 1). The milk fat obtained during summer feeding contained significantly more of these isomers compared to the fat from the winter feeding and transition period (Table 1). However, fluctuations in the content of these positioning isomers during a year were minor as compared to changes in the total content of *trans* 10 and *trans* 11 isomers (Figure 1A).

The average total content of the C18:1 *trans* isomers determined in milk fat from the summer feeding period accounted for 4.5%, whereas in fat from the winter feeding – for 1.43% and during the transition period – for 3.12% (Table 1). Similar results were reported by Bartnikowska *et al*. [1999]. It was found that butter fat from the winter season contained 1.4% of C18:1 *trans* isomers on average, whereas in that from the summer feeding the content of those isomers reached 3.55%.

The major C18:1 *cis* isomer, oleic acid (*cis* 9 C18:1), constituted on average 92.6% (during winter feeding) and 93.4% (during summer feeding) of the total C18:1 *cis* isomers (Table 1). The lowest content of that acid (16.56%) was determined in fat from December. A change in cows feeding regime from winter to summer one did not result in any significant increase in the content of that acid (Figure 1B). The content of oleic acid in fat from May was higher only by 1.2 percentage units than in fat from April. The oleic acid of milk fat derives not only from blood lipids [Hawke & Taylor, 1983]. Over 50% of the oleic acid is synthesized in the mammary gland from stearic acid in the presence of delta-9 desaturase [Chilliard *et al.*, 2000].

Of the other *cis* isomers of C18:1 acid, the *cis* 11 C18:1 isomer occurred in milk fat in the highest quantities (*ca*. 0.9% of the total fatty acids). Its average content in the compared periods of cow feeding appeared to reach a similar level.

The applied conditions of chromatographic separation enabled separating *trans* isomers of C18:2 acid (*cis,trans*/*trans,cis*). In a group of those isomers, four C18:2 *trans* isomers were identified as: *trans*11*cis*15, *cis*9*trans*12, *trans*9*cis*12 and *cis*9*trans*13 (Table 1).

The content of the total C18:2 *trans* isomers in the analysed samples of milk fat ranged from 0.29% to 1.19% (Table 1). The tendency of seasonal fluctuations in the content of those isomers (Figure 2A) was similar to that of changes in the content of C18:1 *trans* isomers (Figure 1A). During the periods of green forage administration, the mean monthly content of C18:2 *trans* isomers ranged from 0.74% (June) to 1.11% (September), and during winter feeding (January–April, December) – from 0.38% (February) to 0.50% (April). The average content of C18:2 *trans* isomers in milk fat from the transition period (0.86%) was significantly different from that determined in milk fat from the other periods of feeding (Table 1). The results obtained were consistent with those reported by Precht & Molkentin [2000]. According to these authors, the total content of C18:2 *trans* isomers ranged from 0.11% to 1.41%, whereas their mean content in fat from the winter and summer feeding reached 0.46% and 0.87%, respectively.

As indicated in Figures 1A and 2A, the highest content of C18:1 and C18:2 *trans* isomers was demonstrated in milk fat from August and September. These results are consistent with findings of Stołyhwo [1997] who ascribed an increase in the content of *trans* isomers in milk fat reported in the early autumn to a significant concentration of polyenoic fatty acids in grass seeds ripening in that period.

During a year, high fluctuations were observed in the content of C18:2 acid with conjugated bonds (CLA, Figure 2A).

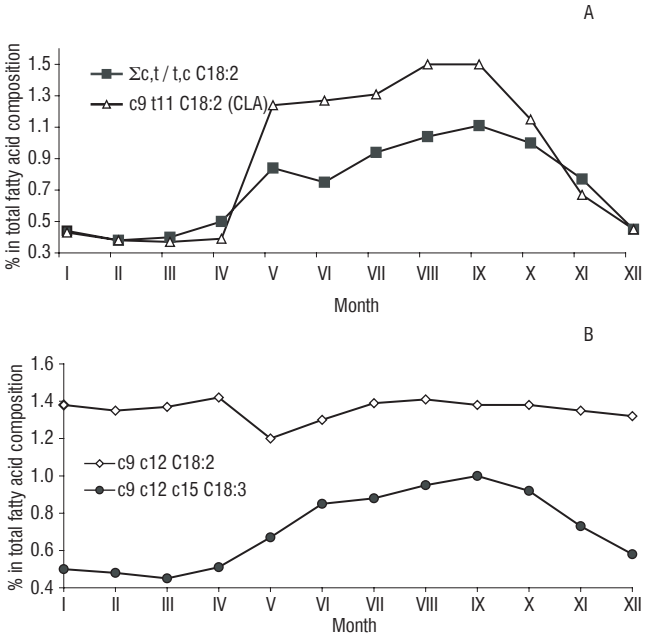

FIGURE 2. Annual changes in the content of (A) *cis, trans/trans, cis* isomers of C18:2 acid and *cis*9*trans*11 C18:2 acid (CLA), and (B) linoleic and linolenic acids in milk fat.

In the reported study, analyses were also carried out for the *cis*9*trans*11 C18:2 acid – the major CLA isomer. The content of this acid in the winter feeding period (January-April) reached *ca.* 0.4% (Figure 2A). A rapid increase (1.24%) in the concentration of *cis*9*trans*11 C18:2 in milk fat occurred in May. During the period of summer feeding its content was found to slightly increase, reaching the highest values in August and September (1.50%), and to finally decrease starting from October. The mean CLA content of milk fat from the period of summer feeding was about three-fold higher as compared to the period of winter feeding (Table 1). Similar concentrations of that acid in milk fat were obtained in previous studies that were carried out with a lower number of samples [Żegarska *et al*., 1996]. According to a study by Precht & Molkentin [1997b] (a few hundreds of milk fat samples were analysed from each period of feeding), the mean content of *cis*9*trans*11 C18:2 accounted for 1.20% in the summer feeding period, for 0.45% during winter feeding and for 0.76% during the transition one. A high seasonal variability in the content of *cis*9*trans*11 C18:2 acid in milk fat was also demonstrated by other authors [Henninger & Ulberth, 1994; Lin *et al.*, 1995; Jahreis *et al*., 1997].

Considering changes in the contents of linoleic (C18:2) and linolenic (C18:3) acids (Figure 2B), it can be noticed that the content of linoleic acid was almost constant during a year. The higher concentration of linoleic acid in green forage did not result in its increase in milk fat. The linoleic acid is intensively hydrogenated in the cow's rumen to *cis*9*trans*11 C18:2, and then to *trans* vaccenic acid and stearic acid [Kelly *et al*., 1998]. The milk fat from May was even characterised by a slight decrease in linoleic acid content. The mean content of linoleic acid in milk fat was found not to differ between the compared feeding periods (Table 1). An almost constant content of linoleic acid in milk fat during a year, determined in this work, confirms results obtained in Germany [Precht & Molkentin, 1997] and France [Ledoux *et al*., 2005].

In contrast, the concentration of the linolenic acid was subject to changes during a year, and both the tendency and range of those changes were similar to fluctuations in the total *trans* isomers of C18:2 acid (Figure 2A). The mean content of linolenic acid was two-fold higher in milk fat from the summer feeding as compared to that in milk fat from the winter feeding (Table 1). The mean annual content of linolenic acid in the analysed samples of milk fat accounted for 0.71%.

The values of correlation coefficients between the content of unsaturated fatty acids with 18 carbon atoms in milk fat (Table 2) indicate that the content of total C18:1 *trans* isomers is highly positively correlated with the content of *cis*9*trans*11 C18:2 (r=0.994). The relation between the concentration of C18:1 *trans* isomers and that of CLA resulted from the fact that the major C18:1 *trans* isomer – vaccenic acid – is produced in cow's rumen from *cis*9*trans*11 C18:2 acid, whereas in the mammary gland tissue the *cis*9*trans*11 C18:2 acid is synthesised from the vaccenic acid [Kelly *et al*., 1998; Griinari *et al*., 2000; Palmquist, 2001]. A positive correlation between vaccenic acid and CLA contents in fat milk was also found in research carried out Jiang *et al*. [1996] and Jahreis *et al*. [1997], r=0.78 and r=0.85, respectively.

The total content of C18:2 *trans* isomers was highly positively correlated with the content of linolenic acid (r=0.956). Precht & Molkentin [2000] obtained a high correlation coefficient (r=0.89) between the content of one of the major isomers of that group, *i.e. trans*11*cis*15 C18:2 isomer, and C18:3 acid content. According to those authors, such a high value of the positive correlation indicates the probable course of bio-hydrogenation of linolenic acid: *c*9*c*12*c*15 C18:3 → *c*9*t*11*c*15 C18:3 → *t*11*c*15 C18:2 → *t*11 C18:1. Results compiled in Table 2 point to a lack of any dependencies of C18:1 *trans* isomers and CLA contents with the concentration of linoleic acid in milk fat.

Summarising, it should be emphasized that a higher content of C18:1 *trans* isomers and CLA in the milk fat from the period of summer feeding of the cows, as compared to that in milk fat from the winter season, determines the nutritive value and technological properties of fat. Bearing in mind the multidirectional health-promoting action of CLA as well as the fact that the major C18:1 *trans* isomer, vaccenic acid, is a precursor of *cis*9*trans* 11C18:2 acid (CLA), it can be stated that the milk fat obtained during the summer feeding period is more favourable in terms of its nutritive value. The physical properties of *trans* unsaturated fatty acids are similar to those of the saturated acids. For example the melting point of *c*9 C18:1 is 13.2°C, whereas that of *t*9 C18:1 – 46.5°C, and that of *t*11 C18:1 – 44.0°C. Hence, the melting point of the milk fat from the summer feeding period is higher, and the consistency of butter is less softer than in the case when all unsaturated fatty acids contain double bonds with *cis* configuration.

CONCLUSIONS

The study indicates that the range of annual changes in the contents of *trans* isomers of unsaturated fatty acids with 18 carbon atoms is considerably higher as compared to that determined for *cis* isomers of those acids. The greatest fluctuations as affected by the period of cow feeding were reported for the content of *cis*9*trans*11 C18:2 acid and total *trans* iso-

TABLE 2. Coefficients of correlation between contents of unsaturated fatty acids with 18 carbon atoms in milk fat.

Fatty acids	Σ *trans C18:1*	Σ *c,t / t,c C18:2*	*c* 9 *c*12 C18:2	*c* 9 *c*12 *c*15 C18:3	*cis* 9 C18:1
Σ *trans C18:1*	-	0.902**	- 0.124	0.899**	0.914**
Σ *c,t / t,c C18:2*	0.902**	-	0.040	0.956**	0.785**
c 9 *c*12 C18:2	- 0.124	0.040	-	0.102	0.055
c 9 *c*12 *c*15 C18:3	0.899**	0.956**	0.102	-	0.801**
*c*9 *t*11 C18:2 (CLA)	0.994**	0.936**	- 0.122	0.928**	0.887**

* – significantly different at p≤0.01; number of samples n=96

mers of C18:1 acid. The content of linoleic acid remained at a similar level during the year, whereas the content of linolenic acid from the period of summer feeding was twice as much as that in milk fat from the winter feeding period.

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ROCZNE ZMIANY ZAWARTOŚCI NIENASYCONYCH KWASÓW TŁUSZCZOWYCH O 18 ATOMACH WĘGLA, WŁĄCZNIE Z KWASEM *CIS9TRANS*11 C18:2 (CLA), W TŁUSZCZU MLEKOWYM

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Skład kwasów tłuszczowych tłuszczu mlekowego w okresie roku oznaczano metodą chromatografii gazowej stosując 100 m kolumnę kapilarną z wysokopolarną fazą ciekłą. Próbki do badań stanowił tłuszcz wydzielony metodą ekstrakcyjną z 30% niepasteryzowanej śmietanki.

Badania wykazały, że zakres zmian zawartości izomerów *trans* w tłuszczu mlekowym w okresie roku był znacznie większy niż zakres zmian zawartości izomerów *cis* tych kwasów. Udział izomerów *trans* C18:1 oraz kwasu *cis*9*trans*11 C18:2 (CLA) był ok. 3-krotnie, a izomerów *trans* kwasu C18:2 ok. 2.5 razy wyższy w tłuszczu mlekowym w okresie pastwiskowego żywienia krów w porównaniu z ich zawartością w tłuszczu z okresu żywienia oborowego. Zawartość kwasu *cis*9*trans* 11 C18:2 w tłuszczu mlekowym w okresie roku mieściła się w granicach 0.39 do 1.76% ogólnego składu kwasów tłuszczowych. Średnia zawartość kwasu oleinowego w tłuszczu mleka z okresu żywienia pastwiskowego wynosiła 20.63% i była wyższa o 2.6 jednostki procentowej w porównaniu z zawartością w tłuszczu z okresu żywienia oborowego (tab. 1, rys. 1B). Zawartość kwasu linolowego w składzie kwasów tłuszczowych tłuszczu była prawie stała w okresie roku. Udział kwasu linolenowego mieścił się w granicach od 0.38 do 1.46%, a jego zawartość w tłuszczu mleka z okresu żywienia pastwiskowego przewyższała dwukrotnie zawartość stwierdzoną w tłuszczu mleka krów żywionych paszą oborową (tab. 1, rys. 2B).