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Variation in the activity of stearoyl-CoA desaturase enzyme and endogenous precursors of unsaturated fatty acids in cow's milk during grazing season

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Abstract: *Variation in the activity of stearoyl-CoA desaturase enzyme and endogenous precursors of unsaturated fatty acids in cow's milk during grazing season.* The aim of this study was to determine variation in the activity of stearoyl-CoA desaturase enzyme and endogenous precursors of unsaturated fatty acids in cow's milk during the grazing season. The study was carried out in 10 certified organic farms The major criterions of choosing organic farms to the experiment were as follows: obtaining certificate for at least five years and abidance of the organic farming standards, possessing a herd of Black and White Polish Holstein-Friesian cows (numbering more than 30 cows). The highest concentrations of CLA *cis*9, *trans*11 isomer, TVA and LA in milk fat has been reported in June, slightly lower in July and August. While the lowest level has been recorded in May, at the beginning of grazing period. Significant correlations between MUFA and SCD ($r^2 = 0.828$, $P < 0.01$) has been recorded. We concluded, that monitoring of SCD activity can be used as a tool to achieve high unsaturation of milk fat, during grazing period.

Key words: CLA, SCD, TVA, LA, grazing, bovine milk

INTRODUCTION

In recent years, scientific and public interest are relating with nutritional quality of milk, which play significant role in maintaining of human health. Milk and milk products are desirable and valuable source of food. The nutritional quality of dairy products is strongly correlated with the quality of milk fat and relates to a high concentration of unsaturated fatty acids, as well as high content of conjugated dienes of linoleic acid (C18:2 c9, t11; CLA). Oleic acid (C18:1 c9; OA) is important representative of monounsaturated fatty acid (MUFA) found in adipose tissue and in triacylglycerols of cow's milk fat. Dairy products are the major dietary source of CLA, but CLA is also found in the meat from ruminants. CLA is a term used to describe positional (carbon 6,8 to 12,14) and geometric (*cis*-*cis* (c), *cis*-*trans* (c,t), *trans*-*cis* (t, c) and *trans*-*trans* (t, t)) isomers of linoleic acid (C18:2 n6 c9, c12; LA). The content of major CLA isomers in ruminants milk fat could be precisely determining both by standards method e.g.: gas chromatography with a flame – ionization detector (GC-FID) and silver ion high performance liquid chromatography (Ag^+HPLC) or by more advanced techniques e.g. gas chromatography – mass spectrometry (GC-MS) (Radzik-Rant et al. 2012). Stearoyl-CoA desaturase (SCD) catalyzes the introduction of the first *cis*-double bond in the delta-9 position (between carbons 9 and 10) in several fatty acyl-CoA substrates. Activity of SCD enzyme is regulated by many different factors, e.g.: diet, hormones, temperature, metals, peroxisomal proliferators, vitamin A and developmental processes (Bernard et al. 2001 by Ntambi 1995, 1999). In cow's milk, OA, palmitoleic acid (C16:1 c9) and substantial part of CLA are synthesised endogenously, in the mammary gland through the action of mammary delta-desaturase on stearic (C18:0), palmitic (C16:0) and vaccenic (C18:1 t11; TVA) acids (Griinari and Bauman 1999).

Nutrition and herd management practices appears to be the factors which have the highest effect on the CLA concentration in milk and milk products, and an organic diet based on fresh or dried forage, that is rich in CLA precursory fatty acids, may improve the yield of fatty acids with beneficial effects on health (Prandini et al. 2009).

Analysis of the fatty acid composition of milk fat (derived from different species of animals), showed that organic system significantly shapes the antioxidant capacity of milk (Barłowska et al. 2011, Kuczyńska 2011a, b, Markiewicz-Kęszycka et al. 2013). It should be noted that organic milk reduced eczema incidence in infants (Rist et al. 2007, Kummeling et al. 2008). Differences in the levels of SCD activity may be explained by the significant variations in the content of unsaturated fatty acids (MUFA, TVA, CLA, LA etc.) in cow's milk fat in response to the dietary factors (Griinari and Bauman 1999, Chilliard et al. 2000).

High importance is attributed to feeding regime at organic farms, as well as a higher biological value of pastures (a higher proportion of herbs, clovers or *Fabaceae* plants) compared to non-organic ones (Ellis et al. 2006, Collomb et al. 2008, Butler et al. 2011, Kuczyńska et al. 2011a, b, Baars et al. 2012). The concentration of essential fatty acids (EFAs) can be increased in cow's milk through the diet manipulation by supplementation of cow's diet various types of oils or seeds (Reklewska et al. 2002, Puppel et al. 2013). An increasing interest in enhancing CLA content in milk, particularly C18:2 *cis*9, *trans*11, has been linked to beneficial health effects of this isomer. CLA arises anti-carcinogenic, anti-diabetic, anti-atherogenic, anti-lipogenic and immunomodulatory functions observed in clinical and biological research (Pariza 1999, Parodi 1999). In addition CLA inhibit chemically induced tumors, prevent atherosclerosis and improve the protein-to-fat ratio in experimental animals.

The aim of this study was to determine variation in the activity of stearoyl-CoA desaturase enzyme and endogenous precursors of unsaturated fatty acids in cow's milk during the grazing season.

MATERIAL AND METHODS

The study was carried out in 10 certified organic farms The major criterions of choosing organic farms to the experiment were as follows: obtaining certificate for at least five years, abidance of the organic farming standards, and possessing a herd of Black and White Polish Holstein-Friesian cows (numbering more than 30 cows). Selected farms were located in

Mazovia region in close neighborhood, to provide comparable climate and soil conditions. Representative milk samples (from 165 cows) were collected from each cow during milking by means of a milk meter in the milking parlor during grazing seasons (from May to August). At the initial grazing period (May) cows were grazing on pasture (*ad libitum*) and additionally received 10 kg of grass silage and 2 kg of concentrate per cow daily. Then, from June to August basis diet of cows constituted a pasture. Cows were strip-grazed on a multispecies pasture (*Dactylis glomerata* L. 16%; *Phalaris arundinacea* L. 6%; *Agrostis stolonifera* L. 3.4%; *Poa pratensis* L. 14.5%; *Lolium perenne* L. 14%; *Festuca rubra* L. 4.3%; *Trifolium repens* 15.5%; *Trifolium pratense* L. 4.5%; *Poa annua* and *P. trivialis* 8.3%. Five quadrants (1 m^2 each) of pasture (from each organic farms) were cut one day of each week pre- and post-grazing to determine chemical composition.

Combined milk from morning and evening milking was placed in sterile bottles, preserved with Mlekostat CC (660 samples of milk; 165 samples of milk per month). After milking, samples were immediately submitted to the Cattle Breeding Division (Milk and Meat Testing Laboratory of Warsaw University of Life Sciences) for analysis.

Analyzed of health-promoting compounds of cow's milk lipid fraction included determination concentrations of CLA, TVA and LA. Extraction of crude fat was performed according to Röse-Gottlieb procedure (AOAC 1995), at a room temperature. Fatty acid methylation was performed according to the transesterification method by EN ISO 5509:2000. Analysis of fatty acid methyl esters (FAME) was carried out with a gas chromatograph (GC instrument type 7890A Agilent Technologies) with Agilent ChemStation, using a Varian CP-SIL 88 fused-silica capillary column (100 m length, 0.25 mm internal diameter and 0.20 μm film thickness). The separation of one micro liter of each sample was performed at pre-programmed temperature: column temperature was kept at 130°C, for 1 min, then increased from 130 to 175°C at rate of 5.5°C per min (kept at 175°C for 8 min), and then to 215°C at rate of 2.75°C per min, and was kept at 215°C for 10 min. Subsequently temperature increased from 215 to 230°C at rate of 20°C per min and was kept at 230°C for 10 min. Total run time was 52.477 min. Purified helium was used as a carrier gas, with a head pressure of 49.6 kPa and a constant column flow of 1.5 ml per min. The flow rate of the carrier gas was average velocity to 23.535 cm per sec. The injection system (Agilent Technologies type G 4513A) used a split ratio of 1 : 100 and an injector temperature 180°C. The detection by a flame-ionization detector (FID) detector was temperature at 250°C. Peaks of individual fatty acids were identified by using pure fatty acids standards: PUFA 1 from marine, composed of 18 fatty acid standards from C14:0 to C24:1 and with individual C18:1 *trans*11 and CLA standards (Sigma-Aldrich). Additionally, selected saturated and monounsaturated fatty acids were analyzed in order to formulate index of activity of stearoyl-CoA desaturase (SCD) enzyme as an indicators of endogenous precursors of unsaturated fatty acids in cow's milk. Activity of SCD enzyme was assessed and calculated as proposed by Smith et al. (2002), using the following formula: (C16:1 + C18:1) / (C14:0 + C16:0 + + C18:0 + C16:1 + C18:1).

The data obtained were analyzed statistically using analysis of variance (least squares) by means of the SPSS 21 packet software.

The model used for analyzed milk samples was

$$Y_{ijk} = \mu + A_i + e_{ij}$$

where:
Y_{ijk} – dependent variable;
μ – general mean;
A_i – month effect (May, June, July, August);
e_{ij} – standard error.

RESULTS AND DISCUSSION

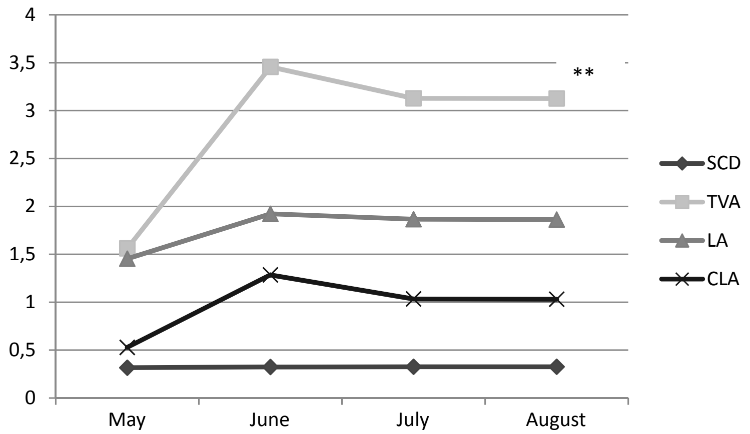
The nutritional requirements of the animals and the nutritive value of their diet were calculated in accordance with the nutrition standards set out in the INRATION 4.0 software. Table 1 summarizes the feeding characteristics during grazing period from May to August.

In the current study, activity of stearoyl-CoA enzyme and endogenous precursors of unsaturated fatty acids of cow's milk was weakening with the passage of the grazing season (Fig. 1).

Generally, the highest concentrations of CLA *cis*9, *trans*11 isomer, TVA and LA in milk fat has been reported in June, slightly lower in July and August. While the lowest level has been recorded in May, at the beginning of grazing period. The first time in the 1930s already, Both et al., noted that summer milk had a greater absorbance at 233 nm than milk produced in the winter. In 1963, Riel noted that summer milk fat had more conjugated dienoic acid than winter milk fat. Ruminants that are exlusively pasture fed produce greater levels of TVA and CLA compared to those fed indoors (Nałęcz-Tarwacka 2006), although this effect is depended on the specific diet composition (Puppel et al. 2013). The CLA content mirrored the TVA rather than LA content with a drop TVA in TVA

TABLE 1. Chemical composition and nutritional value of pastures used in cow feeds (% of DM)

Traits	Pasture			
	May	June	July	August
Ash	8.21	8.33	8.81	8.89
Crude protein	14.2	18.52	18.82	19.23
Ether extract	2.4	3.89	4.13	3.56
Crude fibre	27.82	26.62	28.62	29.01
UFL	0.85	0.84	0.91	0.89
PDIN	88.96	115.23	115.75	102.56
PDIE	84.92	96.53	95.62	93.24
NDF	42.30	48.56	52.84	45.63
ADF	30.4	31.26	40.3	28.96
LA	11.82	43.75	42.5	28.95
LNA	33.3	60.6	52.16	45.15



** May different from June, July and August for TVA, LA and CLA by contrast (P ≤ 0.01).

FIGURE 1. Variation in the activity of SCD enzyme and the concentrations of TVA, LA, and CLA (g per 100 g of fat) in cow's milk during the grazing period

beginning in June and occurring before the drop in CLA in August. This result is partially explained by the fact CLA in milk is mainly from delta-9 desaturasion of TVA in the mammary gland in ruminants. TVA of cow's milk fat is primarily from rumen biohydrogenation in the pathway LA → CLA *cis*9, *trans*11 → TVA → C18:0 → C16:0.

Soyeurt et al. (2008) reported positive genetic correlations between the indices of C14, C16 C18 (0.72, 0.62 and 0.97, respectively) and monounsaturated fatty acids (MUFA), and showed that proportion of MUFA is linked to the activity of SCD.

In Table 2 significant correlations between activity of SCD enzyme and selected indexes of delta-9 desaturase, such as: C14:1 index, C16:1 index and C18:1 index, (0.144, 0.243 and 0.638, respectively) has been presented. Increasing the concentration of C18:0 and C18:1 acids and lower level of C16:0 acid are typical for cows which are grazed on pasture. In the case of cows fed TMR

TABLE 2. Relationships between selected indexes of delta-9 desaturase and month of grazing, and correlations between activity of SCD enzyme and selected indexes of delta-9 desaturase

Traits	May		June		July		August		Correlations	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE	SCD	MUFA
C14:1index	0.13^{ABC}	0.003	0.10^{A}	0.002	0.11^{B}	0.002	0.11^{C}	0.002	0.14**	0.149**
C16:1 index	0.06^{ABC}	0.003	0.06^{A}	0.002	0.05^{B}	0.002	0.05^{C}	0.002	0.24**	–0.049
C18:1index	1.99^{Abc}	0.057	1.78^{A}	0.034	1.85^{b}	0.035	1.84^{c}	0.035	0.63**	0.402**
SCD	0.31	0.005	0.32	0.003	0.32	0.003	0.32	0.003	1	0.828**
MUFA	27.33^{ABC}	0.608	29.63^{A}	0.364	29.77^{B}	0.363	29.8^{C}	0.375	0.82**	1

Values in the rows marked with the same letters differ significantly ^{A,B,C} $P \leq 0.01$; ^{a,b,c} $P \leq 0.05$.
**Significant correlaction has been reported.

diet an inverse relationship has been demonstrated (Kelly et al. 1998, Nałęcz--Tarwacka 2006, Kuczyńska 2011).

CONCLUSION

In the current study significant correlations between MUFA and SCD ($r^2 = 0.828$, $P < 0.01$) has been recorded. We concluded, that monitoring of SCD activity can be used as a tool to achieve high unsaturation of milk fat, during grazing period.

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Streszczenie: *Wpływ okresu wypasu pastwiskowego na poziom indeksu enzymu stearoilo-CoA desaturazy i jego endogennych prekursorów nienasyconych kwasów tłuszczowych w mleku krowim.* Celem pracy było określenie zmienności w aktywności enzymu delta-9 desaturazy SCD i koncentracji endogennych prekursorów nienasyconych kwasów tłuszczowych w mleku krów w okresie wypasu pastwiskowego. Badania przeprowadzono w 10 certyfikowanych gospodarstwach ekologicznych. Główne kryteria wyboru gospodarstw ekologicznych do eksperymentu to: posiadanie certyfikatu od co najmniej pięciu lat i przestrzeganie standardów z ekologicznej produkcji, utrzymywanie minimum 30 krów rasy polskiej holsztyńsko-fryzyjskiej w stadach. Najwyższe koncentracje: izomeru CLA c9, t11, TVA i LA w tłuszczu mlekowym wykazano w czerwcu, nieco niższe w lipcu i sierpniu. Najniższe poziomy natomiast zaobserwowano w maju, w początkowym okresie wypasu pastwiskowego. Wykazano istotną korelację ($p \leq 0,01$) między MUFA i SCD ($r^2 = 0,828$). Podsumowując, monitorowanie aktywności SCD może być użyteczne jako narzędzie w celu zwiększania nienasycenia tłuszczu mleka pozyskiwanego w trakcie wypasu pastwiskowego.

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