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THE EFFECT OF PARITY ON THE FATTY ACID PROFILE OF MILK FROM HIGH-YIELDING COWS

Zenon Nogalski, Beata Jagłowska, Zofia Wielgosz-Groth, Paulina Pogorzelska-Przybyłek, Monika Sobczuk-Szul, Magdalena Mochol

University of Warmia and Mazury in Olsztyn, Poland

Abstract. The objective of this study was to determine the effect of parity on the fatty acid profile of milk from 42 high-yielding cows, including 15 primiparous cows, 15 cows in their second lactation and 12 cows in their third lactation. Milk yield was evaluated and milk samples (462 in total) were collected between lactation days 6 and 60, at five-day intervals. The concentrations of 43 fatty acids were determined by gas chromatography in extracted fat. Cows in their third lactation were characterized by the highest milk yield – over 305-day lactation, they produced 13,160 kg of milk on average. Milk from primiparous cows had the most desirable fatty acid profile. It contained more (by ca. 12–17%) n-3 fatty acids and less (by 2.89–5.46%) fatty acids that adversely affect human health, compared with the other groups. Among essential fatty acids, differences were noted with respect to CLA (+9.5–9.8%), LNA (+12.7–18.7%) and DHA (+21–23%) to the advantage of the milk fat of primiparous cows.

Keywords: CLA, dairy cows, essential fatty acids, parity

INTRODUCTION

Fatty acids contained in products of animal origin, such as milk and meat, are important nutritional factors known to deliver health benefits. Fatty acids assist hormone production, help boost fertility and promote the development of a healthy nervous system [Kolanowski 2007]. Milk fat is the main energy component of milk and the most easily digested animal fat in human diet. Milk fat contains from 400 [Jansen 2002] to around 500 fatty acids [Reklewska et al. 2003]. Monounsaturated fatty acids, including oleic acid OA (n-*9*) and vaccenic acid, inhibit cholesterol absorption, reduce blood viscosity and lower blood pressure [Santon et al. 1997, Simopoulos 2008]. Linoleic acid LA (n-*6*) and linolenic acid LNA (n-*3*), present in the maximum amount of 5%, help control insulin levels and are a source of local hormones, the eicosanoids [Kolanowski 2007]. Milk fat contains conju-

Corresponding author – Adres do korespondencji: dr hab. Zenon Nogalski, Professor at UWM, University of Warmia and Mazury in Olsztyn, Department of Cattle Breeding and Milk Quality Evaluation, Oczapowskiego 5, 10-740 Olsztyn-Kortowo, Poland, e-mail: zena@uwm.edu.pl

gated linoleic acid (CLA) which has antioxidant properties. Due to its high content of saturated fatty acids (SFA) and the presence of cholesterol, milk fat is considered a risk factor for atherosclerosis by some doctors and dieticians. However, it remains a rich source of many fatty acids known for their health-promoting properties [Reklewska et al. 2003].

Fatty acid concentrations in cow's milk are mostly determined by the type of feed offered to animals, and fresh green forage has a more beneficial influence on the fatty acid profile of milk fat than preserved feeds [Reklewska et al. 2003, Nałęcz-Tarwacka 2006]. Parity is yet another important extra-nutritional factor affecting milk fat composition. Thomson and Van der Poel [2000] demonstrated that milk from primiparous cows contains higher levels of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids than milk from older cows. According to Townsend et al. [1997], cow's age has no effect on the fatty acid profile of milk, and a higher milk fat content results from higher SFA concentrations.

The onset of lactation is associated with a prolonged period of negative energy balance (NEB) which results from the difference between the cow's energy demand for milk production and the energy gained from feed intake [Berry et al. 2007, Nogalski and Górak 2008, Gross et al. 2011]. Changes in milk fat composition over lactation imply shifts in the activity of fatty acid synthesis pathways, and are related to changes in the cow's energy status [Gross et al. 2011]. Van Knegsel et al. [2005] demonstrated that during NEB, *de novo* synthesis of fatty acids (C6:0 do C14:0) is reduced while body fat reserves are mobilized. Young growing cows with high maintenance requirements are at particular risk of energy deficiency in early lactation, leading to changes in milk composition. Due to their high genetic potential, high-yielding cows are able to produce large amounts of milk, but they enter a state of NEB around calving.

The available literature provides scant information on the effect of cow's age on milk fat composition [Kelsey et al. 2003, Nałęcz-Tarwacka 2006]. The majority of studies conducted to date have focused on the effects of various feed additives on the fatty acid profile of bovine milk in cattle herds with an average milk yield of up to 10,000 kg. The objective of this study was to determine the effect of parity on the fatty acid profile of milk from high-yielding cows.

MATERIALS AND METHODS

Experimental materials

A total of 42 cows, including 15 primiparous cows, 15 cows in their second lactation and 12 cows in their third lactation, were selected from a herd of 330 Holstein-Friesians (average milk yield of 12360 kg for 305-day lactation) based on a similar calving date (from 16 January 2008 to 14 March 2008). The cows were kept in a free-stall barn throughout the year, and they were milked in a parallel parlor three times a day. The animals were fed a total mixed ration (TMR) based on maize silage, grass and alfalfa haylage, and brewer's spent grain. In the complete diet, the concentrate was supplemented with protein, minerals, vitamins and milk production enhancers (protein and rumen-protected fat, active yeast cultures and other energy supplements). The TMR was formulated to produce 45–50 l of milk

(7.15 MJ · kg^{-1} net energy [lactation] and 18% crude protein). Dry cows and heifers on pre-calving days 60–45 were kept in a deep litter free-stall barn, and they were divided into two groups: 1) dry cows (from 6–8 weeks ante partum to 3 weeks ap, 2) transition cows (three weeks ap to one week post partum). Dry cows were fed a TMR of grass and straw haylage supplemented with minerals in the dry period. Transition cows were administered lactation rations with an increasing share of concentrate. Feed was offered twice daily, using mixer wagons, and it was gathered up several times per day (including at night) to ensure *ad libitum* access.

Trait characteristics

Milk yield in kg ECM[1] and the average content of fat and protein over 305-day lactation were calculated for each cow, as follows.

[1]*Energy Corrected Milk* – milk with a standardized energy content [Sjaunja et al. 1990],

$$ECM\,(kg) = \text{milk}\,(kg) \cdot \frac{(0.383 \cdot fat\,(\%) + 242\ \text{protein}\,(\%) + 0.7832)}{3.140}$$

Between lactation days 6 and 60, at five-day intervals, milk samples were collected from clinically healthy cows during morning milking. The proximate chemical composition of milk was determined by infrared spectrophotometry, using the Milco-scan 133 B analyzer (Foss Electric). Fat was extracted from 462 milk samples by the method proposed by Röse Gottlieb (AOEC 1990), and the concentrations of 43 fatty acids were determined by gas chromatography, in the Varian CP 3800 gas chromatograph equipped with a split/splitless injector and a flame-ionization detector (FID). One µl ester samples were placed on a capillary column with a length of 100 m and an internal diameter of 0.25 mm, with the CP-sil88 stationary phase. Helium was used as the carrier gas, injector temperature was 260˚C, and the total time of a single analysis was 68 min. Fatty acids were identified by comparing their retention times with those of commercially available reference standards purchased from Supelco, Inc.

Health examinations were performed once a week. Animal handling and sampling procedures performed for the needs of this study had been approved by the local ethics committee in Olsztyn (decision No. 74/2007).

Statistical analysis

The results were processed using Statistica®9.0 software [Statsoft Inc. 2010]. The effect of successive lactations on milk yield and the fatty acid composition of milk fat was determined by the least squares method, according to the below model:

$$Y_{ij} = m + A_i + e_{ij}$$

where:

Y_{ij} – values of the analyzed parameters,

m – population mean,

A_i – effect of the i^{th} lactation,

e_{ij} – random error.

Differences between means were estimated using Tukey’s test.

RESULTS AND DISCUSSION

In the group of the oldest cows, the average total output over 305-day lactation was 13,160 kg ECM (Table 1). The average difference between those cows and primiparous cows reached 1791 kg, and it was statistically significant ($P \leq 0.01$). Over that time, average milk production determined for the Polish population of Holstein-Friesian cattle was 6,903 kg ECM [Ocena i hodowla bydła mlecznego 2009]. The very high milk production in the second and third lactation, noted in the present experiment, resulted from a peak daily milk yield of 50–55 kg. The very high productivity of older cows and a high fat content of their milk (4.21–4.29%) could be due to more intense fat reserve mobilization in early lactation (Berry et al. 2007). In the period of NEB, cows with a high genetic potential for milk production mobilize their body fat reserves, which increases both milk yield and milk fat content [Schroeder and Staufenbiel 2006].

Table 1. Characteristics of production traits, LSM±SE
Tabela 1. Charakterystyka cech produkcyjnych, LSM±SE

Traits Cechy	First lactation Pierwsza laktacja		Second lactation Druga laktacja		Third lactation Trzecia laktacja	
	LSM	SE	LSM	SE	LSM	SE
ECM, kg	$11{,}369^{B}$	334.3	$12{,}690^{A}$	380.8	$13{,}160^{A}$	404.8
Fat, % Tłuszcz, %	4.02^{b}	0.12	4.21	0.09	4.29^{a}	0.08
Protein, % Białko, %	3.27	0.03	3.24	0.04	3.23	0.04

Means within a row with different superscripts differ: A, B – $P \leq 0.01$; a, b – $P \leq 0.05$.
ECM – Energy corrected milk.
Średnie w wierszach oznaczone różnymi literami różnią się: A, B – $P \leq 0,01$; a, b – $P \leq 0,05$.
ECM – mleko o standardowej zawartości energii.

Parity had a significant effect on the fatty acid profile of milk fat during the first two months of lactation (Table 2). The milk fat of primiparous cows had a lower proportion of short-chain (SCFA) and medium-chain fatty acids (MCFA), and a higher proportion of long-chain fatty acids (LCFA), compared with the milk fat of older cows. The fatty acid profile of milk fat is determined by shifts in the activity of fatty acid synthesis pathways. Under severe NEB, the concentrations of SCFA and MCFA decrease, while the content of LCFA increases [Stoop et al. 2009]. SCFA are an important source of energy for milk consumers as they are rapidly synthesized in the digestive tract and metabolized in the liver. Thus, they should have a high share of the total fatty acid pool. Milk from multiparous cows contained slightly higher quantities of SFA than milk from primiparous cows, but the noted differences were statistically non-significant. A similar trend was reported by Thomson and Van der Poel [2000], and Nałęcz-Tarwacka [2006]. In our study, MUFA and PUFA concentrations were higher in milk from primiparous cows, compared with older cows. These results differ from the findings of Thomson and Van der Poel [2000] who reported significantly higher MUFA and PUFA levels in the youngest cows. In the current study, milk from primiparous cows had a higher content of n-3 fatty acids (relative to all other cows) and n-6 fatty acids (relative to cows in their second lactation). The elevated levels of n-3 fatty acids in milk from primiparous cows could be due to increased supply of LNA to the rumen. LNA acts a substrate for both trans-vaccenic acid (TVA), which is converted to CLA, and LCFA formed by further desaturation and elongation [Stoop et al. 2009].

Table 2. Effect of parity on the concentrations of selected fatty acid groups in milk fat
Tabela 2. Zawartość wybranych grup kwasów tłuszczowych w mleku w zależności od laktacji

Fatty acid g · 100 g^{-1} milk fat Kwasy tłuszczowe g · 100 g^{-1} tłuszczu mleka	First lactation Pierwsza laktacja		Second lactation Druga laktacja		Third lactation Trzecia laktacja	
	LSM	SE	LSM	SE	LSM	SE
SFCA	7.00	0.15	7.36	0.24	7.51	0.17
MCFA	42.65^{b}	0.54	45.15^{a}	0.77	43.68	0.53
LCFA	50.54^{a}	0.69	47.52^{b}	0.99	48.78^{b}	0.68
SFA	59.34	0.64	59.84	0.91	59.63	0.75
UFA	40.85	0.67	40.18	0.92	40.34	0.75
MUFA	36.46	0.59	36.07	0.88	36.26	0.75
PUFA	4.39	0.15	4.11	0.14	4.08	0.11
n-3	0.412^{a}	0.006	0.352^{b}	0.007	0.367^{b}	0.008
n-6	2.55^{a}	0.033	2.42^{b}	0.039	2.54^{a}	0.038
n-6/n-3	6.20^{a}	0.059	6.87^{b}	0.160	6.92^{b}	0.116
NA	37.19^{B}	0.20	39.34^{A}	0.26	38.63^{A}	0.19

Means within a row with different superscripts differ: A, B – $P \leq 0.01$; a, b – $P \leq 0.05$.
SCFA – short-chain fatty acids; MCFA – medium-chain fatty acids; LCFA – long-chain fatty acids; SFA – saturated fatty acids; UFA – unsaturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; NA – fatty acids adversely affecting human health.
Średnie w wierszach oznaczone różnymi literami różnią się: A, B – $P \leq 0,01$; a, b – $P \leq 0,05$.
SCFA – kwasy tłuszczowe (KT) krótkołańcuchowe; MCFA – KT średniołańcuchowe; LCFA – KT długołańcuchowe; SFA – KT nasycone; UFA – KT nienasycone; MUFA – KT jednonienasycone; PUFA – KT wielonienasycone; NA – KT o negatywnym oddziaływaniu na zdrowie człowieka.

In the present experiment, the n-6/n-3 fatty acid ratio exceeded 6.0 (Table 2). In a study by Nałęcz-Tarwacka [2006], milk from cows fed a TMR contained more n-3 fatty acids and less n-6 fatty acids, in comparison with the values noted in our study, and the n-6/n-3 fatty acid ratio reached 2.96. It should be stressed, however, that in our study milk production over 305-day lactation was by around 3500 kg higher, which could explain the differences in the levels of some fatty acids [Kelsey et al. 2003]. There is no doubt that n-3 and n-6 fatty acids deliver health benefits [Marciniak-Łukasiak 2011]. The optimal n-6/n-3 dietary ratio ranges from 2.5:1 to 5:1 [Simopoulos 2008]. The concentrations of lauric, myristic and palmitic acids, which adversely affect human health, were lowest in milk from primiparous cows (Table 2). Average differences between primiparous cows and cows in their second (2.15 g per 100 g fat) and third lactation (1.44 g per 100 g fat) were highly significant. Nałęcz-Tarwacka [2006] also found the lowest concentrations of fatty acids adversely affecting human health in milk from primiparous cows, but the differences between them and older cows did not exceed 1 g per 100 g milk fat. The proportion of fatty acids which adversely affect human health in the total fatty acid pool may be reduced through pasture grazing and avoiding mono diets [Jansen 2002, Nałęcz-Tarwacka 2006].

The fatty acids found in the milk fat of ruminants are the products of rumen fermentation, lipids contained in feed and lipids stored in the body [Stoop et al. 2009]. SCFA (C4-C10) and MCFA (C12-C16) are *de novo* synthesized in the mammary gland, whereas LCFA are absorbed from the bloodstream [Stoop et al. 2009].

The butyric acid content of milk fat increased in successive lactations, and the differences between the values noted in the first and third lactation were significant ($P \leq 0.01$) (Table 3). Primiparous cows were characterized by the most desirable milk composition with regard to the concentrations of essential fatty acids. Their milk had the highest levels of the following acids: OA, LA, CLA, LNA and DHA. The OA content of milk is higher in the summer and when the ration is not based on concentrates [Jensen 2002]. In the current study, OA content was very high, when compared with the results reported by other authors [Jensen 2002, Nałęcz-Tarwacka 2006], which could be due to the high quality and variety of components in the TMR. Our results regarding the CLA content of milk do not support the findings of Stanton et al. [1997] who observed an increase in CLA in successive lactations. According to Kelsey et al. [2003], the CLA content of milk is conditioned primarily by daily milk yield, and to a lesser extent by other factors.

Table 3. Concentrations of essential fatty acids in milk fat depending on lactation
Tabela 3. Zawartość funkcjonalnych kwasów tłuszczowych w mleku krów od 6. do 60. dnia laktacji

Fatty acid $g \cdot 100\ g^{-1}$ milk fat Kwasy tłuszczowe $g \cdot 100\ g^{-1}$ tłuszczu mleka	First lactation Pierwsza laktacja		Second lactation Druga laktacja		Third lactation Trzecia laktacja	
	LSM	SE	LSM	SE	LSM	SE
C 4:0 (BA)	2.717^{B}	0.050	2.842	0.052	2.932^{A}	0.049
C 18:1 trans 11 (TVA)	1.138	0.063	1.439	0.150	1.393	0.089
C 18:1 cis-9 (OA)	29.00	0.544	27.87	0.880	28.47	0.709
C 18:2 (LA)	2.389^{a}	0.032	2.256^{b}	0.040	2.387^{a}	0.036
C 18:2 cis 9 trans 11 (CLA)	0.368	0.009	0.335	0.011	0.336	0.011
C 18:3 (LNA)	0.373^{A}	0.006	0.314^{B}	0.006	0.331^{B}	0.007
C 20:4 (AA)	0.157	0.006	0.159	0.007	0.156	0.005
C 20:5 (EPA)	0.038	0.003	0.037	0.003	0.041	0.002
C 22:6 (DHA)	0.075^{A}	0.003	0.061^{B}	0.003	0.062^{B}	0.003

Means within a row with different superscripts differ: A, B – $P \leq 0.01$; a, b – $P \leq 0.05$.
BA – butyric acid; TVA – trans-vaccenic acid; OA – oleic acid; LA – linoleic acid; CLA – conjugated linoleic acid; LNA – linolenic acid; AA – arachidonic acid; EPA – eicosapentaenoic acid; DHA – docosahexaenoic acid.
Średnie w wierszach oznaczone różnymi literami różnią się: A, B – $P \leq 0,01$; a, b – $P \leq 0,05$.
BA – kwas masłowy; TVA – kwas wakcenowy; OA – kwas oleinowy; LA – kwas linolowy; CLA – skoniugowany kwas linolowy; LNA – kwas linolenowy; AA – kwas arachidonowy; EPA – kwas eikozapentaenowy; DHA – kwas dokozaheksaenowy.

CONCLUSIONS

The oldest cows, in their third lactation, were characterized by the highest milk yield – over 305-day lactation, they produced 13,160 kg of milk on average. Milk from primiparous cows in the first two months of lactation had a more desirable fatty acid profile, in comparison with older cows. Their milk contained more n-3 and n-6 fatty acids, less fatty acids that adversely affect human health as well as more essential fatty acids, such as CLA, OA, LNA and DHA. It cannot be unequivocally stated that high milk production levels and intensive feeding (TMR) have a negative influence on the functional quality of milk fat.

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WPŁYW KOLEJNEJ LAKTACJI NA PROFIL KWASÓW TŁUSZCZOWYCH W MLEKU KRÓW WYSOKOWYDAJNYCH

Streszczenie. Celem podjętych badań było określenie wpływu kolejnej laktacji na profil kwasów tłuszczowych tłuszczu mleka 42 wysokowydajnych krów: 15 pierwiastek, 15 w drugiej laktacji i 12 w trzeciej laktacji. Oceniano ich wydajność mleczną i od 6. do 60. dnia laktacji co pięć dni pobierano próbki mleka (łącznie 462), a następnie ekstrahowano tłuszcz i metodą chromatografii gazowej określano profil 43 kwasów tłuszczowych. Krowy po trzecim wycieleniu charakteryzowały się najwyższą wydajnością i wyprodukowały w laktacji 305-dniowej średnio 13 160 kg mleka. Pierwiastki produkowały natomiast mleko o najkorzystniejszym profilu kwasów tłuszczowych. Zawierało ono więcej o 12–17% kwasów z grupy n-3 oraz o 2,89–5,46% mniej kwasów o negatywnym oddziaływaniu na zdrowie człowieka. Spośród funkcjonalnych kwasów tłuszczowych różnice na korzyść tłuszczu mleka pierwiastek dotyczyły głównie CLA (+9,5–9,8%), LNA (+12,7–18,7%) i DHA (+21–23%).

Słowa kluczowe: CLA, funkcjonalne kwasy tłuszczowe, krowy mleczne, numer laktacji

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