THE EFFECT OF RUMINAL FLUID pH ON MILK FATTY ACIDS COMPOSITION IN CATTLE*

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Abstract

The present study aimed to evaluate the effects of ruminal fluid pH on the fatty acids (FA) profile of bovine milk. The experiment was performed on 250 Polish Holstein-Friesian cows reared in 11 commercial high-yielding dairy farms. Ruminal fluid samples were collected by rumenocentesis, and fat and FA concentrations in milk were analyzed by Fourier transform infrared spectroscopy. According to ruminal fluid pH, cows were categorized into three pH groups: high pH (pH > 5.8), moderate pH (pH 5.8–5.6), and low pH (pH < 5.6). Milk produced by low-rumen pH cows had a decreased fat content as compared to milk from moderate- and high-rumen pH cows ($P \leq 0.05$). Moreover, milk from low-rumen pH cows was characterized by the lowest level of short-chain FA (SCFA; $P \leq 0.05$), and consequently, the highest medium-chain FA to SCFA ratio ($P \leq 0.01$). The regression analysis showed that these traits explained a small proportion of rumen pH variance, which makes them unreliable indicators of subacute ruminal acidosis (SARA) in dairy cows. However, despite the extensive variability in milk fat composition observed in this study, the effect of ruminal pH on SCFA in all the analyzed herds showed the same trend. Future research aimed to identify SARA biomarkers should therefore be conducted using techniques that allow detection of more individual FA in milk, including SCFA.

Key words: dairy cows, Fourier transform infrared spectroscopy, milk fatty acids composition, ruminal acidosis, short-chain fatty acids

Fat is the most variable component of bovine milk, and its composition largely determines the nutritional value of milk for human consumption. The total fat content and the fatty acids (FA) profile of milk are influenced by several factors, the main being cattle breed, individual variability, and stage of lactation (Gottardo et al., 2017; Hanuš et al., 2018). Milk FA profile is also closely related to the feeding of dairy cows and thus provides considerable information on the metabolic and health status of animals (Colman et al., 2010; Vranković et al., 2017).

It is a common practice to feed high-producing dairy cows with highly fermentable diets, which, however, may lead to the decrease in ruminal pH and to the development of subacute ruminal acidosis (SARA). SARA is one of the most common digestive disorders in dairy herds, with a huge negative effect on the economy and animal welfare (Kleen and Cannizzo, 2012). The current methods to diagnose SARA, based on the measurement of ruminal pH by using a stomach oral probe or rumenocentesis, are invasive and not very accurate because of diurnal fluctuations in pH (Duffield et al., 2004). High concentrate diets, which can lead to SARA, were also found

to modify the FA profile of milk (Enjalbert et al., 2008; Colman et al., 2010). The possible mechanisms of this phenomenon include changes in ruminal fermentation and biohydrogenation patterns and, consequently, in the supply of regulators and substrates for milk fat synthesis (Colman et al., 2012; Abdela, 2016) as well as alteration in the expression of genes involved in lipogenesis in both the mammary gland (Tao et al., 2015) and liver (Xu et al., 2018). Previous research has shown that milk FA have potential to serve as biomarkers of SARA in dairy cows (Colman et al., 2010; Jing et al., 2018). However, most experiments evaluating the effects of SARA on milk fat composition were performed on a small number of cows of the same parity and originating from the same herd, and the decrease in pH of ruminal fluid typically resulted from feeding cows with acidosis-inducing experimental diets (Enjalbert et al., 2008; AlZahal et al., 2010; Guo et al., 2013). The FA profile of milk is usually determined by gas chromatography (GC). This technique is very accurate, but expensive and time-consuming, which prevents its application for large-scale routine measurements. In the present study, the concentrations of major

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FA in milk were determined by Fourier transform infrared spectroscopy (FT-IR), which has been shown to have great potential for regular determination of fat composition in milk (De Marchi et al., 2014).

The present study aimed to evaluate the effect of ruminal fluid pH on FA and FA groups in the milk of cows from Polish high-yielding dairy herds, varying in their parity and days in milk (DIM). We anticipate that the findings of this study will provide further information on the potential of FT-IR-predicted milk fat components in identifying cows at risk of SARA.

Material and methods

Ethics statement

The research was performed in accordance with the “Act on the protection of animals used for scientific purpose” of the Republic of Poland and was approved by the Local Ethical Committee in Poznań, Poland, decision no. 32/2014.

Animals

The study included 250 Polish Holstein-Friesian cows from 11 high-yielding dairy farms under routine milk performance recording conducted by the Polish Federation of Cattle Breeders and Dairy Farmers (PFCBDF). All animals were kept in loose housing systems and fed total mixed rations. Further details on the experimental farms are available in Stefańska et al. (2017 a, b, 2018, 2020). From each herd, 18 to 26 cows were selected. They were in early and mid-lactation stage (40 to 150 DIM), their 305-day milk yields were 10 050–12 041 kg, and their parities ranged from 1 to 6. All cows were free from clinical mastitis, metritis, and hoof diseases.

Ruminal fluid sampling and analyses

Ruminal fluid samples were collected 4 to 6 h after morning feeding by rumenocentesis as described in Stefańska et al. (2017 a, b, 2018, 2020). Ruminal fluid pH measurements of all samples were performed immediately after collection by using a CP-104 pH me-

ter (Elmetron, Poland). The cows were then categorized into three pH groups, according to the classification of Nordlund and Garrett (1994): high pH (>5.81), moderate pH (5.8–5.6), and low pH (<5.6). Table 1 shows the average ruminal pH in these groups as well as in cows representing different herds, parities, and stages of lactation.

Milk sampling and analyses

Milk samples were collected from each cow in the morning and afternoon milking sessions during regular milk recording test days. Fat and FA concentrations were measured in the Laboratory of PFCBDF by FT-IR using MilkoScan FT 6000 equipment with diamond cuvettes (Foss, Hillerød, Denmark). The FT-IR absorption spectra consisted of 1060 infrared frequencies (wave numbers) ranging from 925 to 5008 cm⁻¹, which represent infrared light absorption through the milk samples. FA traits, expressed as g/100 g of fat, included 4 individual FA, namely C14:0, C16:0, C18:0, and C18:1, and 8 groups, namely saturated FA (SFA), unsaturated FA (UFA), monounsaturated FA (MUFA), polyunsaturated FA (PUFA), trans-unsaturated FA (TRANSFA), short-chain FA (SCFA), medium-chain FA (MCFA), and long-chain FA (LCFA). The MCFA to SCFA ratio (MCFA/SCFA), the SFA to UFA ratio (SFA/UFA), and the C18 desaturation index (DI18) were also calculated; the latter was determined as a proportion of C18:1 to the sum of C18:0 and C18:1, multiplied by 100.

Statistical analyses

Data were analyzed using SAS statistical software version 9.4 (SAS, 2014). The effects of the studied factors on the concentrations of fat and FA in milk were tested by analysis of variance (ANOVA) using the PROC GLM procedure. The statistical model included the effects of ruminal pH group (high pH, moderate pH, low pH), farm number (from 1 to 11), number of parity (1, 2, ≥3), and DIM group (<90 days, ≥90 days). For parameters showing significant differences, Duncan's multiple range test was used to compare mean values. Additionally, the PROC REG procedure was used to determine the

Table 1. The average ruminal pH (± SEM) for cows representing different farms, pH groups, parities, and DIM groups

Group		Ruminal pH	P	Group		Ruminal pH	P
Farm	1	6.05±0.08	<0.001	Ruminal pH group	High pH	6.37±0.04	<0.001
	2	5.94±0.09			Moderate pH	5.71±0.01	
	3	6.09±0.08			Low pH	5.44±0.02	
	4	6.33±0.07		Parity	1	6.12±0.06	0.060
	5	5.66±0.06			2	5.98±0.06	
	6	5.70±0.06			≥ 3	6.17±0.05	
	7	5.81±0.09		DIM	< 90 days	6.14±0.05	0.166
	8	6.03±0.09			≥ 90 days	6.04±0.05	
	9	7.08±0.11					
	10	6.07±0.07					
	11	6.22±0.07					

relationships between the concentrations of fat and FA in milk and the ruminal pH value. In all analyses, the effects were considered significant at $P \leq 0.05$, and the standard error of the mean (SEM) was adopted as a measure of error.

Results

Figure 1 shows the average concentrations of fat and FA in the milk of cows originating from 11 dairy farms included in this study. In the population studied, the farm effect was significant ($P \leq 0.01$) for all the analyzed traits.

Table 2 presents the estimated effects of ruminal pH group, cows' parity, and DIM on the concentration of fat and FA in milk. With the decrease in pH of ruminal fluid, the concentrations of fat and SCFA decreased ($P \leq 0.05$), whereas the MCFA/SCFA ratio increased ($P \leq 0.01$). A similar trend for SCFA and MCFA/SCFA was observed in all farms included in the study.

The parity of cows affected PUFA, MCFA, and MCFA/SCFA ($P \leq 0.01$) and TRANSFA ($P \leq 0.05$; Table 2). With the advancing age of cows, PUFA and TRANSFA increased, and MCFA and MCFA/SCFA declined. The stage of lactation influenced eight FA traits. Cows in the earlier lactation phase (DIM ranging from 40 to 89) were characterized by higher concentrations of C18:0 ($P \leq 0.01$), LCFA, and MUFA ($P \leq 0.05$) in milk as compared to cows in the later lactation phase (DIM from 90 to 150). The opposite effects were observed for C14:0,

MCFA, and MCFA/SCFA ($P \leq 0.01$), C16:0, and SFA/UFA ($P \leq 0.05$).

Table 3 shows the results of the regression analysis for the effect of pH of ruminal fluid on milk fat and FA concentrations. A significant relationship was found between ruminal pH and C16:0, PUFA, SCFA, MCFA, and MCFA/SCFA ($P \leq 0.01$). However, all regression equations had low R-squared values, varying between 0.029 and 0.215.

Discussion

In the current study, low-rumen pH cows produced milk with lower fat percentage than cows from moderate- and high-rumen pH groups. The decreased milk fat content has often been associated with SARA (Enjalbert et al., 2008; Guo et al., 2013). This is thought to be mainly caused by alterations in ruminal biohydrogenation patterns that lead to the increased production of C18:2 t10, c12 isomer, which is a strong inhibitor of mammary lipid synthesis (Bauman and Grinari, 2003). However, Sandri et al. (2020) recently showed that experimentally induced SARA decreased fat concentration in milk by other mechanisms such as reduced availability of pre-formed FA mobilized from the adipose tissue. Low milk fat content was not always observed in cows induced with SARA, possibly because of short-term decrease in ruminal pH (Krause and Oetzel, 2005).

Figure 1. The average concentrations of fat (g/100 g of milk) and fatty acids (FA; g/100 g of fat) in milk of Polish Holstein-Friesian cows originating from 11 dairy farms. SFA: saturated FA, UFA: unsaturated FA, MUFA: monounsaturated FA, PUFA: polyunsaturated FA, TRANSFA: trans-unsaturated FA, SCFA: short-chain FA, MCFA: medium-chain FA, LCFA: long-chain FA, MCFA/SCFA: MCFA to SCFA ratio, SFA/UFA: SFA to UFA ratio, DI18: unsaturation index calculated as $C18:1/(C18:0 + C18:1) \times 100$

Table 2. Effects of ruminal pH, parity, and days in milk (DIM) on concentrations of fat (g/100 g of milk) and fatty acids (FA; g/100 g of fat) in milk of Polish Holstein-Friesian cows

Trait	Ruminal pH			Parity			DIM		SEM	P	
	high	moderate	low	1	2	≥ 3	< 90 days	≥ 90 days		ruminal pH	parity
Fat	3.16 a	3.25 A	2.93 Bb	3.28	3.18	3.08	3.17	3.09	0.039	0.012	0.877
C14:0	10.44	10.59	10.35	10.94	10.58	10.26	10.07 B	11.01 A	0.114	0.956	0.263
C16:0	27.60	28.64	28.94	30.60	28.57	27.07	27.62 b	28.56 a	0.346	0.594	0.213
C18:0	11.13	10.90	10.91	10.58	10.92	11.25	11.38 A	10.57 B	0.151	0.840	0.536
C18:1	23.03	22.29	24.09	20.47	22.64	23.94	23.15	22.98	0.312	0.549	0.142
SFA	62.79	62.07	61.49	65.79	62.77	61.53	61.89	63.28	0.487	0.987	0.449
UFA	31.18	29.99	33.07	29.18	30.80	32.07	31.55	30.92	0.386	0.341	0.102
MUFA	27.13	25.84	27.47	25.13	26.45	27.70	27.30 a	26.49 b	0.367	0.393	0.246
PUFA	4.00	3.88	3.89	3.45	3.78 B	4.19 A	3.96	3.96	0.051	0.662	0.002
TRANSFA	3.57	3.61	3.74	2.48 B	3.43 b	3.96 Aa	3.63	3.56	0.086	0.793	0.012
SCFA	10.59 a	9.99 a	8.92 b	10.34	9.80	10.43	10.25	10.14	0.111	0.034	0.590
MCFA	38.47	40.73	41.81	41.52 a	41.14 Ab	37.87 B	38.78 B	40.36 A	0.367	0.466	0.004
LCFA	38.68	37.45	40.02	37.17	37.85	39.56	39.33 a	37.76 b	0.467	0.394	0.102
MCFA/SCFA	3.72 B	4.17	4.82 A	4.08	4.35 A	3.72 B	3.90 B	4.09 A	0.058	<0.001	0.008
SFA/UFA	2.11	2.16	1.92	2.32	2.12	2.01	2.06 b	2.13 a	0.454	0.417	0.053
DI18	67.08	66.86	68.52	66.04	67.04	67.73	66.72	68.13	0.033	0.654	0.819

SFA: saturated FA, UFA: unsaturated FA, MUFA: monounsaturated FA, PUFA: polyunsaturated FA, TRANSFA: trans-unsaturated FA, SCFA: short-chain FA, MCFA: medium-chain FA, LCFA: long-chain FA, MCFA/SCFA: MCFA to SCFA ratio, SFA/UFA: SFA to UFA ratio, DI18: unsaturation index calculated as $C18:1/(C18:0 + C18:1) \times 100$.

SEM: standard error of the mean.

a, b: means in columns with different letters differ at $P \leq 0.05$.

A, B: means in columns with different letters differ at $P \leq 0.01$.

Table 3. Regression analysis of the effect of ruminal fluid pH on milk concentrations of fat (g/100 g of milk) and fatty acids (FA; g/100 g of fat) in Polish Holstein-Friesian cows

Trait	Coefficient	Intercept	R ²	P
Fat	NS	NS	NS	0.756
C14:0	NS	NS	NS	0.870
C16:0	-0.017	6.567	0.029	0.007
C18:0	NS	NS	NS	0.973
C18:1	NS	NS	NS	0.201
SFA	NS	NS	NS	0.997
UFA	NS	NS	NS	0.722
MUFA	NS	NS	NS	0.056
PUFA	0.138	5.555	0.043	0.001
TRANSFA	NS	NS	NS	0.089
SCFA	0.091	5.175	0.088	<0.001
MCFA	-0.033	7.416	0.130	<0.001
LCFA	NS	NS	NS	0.137
MCFA/SCFA	-0.271	7.180	0.215	<0.001
SFA/UFA	NS	NS	NS	0.930
DI18	NS	NS	NS	0.494

SFA: saturated FA, UFA: unsaturated FA, MUFA: monounsaturated FA, PUFA: polyunsaturated FA, TRANSFA: trans-unsaturated FA, SCFA: short-chain FA, MCFA: medium-chain FA, LCFA: long-chain FA, MCFA/SCFA: MCFA to SCFA ratio, SFA/UFA: SFA to UFA ratio; DI18: unsaturation index calculated as $C18:1/(C18:0 + C18:1) \times 100$.

NS: values not shown, as P was not significant at 5% level.

Milk fat percentage is generally believed to be a poor predictor of ruminal pH, as it greatly depends on many other nutritional and physiological factors (Lock, 2010). During the first weeks after calving, the effects of SARA on milk fat synthesis may interact with body fat mobilization due to the negative energy balance, which makes it difficult to interpret the milk fat content particularly in early lactation cows (Kleen et al., 2013). In addition, there are other causes of decrease in milk fat content, such as the high level of UFA in the diet (Bauman and Griinari, 2001).

There is growing interest in the use of specific FA as potential indicators of SARA. Milk FA concentrations predicted in this experiment by using the FT-IR method were generally in agreement with those previously determined by GC in the milk of Polish Holstein-Friesian cows (Kuczyńska et al., 2012; Proskura et al., 2019). Some differences observed between the studies (e.g., lower content of C16:0 than that reported by Proskura et al., 2019) could result from different composition of cows' diets as well as different datasets, as we used only high-producing cows that are at the highest risk of developing SARA. The use of different analytical methods might also be a crucial factor. Samková et al. (2020) recently showed that the FT-IR method can be advantageously used, especially for those FA and FA groups that are present in high proportions of the total milk fat. Results for FA present in milk in low proportions should, however, be interpreted with caution, because of lower correlation coefficients between FT-IR and GC values for these traits.

Some authors who analyzed the relationship between SARA and milk FA profile showed that low-rumen pH can reduce the biohydrogenation process of the C18-UFA, leading to higher proportions of certain trans-C18 FA isomers and lower level of C18:0 in milk fat (Enjalbert et al., 2008; Colman et al., 2013; Guo et al., 2013). These findings, in reference to C18:0, were not confirmed in the present study. The increased concentrations of odd- and branched-chain FA (OBCFA) in the milk of acidotic cows were also reported (Enjalbert et al., 2008; Colman et al., 2010, 2012; Guo et al., 2013; Jing et al., 2018). The OBCFA are synthesized by the ruminal bacteria, although the occurrence of their post-ruminal production in the mammary gland was also suggested (Vlaeminck et al., 2015). The FT-IR method used in the present study to determine FA composition did not allow detecting individual OBCFA in milk. However, our previous study performed on the same animals showed greater concentrations of propionate and valerate (precursors of odd-chain FA) as well as iso-butyrate and iso-valerate (precursors of branched-chain FA) in the ruminal fluid of acidotic cows as compared to those in healthy cows (Stefańska et al., 2018).

In the present study, SCFA and, consequently, MCFA/SCFA were the only fat components associated with ruminal pH, and low-rumen pH cows produced milk with the lowest proportion of SCFA and the highest MCFA/SCFA ratio. The same trend of effects of ruminal fluid pH on these traits was observed in all the analyzed farms. Moreover, although MCFA/SCFA was affected by all factors included in this study, SCFA was not influenced by parity and DIM. In previous studies, the effect of SARA on the SCFA group was not analyzed, and its effects on individual short-chain FA were diverse (Enjalbert et al., 2008; Colman et al., 2013; Sandri et al., 2020).

The SCFA are mainly *de novo* synthesized in the mammary gland from acetate and β -hydroxybutyrate, which originate from the microbial fermentation of dietary carbohydrates in the rumen. Previously, we showed increased molar concentrations of volatile FA, including acetate and butyrate, in the ruminal fluid of acidotic cows (Stefańska et al., 2018). The reduced level of SCFA in the milk of low-rumen pH cows might therefore be due to lower expression or activity of enzymes involved in *de novo* FA synthesis rather than due to deficiency of substrates for their production. This hypothesis is also supported by the findings that lipopolysaccharides derived from the rumen of SARA-affected ruminants decrease the expression of certain genes associated with lipid metabolism (Tao et al., 2015; Xu et al., 2018). On the other hand, milk MCFA, which are also thought to be primarily sourced from post-ruminal *de novo* synthesis in the mammary gland, were not associated with ruminal pH groups in the present study, and the regression coefficient indicated an inverse relationship between these parameters. Furthermore, the regression analysis showed that SCFA (as well as MCFA/SCFA, MCFA, PUFA, and C16:0) are unable to stand alone as an indicator of ruminal acidosis,

as they explain a small proportion of rumen pH variance (as indicated by R-squared value).

The poor relationship that we observed between ruminal fluid pH and milk FA profile could result from the use of the FT-IR method, which allowed identifying only a few individual FA in milk. The other reason might be the considerable variability in FA concentrations between herds included in this study, which is most likely due to the different compositions of total mixed ration diets provided in the analyzed farms (Stefańska et al., 2018, 2020). A substantial variation in the average ruminal pH values and milk FA concentrations was observed between herds, even though only farms similar in terms of cows' milk yield as well as housing and feeding systems were selected for the present study.

Differences in milk FA profile were also noted between cows varying in DIM and parity. Changes in FA composition during lactation are related to a large extent to the body energy balance (Ducháček et al., 2014). Cows in the early lactation stage are typically deficient in energy for up to the first 3 months after calving. In this state, they mobilize adipose tissue, which is particularly rich in LCFA such as C18:1 cis-9 and C18:0 (Hostens et al., 2012). This consequently leads to increased proportions of preformed LCFA in milk at the expense of *de novo* synthesized SCFA, as observed in this study in 40–89 DIM cows as compared to that in 90–150 DIM cows. The content of MUFA decreased as the lactation stage progressed. Similar trends in milk FA concentrations of early and mid-lactation cows were found by Stoop et al. (2009), Gottardo et al. (2017), and Vranković et al. (2017). The results of the current study also suggest that the effects of lactation stage on milk fat composition are not induced by changes in ruminal pH, which was similar in cows varying in DIM.

There are contrasting data in the literature on the effect of parity on milk FA composition in cattle. Some authors found that first lactation cows have lower proportions of MCFA such as C16:0 in their milk as compared to later lactation cows (Craninx et al., 2008; Gottardo et al., 2017); this finding could be explained by the metabolically less active mammary gland of primiparous cows (Miller et al., 2006). In the present study, the most significant effect was observed for the MCFA level, which, however, decreased as cows' parity increased. This could be related to a smaller energy deficit in early lactation primiparous cows than in older animals, as shown by de Vries et al. (1999). On the other hand, individual FA as well as SCFA and LCFA concentrations were not associated with parity in the present study.

Conclusion

In our present experiment, the milk FA profile determined using the FT-IR method proved to be an unreliable indicator of decreased pH of the ruminal fluid in dairy cows. Future studies aimed to identify SARA biomarkers should be conducted using techniques that allow detecting more individual FA in milk; the extensive vari-

ability in fat composition in milk should be considered between farms similar in cows' productivity and feeding systems. The results of this study indicate that SCFA are particularly interesting as one of the potential indicators of SARA, as SCFA level decreased with the decrease in ruminal pH in all the analyzed farms, regardless of cows' parity and DIM. However, only high-producing herds and cows were selected for this study; therefore, the results may not be representative for larger dairy cattle population.

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