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DETERMINANTS OF FAT CONTENT AND FATTY ACID COMPOSITION IN MILK OF COWS OF DIFFERENT BREEDS. PART 2: EFFECT OF COWS' AGE

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Abstract. The aim of the study was to analyze the effect of cows' age on the fat content and fatty acid profile in milk of cows of three breeds: Polish Red-White (ZR), Polish Red (RP) and Polish Holstein-Friesian Red-White (RW). The study included 473 cows kept on 17 farms. The cows studied were of different ages, this is in 1st, 2nd, 3rd, ≥ 4 th lactation. Milk samples were collected from each cow during a test milking. The study showed that the milk of younger cows (1st–3rd lactation) of the RP breed and older cows (≥ 4 th lactation) of the ZR breed was characterized by the most favorable fatty acid profile. In the milk fat of both groups of cows, significantly ($p < 0.05$) and highly significantly ($p < 0.01$) the highest contents of many of the tested monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were found, as well as highly significantly ($p < 0.01$) the lowest concentration of some of the tested saturated fatty acids SFA. Milk from RW cows (regardless of the age of the animals) was the milk with the lowest nutritional value in terms of fatty acid composition – significantly ($p < 0.05$) and highly significantly ($p < 0.01$) the lowest concentration of many health-promoting MUFA and PUFA acids and significantly ($p < 0.05$) and highly significantly ($p < 0.01$) the highest content of some SFA acids that are unfavourable to the health of consumers. The lowest fat concentration was determined in the milk of older cows from the ZR breed (≥ 4 th lactation), while the highest fat concentration was noted in the milk of younger cows from the RP breed (1st–3rd lactation). The obtained results provide important information on the health-promoting values of milk produced by cows of the three breeds studied. Moreover, they can become valuable information for both raw milk producers and consumers of so-called functional food.

Keywords: cows lactation number, milk fatty acids, milk fat.

INTRODUCTION

Milk fat is considered an important source of energy and health-promoting nutrients (Haug et al. 2007; Barłowska and Litwińczuk 2009; Kuczyńska et al. 2011, 2013; Tian et al. 2022; Singh et al. 2023). Milk contains about 70% saturated fatty acids (SFA) and about 30% unsaturated fatty acids (UFA). The latter include monounsaturated fatty acids (MUFA; about 26% of fatty acids content) and polyunsaturated fatty acids (PUFA; about 4% of fatty acids content) (García et al. 2014).

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In the literature (Król and Brodziak 2012; Markiewicz-Kęszycka et al. 2013; Pereira 2014), there is a lot of information about the adverse effects of SFA acids (e.g. C14:0 and C16:0 acids) on human health. In turn, unsaturated UFA fatty acids (e.g. MUFA acids) have a beneficial effect, among others, on the human cardiovascular system (Haug et al. 2007; Król and Brodziak 2012; Markiewicz-Kęszycka et al. 2013; Stobiecka et al. 2021).

The composition of milk fat (including its content and fatty acid profile) changes depending on various genetic and environmental factors (Barłowska and Litwińczuk 2009; Kuczaj et al. 2011; Miciński et al. 2012; Schwendel et al. 2015; Singh et al. 2023). One of such factors is the number of lactations (understood as the number of parities or the age of cows) (Palmquist et al. 1993; Jensen 2002; Kelsey et al. 2003; Bajodek et al. 2024), classified as an animal factor (Samková et al. 2012). Differences in the fatty acid composition of milk fat are also influenced by breed, individuality of the cow, number of parities or month of lactation. However, the main factor determining the content of saturated and unsaturated fatty acids in milk is the composition of the feed ration (and thus the season and feeding system) (Węglarz et al. 2007; Kalac and Samková 2010; Kuczaj et al. 2011; Frelich et al. 2012).

The mammary gland of younger cows (cows in the 1st lactation) does not show high metabolic activity and is characterized by lower expression of fatty acid synthase than in slightly older cows in their second or subsequent lactation (Miller et al. 2006). As a result, the milk of these cows contains a lower share of fatty acids synthesized *de novo*: C4:0–C14:0 acids and some C16:0 acids (Harvatine et al. 2009; Bilal et al. 2014). This is the result of increased use of fatty acids from both the feed ration and the body's reserves (Miller et al. 2006).

The results of some studies indicate that the order of lactation (parity) had no significant effect on the fatty acid profile in milk fat (Kelsey et al. 2003; Kala et al. 2018; Samková et al. 2018; Van et al. 2020).

According to many authors (Miciński et al. 2012; Stádník et al. 2013; Kala et al. 2018; Samková et al. 2018; Rodríguez-Bermúdez et al. 2023), the nutritional value of milk obtained from younger cows (primiparous, i.e. cows in their 1st lactation) is more desirable due to the fatty acid composition (lower content of many SFA and higher content of many UFA including CLA) than milk from older cows (≥ 3 rd lactation).

The aim of the study was to analyze the effect of cows' age on the fat content in milk and the composition of fatty acids in the milk fat of cows of three breeds: Polish Red-White (ZR), Polish Red (RP) and Polish Holstein-Friesian Red-White (RW). Cattle of the ZR and RP breeds are covered by a conservation breeding program in Poland.

A research hypothesis was put forward that the fat content in milk and the composition of fatty acids in the milk fat of the cow breeds studied differ depending on the age of the cows.

The results of the study allowed us to indicate which breed of cattle and which age group of cows produced milk with the most expected concentration of fat and fatty acids. This information will allow us to supplement knowledge in this area, which can also be used in cattle improvement, including the modification of the fat metabolism of milk in cows (including the composition of fatty acids in milk fat) in order to improve its health values.

MATERIAL AND METHODS

Animal material

The study involved 473 cows of three breeds: Polish Red-White (ZR; $n = 94$), Polish Red (RP; $n = 144$) and Polish Holstein-Friesian Red-White (RW; $n = 235$), of different age, in the 1st, 2nd, 3rd, 4th and further lactations and kept on 17 farms located in north-eastern, south-western and southern Poland.

Milk samples were obtained in the summer season (July) during a test milking of cows (method of assessing the utility value of cattle A4 and AT4) by a zootechnician. Cows were fed in a traditional barn-pasture system. Cows in the studied farms were healthy, kept in conditions of proper welfare and under constant medical and veterinary supervision.

In order to analyze the influence of the age of the cows of the three breeds on the fat content in milk and the fatty acid composition in milk fat within each breed, two groups were created: group I – “younger cows” in the first three consecutive lactations (1st–3rd lactation; $n = 274$), group II – “older cows” in the 4th or further lactations (≥ 4 th lactation; $n = 199$). The group of older cows consisted of cows used from the 4th to the 13th lactation. The fat concentration in the tested milk at the level of 3.5–4.0% was determined as the most expected.

Milk samples from the tested cows were collected in plastic containers and cooled at 4°C and transported to the laboratory. The samples remained cooled until their fat content was determined and the fat was extracted.

Milk analysis

Milk samples were analyzed for fat content (% fat – Infrared Milk Analyzer 150/Bentley Instruments Inc., Chaska, MN, USA).

The fatty acid profile in milk fat was determined using a gas chromatograph with a flame ionization detector (Agilent Technologies 7890A GC System, Santa Clara, CA, USA). Milk fat was extracted using Folch’s method (Folch et al. 1957). Esterification was carried out using potassium hydroxide (KOH) in a methanol–hexane mixture. The method for determining specific fatty acid esters and the temperature–time conditions for their separation were described by Przybylska and Kuczaj (2024a, 2024b). Agilent ChemStation software (Agilent Technologies) was used to identify the esters of the determined fatty acids.

The content of 28 fatty acids in the milk fat of the tested cows was determined. In addition, the concentration of all identified *trans* isomers of C18:1 fatty acids was determined. The classification of all fatty acids so identified in milk fat was previously described in the work of Przybylska and Kuczaj (2024a, 2024b).

Statistical analysis

The Kolmogorov–Smirnov test was used to verify the normality of the distributions of the studied variables. The multivariate analysis of variance and Bonferroni’s post-hoc multiple comparison tests were used to verify the significance of differences.

Pearson’s Chi-square (χ^2) test was used to compare observed frequencies with expected frequencies assuming the null hypothesis of no relationship between two variables. The description of the analyses of the relationship between variables was presented in the previous works by Przybylska and Kuczaj (2024a, 2024b). The method of analysis of the verification of relationships between breed and age group of cows in order to determine whether they significantly differentiate the results of the studied dependent variables (fat content and specific fatty acids of milk) was previously described by Przybylska and Kuczaj (2024a, 2024b). All analyses were statistically developed using the Statistica v.13.1 package.

RESULTS

The Bonferroni’s post-hoc tests did not show any statistically significant differences in milk fat concentration between the groups of younger cows (1st–3rd lactation) and older cows (≥ 4 th lactation) of the three cattle breeds studied (Table 1).

Table 1. Content (mean ± SD) of fat and fatty acids in milk of Polish Red-White (ZR), Polish Red (RP) and Polish Holstein-Friesian Red-White (RW) cows depending on the age of the cows

Trait [%]	ZR breed cows' age		RP breed cows' age		RW breed cows' age	
	younger (1st–3rd lactation)	older (≥4th lactation)	younger (1st–3rd lactation)	older (≥4th lactation)	younger (1st–3rd lactation)	older (≥4th lactation)
Fat	4.12 ±0.79	3.78 ±0.82	4.56 ±0.88	4.34 ±0.91	4.22 ±0.86	4.23 ±0.76
C4:0	0.85 ±0.44	0.63 ±0.34	0.73 ±0.38	0.76 ±0.43	0.71 ±0.31	0.82 ±0.35
C6:0	1.00 ±0.35	0.83 ±0.27	0.85 ±0.31	0.89 ±0.32	0.92 ±0.23	0.97 ±0.27
C8:0	0.81 ^C ±0.22	0.72 ±0.20	0.67 ^{A,E,F} ±0.22	0.70 ^{E,f} ±0.20	0.80 ^{C,D} ±0.16	0.82 ^{C,d} ±0.19
C10:0	2.10 ±0.59	1.94 ±0.57	1.68 ±0.59	1.79 ±0.54	2.21 ±0.50	2.19 ±0.64
C11:0	0.14 ±0.06	0.18 ±0.06	0.04 ±0.03	0.06 ±0.01	0.28 ±0.72	0.04 0.01
C12:0	2.69 ±0.80	2.54 ±0.74	2.13 ±0.68	2.25 ±0.60	2.92 ±0.74	2.83 ±0.86
C13:0	0.08 ±0.05	0.06 0.02	0.07 ±0.03	0.05 ±0.01	0.12 ±0.06	0.08 ±0.03
C14:0	9.98 ^C ±1.94	9.45 ^{E,f} ±1.83	8.60 ^{A,E,F} ±1.78	9.28 ^{E,F} ±1.77	10.57 ^{B,C,D} ±1.62	10.49 ^{b,C,D} ±2.07
C15:0	1.15 ±0.25	1.09 ±0.23	1.23 ±0.23	1.21 ±0.24	1.22 ±0.32	1.18 ±0.31
C16:0	28.15 ±5.16	26.57 ±4.52	25.68 ±4.50	27.26 ±3.65	30.43 ±4.28	30.89 ±4.39
C17:0	0.70 ±0.18	0.63 ^{C,D} ±0.13	0.78 ^{B,E,F} ±0.16	0.76 ^{B,E,F} ±0.13	0.67 ^{C,D} ±0.14	0.66 ^{C,D} ±0.16
C18:0	11.48 ^e ±2.48	10.81 ^C ±2.11	12.59 ^{B,F,E} ±2.30	11.80 ^E ±2.05	10.20 ^{a,C,D} ±2.25	10.79 ^C ±2.35
C20:0	0.16 ±0.06	0.15 0.06	0.17 ±0.05	0.16 ±0.04	0.17 ±0.04	0.17 ±0.04
Σ SFA	59.10 ±7.00	55.49 ^{E,F} ±6.12	55.16 ^{E,F} ±7.27	56.90 ^{E,F} ±6.47	60.62 ^{B,C,D} ±6.04	61.72 ^{B,C,D} ±7.39
C14:1	1.28 ±0.34	1.46 ±1.06	1.26 ±0.20	1.28 ±0.27	1.45 ±0.30	1.43 ±0.32
C16:1	4.56 ^B ±1.76	6.05 ^{E,A,F,C} ±1.47	4.73 ^B ±1.69	5.32 ^F ±2.07	4.82 ^B ±1.83	4.15 ^{B,D} ±1.67
C17:1	0.37 ^{c,D} ±0.11	0.37 ^{e,C,D} ±0.12	0.45 ^{a,B,E} ±0.10	0.47 ^{A,B,E,f} ±0.10	0.31 ^{b,F,C,D} ±0.11	0.40 ^{E,d} ±0.14
C18:1n9c	20.99 ±4.11	20.27 ±4.37	22.91 ±2.75	21.72 ±3.70	21.38 ±4.46	21.74 ±4.97
C18:1n8c (11c)	0.78 ±0.28	0.75 ±0.28	0.84 ±0.19	0.79 ±0.27	0.96 ±0.30	0.92 ±0.37
C18:1n9t	1.09 ±0.74	1.43 ^{E,F,c,d} ±0.63	1.09 ^b ±0.62	1.11 ^{b,f} ±0.60	1.06 ^B ±0.56	0.77 ^{B,d} ±0.48

Trait [%]	ZR breed cows' age		RP breed cows' age		RW breed cows' age	
	younger (1st–3rd lactation)	older (≥4th lactation)	younger (1st–3rd lactation)	older (≥4th lactation)	younger (1st–3rd lactation)	older (≥4th lactation)
C18:1n7t	2.81 ^{E,f,c} ±1.37	2.94 ^{E,F,c} ±1.25	3.62 ^{a,b,E,F,D} ±1.60	2.85 ^{E,C,F} ±1.21	1.74 ^{A,B,D,C} ±0.97	2.02 ^{B,a,C,D} ±0.94
Other <i>trans</i> C18:1	0.47 ±0.47	0.31 ±0.14	0.29 ±0.13	0.24 ±0.06	0.44 ±0.23	0.42 ±0.11
C18:2n6c	1.27 ±0.39	1.09 ±0.32	1.30 ±0.34	1.16 ±0.24	1.65 ±0.31	1.53 ±0.38
CLA	1.05 ^{c,E,f} ±0.58	1.23 ^{F,E} ±0.58	1.39 ^{a,F,E,d} ±0.71	1.11 ^{F,E,c} ±0.54	0.59 ^{A,B,C,D} ±0.24	0.71 ^{B,C,D,a} ±0.39
C18:3n3	0.73 ±0.29	0.81 ±0.25	0.91 ±0.30	0.90 ±0.20	0.48 ±0.20	0.58 ±0.27
C20:1	0.11 ±0.06	0.07 ^{F,E} ±0.04	0.08 ^{E,F} ±0.04	0.09 ^{E,F} ±0.05	0.13 ^{D,B,C} ±0.05	0.14 ^{B,D,C} ±0.06
C20:4n6	0.10 ±0.05	0.08 0.04	0.09 ±0.04	0.07 ±0.03	0.13 ±0.04	0.11 ±0.04
C20:5n (<i>cis</i> -5,8,11,14,17)	0.07 ±0.03	0.07 ±0.02	0.08 ±0.03	0.07 ±0.03	0.07 ±0.02	0.06 ±0.02
Σ UFA	35.09 ±5.19	36.65 ±4.81	38.81 ±4.72	37.05 ±4.54	34.75 ±5.22	34.44 ±6.44
SFA+UFA	94.19 ±3.11	92.14 ^F ±2.67	93.97 ±3.01	93.95 ±2.98	94.45 ±8.45	96.15 ^B ±1.80

SFA – saturated fatty acids; UFA – unsaturated fatty acids; Σ SFA – total SFA content; Σ UFA – total UFA content; a, b, c, d, e, f, – values marked with lower case letters differ significantly in the rows between the age groups of cows of the ZR, RP and RW breeds ($p < 0.05$); A, B, C, D, E, F – values marked with capital letters differ highly significantly in the rows between the age groups of cows of the ZR, RP and RW breeds ($p < 0.01$); a, A – younger cows (1st–3rd lactation) of the ZR breed; b, B – older cows (≥4th lactation) of the ZR breed; c, C – younger cows (1st–3rd lactation) of the RP breed; d, D – older cows (≥4th lactation) of the RP breed; e, E – younger cows (1st–3rd lactation) of the RW breed; f, F – older cows (≥4th lactation) of the RW breed; SD – standard deviation.

However, statistically significant differences ($p < 0.05$, $p < 0.01$) were noted in the content of many tested SFA acids (C8:0, C14:0, C17:0, C18:0), UFAs (C16:1, C17:1, C18:1n9t, C:18:1n7t, CLA, C20:1) and in the total content of all tested SFAs and in the total content of tested SFAs and UFAs (Table 1) between the groups of younger and older cows of the three tested cattle breeds.

The highest concentration of C8:0 acid (caprylic acid) was noted in milk fat among the group of older cows of the RW breed (0.82%). Slightly less of this acid was found in the tested milk from the group of younger cows for the ZR (0.81%) and RW (0.80%) breeds, respectively. The lowest C8:0 acid was noted in the milk fat of RP cows – 0.67% in group I – younger cows and 0.70% in group II – older cows, respectively, among all the animals tested. The difference between the highest and lowest content of caprylic acid turned out to be highly significant ($p < 0.01$).

The highest content of C14:0 acid (myristic acid) was found in the milk fat of both age groups of RW cows (younger cows: 10.57% and older cows: 10.49%) compared to the other animals tested. Similarly to the C8:0 acid, the least C14:0 acid was found in the milk fat of RP cows – 8.60% in group I – younger cows and 9.28% in group II – older cows, respectively. It should be mentioned that this was a significantly lower concentration ($p < 0.01$) compared to the content of this acid in the milk fat of group I – younger cows and group II – older cows of

the RW breed. A highly significantly ($p < 0.01$) higher content of myristic acid was also found in the milk fat of younger ZR cows (9.98%) than in the milk fat of younger RP cows (8.60%).

In the case of C17:0 acid (margaric acid), the highest concentration was found in the milk fat of RP cows, unlike in the case of C8:0 and C14:0 acids – for group I – younger cows: 0.78% and for group II – older cows: 0.76%. The values found were highly significantly ($p < 0.01$) higher compared to the content of C17:0 acid in the tested milk of younger cows of the RW breed (0.67%) as well as older cows of the ZR breed (0.63%) and RW (0.66%). The lowest concentration of C17:0 acid was found in the milk fat of older cows of the ZR breed (0.63%).

In the case of C18:0 acid (stearic acid), similarly to C17:0 acid, its highest concentration was found in the milk of younger cows (12.59%) and older cows (11.80%) of the RP breed. Younger cows (1st–3rd lactation) of the RP breed produced milk with a highly significantly higher ($p < 0.01$) content of stearic acid in comparison with the milk from younger cows of the RW breed (10.20%) and older cows of the ZR (10.81%) and RW (10.79%) breeds. Milk from RW cows had the lowest content of C18:0 acid.

The highest concentration of C16:1 acid (palmitoleic acid) was noted in the milk fat of older cows of the ZR breed (6.05%). It turned out that the concentration of this acid was highly significantly higher ($p < 0.01$) than in the milk of younger cows of all tested cattle breeds – ZR (4.56%), RP (4.73%) and RW (4.82%). The difference between the highest (older cows of the ZR breed) and the lowest (older cows of the RW breed – 4.15%) concentration of C16:1 acid among the tested cow breeds turned out to be highly significant ($p < 0.01$).

Milk of RP cows in both age groups was characterized by the highest concentration of C17:1 acid (heptadecanoic acid) – in older cows: 0.47% and in younger cows: 0.45%, respectively. The values found were highly significant and significantly higher than those determined in milk fat of ZR cows (for both age groups; $p < 0.01$, $p < 0.05$) and in fat of younger RW cows (0.31%; $p < 0.01$, $p < 0.05$). It was observed that the milk of the younger RW cows was characterized by the lowest concentration of this acid among all the animals tested.

In the case of C18:1n9t acid (elaidic acid), its highest concentration was determined in the milk fat of older cows of the ZR breed (1.43%). On the other hand, the lowest content of this acid was noted for older cows of the RW breed (0.77%). The difference between the highest and lowest content of C18:1n9t acid turned out to be highly significant ($p < 0.01$). It is worth mentioning that the milk fat of older cows of the ZR breed was characterized by a significantly higher content of this acid than the milk fat of both age groups of cows of the RP breed (younger cows: 1.09%, older cows 1.11%; $p < 0.05$) and both age groups of cows of the RW breed (younger cows: 1.06%, older cows: 0.77%; $p < 0.01$).

Analyzing the concentration of C18:1n7t acid (trans 7 oleic acid) in the milk fat of cows of the studied breeds, its highest concentration was determined in younger cows of the RP breed (3.62%). It turned out that this content was significantly and highly significantly higher in comparison with all other age groups of cows of the following breeds: ZR (younger cows: 2.81% and older cows: 2.94%; $p < 0.05$), RW (respectively: 1.74% and 2.02%; $p < 0.01$) and RP (older cows: 2.85%; $p < 0.01$). The lowest concentration of C18:1n7t acid was determined in the milk of older cows of the RW breed (2.02%).

The highest concentration of CLA (conjugated linoleic acid) was found in the milk fat of younger cows of the RP breed (1.39%). This concentration was significantly ($p < 0.05$) higher than in the milk of younger cows of the ZR breed (1.05%) and in the group of older cows of the RP breed (1.11%) and highly significantly ($p < 0.01$) higher than in cows of both age groups of the RW breed (younger cows: 0.59, older cows: 0.71%). Milk of younger cows of the RW breed contained the lowest concentration

of CLA. The difference between the highest and lowest concentration of this acid in milk fat was highly significant ($p < 0.01$).

As a result of the conducted analyses, the highest content of C20:1 acid (gadoleic acid) was found in the milk fat of older (0.14%) and younger (0.13%) cows of the RW breed. These concentrations were highly significantly ($p < 0.01$) higher compared to the content of this acid in milk from older cows of the ZR breed (0.07%) and younger (0.08%) and older (0.09%) cows of the RP breed. The lowest concentration of C20:1 acid was characteristic of the milk fat of older cows of the ZR breed.

The highest concentration of SFA acids was found in milk fat of RW cows (older cows: 61.72% and younger cows: 60.62%) (Table 1). The above contents were found to be highly significantly ($p < 0.01$) higher compared to the lowest concentrations of SFAs tested: in younger RP cows (55.16%) and older cows of the following breeds: ZR (55.49%) and RP (56.90%).

The highest total content of the tested SFA and UFA acids (Table 1) was found in milk fat from older cows of the RW breed (96.15%). In turn, the lowest total content of the determined SFAs and UFAs was characterized by milk fat from older cows of the ZR breed (92.14%). The difference between the values found was highly significant ($p < 0.01$).

DISCUSSION

Young cows (in their 1st lactation, i.e. first-time mothers) of the Czech Fleckvieh breed (dairy and meat breed) and Holstein cows included in the studies by Samková et al. (2018) and Kala et al. (2018) produced milk with a higher fat concentration than older cows (in their 3rd or further lactations). The results of our own research regarding the fat content in the milk of ZR and RP cows are generally consistent with the above statements. Younger cows of the ZR and RP breeds (1st–3rd lactation) produced milk with a higher fat concentration compared to older cows (≥ 4 th lactation). The studies by Nogara et al. (2024) (Holstein cows kept in Brazil) also confirm a higher fat concentration in the milk of younger cows (1st–3rd lactation). In turn, the analyses by Otwinowska-Mindur et al. (2018) contradict the aforementioned results of studies by other authors (Kala et al. 2018; Samková et al. 2018; Nogara et al. 2024) and our own research on cows of the ZR and RP breeds. In the studies by Otwinowska-Mindur et al. (2018), younger cows (1st–3rd lactation) of the Polish Holstein-Friesian breed produced milk with a lower fat concentration than older cows (≥ 4 th lactation). Similarly, younger cows (1st lactation) of the Holstein breed kept in Belgium (Liège province) covered by the studies by Van et al. (2020) were also characterized by milk with a lower fat content than the milk of slightly older cows (≥ 2 nd lactation).

The concentration of fat in milk of RW cows found in own studies does not confirm the results of the studies of the cited authors (Kala et al. 2018; Otwinowska-Mindur et al. 2018; Samková et al. 2018, Nogara et al. 2024). Namely, the fat content in milk of RW cows is practically the same in the group of younger and older cows (insignificantly more by 0.01% of fat in milk of older cows). The differences in fat content in milk shown in own studies and in studies of other authors (Kala et al. 2018; Otwinowska-Mindur et al. 2018; Samková et al. 2018) did not differ statistically significantly.

Van et al. (2020) found significantly ($p < 0.05$) higher concentrations of fatty acids: C18:1n9c acid (similar to the studies of Kala et al. 2018 and Rodríguez-Bermúdez et al. 2023), the tested PUFAs (similar to the results of the studies by Miciński et al. 2012; Samková et al. 2018) and OBCFA acids (odd-chain branched fatty acids; a type of saturated fatty acids) and C18:0 acid in the milk fat of slightly older cows (≥ 2 lactation). Van et al. (2020) noted in the

milk of young (in the 1st lactation) Holstein cows (Belgium, province of Liège) a significantly ($p < 0.05$) lower contents of 1) C4:0-C14:0 acids – similar to the results of studies by other authors (Kala et al. 2018; Samková et al. 2018; Rodríguez-Bermúdez et al. 2023; and for C4:0 acids similar to the results of Miciński et al. 2012) and 2) C16:0 acid – similar to the results of other studies (Miciński et al. 2012, 2018; Samková et al. 2012; Bilal et al. 2014; Rodríguez-Bermúdez et al. 2023).

Our own observations indicate that the milk of young cows (1st–3rd lactation) of the ZR and RP breeds was characterized by a higher concentration of some of the tested MUFA acids (C18:1n9c, C18:1n8c (11c)) compared to older cows (≥ 4 th lactation). Additionally, in the milk of young cows of the RP breed, similarly to the aforementioned MUFA acids, a highly significantly ($p < 0.01$) higher concentration of C18:1n7t acid was determined than in the milk of older cows. A similar predominance of C18:1n9t acid content was found in the milk fat of RW cows from the group of young cows. The observations of Rodríguez-Bermúdez et al. (2023) confirm the high content of MUFA and C18:1 acids in the milk of young Holstein cows (kept in Galicia, Spain) in their 1st lactation. The results of the cited author (Rodríguez-Bermúdez et al. 2023) also confirm the lower level of these acids determined in the milk fat of cows in the 3rd and further lactations (older cows). The same conclusions were obtained by Miciński et al. (2012) for the milk of Polish Holstein-Friesian cows and Samková et al. (2018) for the milk obtained from Czech Fleckvieh cows and Holstein cows.

According to the cited researchers (Samková et al. 2018; Rodríguez-Bermúdez et al. 2023), Holstein cows from the group of older cows (≥ 3 lactation) also had a lower level of C16:0 acid than cows in the 1st lactation. These conclusions are confirmed by the content of C16:0 acid in the milk fat of RP and RW cows found in our own studies (more of this acid was found in the milk of younger cows (1st–3rd lactation) than in the milk of older cows (≥ 4 th lactation)); these differences turned out to be statistically insignificant. On the other hand, Kala et al. (2018) obtained different results compared to those found by other researchers (Miciński et al. 2012; Samková et al. 2018; Rodríguez-Bermúdez et al. 2023) and compared with the results of our own studies regarding the level of C16:0 acid in the milk fat of RP and RW cows. They found a higher content of C16:0 acid in the milk fat of Czech Fleckvieh cows and Holstein cows in the 3rd or further lactation compared to the milk fat of cows in the 1st lactation (this difference was statistically insignificant).

Many authors (Miciński et al. 2012; Kala et al. 2018; Samková et al. 2018; Rodríguez-Bermúdez et al. 2023) observed a higher content of acids from the SFA group (also SCFA) in the milk fat of older cows (≥ 3 lactation). The observations of the cited authors are confirmed by the higher concentration of C4:0 and C6:0 acids (SCFA acids) and C8:0 in the milk of older cows of the RP and RW breeds (statistically insignificant difference) obtained in own studies. The contents of C10:0, C11:0 and C12:0 acids were higher for older cows of the RP breed (and additionally for older cows of the ZR breed for C11:0 acid); the observed differences turned out to be statistically insignificant. Kala et al. (2018) found significant differences ($p < 0.05$) only in the concentration of SFAs (also SCFAs) between the milk of younger cows (in the 1st lactation) and older cows (≥ 3 rd lactation).

The described results of own research and those of other cited researchers (Samková et al. 2018; Rodríguez-Bermúdez et al. 2023) are also consistent with the observations of the authors (Miciński et al. 2012; Samková et al. 2012; Bilal et al. 2014), in which younger cows (cows in the 1st lactation) produced milk with higher proportions of many UFA acids (including oleic acid) and lower content of many SFA acids (including C16:0 acid) compared to the milk fat of older cows.

The observations of Samkova et al. (2018) and Miciński et al. (2012) show that the milk of cows in the 1st lactation was characterized by the highest concentration of all the determined PUFA acids, C18:3n3 and CLA acids compared to the milk of older cows (≥ 3 lactation). Our own observations confirm this, but only in the case of the milk of RP cows (for CLA, the difference between younger and older cows was significant ($p < 0.05$); for C18:3n3 acid, this difference was statistically insignificant). The conclusions of Samkova et al. (2018) regarding the predominance of the mentioned PUFA acids are contradicted by the results of our own studies for ZR and RW cows. Namely, the milk fat of older ZR and RW cows contained more CLA than the fat in the milk of younger cows. It should be emphasized that in own research, unlike in the study of Samkova et al. (2018), more C18:2n6c acid was noted in the milk of younger cows (1st–3rd lactation) of the following breeds: ZR, RP and RW. In the study of Samkova et al. (2018), Czech Fleckvieh cows and Holstein cows had more of this acid in the milk fat of older cows (≥ 3 rd lactation). However, in both cases these differences were statistically insignificant.

Kala et al. (2018) found a higher content of the following UFA acids in the milk of younger (1st lactation) Czech Fleckvieh and Holstein cows: total concentration of the determined MUFA acids (similar to the study by Miciński et al. 2012), C:18:1n9c acid (the same as in our own study in ZR and RP cows and in the study by Rodríguez-Bermúdez et al. 2023) and C18:3n3 acid (the same as in RP cows in our own study and in the study by Miciński et al. 2012 and Samkova et al. 2018). Kala et al. (2018) noted the same concentration of C18:2n6c acid in both young (1st lactation) and older cows (≥ 3 rd lactation). However, the results of own research (for the ZR, RP and RW breeds) and the results of other studies (Miciński et al. 2012; Samková et al. 2018) do not confirm these observations of the cited researcher.

CONCLUSIONS

1. The age of cows had a significant effect ($p < 0.05$, $p < 0.01$) on the fatty acid profile in milk fat and a statistically insignificant effect on the fat content in milk of the studied cow breeds: ZR, RP and RW.
2. The research hypothesis was confirmed that the composition of fatty acids in milk fat and the fat content in milk of the studied cow breeds differed depending on the age of the cows. In the case of fat content in milk, these were statistically insignificant differences.
3. Younger RP cows (1st–3rd lactation) produced milk with the most desirable fatty acid content among all cow breeds tested. Milk fat from RP cows had significantly ($p < 0.05$) and highly significantly ($p < 0.01$) the highest content of health-promoting acids: C18:1n7t, CLA, C18:3n3 and highly significantly ($p < 0.01$) the lowest concentration of health-unfavorable acids: C8:0 and C14:0.
4. RW cows (regardless of age) produced milk with the least favorable fatty acid profile. The milk fat of these cows was characterized by significantly ($p < 0.05$) and highly significantly ($p < 0.01$) the lowest content of acids desirable in the human diet: C18:1n9t, C18:1n7t, CLA, C18:3n3 and acids: C16:1, C17:1, as well as significantly ($p < 0.05$) and highly significantly ($p < 0.01$) the highest concentrations of acids unfavorable for consumer health: C8:0, C14:0.
5. Older ZR cows (≥ 4 th lactations) produced milk with an averagely attractive fatty acid composition among the cattle breeds studied. Milk fat from ZR cows had a high content of health-promoting acids: C18:1n9t, C18:1n7t, CLA, C16:1 and a highly significant ($p < 0.01$) lowest content of the undesirable C17:0 acid in the human diet.

6. Older ZR cows (≥ 4 th lactations) produced milk with the most expected fat concentration. In contrast, younger RP cows (1st–3rd lactations) produced milk with the least expected fat content in milk.

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UWARUNKOWANIA ZAWARTOŚCI TŁUSZCZU I KOMPOZYCJI KWASÓW TŁUSZCZOWYCH W MLEKU KRÓW RÓŻNYCH RAS. CZĘŚĆ 2: EFEKT WIEKU KRÓW

Streszczenie. Celem badań była analiza wpływu wieku krów na zawartość tłuszczu i profil kwasów tłuszczowych w mleku krów trzech ras: polskiej czerwono-białej (ZR), polskiej czerwonej (RP) oraz polskiej holsztyńsko-fryzyjskiej odmiany czerwono-białej (RW). Badaniami objęto 473 krowy utrzymywane w 17 gospodarstwach. Badane krowy były w różnym wieku, tj. w 1., 2., 3. i ≥ 4 . laktacji. Próby mleka pobierane od każdej krowy pozyskiwano podczas próbnego udoju. Przeprowadzone badania wykazały, że mleko młodszych krów (1.–3. laktacja) rasy RP oraz starszych krów (≥ 4 . laktacja) rasy ZR charakteryzowało się najkorzystniejszym profilem kwasów tłuszczowych. W tłuszczu mlekowym obu grup krów stwierdzono istotnie ($p < 0.05$) i wysoce istotnie ($p < 0.01$) największe zawartości wielu badanych kwasów tłuszczowych jednonienasyconych MUFA i wielonienasyconych PUFA oraz wysoce istotnie ($p < 0.01$) najniższe stężenie niektórych badanych nasyconych kwasów tłuszczowych SFA. Mleko krów rasy RW (niezależnie od wieku zwierząt) miało najmniejsze wartości odżywcze pod względem składu kwasów tłuszczowych – istotnie ($p < 0.05$) i wysoce istotnie ($p < 0.01$) najmniejszą koncentrację wielu prozdrowotnych kwasów MUFA i PUFA oraz istotnie ($p < 0.05$) i wysoce istotnie ($p < 0.01$) największą zawartość niektórych kwasów SFA niekorzystnych dla zdrowia konsumentów. Najmniejsze stężenie tłuszczu oznaczono w mleku starszych krów rasy ZR (≥ 4 . laktacja), natomiast największe stężenie tłuszczu zanotowano w mleku młodszych krów rasy RP (1.–3. laktacja). Uzyskane wyniki dostarczają ważnych informacji na temat walorów prozdrowotnych mleka produkowanego przez krowy badanych trzech ras. Ponadto mogą stać się cennymi informacjami zarówno dla producentów mleka surowego, jak i konsumentów tzw. żywności funkcjonalnej.

Słowa kluczowe: numer laktacji krów, kwasy tłuszczowe mleka, tłuszcz mleka.