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DETERMINANTS OF FAT CONTENT AND FATTY ACID COMPOSITION IN MILK OF COWS OF DIFFERENT BREEDS. PART 1: EFFECT OF LACTATION PHASE

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Abstract. The aim of the study was to analyze the effect of the lactation phase on the concentration of fat and fatty acids in the milk of cows of three breeds: Polish Red-White (ZR), Polish Red (RP) and Polish Holstein-Friesian Red-White (RW). The study was conducted in 17 herds on a total of 473 cows kept in a traditional barn-pasture. The analyses showed that the milk of RP cows from the 3rd phase of lactation and ZR cows from the 2nd phase of lactation was characterized by the best fatty acid profile – significantly and highly significantly (p < 0.05, $p \le 0.01$) the highest contents of some tested monounsaturated MUFA and polyunsaturated PUFA acids and significantly and highly significantly (p < 0.05, $p \le 0.01$) the lowest contents of some tested SFA acids. Milk with the most expected fat content came from ZR cows from the 1st and 2nd phase of lactation and RW cows from the 1st phase of lactation. RW cows in the 2nd phase of lactation produced milk with the lowest nutritional value – significantly and highly significantly (p < 0.05, $p \le 0.01$) the lowest concentration of some health-promoting MUFA and PUFA acids and significantly and highly significantly (p < 0.05, $p \le 0.01$) the highest concentration of many saturated SFA acids unfavorable for the consumer's health. RW cows in the 3rd phase of lactation produced milk with the highest fat content. The research results can be used in improving cattle in terms of improving the fatty acid composition of produced milk. and thus improving its health-promoting values.

Key words: cows lactation phase, fatty acid composition, fat content in milk.

INTRODUCTION

Milk and dairy products are important components of the human diet, being a source of exogenous amino acids, fat, minerals and vitamins. Milk fat is characterized by a complex composition of fatty acids with a different number of carbon atoms (even and odd), different degrees of saturation (saturated fatty acids, SFA; unsaturated fatty acids, UFA; monounsaturated fatty acids, MUFA; polyunsaturated fatty acids, PUFA), with different configurations of the *cis* and *trans* type of some acid isomers, having straight and branched chains. Many of them have numerous biological and nutritional values important for human health (Kuczyńska et al. 2013).

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It is currently emphasized that the chemical composition of milk and milk fat (including a favorable profile of fatty acids) are important components in the human diet with desirable health-promoting properties. The most important fatty acids of milk fat that have a health-promoting effect on the human body are, among others: C18:1 cis-9 (oleic acid, OA); C18:2 cis-9,cis-12 (linoleic acid, LA); C18:2 cis-9,trans-11 (conjugated linoleic acid, CLA); C18:3 cis-9,cis-12,cis-15 (alpha-linolenic acid, ALA); C18:1 trans-11 (trans-vaccenic acid, TVA); C20:4 (arachidonic acid, AA); C18:3 (gamma-linolenic acid, GLA); C20:3 (dihomo-gamma-linolenic acid, DGLA); C20:5 (eicosapentaenoic acid, EPA); C22:5 (docosapentaenoic acid, DHA) (Kuczyńska et al. 2013).

The main determinants of the fat content in milk and the fatty acid profile are, among others: species, breed and genotype of animals, season and feeding system, number and phase of lactation (Barłowska and Litwińczuk 2009; Kuczaj et al. 2011; Miciński et al. 2012; Schwendel et al. 2015). The research results of many authors indicate the significant importance of the lactation phase on the composition of fatty acids in cow's milk (Auldist et al. 1998; Kay et al. 2005; Samková et al. 2012; Bilal et al. 2014; Rodríguez-Bermúdez et al. 2023; Bajodek et al. 2024). The content of most fatty acids in cow's milk changes during lactation (Bilal et al. 2014) and is determined by the cow's physiology and energy balance (Stoop et al. 2009; Gross et al. 2011; Samková et al. 2018). Cows starting lactation have a negative energy balance (NEB), which causes the activation of fatty acids of adipose tissue and their presence in milk (Walsh et al. 2011; Samková et al. 2012; Bilal et al. 2012; Bilal et al. 2014). The lactation phase is also an important factor influencing the concentration of milk fat in milk. The results of studies by many authors (Auldist et al. 1998; Bilal et al. 2014; Vranković et al. 2017) indicate that the highest fat content in milk occurs in the late phase of lactation.

The aim of the study was to determine the effect of the lactation phase on the fat content and fatty acid composition of milk of three cow 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 the conservation breeding program in Poland.

The hypothesis was put forward that the fat content in milk and the fatty acid composition of the milk fat of the tested cow breeds differ depending on the lactation phase. The obtained research results will allow to indicate which cattle breed and in which phase of lactation produces milk with the most expected fat concentration and fatty acid composition. This information can be used to improve cattle and to popularize the breeding of conservative cattle breeds due to their production of milk with high nutritional value and significant health-promoting properties.

MATERIAL AND METHODS

Research material

The research material consisted of 473 milk samples obtained from cows of three breeds: Polish Red-White (ZR; n = 94), Polish Red (RP; n = 144) and Polish Holstein-Friesian Red-White (RW; n = 235). The milk came from the summer season (July) and was obtained during the so-called test milking of cows (method of assessing the utility value of cattle A4 and AT4) by the zootechnician. The cows were fed in a traditional barn-pasture system. The farms (n = 17) from which the studied cows came were located in north-eastern, south-western and southern Poland and were included in the assessment of the utility value of dairy cattle.

Taking into account the lactation phase within each breed of cows, they were divided into 3 groups: I – cows in the 1st phase of lactation (\leq 100 DIM, n = 136), II – cows in the 2nd

phase of lactation (101–200 DIM, n = 156), III – cows in the 3rd phase of lactation (\geq 201 DIM, n = 181). The fat content in the tested milk of 3.5–4.0% was defined 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. They remained cooled until their fat content was determined and the fat was extracted.

At the time of milk sampling, the cows were healthy. The animals were provided with constant medical and veterinary care and were kept in conditions of proper welfare. The milk in the tested barns met the requirements of the highest quality class, this is extra class (PN-A-86002: 1999).

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 individual fatty acid esters and the temperature-time conditions for their separation were pre-viously described by Przybylska and Kuczaj (2024a, 2024b). Agilent ChemStation software (Agilent Technologies) was used to identify esters of the analyzed fatty acids.

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

Statistical analyses

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

To compare observed frequencies with expected frequencies assuming the null hypothesis of no relationship between two variables, Pearson's Chi-square (χ^2) test was used. The description of the analyses of the relationship between variables was presented in the previous works by Przybylska and Kuczaj (2024a, 2024b). Analyses verifying the relationships between breed and lactation phase in order to determine whether they significantly differentiate the results of the analyzed dependent variables (fat content and individual tested fatty acids of milk) were previously described by Przybylska and Kuczaj (2024a, 2024b). All analyses were performed using the Statistica v.13.1 package.

RESULTS

The post-hoc Bonfferoni's multiple comparison tests showed statistically significant differences ($p \le 0.01$, p < 0.05) in milk fat content between the lactation phases of the studied cow breeds (Table 1). It turned out that RW cows in the 3rd phase of lactation produced milk with the highest fat concentration (4.61%) among all the studied animals. This content was highly significantly higher ($p \le 0.01$) compared to the 1st and 2nd phase of lactation for cows of the following breeds: RW and ZR. In turn, milk from the 1st phase of lactation of RW cows was characterized by the lowest fat concentration (3.56%) among all the cow breeds

Trait	ZR breed lactation phase				RP breed lactation phase	•	RW breed lactation phase			
[%]	1	2	3	1	2	3	1	2	3	
Fat	3.84 ^{I,D,f}	3.75 ^{I,D,e,F}	4.29 ^G	4.53 ^{A,B,G}	4.37 ^{ь,G}	4.48 ^{a,B,G}	3.56 ^{C,H,I,E,D,F}	4.10 ^{G,I}	4.61 ^{A,B,G,F}	
	±0.79	±0.86	±0.71	±0.75	±0.88	±1.06	±0.68	±0.75	±0.70	
24:0	0.75	0.67	0.73	0.86	0.78	0.59	0.79	0.67	0.75	
	±0.39	±0.37	±0.45	±0.44	±0.43	±0.27	±0.36	±0.32	±0.30	
26:0	0.90	0.89	0.91	0.98 [⊧]	0.90	0.73 ^{g,I,D}	0.94 ^f	0.92	0.93 ^f	
	±0.32	±0.28	±0.37	±0.34	±0.33	±0.21	±0.30	±0.22	±0.23	
28:0	0.72	0.78 [⊧]	0.76 ^f	0.77 [⊧]	0.69 ^h	0.58 ^{B,c,G,H,I,D}	0.81 [⊧]	0.82 ^{e,F}	0.78 [⊧]	
	±0.22	±0.19	±0.23	±0.24	±0.21	±0.15	±0.21	±0.14	±0.15	
C10:0	1.86 ^н	2.12 [⊧]	2.04 [⊧]	1.93 ^{f,H}	1.78 ^{н,ı,g}	1.49 ^{c,G,d,B,H,I}	2.20 ^{F,E}	2.38 ^{A,E,D,F}	2.11 ^{e,F}	
	±0.58	±0.55	±0.60	±0.65	±0.52	±0.43	±0.69	±0.46	±0.47	
C11:0	0.16	0.19	0.12	0.06	0.05	0.03	0.11	0.43	0.05	
	±0.04	±0.06	±0.05	±0.03	0.01	±0.01	±0.04	±0.99	0.04	
C12:0	2.36 [⊢]	2.80 ^{e,F}	2.67 [⊧]	2.38 ^н	2.24 ^{G,H,I,b}	1.95 ^{с,н,і,с}	2.84 ^{E,F}	3.13 ^{A,E,F,D}	2.79 ^{E,F}	
	±0.71	±0.73	±0.81	±0.75	±0.58	±0.53	±0.94	±0.79	±0.65	
C13:0	0.07 ^G	0.07 ^{G,h}	0.08 ^G	0.07 ^G	0.06 ^{G,H}	0.06 ^{G,h}	0.13 ^{A,B,C,I,D,E,F}	0.11 ^{b,E,f}	0.07 ^G	
	±0.03	±0.04	0.03	±0.03	±0.02	±0.02	±0.07	±0.06	±0.02	
C14:0	9.05	10.07	9.92	9.10	9.32	8.34	10.33	11.13	10.36	
	±1.85	±1.68	±2.06	±1.88	±1.80	±1.58	±2.29	±1.52	±1.50	
C15:0	1.02 ^{H,g,F,E}	1.14	1.20	1.07 ^{h,F}	1.27 ^A	1.31 ^{A,D}	1.24ª	1.27 ^{A,d}	1.16	
	±0.25	±0.27	±0.10	±0.19	±0.21	±0.25	±0.36	±0.33	±0.29	
216:0	26.71 ^{ı,G,H}	26.95 ^{с,н}	28.26 ^{H,f}	27.69 ^{H,f}	27.34 ^{g,H,f}	24.22 ^{с,G,H,I,d,e}	30.48 ^{B,A,e,F}	32.45 ^{C,A,B,I,D,E,F}	29.60 ^{a,H,I}	
	±4.38	±4.14	±6.25	±4.27	±4.06	±3.15	±4.54	±4.38	±3.84	
C17:0	0.69 ^f	0.62 ^{d,g,E,F}	0.68	0.74⁵	0.77 ^{в,і,н}	0.80 ^{а,в,і,н}	0.73⁵	0.65 ^{⊑,ϝ}	0.65 ^{E,F}	
	±0.19	±0.13	±0.12	±0.13	±0.14	±0.15	±0.16	±0.13	±0.14	
C18:0	11.81	10.17	11.35	12.25	12.11	12.18	10.50	9.61	10.69	
	±2.46	±1.66	±2.40	±2.63	±2.11	±1.88	±2.67	±2.27	±2.01	
C20:0	0.14 ^{I,C,f,e}	0.13 ^{C,f,I,E}	0.19 ^{A,B}	0.15	0.18 ^{а,в}	0.18 ^{b,a}	0.16	0.16	0.18 ^{a,B}	
	±0.04	±0.04	0.08	±0.04	±0.04	±0.06	±0.04	±0.04	±0.04	
ΣSFA	56.09 ^н	56.46 [∺]	58.78⁵	57.96 ^{f,h}	57.40 ^{н, г}	52.41 ^{I,G,H,d,C,e}	60.81 [⊧]	63.02 ^{A,E,B,F,d}	59.90 [⊧]	
	±6.24	±6.09	±7.98	±6.96	±7.10	±5.00	±7.81	±5.83	±5.80	
C14:1	1.17	1.66	1.32	1.15	1.32	1.33	1.30	1.45	1.50	
	±0.30	±1.30	±0.31	±0.24	±0.21	±0.25	±0.36	±0.31	±0.26	
C16:1	5.46	5.84	4.91	4.69	4.96	5.51	4.16	4.81	4.78	
	±1.84	±1.46	±1.91	±1.96	±2.04	±1.64	±1.43	±1.93	±1.89	
C17:1	0.40	0.36	0.35	0.47	0.46	0.45	0.38	0.31	0.32	
	±0.14	±0.09	±0.10	±0.13	±0.08	±0.09	±0.13	±0.13	±0.12	
C18:1n9c	22.14	19.11	20.40	23.13	21.44	22.59	23.00	19.83	21.59	
	±5.18	±3.55	±2.87	±4.39	±2.47	±2.93	±5.79	±4.49	±3.67	
C18:1n8c (11c)	0.85 ^G	0.70 ^{H,G}	0.73 ^G	0.86 ^G	0.76 ^{G,h}	0.83 ^G	1.16 ^{A,B,C,h,I,E,D,F}	0.95 ^{B,g,e}	0.84 ^G	
	±0.38	±0.20	±0.17	±0.23	±0.26	±0.20	±0.39	±0.34	±0.21	
C18:1n9t	1.30	1.37	1.22	0.89	1.04	1.39	0.82	0.94	1.08	
	±0.72	±0.58	±0.82	±0.55	±0.61	±0.55	±0.40	±0.50	±0.62	
C18:1n7t	2.63 ^{н,ғ}	3.26 ^{с,н,}	2.72 ^{H,f}	2.71 ^{ı,ғ,н}	3.15 ^{G,H,I}	3.80 ^{A,c,G,H,D,I}	1.93 ^{в,е,ғ}	1.51 ^{A,B,C,E,F,D}	1.92 ^{B,d,E,I}	
	±1.13	±1.11	±1.66	±1.33	±1.40	±1.47	±1.24	±0.85	±0.85	
Other <i>trans</i>	0.41	0.31	0.33	0.24	0.25	0.29	0.44	0.49	0.40	
C18:1	±0.42	±0.12	±0.15	±0.09	±0.05	±0.14	±0.12	±0.32	±0.16	
C18:2n6c	1.21	1.11	1.17	1.23	1.17	1.29	1.74	1.64	1.55	
	±0.39	±0.35	±0.33	±0.28	±0.26	±0.36	±0.28	±0.36	±0.33	
CLA	0.98 ^{b,G,F,H}	1.38 ^{a,G,H,I,D}	1.06 ^{G,H,F}	0.93 ^{b,g,h,f}	1.25 ^{G,H,I}	1.56 ^{A,C,G,H,I,D}	0.52 ^{A,B,C,D,E,F}	0.51 ^{B,A,C,D,E,F}	0.73 ^{в,е,ғ}	
	±0.47	±0.60	±0.62	±0.48	±0.64	±0.63	±0.19	±0.18	±0.34	
C18:3n3	0.76 ^{G,H,I,e,F}	0.81 ^{G,I,f,H}	0.77 ^{H,f,I,G}	0.77 ^{G,H,I,F}	0.94 ^{a,G,H,I}	0.99 ^{A,b,c,G,H,I,D}	0.47 ^{A,B,C,D,E,F}	0.43 ^{A,B,C,D,E,F}	0.56 ^{A,B,C,D,}	
	±0.28	±0.24	±0.30	±0.22	±0.22	±0.27	±0.20	±0,20	±0.24	

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 lactation phase	

C20:1	0.09	0.07	0.10	0.09	0.08	0.07	0.12	0.14	0.13
	±0.05	±0.04	±0.06	±0.05	±0.04	±0.04	±0.05	±0.05	±0.06
C20:4n6	0.10	0.07	0.09	0.09	0.07	0.08	0.13	0.12	0.12
	±0.06	±0.04	0.04	±0.04	±0.02	±0.03	±0.04	±0.03	±0.04
C20:5n	0.07	0.06	0.07	0.07	0.07	0.08	0.06	0.05	0.07
<i>(cis</i> -5,8,11,14,17)	±0.03	±0.02	0.03	±0.04	±0.03	±0.03	±0.03	±0.02	0.02
ΣUFA	37.14 ^н	35.70 ^f	34.93 [⊧]	37.18 [∺]	36.79 ^н	40.06 ^{b,C,G,H,I}	35.67 [⊧]	32.71 ^{A,D,E,F}	35.19 [⊧]
	±5.16	±4.46	±5.38	±5.25	±4.57	±3.50	±7.13	±5.26	±4.58
SFA+UFA	93.23	92.16	93.71	95.15	94.19	92.47	95.13	94.42	95.09
	±3.12	±2.71	±3.11	±2.85	±3.06	±2.41	±9.84	±10.32	±2.12

SFA – saturated fatty acids; UFA – unsaturated fatty acids; Σ SFA – total SFA content; Σ UFA – total UFA content; a, b, c, d, e, f, g, h, i – values marked with lower case letters differ significantly in the rows between the lactation phases of ZR, RP and RW cows (p < 0.05); A, B, C, D, E, F, G, H, I – values marked with capital letters differ highly significantly in the rows between the lactation phases of ZR, RP and RW cows (p < 0.01); a, A – 1st phase of lactation of ZR cows; b, B – 2nd phase of lactation of ZR cows; c, C – 3rd phase of lactation of ZR cows; d, D – 1st phase of lactation of RP cows; e, E – 2nd phase of lactation of RP cows; f, F – 3rd phase of lactation of RP cows; g, G – 1st phase of lactation of RW cows; h, H – 2nd phase of lactation of RW cows; i, I – 3rd phase of lactation of RW cows; SD – standard deviation.

tested (highly significantly ($p \le 0.01$) lower fat content compared to all phases of lactation of RP cows and to the 3rd phase of lactation of ZR cows and to the 2nd and 3rd phase of lactation of RW cows).

Among the saturated fatty acids (SFA) tested, statistically significant differences ($p \le 0.01$, p < 0.05) were found between the subsequent lactation phases of the tested cow breeds in the case of acids: C6:0, C8:0, C10:0, C12:0, C13:0, C15:0, C16:0, C17:0, C20:0 (Table 1).

The highest concentration of C6:0 acid (caproic acid) was determined in the milk of RP cows from the 1st phase of lactation (0.98%) (Table 1). This content was highly significantly ($p \le 0.01$) higher only in comparison with the milk from the 3rd phase of lactation of this breed (0.73%). It should be mentioned that the lowest content of C6:0 acid among all the cows tested was characterized by the milk of RP cows from the 3rd phase of lactation (significantly (p < 0.05) lower acid concentration compared to the milk from the 1st and 3rd phase of lactation of the RW breed).

The highest content of C8:0 acid (caprylic acid) was noted in the fat of the milk of RW cows in the 2nd phase of lactation (0.82%). It turned out that the level of this acid was significantly and highly significantly higher than in the milk fat of RP cows in the 2nd (p < 0.05) and 3rd ($p \le 0.01$) phase of lactation. The lowest content of C8:0 acid was found in the milk fat of RP cows from the 3rd phase of lactation (0.58%).

Similarly to C8:0 acid, the highest content of C10:0 acid (capric acid) was found in the milk of RW cows produced during the 2nd phase of their lactation (2.38%). A highly significant ($p \le 0.01$) higher concentration of this acid was noted than in the milk of ZR cows in the 1st phase of lactation and RP cows in all phases of lactation. It was also found that, similarly to C8:0 acid, milk from the 3rd phase of lactation of RP cows (1.49%) was characterized by a highly significantly and significantly lower content of C10:0 acid than milk from the 2nd and 3rd phase of lactation of the ZR breed ($p \le 0.01$), 1st phase of lactation of the RP breed (p < 0.05), all phases of lactation of the RW breed ($p \le 0.01$) (Table 1).

Once again (analogy to C8:0 and C10:0 acids), it was noted that milk from the 2nd phase of lactation of RW cows was characterized by the highest content of C12:0 acid (lauric acid; 3.13%) (Table 1). It turned out that this concentration was highly significantly (p ≤ 0.01) higher in comparison with milk from the 1st phase of lactation of ZR cows and all phases of lactation of RP cows. The lowest concentration of C12:0 acid was noted (identically as for C8:0 and C10:0 acids) in milk obtained from RP cows from the 3rd phase of lactation (1.95%). This content was highly significantly (p ≤ 0.01) lower in comparison with the 3rd phase of lactation of ZR cows and all phases of lactation of ZR cows and all phases of lactation (1.95%). This content was highly significantly (p ≤ 0.01) lower in comparison with the 3rd phase of lactation of ZR cows and all phases of lactation of ZR cows and all phases of lactation of RW cows.

In the case of C13:0 acid, its highest content was determined in the milk fat of RW cows in the 1st phase of lactation (0.13%). The concentration of this acid turned out to be highly significantly ($p \le 0.01$) higher compared to the fat of milk produced in all stages of lactation of both the ZR and RP breeds and from the 3rd stage of lactation of RW cows. It should be emphasized that the content of this acid was similar for most of the studied groups, constituting at the same time the lowest recorded concentration of this acid (Table 1).

The highest concentration of C15:0 acid was found in the milk of RP cows from the 3rd stage of lactation (1.31%). It turned out that the content of this acid was highly significantly higher ($p \le 0.01$) than its lowest recorded concentrations, that is in milk from the 1st phase of lactation of both ZR cows (1.02%) and RP cows (1.07%) (Table 1).

Similarly to C8:0, C10:0 and C12:0 acids, the highest concentration of C16:0 acid (palmitic acid) was found in the milk fat of RW cows in the 2nd phase of lactation (32.45%). It turned out that this content was highly significantly ($p \le 0.01$) higher compared to the

concentration of this acid in the milk fat of all phases of lactation of cows of the breeds: ZR and RP and from the 3rd phase of lactation of RW cows. The lowest content of C16:0 acid was found in the milk fat of RP cows in the 3rd phase of their lactation (24.22%). It turned out that this concentration was highly significantly ($p \le 0.01$) and significantly (p < 0.05) lower than in the milk fat of the six groups of cows tested within the breeds included in the study (Table 1).

In the case of C17:0 acid (margaric acid), its highest content, similarly to C15:0 acid, was found in the milk of RP cows in the 3rd phase of lactation (0.80%). It was a significantly and highly significantly higher concentration compared to the milk of ZR cows from the 1st (p < 0.05) and 2nd ($p \le 0.01$) phase of lactation and to the milk of RW cows from the 2nd and 3rd phase of lactation ($p \le 0.01$). In turn, the lowest content of C17:0 acid was characterized by the milk fat of ZR cows in the 2nd phase of lactation (0.62%). This concentration turned out to be highly significantly ($p \le 0.01$) and significantly (p < 0.05) lower than that determined in the milk of RP cows from all stages of their lactation and in the milk of RW cows from the 1st stage of lactation (p < 0.05).

It turned out that the 3rd phase of lactation of ZR cows was associated with the highest content of C20:0 acid (arachidic acid) in their milk (0.19%). The lowest content of C20:0 acid was found in the milk of ZR cows from the 2nd phase of their lactation (0.13%). It was found that this concentration was highly significantly and significantly lower than in the milk of ZR cows from the 3rd phase of lactation ($p \le 0.01$), RP cows from the 2nd and 3rd phase of lactation ($p \le 0.01$, p < 0.05) and RW cows from the 3rd phase of lactation ($p \le 0.01$).

Among the unsaturated fatty acids (UFA) studied, statistically significant differences ($p \le 0.01$, p < 0.05) were found between the lactation phases of the studied cow breeds in the case of acids: C18:1n8c (11c), C18:1n7t, CLA, C18:3n3 (Table 1).

Highly significant ($p \le 0.01$) and significantly (p < 0.05) the highest content of C18:1n8c (11c) acid was noted in milk from the 1st phase of lactation of RW cows (1.16%) among all the studied cow breeds. It was noted that the 2nd phase of lactation most often (in ZR and RP cows) determined the lowest content of C18:1n8c (11c) acid. In the milk fat of ZR cows, it was the lowest concentration of this acid among all the studied cows (0.70%). In turn, in RW cows, the lowest content (0.84%) was observed in the 3rd phase of lactation. This concentration was highly significantly ($p \le 0.01$) lower only than the concentration in milk from the 1st phase of lactation of RW cows.

In the case of C18:1n7t acid (trans 7 oleic acid), the highest concentration was noted in milk from the 3rd phase of lactation of RP cows (3.80%). This content was highly significantly higher in as many as five cases ($p \le 0.01$) when comparing the concentration of this acid in the milk fat of ZR and RP cows in the 1st phase of lactation and RW cows in all phases of lactation. The lowest concentration of C18:1n7t acid was noted in the milk fat of RW cows (1.51%) in the 2nd phase of lactation. This content turned out to be highly significantly the lowest ($p \le 0.01$) in comparison to as many as six groups of animals studied. These were groups of cows of the ZR and RP breeds in all phases of lactation.

The highest concentration of CLA (conjugated linolenic acid) belonging to the group of PUFA acids was found in the milk of RP cows in the 3rd phase of lactation (1.56%). This content turned out to be highly significantly ($p \le 0.01$) higher than in the milk of as many as six groups of cows within the studied breeds. An equally high content of CLA was found in the milk fat of ZR cows (1.38%) in the 2nd phase of lactation. In turn, the lowest level of CLA was found in the 1st (0.52%) and 2nd (0.51%) phase of lactation of RW cows. Milk from the 2nd phase of lactation of RW cows had the lowest content of this acid among all the groups

of cows studied. This level was found to be highly significantly ($p \le 0.01$) lower than the concentration of this acid in the milk fat of ZR and RP cows in all phases of lactation.

Similarly to CLA, the highest concentration of C18:3n3 acid (α -linolenic acid) was noted in milk from the 3rd phase of lactation of RP cows (0.99%). It turned out that this content was highly significantly and significantly higher than the concentration of this acid in the milk fat of the following groups of cattle: ZR cows in all stages of lactation (p < 0.05, p ≤ 0.01), RP cows in the 1st stage of lactation (p ≤ 0.01) and RW cows in all stages of lactation (p ≤ 0.01). As a result of the conducted analyses, it turned out that the lowest amount of C18:3n3 acid was determined in the milk fat of RW cows in the 2nd phase of lactation (0.43%). This content was significantly (p ≤ 0.01) lower than in ZR and RP cows in each of their lactation phases.

The highest concentration of the sum of the tested SFA acids was found in the milk fat of RW cows in the 2nd phase of lactation (63.02%). This content was highly significantly and significantly higher than in the milk fat of ZR cows in the 1st ($p \le 0.01$) and 2nd ($p \le 0.01$) phase of lactation and RP cows in all phases of lactation (p < 0.05, $p \le 0.01$). The lowest concentration of the sum of the tested SFA acids was found in the milk fat of RP cows in the 3rd phase of lactation (52.41%). This content turned out to be highly significantly and significantly lower compared to the milk fat of the following groups of cows: ZR breed in the 3rd phase of lactation ($p \le 0.01$), RP breed in the 1st and 2nd phase of lactation (p < 0.05) and RW breed in all phases of lactation ($p \le 0.01$).

The highest content of the summed tested UFA acids was found in the milk fat of RP cows in the 3rd phase of lactation (40.06%). It turned out that this content was significantly and highly significantly higher than in the milk fat of ZR cows in the 2nd (p < 0.05) and 3rd ($p \le 0.01$) phase of lactation and RW cows in all phases of lactation ($p \le 0.01$). In turn, the lowest amount of all tested UFA acids was determined in the milk of RW cows in the 2nd phase of their lactation (32.71%). It was noted that the milk of these cows was characterized by a highly significantly ($p \le 0.01$) lower sum of analyzed UFA acids than the milk of RP cows in all phases of lactation and ZR cows in the 1st phase of lactation (Table 1).

DISCUSSION

Many researchers (Auldist et al. 1998; Bilal et al. 2014; Vranković et al. 2017) indicate the highest fat content in the 3rd phase of lactation, while the lowest in the 1st phase of lactation. The beginning of lactation is associated with a high energy demand that affects the fat concentration in milk (Bauman et al. 2006) and the proportions of individual fatty acids in milk (Stádník et al. 2013). The results of our own research confirm the above statements of the cited authors, but only for the milk of ZR and RW cows. The fat content in the milk of RP cows contradicts this statement. Namely, the highest fat concentration in the milk of RP cows was determined in the 1st phase of lactation (4.53%; $p \le 0.01$), while the lowest occurred in its 2nd phase (4.37%; p < 0.05, $p \le 0.01$). In the case of the study by Vranković et al. (2017), the 3rd phase of lactation of Holstein cows (Croatia, Đakovo) was not taken into account.

According to Kay et al. (2005), the greatest changes in the content of fatty acids in milk take place during the 1st phase of lactation. A similarity was found in our own research to the determined concentration of some fatty acids noted by Kay et al. (2005). Namely, the content of C10:0 acid in the milk of ZR cows in the 1st phase of lactation (1.86%) was very similar to the average content of this acid in the milk fat of American Holsteins (US Holstein) (1.88%) in the first sixteen weeks of their lactation (1st phase of lactation). It is worth noting that the content of C14:0 acid in the milk fat of ZR cows in the 1st phase of lactation (9.05%) was

almost identical to the content of this acid determined in the sixteenth week of lactation of American Holsteins (9.01%). Moreover, once again, a great similarity was observed between the milk of ZR cows and the cows studied by Kay et al. (2005) in terms of the concentration of C17:0 acid. Namely, ZR cows produced milk characterized by a concentration of this acid of 0.69% in the 1st phase of lactation. In turn, the milk of American Holsteins in the 1st week of lactation was characterized by the content of this acid at the level of 0.68%. It should be emphasized that RW cows (similarly to American Holstein cows) were characterized by milk with a similar concentration of C18:1n9c acid and CLA in the 1st phase of lactation. Moreover, a similar content of milk fat was found in RW cows and cows studied by Kay et al. (2005) (respectively: 3.56% and on average for both groups studied: 3.60%).

Changes in the fatty acid profile during lactation depend on different activities of their synthesis pathways (nutrition, *de novo* synthesis in the mammary glands of cows, rumen biohydrogenation and adipose tissue mobilization) (Kay et al. 2005; Bauman et al. 2006; Węglarz et al. 2007; Stoop et al. 2009; Gross et al. 2011; Kuczaj et al. 2011; Frelich et al. 2012; Samková et al. 2012). However, the main factor determining the content of saturated and unsaturated fatty acids in milk is the composition of the feed ration (Kalač and Samková 2010; Kuczaj et al. 2011; Frelich et al. 2012).

The 1st week of lactation is associated with the lowest content of mainly C16:0 acid derived from *de novo* synthesis (Samková 2008; Miciński et al. 2012; Vranković et al. 2017; Rodríguez-Bermúdez et al. 2023). The content of C16:0 acid increases with the improvement of energy balance until the 12th week of lactation. In turn, the level of C18:1n9c acid decreases until the 12th week of lactation (Kay et al. 2005; Stoop et al. 2009; Gross et al. 2011). This is because the beginning of lactation is characterized by high absorption of long-chain fatty acids by the mammary gland of cows (causing an increase in their content in milk), which, by inhibiting acetyl-CoA carboxylase, affect the *de novo* synthesis of fatty acids (C4:0-C14:0 acids and some C16:0 acids), reducing their concentration (Gross et al. 2011). As lactation progresses, the energy balance improves, influencing the change in the fatty acid profile (Gross et al. 2011).

This rule is confirmed in the presented own studies for ZR cows. An increasing trend was observed in the milk of cows of this breed as lactation progressed: the lowest concentration of C16:0 acid in the 1st phase of lactation – 26.71%, then the acid content increased slightly (26.95%), to reach the highest level in the 3rd phase (28.26%). However, the results of other studies (Samková 2008; Bilal et al. 2014; Rodríguez-Bermúdez et al. 2023), in contrast to the presented own studies, again indicate a decrease in the content of C16:0 acid in milk in the 3rd phase of lactation. The course of C16:0 acid concentration in milk fat in RW cows as lactation progresses is similar to that presented by many authors (Samková 2008; Miciński et al. 2012; Bilal et al. 2014; Rodríguez-Bermúdez et al. 2023) – the level of C16:0 acid increased in the milk of RW cows in the 2nd phase of lactation (32.45%), to reach a concentration similar to that at the beginning of lactation (30.48%) in the 3rd phase (29.60%). However, in other studies (Samková 2008; Miciński et al. 2017; Rodríguez-Bermúdez et al. 2012), slightly differently than for RW cows, the initial phase of lactation determines the lowest content of C16:0 acid.

The distribution of C17:0 acid concentration in milk fat of RW cows during lactation was confirmed in the study by Craninx et al. (2008) involving Holstein cattle kept in Belgium (Flanders). In own studies, the highest content of this acid was found in the milk of RW cows at the beginning of lactation (1st phase: 0.73%; p < 0.05). In the 2nd phase of lactation, the acid content was noted at 0.65% (p ≤ 0.01), which was maintained at a constant level

until the 3rd phase of lactation (0.65%; $p \le 0.01$). The concentration of C17:0 acid for ZR cows during lactation was not fully confirmed in the study by Craninx et al. (2008). The 1st phase of lactation was associated with the highest content of C17:0 acid (0.69%; p < 0.05), while the 2nd phase with its decrease (0.62%; $p \le 0.01$, p < 0.05). However, the 3rd phase of lactation, unlike in the studies by Craninx et al. (2008) and analogously to the studies by Bilal et al. (2014), was characterized by a renewed increase in the content of C17:0 acid in milk fat (up to 0.68%) in ZR cows. The analysis presented by some researchers (Craninx et al. 2008; Bilal et al. 2014) was contradictory with the concentration of C17:0 acid in milk fat of RP cows.

In the case of C18:0 acid (similar to C17:0 acid), in the studies of Bilal et al. (2014) and in our own studies (mainly for RP cows) a tendency was found for a high concentration of this acid in milk fat in the first days of lactation, its decrease in the 2nd phase of lactation and again a slight increase in the 3rd phase of lactation. A similar regularity was noted in the studies of Rodríguez-Bermúdez et al. (2023) for Holstein cows kept in Galicia (Spain).

Analyzing changes in the content of C14:0 acid as lactation progressed, confirmation was found in the results of studies by many authors (Craninx et al. 2008; Bilal et al. 2014; Rodríguez-Bermúdez et al. 2023) only in the case of ZR cows. The concentration of C14:0 acid in the 1st phase of lactation was the lowest (9.05%), then in the 2nd phase this content was higher (10.07%) and in the 3rd phase of lactation it decreased slightly – 9.92%.

Many authors (Craninx et al. 2008; Bilal et al. 2014; Vranković et al. 2017) also observed an upward trend in the content of C15:0 acid in the milk of the studied cattle. In own studies, such a trend was also observed in the milk of cows of the ZR and RP breeds. Namely, the lowest content of C15:0 acid was associated with the 1st phase of lactation of these cows (respectively: 1.02% and 1.07%; $p \le 0.01$, p < 0.05). The acid concentration increased in the 2nd phase (1.14% and 1.27%; $p \le 0.01$, respectively) and reached an even higher level in the 3rd phase of lactation (1.20% and 1.31%; $p \le 0.01$, respectively). In the analyses of Craninx et al. 2008 and Bilal et al. 2014, the content of C15:0 acid in milk fat did not increase/ decrease much or remained at a constant high level in the 3rd phase of lactation.

Similar upward trends for about the first 100 days of lactation and then a slight increase or maintaining at a constant high level during the 2nd and 3rd phase of lactation were observed by Bilal et al. (2014) in the milk of Canadian Holsteins for the following acids: C6:0, C8:0, C10:0, C11:0, C12:0. Confirmation of these analyses is found in own studies for ZR cows (for C8:0, C10:0, C12:0 acids). In turn, the distribution of the content of C8:0, C10:0, C12:0 acids during lactation in the milk of RP cows is not confirmed in the studies of Bilal et al. (2014). The results of other studies (Vranković et al. 2017) indicated a highly significant (p < 0.01) low level of C10:0 and C12:0 acids at the beginning of lactation in the tested Holstein cows kept in Croatia (Đakovo). The content of these acids in milk fat increased in the 2nd phase of their lactation (an increasing trend was also observed for these acids in the studies by Bilal et al. 2014). On the other hand, Miciński et al. (2012) found in their studies on Polish Holstein-Friesian (HO) cows the lowest concentration of C12:0 acid in the 1st week of lactation (day 6) and its highest concentration at the end of the 1st phase of lactation (day 90). The end of the 3rd phase of lactation was associated with a decrease in the C12:0 acid content in cows of this breed to the level from about day 30–90 of lactation.

The results of own studies for fatty acids from the C18:1 group are mostly confirmed by analyses presented by other researchers (Samková 2008; Stádník et al. 2013; Rodríguez-Bermúdez et al. 2023). The results of studies by other authors (Lake et al. 2007; Samková 2008; Stádník et al. 2013; Rodríguez-Bermúdez et al. 2023) indicate that the highest content of

C18:1 acids in milk is characteristic of the first days of lactation (especially the first 30 days). As lactation progresses, the concentration of these acids in milk fat decreases and only in the 3rd phase is their content increased again (but to a level lower than in the initial days of lactation). An identical trend in the distribution of C18:1 acids content was noted by Rodríguez-Bermúdez et al. (2023). The same tendency to reach the above-described concentration levels as lactation progressed was noted in own studies for the following acids: C18:1n9c (ZR, RP and RW cow breeds), C18:1n8c (11c) (ZR and RP cow breeds; $p \le 0.01$, p < 0.05), C18:1n9t (ZR cow breed), C18:1n7t (ZR cow breed, but unlike Samková (2008) a slightly higher level of this acid was noted in the 3rd phase of lactation than in its 1st phase; RW cow breed; $p \le 0.01$, p < 0.05), other identified C18:1 acids (ZR cow breed).

Many researchers (Auldist et al. 1998; Åkerlind et al. 1999; Kay et al. 2005; Samková 2008; Stoop et al. 2009; Miciński et al. 2012; Samková et. al. 2012) claim that the highest content of VA (vaccenic acid) and CLA acids is determined at the end of lactation. These observations were confirmed in own studies for RP cows. The concentration of these acids in milk fat in the 1st phase of lactation was 2.71% ($p \le 0.01$, p < 0.05) and 0.93% ($p \le 0.01$), respectively. In the 2nd phase of lactation, the concentration of these acids increased and in the 3rd phase their highest contents were determined at the level of 3.80% for vaccenic acid ($p \le 0.01$, p < 0.05) and 1.56% for CLA ($p \le 0.01$), respectively. Similarly, in the milk of RW cows the highest content of CLA was noted in the 3rd phase of lactation (0.73%; $p \le 0.01$). The 1st and 2nd phases of lactation were characterized by a relatively constant level of this acid (0.52% and 0.51%, respectively; $p \le 0.01$) – a similar situation is presented by Samková (2008) also in relation to CLA. In turn, Vranković et al. (2017) found a highly significant (p < 0.01) highest level of CLA in milk at the beginning of lactation and its decrease in the 2nd phase of lactation. However, other authors (Kelsey et al. 2003; Kgwatalala et al. 2009) do not confirm in their studies a significant influence of the lactation phase on the CLA content in milk fat.

Vranković et al. (2017) found a highly significant (p < 0.01) decrease in the level of C18:2n6c acid (linolenic acid) in the milk of Holstein cows (Croatia, Đakovo) on the 150th day of their lactation (1.63%) compared to the beginning of lactation, when this level was higher (2.25%; on the 30th day of lactation). Considering, as Vranković et al. (2017), only the first two phases of lactation, a similar downward trend in the content of C18:2n6c acid in milk fat was found in own studies for all the tested cow breeds.

Rodríguez-Bermúdez et al. (2023) found that milk fat from the 3rd phase of lactation is characterized by a significantly higher content of UFA acids than from the 2nd phase of lactation. Our own studies confirm this mainly in the case of RP and RW cows. Namely, the advantage of the 3rd phase of lactation over its 2nd phase was noted in the content of UFA acids such as: C18:1n8c (11c) (for ZR, RP breeds); C18:1n7t (for RP, RW breeds); CLA (for RP and RW breeds); C18:3n3 (for RP and RW breeds) and in the content of the UFA acids tested (for RP and RW breeds).

The results of many studies (Gross et al. 2011; Kala et al. 2018) indicate that milk from the 1st phase of lactation (specifically from its beginning – between days 10 and 30) is characterized by a more beneficial fatty acid composition for health (due to, among others, the high level of acids in milk fat: C18:1n9c and LA).

According to Bilal et al. (2014), whose studies included Canadian Holsteins, the level of C18:1n9c acid (oleic acid) in their milk was high at the beginning of lactation (similarly in the studies by Vranković et al. (2017) in Holstein cows bred in Croatia – Đakovo). In the analyses by Bilal et al. (2014), the content of this acid was decreasing until the beginning of the 2nd phase of lactation (around the 100th day of lactation), in which the concentration

of this acid in milk remained at a relatively constant level. Only in the 3rd phase of lactation did Bilal et al. (2014) note an upward trend in the content of this acid. An analogy was noticed in own research. Namely, for all the tested cow breeds, the highest oleic acid content in milk fat was found in the 1st phase of lactation. After the 100th day of lactation, a lower level of oleic acid was determined in the milk of cows of all breeds. In turn, in the 3rd phase of lactation (analogously to Canadian Holsteins in the study by Bilal et al. 2014), there was a relatively small increase in C18:1n9c acid in milk fat for the tested cow breeds. The results of our own research on the levels of MUFA concentration (including C18:1n9c acid) in milk fat during lactation are also confirmed by the observations of other authors (Stádník et al. 2013; Vranković et al. 2017; Rodríguez-Bermúdez et al. 2023).

CONCLUSIONS

- 1. The lactation phase significantly (p < 0.05, $p \le 0.01$) affected the fatty acid profile in the fat of the tested milk and the fat content in the milk of the three cow breeds.
- 2. The research hypothesis was confirmed that the fat content and fatty acids in the milk of the three cow breeds studied differed depending on the lactation phase.
- 3. ZR cows in the 1st and 2nd phase of lactation and RW cows in the 1st phase of lactation produced milk with the most expected fat content. On the other hand, RW cows in the 3rd phase of lactation produced milk with the least expected fat concentration.
- 4. RP cows in the 3rd phase of lactation and ZR cows in the 2nd phase of lactation produced milk with the most desirable fatty acid profile. Milk fat contained significantly (p < 0.05) and highly significantly (p ≤ 0.01) the highest contents of acids with health-promoting effects: C18:1n7t, CLA, C18:3n3 and significantly (p < 0.05) and highly significantly (p ≤ 0.01) the lowest contents of acids unfavorable for consumer health: C6:0, C8:0, C10:0 and C12:0 (in RP cows) and C17:0 and C20:0 (in ZR cows).</p>
- RW cows in the 2nd phase of lactation produced milk with the least favorable fatty acid profile. Milk fat had a highly significantly (p ≤ 0.01) lowest concentration of acids beneficial to consumer health: C18:1n7t, CLA and C18:3n3 and significantly (p < 0.05) and highly significantly (p ≤ 0.01) the highest content of undesirable acids: C8:0, C10:0, C12:0, C16:0.

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UWARUNKOWANIA ZAWARTOŚCI TŁUSZCZU I KOMPOZYCJI KWASÓW TŁUSZCZOWYCH W MLEKU KRÓW RÓŻNYCH RAS. CZĘŚĆ 1: EFEKT FAZY LAKTACJI

Streszczenie. Celem badań była analiza wpływu fazy laktacji na koncentrację tłuszczu i kwasów tłuszczowych w mleku krów trzech ras: polskiej czerwono-białej (ZR), polskiej czerwonej (RP) oraz polskiej holsztyńsko-fryzyjskiej odmiany czerwono-białej (RW). Badania przeprowadzono w 17 stadach na łącznej liczbie 473 krów utrzymywanych w tradycyjnym systemie alkierzowo-pastwiskowym. Przeprowadzone analizy wykazały, że mleko krów rasy RP z 3. fazy laktacji oraz krów rasy ZR z 2. fazy laktacji cechowało się najlepszym profilem kwasów tłuszczowych - istotnie i wysoce istotnie (p < 0.05, p ≤ 0.01) najwyższą zawartością niektórych badanych kwasów jednonienasyconych MUFA i wielonienasyconych PUFA oraz istotnie i wysoce istotnie (p < 0.05, $p \le 0.01$) najniższą zawartością niektórych oznaczanych kwasów SFA. Mleko o najbardziej oczekiwanej zawartości tłuszczu pochodziło od krów rasy ZR z 1. i 2. fazy laktacji oraz krów rasy RW z 1. fazy laktacji. Krowy rasy RW w 2. fazie laktacji produkowały mleko o najmniejszych wartościach odżywczych - stwierdzono istotnie i wysoce istotnie (p < 0.05, p ≤ 0.01) najmniejszą koncentrację niektórych prozdrowotnych kwasów MUFA i PUFA oraz istotnie i wysoce istotnie (p < 0.05, p ≤ 0.01) największą koncentrację wielu niekorzystnych dla zdrowia konsumenta nasyconych kwasów SFA. Krowy rasy RW w 3. fazie laktacji produkowały mleko o najwyższej zawartości tłuszczu. Wyniki badań mogą być wykorzystane w doskonaleniu bydła w zakresie polepszenia kompozycji kwasów tłuszczowych produkowanego mleka, a tym samym poprawy jego walorów prozdrowotnych.

Słowa kluczowe: faza laktacji krów, skład kwasów tłuszczowych, zawartość tłuszczu w mleku.