

Relationship between the content of chlorinated hydrocarbons and fatty acid composition of milk fat

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Abstract

Introduction: Reports that the presence of persistent organic pollutants in fat may affect fatty acid metabolism prompted this research aiming to study the relationship between the contents of γ -HCH and DDT, DDE, DDD, and Σ DDT, and fatty acid composition of milk fat. **Material and Methods:** The material consisted of 50 samples of cow and mare milk, collected in 2015. Ludwicki's and the Röse-Gottlieb and IDF Standard methods were used to prepare the samples. Statistical analyses were conducted using Statistica 12.0. **Results:** There was a negative correlation between the content of γ -HCH and C16:1, C17:1, C18:1*c9*, C18:1*c9c12*, and Σ MUFA in cow milk fat and C13:0, C14:0, and C10:1 in mare milk fat. A positive correlation was observed between γ -HCH and C6:0 to C12:0, C14:0, C18:1*t16*, and Σ SFA in cow milk fat, and between this compound and C14:0*iso*, C16:1, C17:1, C18:1*c9,11*, and Σ MUFA in mare milk fat. A negative correlation between the contents of Σ DDT and C16:1, C17:1, C18:1*c9,11,13* and Σ MUFA in cow milk fat and C16:0*iso*, C17:0, and C18:3 in mare milk fat was noted. A positive correlation was found between the contents of Σ DDT and saturated and polyunsaturated fatty acids and Σ SFA and Σ PUFA in cow milk fat, and C18:2*c9c12* in mare milk fat. **Conclusion:** The correlation between the content of selected organochlorine compounds and the composition of fatty acids in cow and mare milk fat indicates the strong influence of these environmental pollutants on the nutritional value of milk fat.

Keywords: cow milk, mare milk, DDT, γ -HCH.

Introduction

Milk fat is a valuable component of human diet with health benefits. The fatty acids contained in milk fat play an important biological role (2, 29). Short-chain saturated fatty acids, including butyric acid (C 4:0), are considered potential inhibitors of tumour cell growth. Conjugated linoleic acid (CLA) present in milk fat exhibits antioxidant and immunomodulating effects in the human body, and furthermore has documented antiatherogenic and anticarcinogenic activity. From polyunsaturated acids, mainly linoleic acid (LA n-6) and linolenic acid (ALA n-3), long-chain derivatives are produced: arachidonic acid (n-6), eicosapentaenoic acid EPA (n-3), and docosahexaenoic acid DHA (n-3). Nutritional values of food depend on the proportion of n-6 to n-3 fatty acids, where the optimal proportion in milk fat is average 3-4:1. Fatty acid composition in milk fat is influenced by many

factors, such as the animal's nutrition, breed, lactation, and age (22, 26, 30).

The fact that milk fat accumulates many environmental contaminants, including organochlorine compounds such as chlorinated hydrocarbons (18, 21, 27, 28), is disturbing. Persistent organic pollutants like γ -hexachlorocyclohexane (γ -HCH, lindane) and p,p'-dichlorodiphenyltrichloroethane (p,p'-DDT, 1,1,1-trichloro-2,2-bis (2-chlorophenyl-4-chlorophenyl) ethane) and its metabolites: p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE, 1,1-dichloro-2,2-bis (4-chlorophenyl) ethylene) and p,p'-dichlorodiphenyldichloroethane (p,p'-DDD, 1,1-dichloro-2,2-bis (4-chlorophenyl) ethane) are included in the list of the most toxic agents called "the dirty dozen" composed by the Stockholm Convention on Persistent Organic Pollutants (POPs). Chlorinated hydrocarbons classify as environmental oestrogens which change cellular hormonal activity. They suppress

immune system activity, impede cognitive or behavioural function in children, and increase the number of congenital malformations. These compounds are considered to be mutagens and carcinogens (19, 31). Persistent organic pollutants such as PCBs and organochlorine compounds including DDT cause developmental defects through neurotoxicity (5). There are epidemiological and experimental studies on the association of exposure to POPs with insulin resistance and related metabolic disorders like obesity, diabetes, and metabolic syndrome (8, 17). Persistent organic compounds like chlorinated hydrocarbons and polychlorinated biphenyls have lipophilic properties and the ability to accumulate in the body, in particular in fat. Many research papers confirm that these compounds induce changes in fat metabolism (especially physiologically important fatty acids) and inhibit enzyme activity (1, 6, 7, 10). Radosavljevic *et al.* (25) found synergistic effects of lindane (γ -HCH) and alcohol on hepatic enzyme levels and fatty acid metabolism disorders in the fat of the experimental animals. Matsusue *et al.* (16) found that coplanar PCBs have a significant effect on the reduced synthesis of physiologically essential long-chain unsaturated fatty acids, such as arachidonic acid in rat liver, by suppressing δ -5 and δ -6 desaturase activities and thus allowing the ω -6 parent fatty acid, linoleic acid, to accumulate. A 50% reduction of arachidonic acid (C20:4) and a significant increase in oleic (C18:1) and linoleic acids (C18:2) in rat liver caused by PCB 126 was demonstrated (15). In these reports, the influence of PCB and γ -HCH on fatty acid composition was investigated using experimental animals; however, there is a lack of studies concerning the influence of the organochlorine compounds on the fatty acid composition of farm animal milk fat and its nutritional quality. Therefore, the aim of this research was to study the relationship between the contents of chlorinated hydrocarbons and fatty acid composition in cow and mare milk fat.

Material and Methods

Material. The research material consisted of 50 samples of milk: 30 from cows and 20 from mares, collected from May to July 2015. The samples of cow milk were collected from two certified organic farms, while the mare milk originated from an individual homestead located in the Warmia and Mazury region (north-eastern Poland). The samples of cow milk were collected from the Holstein-Friesian breed, while the mare milk was drawn from the Polish Cold-blooded breed. The samples were collected and prepared according to the PN-EN ISO 707:2009 standard (24).

Fat extraction. Fat was extracted from milk by the Röse-Gottlieb method (23). The sample of milk was mixed with ammonia, alcohol, diethyl ether, and

petroleum ether. After separation and distillation of the ether fractions, the fat was obtained.

Determination of chlorinated hydrocarbon concentration. The samples of extracted fat were prepared by Ludwicki's method (14), which involves decomposition of lipids by concentrated sulphuric acid and release of organic insecticides to the hexane layer (14). The separation and quantitative determination of p,p'-DDT (1,1,1-trichloro-2,2-bis-(chlorophenyl)ethane) and its metabolites (p,p'-DDD and p,p'-DDE) and γ -HCH (γ -hexachlorocyclohexane) were conducted with gas chromatography using an electron capture detector (ECD), under the following conditions: an Agilent Technologies 6890N ECD/gas chromatograph equipped with a capillary column (25 m \times 0.32 mm) filled with a DB-1701 liquid phase. Film thickness was 0.25 μ m and separation temperatures were 280°C for the detector, 250°C for the injector, and 200°C for the column. Helium was used as the carrier gas supplied at a flow of 2.5 cm³/min. The detection limits (μ g/kg) were 0.50 for DDT, 0.50 for DDE, 0.20 for DDD, and 0.20 for γ -HCH; recovery rates were 96% for DDT, 97% for DDE, 93% for DDD, and 90% for γ -HCH. The content of chlorinated hydrocarbon residues was calculated by comparing the peak areas of the samples examined with that of the applied standard using Unicam 4880 computer software. The separation was conducted on the basis of reference material (Pesticide Std. Mixes A-1 and B-1, cat. nos. 47977 and 47978, Supelco, part of Sigma Aldrich, USA). The obtained results were presented as mean values of three parallel determinations, expressed as μ g/kg fat.

Determination of fatty acids composition. The fatty acid composition was determined after the acids were transformed into methyl esters according to the IDF Standard method. Solvent n-hexane and 2M KOH in methanol were added to the fat sample and the mixture was shaken, then NaHSO₄ was added and the mixture was centrifuged. The methyl esters obtained in the process were analysed (9). Chromatographic separation was performed using a Hewlett-Packard 6890 gas chromatograph with a flame-ionisation detector (FID) and a capillary column of length 100 m and internal diameter 0.25 mm. The liquid phase was Supelcowax 10 (Supelco, part of Sigma-Aldrich, USA) and the film thickness was 0.25 μ m. The conditions of separation were as follows: helium carrier gas, 1.5 mL/min flow rate, 60°C column temperature, 5°C/min increase to 180°C, and 250°C detector temperature. Methyl esters of acids were identified according to their retention times, which were compared with those of the mixture of methyl esters of fatty acids in the standard Supelco 37 Component FAME Mix (10 mg/mL in methylene chloride). For the calculation of the percentage share of fatty acids, the Chemostation computer programme (Agilent, USA) was used. Amounts of fatty acids were expressed as a weight percentage of total methyl esters of fatty acids.

Statistical analysis. Statistical analyses including calculation of mean values (X) and standard deviations (SD) were conducted using Microsoft Excel software. The normal distribution of data (Shapiro–Wilk test) and variance homogeneity (Levene's test) were investigated. If the conditions were fulfilled, an analysis of variance was conducted, otherwise a non-parametric Kruskal–Wallis test was applied (analysis at the level of significance of $P \leq 0.05$) to calculate the significance of differences between the mean values. To investigate the relationship between chlorinated hydrocarbons content and fatty acids composition in milk fat, a correlation analysis was performed and Pearson's correlation coefficients were calculated using correlation matrix modulus and Statistica 12.0 software (StatSoft Inc., Tulsa, Oklahoma, USA).

Results

The content of selected chlorinated hydrocarbons in cow and mare milk is presented in Table 1. Compounds such as γ -HCH, DDE, and DDT were present in 100% of the tested milk fat samples. The DDD content in all tested samples of cow milk fat was below the detection level, whereas this metabolite was found in 74% of the mare milk fat samples. The contents of γ -HCH, DDD, DDT, and Σ DDT differed significantly in cow and mare milk. In mare milk (4.61 $\mu\text{g}/\text{kg}$ of fat) the concentration of γ -HCH was ten times higher than in cow milk (0.46 $\mu\text{g}/\text{kg}$ of fat). The mean content of metabolite DDE in cow and mare milk was similar and reached the levels of 4.65 and 3.9 $\mu\text{g}/\text{kg}$ of fat respectively. In the case of cow milk fat, metabolite DDD was not detected, while in mare milk it was found only in 46% of the analysed samples and amounted to 0.21 $\mu\text{g}/\text{kg}$ of fat. The mean content of DDT in mare milk fat (11.06 $\mu\text{g}/\text{kg}$ of fat) was twice as high as in cow milk (4.56 $\mu\text{g}/\text{kg}$ of fat). A similar situation was observed in the case of Σ DDT (DDE+DDD+DDT). In mare milk Σ DDT was determined at the level of 15.17 $\mu\text{g}/\text{kg}$ of fat, whereas in cow milk 9.2 $\mu\text{g}/\text{kg}$ of fat was found.

The proportions of particular fatty acids in cow and mare milk fat are presented in Table 2. Significant differences in fatty acids constituents were found between the samples of cow and mare milk fat. Cow milk fat was richer in saturated fatty acids like C4:0

(3.27%), C6:0 (2.22%), C16:0 (26.22%), and C18:0 (11.41%), while in mare milk fat a higher proportion of the fatty acids C8:0 (2.84%), C10:0 (7.09%), and C12:0 (6.10%) was found.

In the group of unsaturated fatty acids in mare milk fat, the proportion of C16:1 (5.11%), C18:1*cis* 9 (22.46%), C18:2*cis*9*cis*12 (11.70%), and C18:3 (8.11%) was significantly higher than in cow milk fat. Also, a higher proportion of cow milk–typical C18:1*trans*10+*trans*11 (3.55%) and C18:2*cis*9*trans*11 (CLA) (1.18%) fatty acids was determined in cow milk.

Significant differences in SFA and PUFA content in tested milk fat were reported. The fat of mare milk is the richest source of polyunsaturated fatty acids (19.90%), while in the cow milk these compounds were determined at the level of 4.42%. Cow milk fat was characterised by a higher percentage contribution of SFA (66.93%) compared to mare milk fat (48.51%). Among the saturated fatty acids deserving attention were short chain acids (C4:0, C6:0, C8:0, and C10:0) due to their health-giving properties. The total amount of these fatty acids in the analysed milk fat was about 10%, while in cow milk fat C4:0 and C6:0 together constitute half of the fatty acids. In mare milk fat C8:0 and C10:0 were dominant.

The relationships between the contents of γ -HCH, DDT, DDE, DDD, and Σ DDT, and the fatty acid composition of cow and mare milk fat was found and are presented in Table 3.

A negative correlation between the contents of γ -HCH, MUFA (C16:1, C17:1, C18:1*c*9), PUFA C18:2*c*9*c*12, and Σ MUFA in cow milk fat was noted. A positive correlation was established between the contents of γ -HCH, saturated fatty acids C6:0, C8:0, C10:0, C11:0, C12:0, C14:0, and Σ SFA. In mare milk fat a negative correlation between the contents of γ -HCH and fatty acids C13:0, C14:0, and C10:1 was determined, while a positive correlation was observed in the case of C14:0*iso*, C16:1, C17:1, C18:1*c*9, C18:1*c*11, and Σ MUFA. In cow milk fat a positive correlation was found between the contents of DDE metabolite and most saturated fatty acids except for C4:0 and C16:0, where a negative correlation emerged. A positive correlation was seen between DDE and Σ MUFA, Σ PUFA, and Σ PUFA, with the exception of the C16:1, C17:1, and C18:1*c*13 acids, where a negative correlation was determined.

Table 1. Content of chlorinated hydrocarbons in cow and mare milk fat ($\mu\text{g}/\text{kg}$ of fat)

Chlorinated hydrocarbons	Cow milk			Mare milk		
	Mean	\pm SD	Range	Mean	\pm SD	Range
γ -HCH	0.46	\pm 0.32 b	(0.13–1.05)	4.61	\pm 1.16 a	(2.9–6.62)
DDE	4.65	\pm 1.65 a	(2.96–7.92)	3.90	\pm 2.6 a	(1.52–10.56)
DDD	BDL	\pm ND	(BDL)	0.21	\pm 0.25	(BDL–0.62)
DDT	4.56	\pm 3.06 b	(0.27–10.31)	11.06	\pm 1.57 a	(8.23–13.15)
Σ DDT	9.20	\pm 4.06 b	(3.47–15.69)	15.17	\pm 3.2 a	(10.71–22.24)

a, b – mean values in rows with different letters differ significantly at $P < 0.05$;
BDL – below detection level; ND – not determined; SD – standard deviation

Table 2. Percentage contribution of fatty acids of cow and mare milk fat (%)

Fatty acid	Cow milk			Mare milk		
	Mean	±SD	Range	Mean	±SD	Range
C4:0	3.27	±0.28 a	(2.78–3.76)	0.17	±0.07 b	(0.05–0.28)
C6:0	2.22	±0.20 a	(1.81–2.43)	0.31	±0.26 b	(0.01–1.02)
C8:0	1.41	±0.14 b	(1.16–1.59)	2.84	±1.51 a	(0.05–5.45)
C10:0	3.21	±0.45 b	(2.49–3.82)	7.09	±3.05 a	(1.79–13.69)
C11:0	0.05	±0.02 a	(0.03–0.08)	0.02	±0.03 b	(0.00–0.09)
C12:0	3.57	±0.51 b	(2.73–4.25)	6.1	±2.46 a	(2.13–10.42)
C13:0 <i>iso</i>	0.07	±0.02 a	(0.05–0.09)	0.07	±0.10 a	(0.0–0.35)
C13:0	0.20	±0.03 a	(0.14–0.24)	0.11	±0.08 b	(0.02–0.33)
C14:0 <i>iso</i>	0.21	±0.07 a	(0.07–0.28)	0.05	±0.03 b	(0.03–0.17)
C14:0	11.01	±1.49 a	(8.16–12.83)	6.60	±1.79 b	(4.02–9.36)
C15:0 <i>iso</i>	0.43	±0.15 a	(0.18–0.58)	0.06	±0.05 b	(0.0–0.20)
C15:0 <i>aiso</i>	0.76	±0.31 a	(0.27–1.12)	0.08	±0.09 b	(0.0–0.36)
C15:0	1.45	±0.46 a	(0.81–1.94)	0.26	±0.16 b	(0.0–0.74)
C16:0 <i>iso</i>	0.40	±0.11 a	(0.22–0.53)	0.13	±0.04 b	(0.0–0.23)
C16:0	26.22	±2.91 a	(22.63–31.15)	22.3	±1.69 b	(20.0–26.75)
C17:0	0.88	±0.07 a	(0.79–0.98)	0.31	±0.15 b	(0.2–0.83)
C18:0	11.41	±1.88 a	(9.13–14.95)	1.94	±0.48 b	(0.75–2.46)
C20:0	0.18	±0.07 a	(0.03–0.31)	0.08	±0.04 b	(0.01–0.21)
C10:1	0.26	±0.04 b	(0.16–0.32)	0.78	±0.65 a	(0.05–2.54)
C12:1	0.07	±0.03 a	(0.02–0.12)	0.03	±0.03 b	(0.0–0.10)
C14:1	0.81	±0.12 a	(0.61–1.01)	0.30	±0.21 b	(0.0–0.92)
C16:1	1.46	±0.5 b	(0.75–2.22)	5.11	±1.51 a	(2.76–8.14)
C17:1	0.30	±0.10 a	(0.18–0.5)	0.35	±0.13 a	(0.16–0.61)
C18:1 <i>cis 9</i>	19.61	±3.34 b	(15.30–25.24)	22.46	±4.57 a	(14.51–30.0)
C18:1 <i>cis 11</i>	1.27	±2.29 a	(0.45–8.51)	1.69	±0.44 a	(1.0–2.39)
C18:1 <i>cis 12</i>	0.14	±0.04	(0.1–0.26)	0.0	±0.0	(ND)
C18:1 <i>cis 13</i>	0.11	±0.06 a	(0.05–0.23)	0.18	±0.26 a	(0.01–1.07)
C18:1 <i>trans6</i>	0.15	±0.09 a	(0.00–0.28)	0.01	±0.01 b	(0.0–0.04)
C18:1 <i>trans9</i>	0.28	±0.04 a	(0.20–0.33)	0.05	±0.02 b	(0.02–0.12)
C18:1 <i>trans10+trans11</i>	3.55	±1.59 a	(0.87–5.63)	0.27	±0.44 b	(0.0–1.57)
C18:1 <i>trans12</i>	0.21	±0.04 a	(0.16–0.27)	0.01	±0.01 b	(0.0–0.03)
C18:1 <i>trans16</i>	0.32	±0.07 a	(0.23–0.45)	0.02	±0.09 b	(0.0–0.43)
C20:1	0.13	±0.03 b	(0.09–0.19)	0.39	±0.20 a	(0.01–0.94)
C18:2 <i>cis9cis12</i>	1.31	±0.17 b	(1.04–1.54)	11.70	±4.18 a	(3.47–19.76)
C18:2 <i>trans13cis9</i>	0.19	±0.06 a	(0.14–0.32)	0.01	±0.02 b	(0.0–0.08)
C18:2 <i>trans12cis9</i>	0.25	±0.07 a	(0.17–0.41)	0.04	±0.04 b	(0.0–0.14)
C18:2 <i>trans9cis12</i>	0.07	±0.03 a	(0.03–0.12)	0.01	±0.02 b	(0.0–0.08)
C18:2 <i>trans11cis15</i>	0.54	±0.27 a	(0.12–0.95)	0.01	±0.02 b	(0.0–0.6)
C18:2 <i>cis9trans11 CLA</i>	1.18	±0.69 a	(0.41–2.51)	0.02	±0.02 b	(0.0–0.05)
C18:3	0.85	±0.10 b	(0.73–1.05)	8.11	±3.43 a	(1.5–13.3)
ΣSFA	66.93	±4.85 a	(58.39–71.65)	48.51	±8.97 b	(35.96–66.56)
ΣMUFA	28.65	±4.63 a	(23.97–38.30)	31.65	±5.58 a	(22.86–41.31)
ΣPUFA	4.42	±1.08 b	(3.22–6.45)	19.90	±6.27 a	(8.05–28.05)

a, b – mean values in rows with different letters differ significantly at $P < 0.05$;
 ND – not determined; SD – standard deviation

Table 3. Correlation between chlorinated hydrocarbons content and fatty acid composition in cow and mare milk fat

Fatty acid	Cow milk				Mare milk				
	γ -HCH	DDE	DDT	Σ DDT	γ -HCH	DDE	DDD	DDT	Σ DDT
C4:0	NS	-0.52**	NS	NS	NS	NS	NS	NS	NS
C6:0	0.37*	NS	NS	NS	NS	NS	NS	NS	NS
C8:0	0.43*	NS	NS	NS	NS	NS	NS	NS	NS
C10:0	0.47**	NS	0.35*	NS	NS	NS	NS	NS	NS
C11:0	0.56***	0.49**	NS	0.34*	NS	NS	NS	NS	NS
C12:0	0.49**	NS	0.49**	0.43*	NS	NS	NS	NS	NS
C13:0 <i>iso</i>	NS	NS	0.36*	NS	NS	NS	NS	NS	NS
C13:0	NS	0.59***	NS	NS	-0.80**	NS	NS	NS	NS
C14:0 <i>iso</i>	NS	0.71***	NS	0.39**	0.73*	NS	NS	NS	NS
C14:0	0.46**	NS	0.66***	0.61***	-0.72*	NS	NS	NS	NS
C15:0 <i>iso</i>	NS	0.68***	NS	0.47*	NS	NS	NS	NS	NS
C15:0 <i>aiiso</i>	NS	0.70***	NS	0.48**	NS	NS	NS	NS	NS
C15:0	NS	0.71***	NS	0.51**	NS	NS	NS	NS	NS
C16:0 <i>iso</i>	NS	0.69***	NS	0.43*	NS	-0.72*	NS	NS	-0.64*
C16:0	NS	-0.41*	0.36*	NS	NS	NS	NS	NS	NS
C17:0	NS	0.56***	NS	NS	NS	NS	NS	NS	-0.64*
C18:0	NS	0.36*	-0.40*	NS	NS	NS	NS	-0.69*	NS
C20:0	NS	NS	NS	NS	NS	NS	NS	NS	NS
C10:1	NS	0.54***	0.74***	0.79***	-0.67*	NS	NS	NS	NS
C12:1	NS	0.68***	NS	0.43*	NS	NS	NS	NS	NS
C14:1	NS	NS	NS	NS	NS	NS	NS	NS	NS
C16:1	-0.47**	-0.57***	NS	-0.39*	0.71*	NS	NS	NS	NS
C17:1	-0.55***	-0.46**	-0.46**	-0.54***	0.63*	NS	NS	NS	NS
C18:1 <i>c9</i>	-0.49**	NS	-0.39*	-0.43**	0.64*	NS	NS	NS	NS
C18:1 <i>c11</i>	NS	NS	-0.47**	-0.49**	0.75*	NS	NS	NS	NS
C18:1 <i>c12</i>	NS	NS	-0.41*	NS	ND	ND	ND	ND	ND
C18:1 <i>c13</i>	NS	-0.41*	-0.63***	-0.64***	NS	NS	NS	NS	NS
C18:1 <i>t6</i>	NS	0.68***	NS	0.47**	NS	NS	NS	NS	NS
C18:1 <i>t9</i>	NS	NS	NS	NS	NS	NS	NS	NS	NS
C18:1 <i>t10+t11</i>	NS		NS	0.42*	NS	NS	NS	NS	NS
C18:1 <i>t12</i>	NS	0.55***	NS	NS	NS	NS	NS	NS	NS
C18:1 <i>t16</i>	0.36*	0.62***	NS	NS	NS	NS	NS	NS	NS
C20:1	NS	0.62***	NS	0.49**	NS	NS	NS	NS	NS
C18:2 <i>c9c12</i>	-0.39*	-0.67***	NS	NS	NS	0.70*	NS	NS	0.73*
C18:2 <i>t13c9</i>	NS	0.48**	NS	0.36*	NS	NS	NS	NS	NS
C18:2 <i>t12c9</i>	NS	NS	NS	NS	NS	NS	NS	NS	NS
C18:2 <i>t9c12</i>	NS	0.39*	NS	0.41*	NS	NS	NS	0.87***	NS
C18:2 <i>t11c15</i>	NS	0.64***	NS	0.42*	NS	0.65*	NS	NS	NS
C18:2 <i>c9t11</i>	NS	0.47**	0.36*	0.47**	NS	NS	NS	NS	NS
C18:3	NS	NS	NS	NS	NS	-0.77**	NS	NS	-0.81**
Σ SFA	0.55***	NS	0.39*	0.36*	NS	NS	NS	NS	NS
Σ MUFA	-0.52**	NS	-0.48**	-0.46**	0.67*	NS	NS	NS	NS
Σ PUFA	NS	0.41*	NS	0.42*	NS	NS	NS	NS	NS

* correlation significant at $P < 0.05$; ** correlation significant at $P < 0.01$; *** correlation significant at $P < 0.001$; NS – not significant

In mare milk fat a positive correlation between the content of DDE metabolite, C18:2*c9c12*, and C18:2*t11c15* was found, whereas a negative one was determined in the cases of C16:0 and C18:3. In cow milk fat, DDT correlated positively with the saturated acids C10:0, C12:0, C13:0*iso*, C14:0, C16:0, Σ SFA, and C18:2*c9t11*, while it did so negatively with the C18:0 and C18:1*c9-c13* acids and Σ MUFA. In mare milk fat a negative correlation occurred only with C18:0 and a positive one only with C18:2*t9c12*. A positive correlation was brought to light between the contents of Σ DDT and most saturated, monounsaturated, and polyunsaturated fatty acids, and between Σ DDT and Σ SFA and Σ PUFA, while a negative correlation was recognised between the contents of Σ DDT, C16:1, C17:1, C18:1*c9*, *c11*, *c13*, and Σ MUFA in cow's milk fat. In the case of mare's milk fat, a correlation was observed between the contents of Σ DDT and C16:0*iso*, C17:0, C18:3 (negative), and C18:2*c9c12* (positive).

Discussion

The presence of chlorinated hydrocarbons was found in all milk fat samples analysed in the study. The content of γ -HCH, DDT, DDE, and DDD varied both within one kind of milk and across milk types of different animal species. Analysing the results of the research concerning chlorinated hydrocarbons carried out in the same region in previous years, Σ DDT can be observed trending downward by content in farm animal milk over the years (20, 27). In 2000–2001 in mare milk it was 36.7–96.3 $\mu\text{g}/\text{kg}$ (20), whereas in the presented study it was 15.17 $\mu\text{g}/\text{kg}$. The content of γ -HCH in the milk of mares also manifests a downward trend: samples obtained in 2000–2001 had content ranging from 6.7 to 15.1 $\mu\text{g}/\text{kg}$ of fat, while in this study the mean γ -HCH content was 4.61 $\mu\text{g}/\text{kg}$ of fat. In 2004, Świstowska *et al.* (28) showed that the content of γ -HCH in mare milk ranged from 0.10 to 1.34 $\mu\text{g}/\text{kg}$, whereas the amount of DDT fell between 10.84 and 27.67 $\mu\text{g}/\text{kg}$. The analysis performed in 2010 in the same region showed that the contents of γ -HCH and Σ DDT in mare milk fat were 4.69 ± 1.33 and 16.72 $\mu\text{g}/\text{kg}$, respectively (21). These results were similar to those obtained in this study.

The residues of γ -HCH and Σ DDT in cow milk fat in the 1990s were 12.0 and 438.0 $\mu\text{g}/\text{kg}$ of fat, respectively (27). The mean content of γ -HCH in cow milk samples collected in 2010 in the same region was 9.15 $\mu\text{g}/\text{kg}$ of fat from values ranging from 2.92 to 22.75 $\mu\text{g}/\text{kg}$, and the mean content of Σ DDT was 18.92 $\mu\text{g}/\text{kg}$ from values ranging from 6.09 to 53.12 $\mu\text{g}/\text{kg}$ of fat (21). Nowadays, the mean content of γ -HCH and Σ DDT in cow milk fat is comparable to that from 2010, the fat containing 0.46 and 9.20 $\mu\text{g}/\text{kg}$, respectively.

The significant difference in chlorinated hydrocarbon content in cow and mare milk fat may be associated with different species-specific exposure to these compounds in the environment and in animal feed (11, 21). Significant differences in the constituent percentage of different fatty acids were found between cow and mare milk fat. The differences in composition of fatty acids in mare and cow milk are related, among other factors, to the animal species and the different anatomical structures of their gastrointestinal tracts, which necessitate different digestive mechanisms (29). Acids typical for cow milk include butyric acid, natural trans isomer CLA (C18:2*cis9trans11*), and vaccenic acid (C18:1*trans11*). CLA is produced in the rumen with the participation of bacterial enzymes in the hydrogenation of α -linoleic and linoleic acids (13). Other authors' studies also support similar trends in fatty acid compositions in cow and mare milk fat (26).

Correlations between the contents of γ -HCH, DDT, DDE, DDD, and Σ DDT on the one hand, and most fatty acids and their sums (Σ SFA, Σ MUFA, and Σ PUFA) on the other were found in analysed milk fat (particularly in relation to cow milk fat). The negative correlation which was determined between γ -HCH, DDE, DDT, and Σ DDT and the fatty acid constituents in cow milk fat can demonstrate the negative impact of these pollutants on the content of MUFA (C16:1, C17:1, and C18:1*c9-c13*), Σ MUFA, and C18:2*c9c12*. In mare milk fat, a strong negative correlation was found in the cases of the contents of γ -HCH and C13:0 ($r = -0.80$) and C14:0 ($r = -0.72$), the content of DDE and C16:0*iso* ($r = -0.72$) and C18:3 ($r = -0.77$), and the contents of Σ DDT and C18:3 ($r = -0.81$).

A negative correlation was found between the content of γ -HCH and contents of DDE and C18:2 linoleic acid (*cis9*, *cis12*) in cow milk fat, while in mare milk fat a strong positive correlation was found between DDE and Σ DDT and linoleic acid ($r = 0.70$ and $r = 0.73$, respectively). This study should be continued, due the fact that C18:1*c9c12* acid is metabolised in the body to a number of products important for the proper functioning of the metabolism. It is considered an essential component of the diet, because it is not synthesised by the body (30).

In mare milk fat, a strong negative correlation between the contents of DDE and Σ DDT and C18:3 acid ($r = -0.77$ and $r = -0.81$ respectively) was observed. As is well known, a balanced diet rich in MUFA and PUFA helps to prevent many ailments and illnesses. Wcisło and Rogowski (30) presented the beneficial effects of unsaturated fatty acids in the treatment of inflammatory conditions, allergies, and rheumatoid arthritis (30). According to the recommendations of the European Food Safety Authority (EFSA), at least 2 g of α -linolenic acid (C18:3) should be consumed daily.

Many research papers confirm that organochlorine compounds induce changes in fatty acid metabolism

(1, 6, 10, 12), which corresponds to the results of the presented study.

The residues of chlorinated hydrocarbons like DDT and γ -HCH in the natural environment are still a present problem. Although in Poland insecticide preparations containing DDT were withdrawn from production in 1976 and the use of lindane as an active substance of plant protection has been banned since 1988, their residues are still determined in food of animal origin. Each milk fat sample tested in the study, from both cows and mares, contained γ -HCH and DDT, which confirms their widespread occurrence. The amounts of γ -HCH and Σ DDT found in mare and cow milk fat (after calculating the results to mg/kg of milk) were below maximum residue levels (MRL), which are 0.04 mg/kg of milk for Σ DDT and 0.01 mg/kg of milk for γ -HCH. These MRLs are established in legislation as Commission Regulation (EC) No 149/2008 of 29 January 2008 for Σ DDT (3) and Commission Regulation (EU) 2017/ 978 of 9 June 2017 for γ -HCH (4). However, long-term, low-dose exposure is increasingly linked to deleterious effects on human health, such as immune suppression, hormone disruption, diminished intelligence, reproductive abnormalities, cancer, and influence on fatty acid metabolism.

In conclusion, the correlation between the content of selected organochlorine compounds and composition of fatty acids in mare and cow milk fat indicates the strong influence of these environmental pollutants on the nutritional value and health-promoting properties of milk fat. A higher number of significant correlations between organochlorine compounds and the proportion of fatty acids were noted in cow milk than in mare milk, which might be attributed to differences in the proportion of particular fatty acids in the analysed milk fat. Determining the extent to which chlorinated hydrocarbons can influence the proportions of individual fatty acids should be also continued to ascertain the content of these compounds in milk fat obtained from other animal species and breeds. The confirmation of the influence of chlorinated hydrocarbons on fatty acid composition indicates the need to continue research, also taking into account the content of these pollutants in feed.

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