

## Fatty acid profile in 4 types of fat depots in Polish Holstein-Friesian and Limousine × Polish Holstein-Friesian bulls

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**Abstract:** The aim of this study was to determine the fatty acid profile in 4 types of fat deposits in Polish Holstein-Friesian (PHF) and Limousine × Polish Holstein-Friesian (LM × PHF) bulls. The experimental materials included samples of intramuscular, intermuscular, subcutaneous, and internal fat collected from the carcasses of 28 PHF bulls and 26 LM × PHF bulls. Determined fatty acids (FAs) were divided into saturated (SFAs) and unsaturated (UFAs) FAs, including monounsaturated (MUFAs) and polyunsaturated (PUFAs) FAs. The UFA/SFA, MUFA/SFA, PUFA/SFA, and PUFA n-6/PUFA n-3 ratios were calculated. Internal fat was characterized by the highest SFA concentrations, and subcutaneous fat had the highest MUFA content. Intramuscular fat was marked by a high proportion of PUFA and the highest PUFA/SFA ratio. The subcutaneous fat of PHF bulls had the highest n-6/n-3 PUFA ratio.

**Key words:** Beef cattle, fat deposit, fatty acids, gas chromatography

### 1. Introduction

Consumers attach importance to the quality of food, including beef. The content, distribution, and composition of fat in beef carcasses is also an important topic for human nutrition (1). In Poland, beef production is based mainly on dairy cattle herds. Black-and-white cows are also increasingly crossbred with beef bulls to improve fattening performance, slaughter value, and meat quality. Breed affects the distribution of fat within the carcass (2), while fat quality is determined by the levels and proportions of fatty acids (FAs). The FA profile of beef is influenced by 3 main factors: the breed of animals, age at slaughter, and nutritional regime (3–8). According to Aldai et al. (9), the amount, location, and composition of fat in cattle are important because internal, intermuscular, and subcutaneous fat is less valuable than intramuscular fat, since intramuscular fat content affects the flavor and tenderness of beef. The taste and tenderness that fat adds to beef, however, cannot be considered in isolation from its health effects and related consumer concerns (10–12). Animal fats, including bovine fat, are believed to contain relatively high concentrations of nutritionally undesirable saturated FAs (SFAs) and high cholesterol

levels (13). However, only select SFAs have adverse effects on human health, including an increased risk of hypercholesterolemia, thrombogenesis (C14:0 and C16:0), thrombosis, and ischemic heart disease. The results of recent research show that animal fats also contain biologically active substances that deliver health benefits. In particular, n-3 polyunsaturated FAs (PUFAs) and conjugated linoleic acids (CLAs) may be beneficial to human health (10,14,15).

According to Callow (16), subcutaneous fat and muscles even with a similar anatomical location may have different FA profiles. The present study, therefore, was undertaken to determine the FA profile in different types of fat deposits in Polish Holstein-Friesian (PHF) and Limousine × Polish Holstein-Friesian (LM × PHF) bulls due their different slaughter value and meat quality.

### 2. Materials and methods

#### 2.1. Experimental material

The experimental materials included fat samples collected from the carcasses of 28 PHF black-and-white bulls and 26 crossbred beef bulls produced by mating PHF black-and-white cows with LM bulls (LM × PHF). Feeding was

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typical for northeast Poland: semiintensive fattening based on pasture (spring and summer) and on forage (autumn and winter). The average age of bulls was  $21.10 \pm 2.94$  months, and their average body weight at slaughter was  $645 \pm 9.8$  kg. Slaughter and postslaughter processing were carried out in accordance with the relevant meat industry regulations. After 96 h of carcass chilling, samples of 4 types of fat deposits were collected. Intramuscular fat samples were collected from the loin (*m. longissimus dorsi*, between the 11th and 13th thoracic vertebra). Samples of intermuscular and subcutaneous fat were collected from the leg, and internal fat samples from the kidney region. Vacuum-packaged samples were transported (4 °C) to the laboratory of the Department of Cattle Breeding and Milk Quality Evaluation, University of Warmia and Mazury, Olsztyn.

## 2.2. Fat extraction

Fat was extracted from ground meat samples by the Soxhlet method using the Büchi B-811 extraction system (17), with hexane as a solvent.

## 2.3. Fatty acid profile

FA methyl esters were obtained by dissolving the extracted fat in a methanol-chloroform- $H_2SO_4$  mixture, followed by methylation according to the modified Peisker method (18) and standard PN-EN ISO 5509:2001 (19). The percentage share of 31 FAs was determined by gas chromatography, using the Varian CP 3800 system with a split/splitless injector and a flame-ionization detector (20). Samples (1  $\mu$ L) of FA methyl esters were placed on a CP-Sil 88 capillary column (length: 100 m, inner diameter: 0.25 mm). Data were processed using the GALAXIE Chromatography Data System. FAs were identified by comparing their retention times with those of commercially available reference standards purchased from Supelco, Inc. Analyses of samples and reference standards were performed under identical conditions, i.e. carrier gas: helium, injector temperature: 260 °C, detector temperature: 260 °C, initial oven temperature: 110 °C raised to 249 °C. The FAs were divided into the following categories: SFAs, unsaturated FAs (UFAs) including monounsaturated FAs (MUFAs) and PUFAs, desirable hypocholesterolemic FAs (DFAs), and undesirable hypercholesterolemic FAs (OFAs). The following ratios were calculated: UFA/SFA, MUFA/SFA, PUFA/SFA and PUFA n-6/PUFA n-3.

## 2.4. Statistical analysis

The results were processed statistically using the STATISTICA data analysis software system, ver. 9.0 (21). One-way and two-way analysis of variance with interactions was performed. The significance of differences between mean values in groups was estimated by the least significant difference test.

## 3. Results

In this study, the differences in the levels of particular FAs between both breeds were insignificant (Table 1), but the concentrations of the majority of FAs tended to be higher in fat samples from LM  $\times$  PHF bulls in comparison with PHF bulls. Significant ( $P \leq 0.01$ ) differences were noted with respect to the levels of iso-C14:0, C15:0, iso-C16:0, C17:0, C18:3 n-3, and CLA. In the present study, oleic acid (C18:1 n-9) and palmitic acid (C16:0) were present in the largest amounts, and their proportion was higher in fat samples from PHF bulls (34.22% and 27.845%, respectively). Despite a lower oleic acid content, samples from crossbred bulls had higher concentrations of other functional FAs. The levels of  $\alpha$ -linolenic acid (C18:3 n-3) and CLA were significantly ( $P \leq 0.01$ ) higher in fat samples from LM  $\times$  PHF bulls in comparison with PHF bulls. The C18:3 n-3 content was significantly ( $P \leq 0.01$ ) higher in fat samples from crossbred beef bulls than in those from PHF bulls.

Intramuscular fat had the lowest content of myristic acid (C14:0) and palmitic acid (C16:0) (Table 1). The lowest CLA levels were also found in intramuscular fat, whose content is an important determinant of beef quality. Subcutaneous fat had a significantly higher CLA content (0.404%). Despite the lowest CLA content, intramuscular fat had significantly ( $P \leq 0.01$ ) higher concentrations of PUFAs than the other analyzed fats. This trend was particularly noticeable with regard to functional FAs, i.e. C20:4 n-6 (0.383%), C20:5 n-3 (0.076%), and C22:5 n-3 (0.193%). The average levels of those FAs in intramuscular fat were several-fold higher than in the remaining types of fat deposits. Similarly as in samples collected from different cattle breeds, in samples of different types of fat the predominant MUFA was oleic acid (C18:1 n-9), which had a significantly ( $P \leq 0.01$ ) higher share of the total FA pool in intramuscular fat and subcutaneous fat (37.841%) than in intermuscular fat (30.871%) and internal fat (39.432%). Subcutaneous fat was characterized by a several-fold higher content of C16:1 (6.40%) and a higher proportion of C17:1 (0.82%) in comparison with the other types of fat deposits.

Subcutaneous fat had the lowest SFA and PUFA content and the highest concentrations of UFAs and MUFAs (Table 2). Intramuscular fat had the highest content of essential PUFAs (4.29%) and a more desirable PUFA/SFA ratio (0.088) compared with the other types of adipose tissue.

## 4. Discussion

The profile of the analyzed FAs was determined by the type of adipose tissue to a greater extent than by bull genotype, which corroborates the findings of Smith et al. (22), who reported that cattle breed was the least important determinant of the FA composition of beef carcasses.

**Table 1.** The influence of genotype on FA profiles (% of total amount of EAs) in different adipose tissues; mean values denoted by different letters in rows within trait are significantly different at: a, b, P ≤ 0.05; A, B, P ≤ 0.01. Mean values are significantly different at: \*P ≤ 0.05; \*\*P ≤ 0.01.

Specification	Influence										
	Subcutaneous fat	Visceral fat	Intermuscular fat	Intramuscular fat	SEM	PHF bulls	LM × PHF bulls	SEM	Breed	Adipose tissue	Interaction breed × adipose tissue
C10:0	0.048 <sup>B</sup>	0.064 <sup>A</sup>	0.055 <sup>A</sup>	0.046 <sup>B</sup>	0.002	0.052	0.054	0.002	ns	**	ns
C12:0	0.075 <sup>b</sup>	0.081	0.089 <sup>Ab</sup>	0.072 <sup>B</sup>	0.002	0.074	0.081	0.002	ns	*	ns
iso-C14:0	0.090 <sup>B</sup>	0.137 <sup>A</sup>	0.136 <sup>A</sup>	0.078 <sup>B</sup>	0.003	0.099 <sup>B</sup>	0.114 <sup>A</sup>	0.003	**	**	ns
C14:0	3.306 <sup>A</sup>	3.199 <sup>Ab</sup>	3.411 <sup>Ab</sup>	2.692 <sup>B</sup>	0.041	3.108	3.140	0.041	ns	**	ns
C14:1	1.751 <sup>A</sup>	0.503 <sup>B</sup>	0.610 <sup>B</sup>	0.660 <sup>B</sup>	0.038	0.940	0.855	0.038	ns	**	ns
anti-iso-C15:0	0.291 <sup>B</sup>	0.460 <sup>A</sup>	0.451 <sup>A</sup>	0.253 <sup>B</sup>	0.009	0.319 <sup>B</sup>	0.378 <sup>A</sup>	0.009	**	**	ns
C15:0	0.585 <sup>B</sup>	0.823 <sup>A</sup>	0.834 <sup>A</sup>	0.519 <sup>B</sup>	0.017	0.616 <sup>B</sup>	0.714 <sup>A</sup>	0.017	**	**	ns
iso-C16:0	0.308 <sup>B</sup>	0.410 <sup>A</sup>	0.408 <sup>A</sup>	0.299 <sup>B</sup>	0.007	0.331 <sup>B</sup>	0.364 <sup>A</sup>	0.007	**	**	ns
C16:0	28.742 <sup>Ab</sup>	27.526 <sup>b</sup>	26.838 <sup>b</sup>	26.639 <sup>B</sup>	0.230	27.845	27.214	0.230	ns	**	ns
C16:1	6.400 <sup>A</sup>	1.922 <sup>C</sup>	2.169 <sup>C</sup>	3.282 <sup>B</sup>	0.126	3.568	3.441	0.126	ns	**	ns
C17:0	0.875 <sup>C</sup>	1.522 <sup>A</sup>	1.493 <sup>A</sup>	1.119 <sup>B</sup>	0.024	1.200	1.261	0.024	ns	**	ns
C17:1	0.820 <sup>A</sup>	0.527 <sup>C</sup>	0.564 <sup>C</sup>	0.729 <sup>B</sup>	0.022	0.631	0.683	0.022	*	**	ns
C18:0	12.174 <sup>C</sup>	25.384 <sup>A</sup>	23.904 <sup>A</sup>	17.584 <sup>B</sup>	0.403	19.187	19.734	0.403	ns	**	ns
C18:1 T6+9	0.520	0.568	0.544	0.419	0.022	0.551	0.486	0.022	ns	ns	ns
C18:1 T10+11	1.274 <sup>B</sup>	2.062 <sup>A</sup>	2.166 <sup>A</sup>	1.181 <sup>B</sup>	0.065	1.590	1.663	0.065	ns	**	ns
C18:1 n-9	37.091 <sup>A</sup>	29.432 <sup>Bb</sup>	30.871 <sup>Ba</sup>	37.841 <sup>A</sup>	0.337	34.220	33.941	0.337	ns	**	ns
C18:1 C11	1.498 <sup>a</sup>	0.827 <sup>b</sup>	0.886 <sup>b</sup>	1.260	0.024	1.155	1.117	0.024	ns	**	ns
C18:1 C12	0.158 <sup>C</sup>	0.215 <sup>A</sup>	0.199 <sup>AB</sup>	0.174 <sup>BC</sup>	0.004	0.174	0.192	0.004	ns	**	ns
C18:1 C13	0.391 <sup>A</sup>	0.144 <sup>B</sup>	0.163 <sup>B</sup>	0.272 <sup>C</sup>	0.008	0.264	0.237	0.008	ns	**	ns
C18:1 T16	0.280 <sup>B</sup>	0.453 <sup>A</sup>	0.444 <sup>A</sup>	0.293 <sup>B</sup>	0.008	0.343	0.373	0.007	ns	**	ns
C18:2 C9 T13	0.314 <sup>A</sup>	0.262 <sup>B</sup>	0.275 <sup>B</sup>	0.252 <sup>B</sup>	0.004	0.270	0.278	0.004	ns	**	ns
C18:2	1.613 <sup>C</sup>	2.024 <sup>B</sup>	2.023 <sup>B</sup>	2.491 <sup>A</sup>	0.033	1.976	2.092	0.032	ns	**	ns
C18:3 n-3	0.465 <sup>B</sup>	0.624 <sup>A</sup>	0.586 <sup>A</sup>	0.598 <sup>A</sup>	0.012	0.538	0.585	0.013	**	**	ns
C20:0	0.151 <sup>B</sup>	0.280 <sup>Ab</sup>	0.248 <sup>Ab</sup>	0.164 <sup>B</sup>	0.005	0.199	0.213	0.005	ns	**	ns
CLA	0.404 <sup>A</sup>	0.276 <sup>B</sup>	0.320 <sup>Ba</sup>	0.257 <sup>Bb</sup>	0.009	0.272 <sup>B</sup>	0.334 <sup>A</sup>	0.009	**	**	ns
C20:1	0.180 <sup>a</sup>	0.145 <sup>b</sup>	0.143 <sup>b</sup>	0.158	0.005	0.156	0.158	0.005	ns	**	ns
C20:2	0.025 <sup>B</sup>	0.028 <sup>b</sup>	0.028 <sup>b</sup>	0.040 <sup>Aa</sup>	0.002	0.027	0.033	0.002	ns	ns	ns
C20:4 n-6	0.043 <sup>B</sup>	0.034 <sup>B</sup>	0.037 <sup>B</sup>	0.383 <sup>A</sup>	0.012	0.118	0.144	0.012	ns	**	ns
C22:0	0.045 <sup>B</sup>	0.085 <sup>A</sup>	0.083 <sup>A</sup>	0.045 <sup>B</sup>	0.005	0.056	0.067	0.005	ns	**	ns
C20:5 EPA n-3	0.014 <sup>B</sup>	0.015 <sup>B</sup>	0.016 <sup>B</sup>	0.076 <sup>A</sup>	0.003	0.025	0.036	0.003	ns	**	ns
C22:5 DPA n-3	0.043 <sup>B</sup>	0.037 <sup>B</sup>	0.042 <sup>B</sup>	0.193 <sup>A</sup>	0.006	0.072	0.090	0.006	ns	**	ns

**Table 2.** The influence of genotype on FA groups and ratios in different adipose tissues; mean values denoted by different letters in rows within trait are significantly different at: a, b,  $P \leq 0.05$ ; A, B,  $P \leq 0.01$ . Mean values are significantly different at: \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ .

Specification	Subcutaneous fat	Visceral fat	Intermuscular fat	Intramuscular fat	SEM	PHF bulls	LM × PHF bulls	SEM	Influence		
									Breed	Adipose tissue	Interaction breed × adipose tissue
SFA	$\bar{x}$ 46.873 <sup>C</sup>	59.628 <sup>Aa</sup>	57.697 <sup>Ab</sup>	49.615 <sup>B</sup>	0.441	53.110	53.218	0.441	ns	**	ns
UFA	$\bar{x}$ 53.125 <sup>A</sup>	40.467 <sup>Cb</sup>	42.370 <sup>Ca</sup>	50.404 <sup>B</sup>	0.440	46.893	46.846	0.440	ns	**	ns
MUFA	$\bar{x}$ 50.363 <sup>A</sup>	36.799 <sup>Cb</sup>	38.758 <sup>Ca</sup>	46.269 <sup>B</sup>	0.444	43.594	43.147	0.444	ns	**	ns
PUFA	$\bar{x}$ 2.922 <sup>C</sup>	3.301 <sup>B</sup>	3.327 <sup>B</sup>	4.290 <sup>A</sup>	0.054	3.299 <sup>B</sup>	3.592 <sup>A</sup>	0.054	**	**	ns
ΣUFA/SFA	$\bar{x}$ 1.174 <sup>A</sup>	0.689 <sup>C</sup>	0.748 <sup>C</sup>	1.034 <sup>B</sup>	0.016	0.925	0.919	0.016	ns	**	ns
ΣPUFA/SFA	$\bar{x}$ 0.063 <sup>B</sup>	0.056 <sup>C</sup>	0.058 <sup>BC</sup>	0.088 <sup>A</sup>	0.001	0.063 <sup>b</sup>	0.069 <sup>a</sup>	0.001	*	**	ns
ΣMUFA/SFA	$\bar{x}$ 1.114 <sup>A</sup>	0.626 <sup>C</sup>	0.684 <sup>C</sup>	0.949 <sup>B</sup>	0.016	0.861	0.848	0.016	ns	**	ns
n-3	$\bar{x}$ 0.479 <sup>B</sup>	0.639 <sup>A</sup>	0.602 <sup>A</sup>	0.674 <sup>A</sup>	0.013	0.563 <sup>b</sup>	0.621 <sup>a</sup>	0.013	**	**	ns
n-6	$\bar{x}$ 1.104 <sup>B</sup>	1.375	1.305	1.471 <sup>A</sup>	0.049	1.318	1.319	0.049	ns	ns	**
n-6/n-3 PUFA ratio	$\bar{x}$ 2.527	2.415	2.538	2.416	0.112	2.528	2.439	0.112	**	ns	*

Garcia et al. (23) also, as in our research, observed higher concentrations of myristic acid in meat from crossbred beef steers than in meat from Holstein steers, while beef from Angus steers had the highest content of this FA. Yoshimura and Namikawa (24) compared Japanese Black and Holstein cattle and found that the beef breed had a higher proportion of C18:1 n-9 and lower proportions of the major SFAs C16:0 and C18:0 in adipose tissue compared with the dairy breed. However, in our experiment the concentrations of C18:1 n-9 and C18:0 were higher in fat samples from PHF bulls in comparison with crossbred beef bulls. In a study by Choi et al. (25), the adipose tissue of Holstein-Friesians had a higher proportion of C18:1 n-9 compared with Welsh Blacks. In the present experiment, C18:3 n-3 content was significantly ( $P \leq 0.01$ ) higher in fat samples from crossbred beef bulls than in those from PHF bulls. Choi et al. (25) also noted a higher proportion of C18:3 n-3 in the adipose tissue of Welsh Blacks as compared with Holstein-Friesians. As suggested by the cited authors, the deposition of C18:3 n-3 is more effective in beef breeds than in dairy breeds or higher amounts of this FA do not undergo biohydrogenation in the rumen of beef cattle.

In our results, intramuscular fat had the lowest content of myristic acid (C14:0) and palmitic acid (C16:0), which are hypercholesterolemic and thrombogenic FAs (23). Significantly higher CLA content (0.404%) was seen in subcutaneous fat, which is consistent with the findings of Aldai et al. (9), who compared 3 types of adipose tissue

(subcutaneous, intramuscular, and intermuscular) in yearling bulls and reported that CLA content was highest in the subcutaneous fat (0.42%), followed by intermuscular fat (0.37%) and intramuscular fat (0.22%). According to Kazala et al. (26) and Raes et al. (4), whose findings are cited in the work of Aldai et al. (9), the above could be due to the fact that CLA isomers are found mostly in the fraction of neutral lipids. Despite the lowest CLA content, intramuscular fat had significantly ( $P \leq 0.01$ ) higher concentrations of PUFAs than the other analyzed fats, both in our experiment and in the studies cited above.

In our study, the PUFA/SFA ratio was similar to that reported by Enser et al. (27) of 0.11 and lower than that noted by Aldai et al. (9) of 0.45. The values of the n-6/n-3 PUFA ratio ranged from 2.415 to 2.538. There are many different views among researchers regarding the “ideal” dietary balance between the 2 series of FAs. According to Bartnikowska and Kulasek (28), the optimal n-6/n-3 PUFA ratio in the human diet is 2–5 to 1, whereas according to Wijendran and Hayes (29) it should oscillate around 6:1. Based on the current nutritional recommendations of the UK Department of Health (30), the n-6/n-3 PUFA ratio should not exceed 4.0. The n-6/n-3 PUFA ratios determined in 4 types of fat deposits and in 2 cattle breeds in the present experiment were consistent with the values recommended by nutrition experts. The lowest n-6/n-3 PUFA ratio was noted in intramuscular fat and in crossbred bulls whose adipose tissue contained larger amounts of

n-3 and n-6 FAs. Choi et al. (25) also demonstrated that the n-6/n-3 ratio was more desirable in Welsh Blacks compared with Holstein-Friesians. The concentrations of PUFAs, including those of the n-3 series, as well as the PUFA/SFA and n-6/n-3 ratios were influenced by breed. The PUFA/SFA ratio in ruminant meat is unfavorably low because dietary UFAs are hydrogenated by rumen microorganisms (25). The recommended value of this ratio is higher than 0.45 (31). In the current study, the PUFA/SFA ratio was slightly higher in fat samples from crossbred beef bulls in comparison with dairy cattle, due to higher PUFA levels in the adipose tissue of the former. Choi et al. (25) also reported a higher PUFA/SFA ratio and higher concentrations of PUFAs in Welsh Blacks compared with Holstein-Friesians.

In conclusion, internal fat was characterized by the highest SFA concentrations and subcutaneous fat had the highest MUFA content. Intramuscular fat, whose content

was relatively low, was marked by a high proportion of PUFA and the highest PUFA/SFA ratio. The subcutaneous fat of PHF bulls had the highest n-6/n-3 PUFA ratio. The analyzed types of adipose tissue were found to have different FA profiles. Our knowledge about the differences in the FA profile of fat deposits, and the genetic factors that influence fat content and quality, may support efforts to regulate and modify the FA composition of beef carcasses.

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