The Fatty Acid Profile of Intramuscular Fat in the *Longissimus Lumborum* Muscle from The Bulls of Black-and-White Holstein-Friesian and Their Cross With the Belgian Blue Breed

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Abstract. *The aim of this study was to analyse and compare the fatty acid profle of intramuscular fat (IMF) in the Longissimus lumborum (LL) muscle from the bulls of Black-and-White Holstein-Friesian (HF) and their cross with the Belgian Blue (BB) breed (HF x BB). HF and HF x BB bulls were raised on the same farm, in a tie-stall barn, under identical conditions. The animals were fed farm-made feed. In autumn and winter, they received hay ad libitum, maize silage and ground cereal grain (approx. 2 kg). In summer, they were fed green forage ad libitum, ground cereal grain and hay. In comparison with HF bulls, the IMF in the LL muscle of crossbred HF x BB bulls was characterized by higher concentrations of polyunsaturated fatty acids (PUFAs) and a higher ratio of PUFAs to saturated fatty acids (SFAs) (PUFA/SFA), which indicates that the BB breed with muscular hypertrophy is suitable for commercial crossing. The IMF of crossbred bulls had a higher content of nutritionally important n-3 and n-6 PUFAs, and eicosapentaenoic acid (EPA). The IMF of HF bulls had a higher concentration of conjugated linoleic acid (CLA) with health-promoting properties, but its content in both groups of bulls was comparable with that determined in other cattle breeds.*

Introduction

High-quality beef can be produced by traditional beef cattle breeds and commercial crossbred cattle (Cuvelier et al., 2006). Commercial crossing with beef breeds contributes to progress in beef production in both quantitative and qualitative terms. A beef cattle breed ideally suited for crossing is difficult to find because each breed has its own advantages and disadvantages. One of such breeds is the Belgian Blue (BB), characterized by muscular hypertrophy known as double muscling. The recessive muscular hypertrophy (*mh*) allele that determines double muscling is a mutant form of the myostatin gene (MSTN) (Charlier et al., 1995).

Research shows that the meat of double-muscled cattle and their crosses has a lower percentage of fat (Cuvelier et al., 2006; Moreno et al., 2008), and a higher content of protein (Keady et al., 2013) and unsaturated fatty acids (UFAs) (Aldai et al., 2008; Wiener et al., 2009). In the opinion of consumers from many countries, visible fat in meat is unacceptable and has adverse health effects (Wood et al., 2008). However, fatty acids accumulated in subcutaneous adipose tissue, intermuscular and intramuscular fat (IMF) considerably influence the processing suitability, sensory attributes and nutritional value of meat (Webb and O'Neill, 2008). The concentrations of individual fatty acids in lipids and phospholipids are determined by animal species (Litwińczuk et al., 2012), feed (Scollan et al., 2006; Aldaiet al., 2010), carcass fat content (Wood et al., 2008), the animal's age (Warren et al., 2008), breed and genotype (Ekine-Dzivenuet al., 2014).

Black-and-White Holstein-Friesians are the most common and the highest-yielding dairy cattle breed in the world. A viable alternative could be crossbreeding between dairy and beef cattle breeds, contributing to the production of high-quality beef. Holstein-Friesians, in particular bulls, have been increasingly used for beef production in recent years. However, it is generally believed that the meat of HF cattle is characterized by lower eating quality than the meat of beef cattle and dairy-beef crosses. Therefore, the HF breed is used mostly for crossing with beef cattle in many countries.

The aim of this study was to analyse and compare the fatty acid profile of IMF in the *Longissimus lumborum* (LL) muscle from the bulls of Black-and-White Holstein-Friesian and their cross with the Belgian Blue breed (HF x BB).

Material and Methods. The experimental materials comprised 10 carcasses of Black-and-White Holstein-Friesian bulls and 10 carcasses of F_1 bulls produced by commercial crossing of HF and Belgian Blue breed (HF x BB). HF x BB bulls were the offspring of double-muscled sires. HF and HF x BB bulls were raised on the same farm, in a tie-stall barn, under identical conditions. The animals were fed farm-made feed. In autumn and winter, they received hay *ad libitum*, maize silage and ground cereal grain (approx. 2 kg). In summer, they were fed green forage *ad libitum*, ground cereal grain and hay.

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The bulls were slaughtered in September, at 21 months of age, at a meat processing plant located at a distance of around 90 km from the farm. They were tied during transport. The bulls were weighed on arrival at the meat processing plant. The average body weight of HF and HF x BB was 650 ± 30 kg and 750 ± 33 kg, respectively. Before slaughter, the animals stayed in lairage, in individual pens with free access to water, for around 20 h. They were stunned with the Radical stunning device. The slaughtering and post-slaughter handling were carried out in accordance with the current meat industry regulations (Council Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing). After slaughter and post-slaughter processing, the carcasses were weighed and classified in the EUROP system. The pH of the LL muscle was measured approximately 45 min post mortem, between the 1st and 2nd lumbar vertebrae. Weighed carcasses were chilled at a temperature of 1–4°C for 72 h. After chilling, the pH of the LL muscle was measured between the 1st and 2nd lumbar vertebrae. The carcasses were divided into primal cuts. Segments of the LL muscle were collected from the right half-carcasses between the 1st and 2nd lumbar vertebrae. The samples were vacuum-packaged and transported in isothermal containers to the laboratory.

Intramuscular fat was extracted by Soxhlet extraction with diethyl ether as the solvent in the Soxtec™ Avanti 2050 Auto Fat Extraction System (FOSS Analytical, Hilleroed, Denmark) (AOAC 2010). Fatty acid methyl esters were obtained by dissolving the extracted fat in a methanol-chloroform-H_2SO_4 mixture (100:100:1 v/v), followed by methylation according to the Peisker method (Żegarska et al., 1991). Fatty acids were identified by comparing their retention times with those of commercially available reference standards purchased from Supelco, Inc. The percentage share of fatty acids was determined by gas chromatography, using the VARIAN CP-3800 system with a split/splitless injector and a flame-ionization detector (FID). Samples (1 µL) of fatty acid methyl esters were placed on a CP-Sil88 capillary column (length: 50 m, inner diameter: 0.25 mm). Analyses of samples and reference standards were performed under identical conditions, i.e., carrier gas – helium, carrier gas flow rate 1.2 mL/min, injector temperature 225°C, detector temperature 250°C, column temperature 200°C. The fatty acids were divided into the following categories: saturated fatty acids (SFAs), unsaturated fatty acids (UFAs), including monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), desirable hypocholesterolemic fatty acids (DFAs) (UFAs + C18:0) and undesirable hypercholesterolemic fatty acids (OFAs) (SFAs - C18:0). The following ratios were calculated: DFA/OFA, UFA/SFA, MUFA/SFA, PUFA/SFA, n-6/n-3 PUFA.

The results were processed statistically using STATISTICA software ver. 13.3 (StatSoft, Inc., 2017). Arithmetic means ($\bar{x}$) and standard deviations (s) for all analysed parameters are presented in the Tables. The mean values were compared by the Student's t-test for independent variables. The significance of differences between means was reported at $P \leqslant 0.01$ and $P \leqslant 0.05$.

Results and Discussion

Table 1 presents hot carcass weight, the percentage content of IMF in the LL muscle and the proportions of individual SFAs in the IMF of HF and HF x BB bulls. In the present study, the average hot carcass weight of HF bulls (350 ± 13 kg) was significantly low-

Table 1. The hot carcass weight (kg), the intramuscular fat (IMF) content (%) of the *Longissimus lumborum* muscle and the concentrations of saturated fatty acids (SFAs) in IMF (% of total fatty acids) (means ± SD)

Parameter	HF bulls	HF x BB bulls	Significance
Hot carcass weight	350 ± 13	438 ± 28	**
IMF content of the LL muscle	2.72 ± 1.12	2.01 ± 0.98	NS
C10:0	0.059 ± 0.003	0.057 ± 0.008	NS
C12:0	0.064 ± 0.005	0.085 ± 0.025	*
C14:0	2.968 ± 0.270	2.979 ± 0.216	NS
C15:0	0.399 ± 0.023	0.459 ± 0.115	NS
C16:0	31.102 ± 0.779	31.779 ± 1.229	NS
C17:0	1.066 ± 0.050	1.097 ± 0.139	NS
C18:0	18.374 ± 0.987	16.950 ± 2.195	NS
C20:0	0.141 ± 0.007	0.148 ± 0.039	NS
C22:0	0.073 ± 0.014	0.141 ± 0.034	**
Saturated fatty acids (SFAs)	54.245 ± 0.926	53.695 ± 2.079	NS

NS: non-significant differences ($P > 0.05$).
*Mean values in rows differ at $P \leqslant 0.05$. **Mean values in rows differ at $P \leqslant 0.01$.

er ($P \leqslant 0.01$) than that of HF x BB bulls (438 ± 28 kg). No significant ($P > 0.05$) differences in the IMF content of the LL muscle were found between the groups.

Both carcass fat content and the ratio of subcutaneous adipose tissue to IMF and intermuscular fat affect meat quality because the two types of adipose tissue differ in the proportions of lipids and phospholipids, and in the concentrations of individual fatty acids (Wood et al., 2008). The percentage of fatty acids in adipose tissue, regardless of its location, is determined by total body fat percentage (fatness). The percentage of adipose tissue in the carcass increases with age, leading to changes in the proportions of fatty acids. The changes in fatty acid composition are also associated with an increase in carcass lean content in beef cattle (Raes et al., 2004). Dairy cattle have a higher content of IMF and intermuscular fat than beef cattle such as BB since the latter deposit fat mostly within subcutaneous adipose tissue (Wood et al., 2008). According to the literature, the meat of double-muscled cattle (Wiener et al., 2009) and their crosses has considerably lower fat content (Keane and Moloney, 2009; Keane, 2010a, 2010b). In a study by Gotoh et al. (2009), the IMF content of the *Longissimus dorsi* (LD) muscle was only 0.6% in BB cattle, compared with 4.7% in HF cattle.

In the present study, the concentrations of lauric acid (C12:0) ($P \leqslant 0.05$) and behenic acid (C22:0) ($P \leqslant 0.01$) in the LL muscle were significantly higher in HF x BB crosses than in HF bulls. The content of the remaining SFAs was similar ($P > 0.05$) in both groups. Moreover, an analysis of the fatty acid profile of IMF in the LL muscle revealed ($P > 0.05$) similar percentages of SFAs in both groups of bulls.

In a study by Schiavon et al., (2011), who analysed the *Longissimus thoracis* (LT) muscle of double-muscled Piemontese bulls, the content of C14:0 fatty acid was similar, the content of C16:0 fatty acid was lower, and the content of C18:0 fatty acid was higher than the respective values determined in both groups in our study. Warren et al. (2008) compared the concentrations of SFAs in the *Longissimus* muscle of Aberdeen Angus and HF cattle and found that the content of C14:0, C16:0 and C18:0 fatty acids in IMF was significantly higher in Aberdeen Angus bulls (slaughtered at 24 months of age) than in HF bulls. Sobczuk-Szul et al. (2014) reported no significant differences in the content of C12:0 and C22:0 fatty acids in the LT muscle of HF bulls and HF x Limousin crosses. In the cited study, the concentrations of C14:0, C15:0 and C16:0 fatty acids in IMF were higher in HF x Limousin crosses than in HF bulls. Brugiapaglia et al. (2014) analysed the concentrations of SFAs in the LT muscle of Piemontese, Friesian and Limousin bulls, and found significant ($P \leqslant 0.05$) differences in the content of C15:0 and C16:0 fatty acids. The effect of breed on the levels of SFAs in the LD muscle of Simmental, Hereford and Charolais cattle was also observed by Ugarković et al. (2013) who reported significant differences in the content of C14:0, C15:0, C16:0 and C17:0 fatty acids among the analysed groups. Horcada et al. (2016) compared the proportions of SFAs in IMF in the LD muscle of young Charolais, Limousin and Retinta cattle and found no significant differences in the content of C12:0 and C14:0 fatty acids, whereas the concentrations of C16:0, C22:0 and C18:0 fatty acids were influenced by breed.

Table 2 presents the percentages of individual UFAs in IMF in the LL muscle of HF and HF x BB

Table 2. The concentrations of unsaturated fatty acids (UFAs) in intramuscular fat (IMF) (% of total fatty acids) in the *Longissimus lumborum* muscle (means ± SD)

Fatty acids	HF bulls	HF x BB bulls	Significance
C14:1	0.586 ± 0.087	0.555 ± 0.169	NS
C16:1	3.459 ± 0.313	4.061 ± 0.919	NS
C17:1	0.884 ± 0.042	1.011 ± 0.124	**
C18:1 cis-9	38.155 ± 0.584	36.559 ± 2.005	**
C20:1	0.078 ± 0.001	0.065 ± 0.009	**
C18:2 n-6	1.881 ± 0.218	3.139 ± 1.228	**
CLA cis-9, trans-11	0.192 ± 0.009	0.159 ± 0.021	**
C18:3 n-3	0.375 ± 0.044	0.429 ± 0.099	NS
C20:2 n-6	0.028 ± 0.004	0.031 ± 0.019	NS
C20:4 n-6	0.065 ± 0.012	0.172 ± 0.107	**
C20:5 n-3 (EPA)	0.054 ± 0.026	0.149 ± 0.102	*
Unsaturated fatty acids (UFAs)	45.755 ± 0.926	46.331 ± 2.085	NS
Monounsaturated fatty acids (MUFAs)	43.160 ± 0.729	42.251 ± 2.788	NS
Polyunsaturated fatty acids (PUFAs)	2.595 ± 0.262	4.079 ± 1.402	**

NS: non-significant differences ($P > 0.05$).
*Mean values in rows differ at $P \leqslant 0.05$.**Mean values in rows differ at $P \leqslant 0.01$.

bulls. IMF in the LL muscle of HF x BB crosses contained significantly ($P \leqslant 0.01$) higher concentrations of margaroleic acid (C17:1), linoleic acid (C18:2 n-6), arachidonic acid (C20:4 n-6) and EPA (C20:5 n-3) ($P \leqslant 0.05$), whereas IMF in the LL muscle of HF bulls had a higher ($P \leqslant 0.01$) content of oleic acid (C18:1 cis-9), gadoleic acid (C20:1) and CLA (C18:2 cis-9, trans-11). In the present study, an analysis of the fatty acid profile of IMF in the LL muscle revealed ($P > 0.05$) similar percentages of UFAs and MUFAs in both groups of bulls, whereas the proportion of PUFAs was significantly ($P \leqslant 0.01$) higher in HF x BB crosses than in HF bulls.

In a study by Litwińczuk et al. (2012), the CLA content of the LL muscle was 0.09% in HF bulls and 0.32% in HF crosses sired by Limousin bulls, and these values are lower and higher, respectively, than those noted in the present experiment. The concentrations of C14:1, C16:1 and C17:1 fatty acids in IMF in the LT muscle were lower in double-muscled Piemontese bulls analysed by Schiavon et al. (2011) than in HF x BB bulls evaluated in this study. Schiavon et al. (2011) reported a higher content of C18:2 fatty acid, and a similar content of CLA, compared with the values noted in HF x BB crosses in the current experiment. In a study by Aldai et al. (2010), the content of CLA (C18:2 cis-9, trans-11) in the LD muscle of double-muscled Asturiana cattle was 0.097 mg/100 g of meat. Heterozygous animals had a higher percentage of CLA in IMF (0.178 mg/100 g of meat), whereas CLA content in cattle with normal muscling was comparable (0.125 mg/100 g of meat) with that noted in double-muscled cattle. Wood et al. (2008) observed a greater increase in carcass fat content with age in beef cattle (Aberdeen Angus) than in dairy cattle (Jersey), accompanied by an increase in the percentage of CLA. Sobczuk-Szul et al. (2014) found no significant differences in the concentrations of C18:2, C22:2 and EPA in IMF in the LD muscle of HF and LM x HF bulls, whereas CLA content was significantly higher in crossbred bulls. Warren et al. (2008) analysed the concentrations of fatty acids in the *Longissimus* muscle of Aberdeen Angus and HF cattle and found that breed had no significant effect on the content of C18:2 n-6 fatty acid. Brugiapaglia et al. (2014) reported a higher percentage of CLA in IMF in the LT muscle of Friesian bulls, compared with Piemontese and Limousin bulls, and no significant differences in EPA content.

The higher content of PUFAs contributed to a more desirable PUFA/SFA ratio ($P \leqslant 0.01$) in the meat of crossbred (HF x BB) bulls (Table 3). In comparison with HF bulls, HF x BB crosses had also significantly higher concentrations of n-3 ($P \leqslant 0.05$) and n-6 ($P \leqslant 0.01$) PUFAs, and a higher ($P \leqslant 0.01$) n-6/n-3 PUFA ratio. No significant ($P > 0.05$) differences in the concentrations of desirable hypocholesterolemic fatty acids (DFAs) and undesirable hypercholesterolemic fatty acids (OFAs) or the DFA/OFA, UFA/SFA and MUFA/SFA ratios were observed between the groups.

Cattle with muscular hypertrophy and low IMF content have higher levels of PUFAs (Aldaiet al., 2008; Wiener et al., 2009; Fiems, 2012), which was also observed in this study in HF x BB bulls. As a result, the PUFA/SFA ratio in double-muscled cattle is also high, ranging from 0.5 to 0.7 (Cuvelier et al., 2006; Scollan et al., 2006; Wiener et al., 2009), compared with approximately 0.25 in animals with normal muscling (Kołczak, 2008).

In our study, the percentage of SFAs was lower and the percentage of UFAs was higher in the LL muscle of HF x BB bulls than in the LT muscle of double-muscled Piemontese bulls evaluated by Schiavon et al. (2011), and in the LT muscle of double-muscled BB bulls analysed by de Smet et al. (2000). In a study by Sobczuk-Szul et al., (2014), the proportions of SFAs (53.110%) and UFAs (46.846%) in

Table 3. The fatty acids groups and ratios in the *Longissimus lumborum* muscle (means ± SD)

Parameter	HF bulls	HF x BB bulls	Significance
Hypocholesterolemic fatty acids DFAs (UFAs + C18:0)	64.129 ± 0.988	63.281 ± 2.099	NS
Hypercholesterolemic fatty acids OFAs (SFAs – C:18:0)	35.871 ± 0.977	36.745 ± 2.455	NS
DFA / OFA ratio	1.788 ± 0.985	1.722 ± 239	NS
UFA / SFA ratio	0.844 ± 0.032	0.863 ± 0.096	NS
MUFA / SFA ratio	0.796 ± 0.027	0.787 ± 0.079	NS
PUFA / SFA ratio	0.048 ± 0.006	0.076 ± 0.019	**
n-3	0.429 ± 0.544	0.578 ± 0.187	*
n-6	1.974 ± 0.845	3.342 ± 1.119	**
n-6 / n-3 PUFA ratio	4.601 ± 0.853	5.782 ± 0.598	**

NS: non-significant differences ($P > 0.05$).
*Mean values in rows differ at $P \leqslant 0.05$.
**Mean values in rows differ at $P \leqslant 0.01$.

the LD muscle of HF bulls were very similar to those noted in the present experiment. In comparison with our findings, Litwińczuk et al. (2012) noted a lower percentage of SFAs (52.24%) and PUFAs (2.62%), and a higher percentage of UFAs (47.75%) and MUFAs (45.135%) in the LT muscle of HF bulls. The cited authors also demonstrated that cattle breed exerted a significant effect on the content of CLA and PUFAs, and the PUFA/SFA ratio in IMF. Aldai et al. (2008) compared the concentrations of n-6 and n-3 PUFAs in the LT muscle of Asturiana bulls with and without the double-muscling phenotype and found that the n-6/n-3 PUFA ratio was significantly higher in double-muscled bulls than in animals with normal muscling. In a study by Sobczuk-Szul et al. (2013), who investigated the fatty acid profile of IMF in the LD muscle of crossbred HF x Limousin bulls, the percentage of DFAs was higher (67.89%) and the percentage of OFAs was lower (32.13%) than the values determined in the current experiment in both groups of bulls.

In the present study, the PUFA/SFA ratio in the LL muscle of HF x BB bulls was similar to that noted by Moreno et al. (2008) in the LD muscle of the same crosses. In our study, the PUFA/SFA ratio was higher and the UFA/SFA and MUFA/SFA ratios were lower than those reported by Sobczuk-Szul et al. (2014) in IMF in the LD muscle of HF bulls and HF x Limousin crosses. Cuvelier et al. (2006) noted a higher PUFA/SFA ratio in IMF in the LT muscle of BB bulls (0.80), compared with Limousin (0.29) and Aberdeen Angus (0.21) bulls. De Smet et al. (2000) demonstrated that a desirable PUFA/SFA ratio and a desirable fatty acid profile of beef were related to a low content of fat in the carcass and in individual muscles. According to the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), the optimal PUFA/SFA ratio in the human diet is 0.45, and the recommended n-6/n-3 PUFA ratio is 5:1 (Kołczak, 2008). Wijendran and Hayes (2004) estimated the optimal n-6/n-3 PUFA ratio in the diet of healthy adults at around 6:1. The present findings confirm that beef has a highly desirable n-6/n-3 PUFA ratio.

It appears that the observed differences in the fatty acid composition of beef may be due to the progress in analytical methods, effective detection and reliable quantitative analysis of fatty acids, in particular long-chain PUFAs. The different proportions of fatty acids in IMF, reported by various authors, result from the fact that they can be modified by many factors such as animal species, breed, age and diet as well as muscle type. The results of studies conducted by Cuvelier et al. (2006), Wiener et al. (2009) and Aldai et al. (2010) as well as the present findings suggest that double muscling is yet another factor influencing the fatty acid profile of beef.

Conclusions

It can be concluded that, in comparison with HF bulls, the IMF in the LL muscle of crossbred HF x BB bulls was characterized by higher concentrations of PUFAs and a higher PUFA/SFA ratio, which indicates that the BB breed with muscular hypertrophy is suitable for commercial crossing. The IMF of crossbred bulls had a higher content of nutritionally important n-3 and n-6 PUFAs, and EPA. The IMF of HF bulls had a higher concentration of CLA with health-promoting properties, but its content in both groups of bulls was comparable with that determined in other cattle breeds. The use of the BB breed with muscular hypertrophy for commercial crossing may affect the fatty acid profile of IMF in the LL muscle.

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