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FATTY ACID PROFILE OF INTRAMUSCULAR FAT IN THE *LONGISSIMUS LUMBORUM* AND *SEMIMEMBRANOSUS* MUSCLES OF BULLS FED DIETS BASED ON VIRGINIA FANPETALS, GRASS AND MAIZE SILAGES*

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Abstract

The aim of this study was to determine the effect of Virginia fanpetals (*Sida hermaphrodita*) silage on the fatty acid profile and the content of selected nutrients and vitamins in the *Longissimus lumborum* (LL) and *Semimembranosus* (SM) muscles of young bulls. Forty Polish Holstein-Friesian bulls aged 16 months were assigned to four dietary treatments (n=10) and were fed different types of silage during a 7-month fattening period. The proportion (g/kg dry matter) of silage in the diets was as follows: (1) grass silage (GS) (600); (2) Virginia fanpetals silage (VFS) (600); (3) VFS (300) and GS (300); and (4) VFS (300) and maize silage (MS) (300). Silage was supplemented with concentrate at 400 g/kg DM in each diet. The animals were slaughtered at the end of the fattening period. The intramuscular fat (IMF) of bulls fed GS had the highest (P<0.05) concentrations of n-3 polyunsaturated fatty acids (PUFAs) and linolenic acid (LNA), whereas the IMF of bulls receiving GS and VFS was characterized by the highest proportion of MUFAs, mostly oleic acid (C18:1 cis 9). In comparison with the LL muscle, the SM muscle contained less IMF (by 40%) with a more nutritionally desirable profile. The SM muscle was characterized by a more desirable mineral composition and a higher concentration of α-tocopherol.

Key words: beef, diet composition, muscle, fatty acid composition, α-tocopherol

Meat is an important component of the human diet as a source of nutrients, vitamins, minerals and essential fatty acids (Pereira and Vicente, 2013). These bio-

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logically active substances are known to have antineoplastic, antioxidant, immune-boosting and antibacterial properties (Decker et al., 2000). Beef and other ruminant products are important dietary sources of conjugated linoleic acid (CLA) and its major isomer cis-9, trans-11 with a range of health-promoting properties (Salter, 2013). The beneficial effects of long-chain n-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), in reducing the risk of cardiovascular disease, cancer and type-2 diabetes, and their critical role in proper brain function, visual development in the fetus and maintenance of neural and visual tissues throughout life have been well recognized (Barceló-Coblijn and Murphy, 2009; Lopez-Huertas, 2010; Scollan et al., 2014). Fatty acids are consumed mostly as the ingredients of intramuscular fat (IMF). The content and composition of IMF in muscles affect the flavor, juiciness and tenderness of meat (Webb, 2006), which are the most important attributes influencing consumer acceptance and purchasing decisions. However, there is a growing concern not only about excessive consumption of fats (in particular animal fats) but also about their composition and impact on human health (Scollan et al., 2014). The fatty acid profile of IMF is particularly important for human health because IMF is not extracted or removed before meat is consumed (De Smet et al., 2004). High dietary intake of saturated fatty acids (SFAs) has been associated with elevated serum levels of total and low-density lipoprotein (LDL) cholesterol, which is the risk factor for cardiovascular disease (Siri-Tarino et al., 2010). For this reason, animal products are criticized on account of their high SFA content, and they are labeled as harmful to health (Wood et al., 2008).

Beef is an excellent source of protein and dietary iron, zinc and magnesium, and it should not be viewed only from the fat content perspective (Cabrera et al., 2010). Calcium, magnesium and phosphorus play a vital role in many bodily functions, in particular bone mineralization. In addition, they are cofactors of many enzyme systems, sustaining muscle and nerve excitation. Vitamins A and E are important natural dietary antioxidants (Arnold et al., 1993; Scollan et al., 2014). Retinol is an essential dietary substance, which is required for rhodopsin formation and vision. At the same time, higher doses of vitamin A have been shown to be teratogenic, and accordingly, the World Health Organization has recommended that the daily intake for pregnant women should not exceed 3.3 mg/day (WHO, 2011). Vitamin E is an essential nutrient which stabilizes PUFAs, and a key determinant of meat quality, in particular in ruminants (Wood et al., 2008).

The fatty acid profile of beef is affected by cattle breed and the type of fat that is deposited within muscles (Wielgosz et al., 2017). The composition of the fatty acids (FA) of animal origin can be influenced by diet (forage and grain), by the digestive system, and by the biosynthetic processes of the animal (Faucitano et al., 2008; Buccioni et al., 2012; Ponnampalam et al., 2018). In ruminants, the fatty acid profile of meat is not directly related to the fatty acid composition of feed (Scollan et al., 2014). However, diets rich in unsaturated fatty acids (UFAs) improve (beneficial for consumers) the PUFA:MUFA (monounsaturated fatty acids) ratio in meat (Oprządek and Oprządek, 2003; Dannenberger et al., 2004). Less intensive fattening systems (less concentrated feed) contribute to a more desirable fatty acid profile of meat (Nogalski et al., 2014). The concentrations of nutritionally important n-3 PUFAs are

higher in the muscles of cattle fed fresh grass than in animals fed conserved forage (Scollan et al., 2006; Ponnampalam et al., 2018). Different types of silage, in particular combinations of several roughage sources, can have a beneficial influence on the proportion of health-promoting fatty acids in the muscles of cattle (Lee et al., 2009; Moloney et al., 2013). Different feeding strategies are applied to increase the content of n-3 PUFAs and to improve the n-6/n-3 PUFA ratio in beef IMF.

New types of feed are also being introduced to increase the fattening performance of cattle and improve the quality of beef. Virginia fanpetals (*Sida hermaphrodita*) is a little-known plant species with forage potential. Botanically, the genus Sida belongs to the family *Malvaceae*. Virginia fanpetals is a polycarpic perennial herb characterized by low soil requirements, which can produce 10–20 t/ha of dry matter annually (Borkowska et al., 2009; Titei, 2015). Virginia fanpetals leaves, which contain mucus-like substances and flavonoids, can be used for pharmaceutical purposes (Borkowska and Styk, 2006). The potential use of Virginia fanpetals as a fodder crop remains insufficiently investigated. The chemical composition of Virginial fanpetals, in particular its protein content, is comparable with that of alfalfa (Tarkowski, 2008). The plant could also be suitable for forage production due to its high yield potential, resistance to lodging and freezing, low soil nutrient requirements, and high drought tolerance (Franzaring et al., 2014).

The present experiment is part of a study investigating the effects of Virginia fanpetals silage (VFS) on animal performance, carcass characteristics and beef quality in growing bulls. The results regarding feed quality, animal performance, carcass characteristics and meat quality have been reported in detail by Nogalski et al. (2020). They found that VFS combined with maize silage improved carcass conformation and fat cover, whereas VFS combined with grass silage improved the sensory properties of beef. The present study focuses on the fatty acid profile and the content of selected nutrients and vitamins in meat from young bulls. It was assumed that VFS fed to finishing bulls would contribute to increasing the content of bioactive compounds in beef, thus delivering health benefits to consumers. Therefore, the aim of this study was to determine the effect of silage, in particular VFS, on the fatty acid profile and the content of selected nutrients and vitamins in meat from young bulls.

Material and methods

Animals and treatments

The experimental material comprised 40 Polish Holstein-Friesian bulls reared at the Agricultural Experiment Station in Bałcyny. The bulls were the offspring of 40 cows and 40 sires. The housing and management conditions of experimental animals have been described in detail in a previous study (Nogalski et al., 2020). The protocol for animal research was approved by the Ethics Committee of the University of Warmia and Mazury (Decision No. 121/2010). Before the experiment, young bulls were raised in a conventional system, and were fed milk replacer, hay and concentrate. Starting from 5 months of age, the animals were fattened semi-intensively, and

were fed *ad libitum* a total mixed ration (TMR) composed of GS (the same GS was used during the feeding trial) supplemented with 2 kg of concentrate (Nogalski et al., 2020). At 16 months of age, when bulls reached a body weight (BW) of around 450 kg, they were assigned to four groups by the analogue method (the animals were at a similar age, and they were divided into groups based on BW) of ten individuals each, and were placed in a monitored fattening facility, in four separate pens on deep bedding. Pen size was 20 × 13 m. The animals had enough space to move freely around, and they had free access to water and salt licks. During a 7-month fattening period, the animals were fed different types of silage (maize silage – MS, grass silage – GS and Virginia fanpetals silage – VFS) supplemented with concentrate (triticale grain, rapeseed meal and premix) (Table 1). The dietary treatments were as follows (Table 2): group 1 (control) – basal diet of GS, group 2 – basal diet of VFS, group 3 – basal diet of VFS+GS (1:1), group 4 – basal diet of VFS+MS (1:1). The animals were fed *ad libitum* TMR composed of silage and concentrate, which was dosed from a self-propelled feed cart. The concentrate to silage ratio, on a DM basis, was 40:60 and the amounts of triticale and rapeseed meal were dosed to obtain an isonitrogenous diet (Table 2). The concentrate contained also 3% of mineral-vitamin premix (Table 2). Virginia fanpetals silage was made from first-harvest biomass cut in the bud formation stage. GS *(Lolium multiflorum)* was made from first-harvest herbage cut in the heading stage and wilted for 24 hours, and MS was made from herbage cut in the dough stage (Table 1). The animals were weighed once a week. Fresh TMR was offered every day, after the leftovers had been removed. The feed intake of each animal was monitored individually using the Roughage Intake Control System (Insentec BV, Marknesse, The Netherlands). All feed samples, collected once a week, were analyzed for the content of basic nutrients – with standard methods (AOAC, 2005), as described in detail in the study by Nogalski et al. (2020).

Table 1. Chemical and fatty acid composition of experimental diets (mean ± standard error)

Specification	MS	GS	VFS	Triticale	Rapeseed meal
1	2	3	4	5	6
Chemical composition					
DM (g/kg fresh)	324±0.96	271±0.95	198±0.89	875±1.12	878±1.05
Organic matter	964±2.12	905±2.31	901±1.56	956±1.25	921±1.58
Crude protein	88.5±1.32	132±1.39	174±1.62	122±1.21	386±1.32
NDF	336±5.32	540±4.32	429±5.12	172±1.36	298±1.98
ADF	194±1.32	317±1.69	315±2.03	42±0.68	212±0.84
ADL	12.4±0.65	26.7±0.62	31.5±0.85	–	–
NFC	503±3.25	199±3.29	278±4.12	634±4.31	237±4.36
pH	3.54±0.09	4.21±0.12	4.57±0.17		
Lactic acid	27.8±7.32	43.8±11.3	65.8±9.25		
Acetic acid	6.3±1.32	12.6±2.62	20.3±2.98		
Butyric acid	0.08±0.03	0.09±0.03	4.4±0.56		
$N\text{-}NH_3$ (g kg^{-1} TN)	33.6±11.4	75.6±14.3	105±15.2		

Table 1 – contd.

1	2	3	4	5	6
Fatty acid profile (g/100 g fatty acids)					
C14:0	0.15±0.01	1.82±0.06	0.87±0.03		
C16:0	13.81±0.21	22.79±0.28	25.91±0.30		
C18:0	1.96±0.09	2.99±0.08	2.29±0.09		
C18:1 n-9 (OA)	23.67±0.07	5.7±0.08	5.8±0.04		
C18:2 n-6 (LA)	51.55±0.12	23.79±0.14	21.89±0.09		
C18:3 n-3 (LNA)	7.76±0.08	38.55±0.14	39.17±0.13		

MS – maize silage; GS – grass silage; VFS – Virginia fanpetals silage; DM – dry matter; NDF – neutral detergent fiber; ADF – acid detergent fiber; ADL – acid detergent lignin; NFC – non-fiber carbohydrate; N-NH_3 – ammonia nitrogen; TN – total nitrogen; OA – oleic acid; LA – linoleic acid; LNA – linolenic acid.

Table 2. Ingredient (% of DM) and chemical composition of experimental diets (mean ± standard error)

Specification	GS	VFS	VFS+GS	VFS+MS
Grass silage	60	–	30	–
Sida silage	–	60	30	30
Maize silage	–	–	–	30
Triticale grain	28	37	33	28
Rapeseed meal	9	–	4	9
Premix[1]	3	3	3	3
DM (g/kg fresh)	344.7±0.98	287.4±0.88	324.8±1.02	345.3±1.08
In g/kg DM				
organic matter	893.6±2.15	894.3±2.32	894.1±2.14	910.1±2.64
crude protein	148.3±1.21	149.5±1.54	147.6±1.36	148.0±1.24
NDF[2]	398.9±4.63	321.0±4.87	359.4±5.23	304.4±5.78
ADF[3]	221.0±1.39	204.5±1.54	211.9±1.69	183.5±1.89
NFC[4]	318.2±3.28	401.4±3.41	361.8±3.58	433.3±3.11

DM – dry matter; MS – maize silage; GS – grass silage; VFS – Virginia fanpetals silage.

[1]Commercial mineral-vitamin premix for fattening cattle (code of product 7619; Cargill Poland Ltd., Warsaw, Poland) consisting of per kg: Ca, 235 g; Na, 79 g; P, 48 g; Mg, 28 g; Fe, 500 g; Mn, 2000 mg; Cu, 375 mg; Zn, 3750 mg; I, 50 mg; Co, 12.5 mg; Se, 12.50 mg; vitamin A, 250.000 IU; vitamin D_3, 50.000 IU; vitamin E, 1000 mg; dl-alpha-tocopherol, 909.10 mg. [2]NDF – neutral detergent fiber; [3]ADF – acid detergent fiber; [4]NFC – non-fiber carbohydrate.

Fat content and fatty acids profile measurements

At the end of the fattening period, the animals were transported to a meat processing plant where they were kept in individual boxes with access to water for 15 to 20 h. For organizational reasons, the animals were slaughtered in two batches (n=20 per batch) at a 7-day interval. To eliminate the potential effect of slaughter batch on research results, the first batch consisted of animals selected randomly, five bulls from each group. Live animals before slaughter and carcasses after slaughter were

weighed with an accuracy of 0.5 kg. The bulls were slaughtered in a commercial slaughterhouse respecting EU regulations (Council Regulation EC No 1099/2009 of 24 September 2009) on the protection of animals at the time of slaughter. The carcasses were classified for conformation and fatness by a trained grader, using the EUROP classification system (Kien, 2004). During carcass dressing, 96 h post mortem, samples of the *Longissimus lumborum (LL)* muscle and the *Semimembranosus (SM)* muscle were collected from the right half-carcass of each animal. Samples of the true cross section of the LL muscle were obtained from the first to third lumbar vertebrae. Samples of the SM muscle were obtained from the center of the muscle. Meat samples weighing approximately 300 g were packaged in polyamide/polyethylene vacuum bags at an ambient temperature of around 4°C, under standard industrial conditions. The samples were transported in an isothermal container to the research laboratory of the Department of Cattle Breeding and Milk Evaluation at the University of Warmia and Mazury in Olsztyn. Fat was extracted from ground meat (Ultra-Turrax, Janke & Kunkel) samples by the Soxhlet method using the Buechi B-811 extraction system, with hexane as a solvent (Morris, 2016). Crude fat content and the percentage of fatty acids were determined based on the following standards: PN-EN ISO 5509 (2001) and PN-EN ISO 5508 (1996). Fatty acid 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 (Żegarska et al., 1991). The percentage of 31 fatty acids was determined by gas chromatography, using the Varian CP 3800 system with a split/splitless injector and a flame-ionization detector. Samples (1 ml) of fatty acid 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. Fatty acids were identified by comparing their retention times with those of commercially available reference standards purchased from Supelco. Inc. (Bellefonte, PA). Analyses of samples and reference standards were performed under identical conditions, i.e. carrier gas – helium, injector temperature – 260°C, detector temperature – 260°C and initial oven temperature – 110°C, raised to 249°C. The fatty acids were divided into the following categories: SFAs, UFAs, including MUFAs and PUFAs. The following ratios were calculated: UFA/SFA, PUFA/SFA and n–6/n–3 PUFA. The fatty acid content was presented as the relative percentage (% of total fatty acids).

Determination of mineral content

Samples of LL and SM muscles were homogenized, specimens of approximately 0.5 g were placed in Teflon-lined pressure vessels and combined with 7 ml of 65% spectrally pure nitric acid (Merck). Each sample was analyzed in duplicate. Means of two replications were used in the calculations. The vessels were sealed, and the samples were mineralized in the Mars Xpress 5 microwave digestion system (Candela, USA). Every mineralization procedure involved 2 blank samples and 2 samples of certified reference material. Mineralized samples were cooled and transferred to 25 ml volumetric flasks. They were analyzed with an atomic absorption spectrometer (Candela, USA) equipped with lamps for different elements (potassium, sodium, magnesium, zinc, iron).

Determination of vitamin A and E content

The content of vitamins A and E was determined based on the applicable standards (PN-EN 12822, 2014), which were slightly modified for the needs of this study. Samples of LL and SM muscles were ground, and 2 g specimens were placed in 25 ml amber glass flasks. The samples were saponified at a temperature of 80°C for 30 minutes in 1.2 ml of 20% ascorbic acid, 0.6 ml of methanol and 5 ml of 60% potassium hydroxide. After cooling, fat was extracted twice with n-hexane, and the extract was evaporated to dryness in a stream of nitrogen. The fat remaining in flasks was dissolved in 1 ml of anhydrous ethanol and filtered into amber glass vials. Chemical compounds were separated by high-performance liquid chromatography (HPLC) using a silica column. The separated compounds were identified by two detectors in tandem (UV-visible photodiode array detector and fluorescence detector). Alpha-tocopherol was detected by fluorescence spectroscopy, and retinol was detected with the use of the UV-visible photodiode array detector. Injection volume was 20 µl. The content of total α-tocopherol and retinol in meat was calculated in duplicate for each muscle, based on the external standard, from a standard curve of peak area vs. concentration. Means of two replications were used in the calculations.

Statistical analysis

The impact of silage type (four levels: GS, VFS, VFS+GS and VFS+MS) on the BW of bulls, age at slaughter and fatness score was analyzed using one-way analysis of variance, whereas the differences between means were analyzed using Tukey's test. Meat samples were taken from each animal with two muscles, for a total of 80 samples. After checking and excluding the effect of the slaughtering session, the effects of silage type (four levels: GS, VFS, VFS+GS and VFS+MS) and muscle type (two levels: LL and SM) on the remaining analyzed parameters were determined by the least squares method using the formula:

$$Yijk = \mu + Ai + Bj + (AB)ij + eijk$$

where: *Yijk* is the value of the analyzed parameter, μ is population mean, *Ai* is the effect of silage type (1–4), *Bj* is the effect of muscle type (1. 2), *(AB)ij* is the silage type × muscle type interaction, and *eijk* is random error. All calculations were performed using Statistica 13.3 software (StatSoft Inc., Tulsa, OK, USA).

Results

Fatty acid profile of intramuscular fat

Maize silage had a considerably lower content of palmitic acid and α-linolenic acid, and a several-fold higher content of oleic acid and linoleic acid than the remaining types of silage (Table 1). The concentrations of the analyzed fatty acids were similar in GS and VFS. The effects of diet on fattening performance (Table 3) have been described in detail in a previous study (Nogalski et al., 2020). Fat cover scores

varied across the dietary treatments, and they were higher in VFS+MS bulls compared with VFS bulls (P<0.05) (Table 3). The dietary treatments had a significant effect on IMF content and VFS+MS bulls had higher IMF content compared to the other treatments (Table 4). In addition, both VFS and VFS+GS bulls had higher IMF content compared to the GS bulls (P<0.05). The SM muscle contained less (P<0.01) IMF (by 0.73% on average) than the LL muscle. Silage type affected (P<0.05) the proportions of SFAs and UFAs in the total fatty acid pool. The IMF extracted from the muscles of bulls fed VFS+GS contained less (P<0.05) SFAs compared with the other groups. The meat of bulls fed VFS+GS had the highest UFA concentrations, and the difference (P<0.05) relative to the remaining groups ranged from 3.20% to 3.79%. Silage type had a significant effect on MUFA levels, and muscle type affected the concentrations of both MUFAs and PUFAs. The proportion of MUFAs was highest (P<0.05) in the IMF of VFS+GS bulls. The LL muscle contained more MUFAs (by 4.17%. P<0.01) and less PUFAs (by 3.58% P<0.01) than the SM muscle. The differences in the proportions of fatty acid groups, affected by the experimental factors, were reflected in the values of the MUFA:SFA ratio. Muscle type also exerted a significant (P<0.01) effect on the PUFA:SFA ratio. The proportion of n-3 PUFAs was significantly (P<0.05) highest in the IMF of GS bulls, compared with VFS, VFS+GS and VFS+MS bulls. The n-6/n-3 PUFA ratio was higher (P<0.05) in the VFS group than in the GS and VFS+MS group. Muscle type affected (P<0.01) both the concentrations of n-3 and n-6 PUFAs and their ratio (n-6/n-3 PUFA). A higher share of n-3 and n-6 acids and a more favorable n-6/n-3 PUFA ratio were found in the fat of the SM muscle. An interaction (P<0.05) between the experimental factors was found for the content of n-6 PUFAs (Figure 1). Intramuscular fat in the SM muscle of VFS+MS bulls contained less (P<0.05) n-6 PUFAs than the IMF of bulls fed GS and VFS+GS. Intramuscular fat in the LL muscle of VFS+MS bulls contained more (P<0.05) n-6 PUFAs than IMF in the LL muscle of VFS bulls.

Table 3. Fattening performance, age, live weight at slaughter and carcass fat content of bulls

Specification	Diet (silage type)				SEM	P-value
	GS	VFS	VFS+GS	VFS+MS		
Initial age (days)	509.2	516.4	512.1	515.6		
Initial BW (kg)[1]	455.1	455.6	477.0	464.3		
Final BW (kg)	696.0	686.5	715.3	708.3		
Final age (days)	724.3	731.5	724.1	727.8		
Daily gain (kg)	1.126	1.079	1.129	1.151		
Hot carcass weight (kg)	358.6	353.1	366.5	372.2		
Fatness score (pts)[2]	5.3 ab	4.9 b	5.4 ab	6.8 a	0.261	0.027

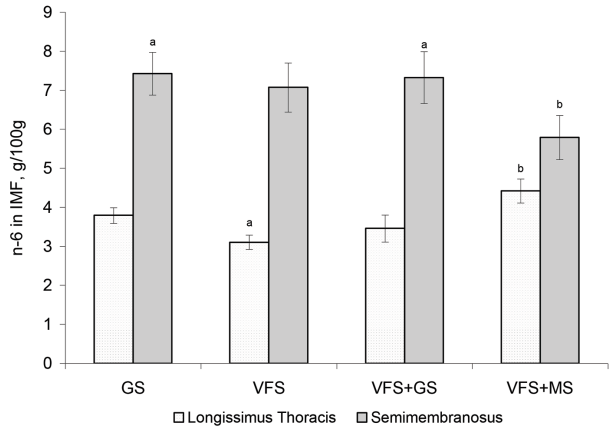
GS – grass silage; VFS – Virginia fanpetals silage; MS – maize silage; SEM – Standard error of the mean.
[1]Body weight; [2]EUROP degree of fat cover: 1 to 15, 1 = very low, 15 = very fat.
a, b, c – values in rows with different letters differ significantly (P≤0.05).

Table 4. The effect of silage type and muscle type on the percentages and ratios of fatty acid groups in intramuscular fat of bulls (g/100 g total fatty acids)

Specification	Type of silage (TS)				Muscle (M)		SEM	Significance (P value)		
	GS	VFS	VFS+GS	VFS+MS	LL	SM		TS	M	TS × M
Intramuscular fat (%)	1.69 Ba	2.06 b	2.39 b	2.68 Aa	2.57 A	1.84 B	0.082	0.025	0.000	0.766
SFAs	45.80 a	45.36 a	42.16 b	45.95 a	44.53	45.11	0.467	0.013	0.551	0.724
UFAs	54.20 b	54.64 b	57.84 a	54.05 b	55.47	54.89	0.469	0.015	0.549	0.732
MUFAs	46.55 b	47.63 b	50.52 a	46.91 b	49.98 A	45.81 B	0.551	0.015	0.000	0.557
PUFAs	7.65	7.06	7.32	7.17	5.50 B	9.08 A	0.322	0.786	0.000	0.087
MUFA/SFA	1.02 b	1.06 b	1.21 a	1.03 b	1.13 a	1.03 b	0.022	0.007	0.014	0.735
PUFA/SFA	0.17	0.16	0.17	0.16	0.12 B	0.20 A	0.007	0.584	0.000	0.100
n-3	2.09 a	1.68 b	1.85 b	1.87 b	1.25 B	2.48 A	0.044	0.041	0.000	0.091
n-6	5.61	5.09	5.39	5.10	3.67 B	6.91 A	0.291	0.742	0.000	0.039
n-6/n-3	2.77 b	3.24 a	2.99 ab	2.83 b	3.09 a	2.85 b	0.111	0.024	0.041	0.346

GS – grass silage; VFS – Virginia fanpetals silage; MS – maize silage; LL – *Longissimus lumborum* muscle; SM – *Semimembranosus* muscle; SEM – standard error of the mean; SFAs – saturated fatty acids; UFAs – unsaturated fatty acids; MUFAs – monounsaturated fatty acids; PUFAs – polyunsaturated fatty acids.

Means followed by different letters differ within rows (within the factor): A, B ($P \leq 0.01$); a, b ($P \leq 0.05$).



GS – grass silage; VFS – Virginia fanpetals silage; MS – maize silage; IMF – intramuscular fat; SEM – standard error of the mean; a, b (P≤0.05).

Figure 1. The effect of the silage type × muscle type interaction on the content of n–6 fatty acids in intramuscular fat (Means ± SEM)

The experimental diets affected the proportions of selected functional fatty acids (Table 5). The highest (P<0.05) concentrations of linolenic acid (C18:3), arachidonic acid (C20:4) and docosapentaenoic acid (C22:5) were noted in the IMF of bulls fed GS, whereas the VFS+GS treatment contributed to a significant increase in oleic acid (C18:1 C9) content (P<0.05). Muscle type had a significant effect on the proportions of most valuable fatty acids. The LL muscle contained higher (P<0.01) amounts of oleic acid and CLA, whereas the concentrations of linoleic acid, linolenic acid, arachidonic acid, eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid were higher (P<0.01) in the SM muscle. In both skeletal muscles, the predominant SFAs were palmitic acid (C16:0) and stearic acid (C18:0) (Table 5). The dietary treatments had no significant effect on the concentrations of three major SFAs, which were affected by muscle type. Intramuscular fat in the LL muscle had a higher (P<0.01) content of myristic acid (C14:0), and IMF in the SM muscle had a higher (P<0.05) content of stearic acid (C18:0).

Contents of minerals and vitamins

An analysis of macronutrients revealed that the muscles of bulls fed GS had higher (P<0.01) potassium content than the muscles of bulls fed VFS (Table 6). The muscles of bulls fed VFS+GS had a higher (P<0.05) concentration of α-tocopherol than the muscles of bulls fed GS and VFS+MS. Muscle type affected the levels of macronutrients in the meat of bulls. The SM muscle had a higher content of magnesium (P<0.05) and potassium (P<0.01), whereas the LL muscle had higher (P<0.05) sodium content. The SM muscle had higher iron content (by 2.8 mg, P<0.01) than the LL muscle. Muscle type exerted varied effects on vitamin content. The LL muscle had a higher (P<0.01) concentration of α-tocopherol, whereas retinol levels were higher (P<0.05) in the SM muscle.

Table 5. The effect of silage type and muscle type on the percentages of fatty acids in intramuscular fat (%, g/100 g total fatty acids)

Specification	Type of silage (TS)				Muscle (M)		SEM	Significance (P value)		
	GS	VFS	VFS+GS	VFS+MS	LL	SM		TS	M	TS × M
C 10:0	0.04	0.04	0.04	0.04	0.04	0.04	0.002	0.756	0.992	0.147
C 12:0	0.06	0.06	0.05	0.06	0.06	0.05	0.052	0.065	0.181	0.220
C 14:0	2.45	2.48	2.28	2.54	2.72 A	2.16 B	0.067	0.456	0.000	0.859
C 14:1	0.45	0.55	0.58	0.46	0.69 A	0.33 B	0.032	0.064	0.000	0.416
C 15:0	0.39 A	0.42 A	0.29 B	0.40 A	0.39	0.37	0.011	0.000	0.249	0.396
C 16:0	25.45	25.29	24.52	25.80	25.59	24.93	0.211	0.197	0.117	0.808
C 16:1	3.29	3.64	3.91	3.42	4.26 A	2.87 B	0.133	0.139	0.000	0.645
C 17:0	1.03 A	1.05 A	0.79 B	1.05 A	0.95	1.02	0.025	0.000	0.085	0.211
C17:1	0.69	0.75	0.68	0.73	0.77 A	0.66 B	0.015	0.228	0.000	0.375
C 18:0	15.76	15.41	13.49	15.52	14.24 b	15.88 a	0.341	0.059	0.015	0.728
C 18:1 T6+9	0.52	0.58	0.48	0.56	0.53	0.54	0.019	0.228	0.662	0.376
C 18:1 (TVA)	1.63	1.80	1.18	1.71	1.59	1.57	0.084	0.051	0.983	0.862
C 18:1 C9 (OA)	37.27 b	37.55 b	40.68 a	37.40 b	39.43 A	36.98 B	0.452	0.012	0.004	0.757
C 18:1 C11	1.72 B	1.74 B	2.01 A	1.64 B	1.70 b	1.86 a	0.041	0.007	0.037	0.502
C 18:1 C12	0.28	0.29	0.30	0.27	0.28	0.30	0.006	0.379	0.158	0.865
C 18:1 C13	0.09	0.10	0.09	0.08	0.09	0.09	0.291	0.051	0.068	0.814
C18:1 T16	0.33	0.33	0.28	0.33	0.32	0.31	0.008	0.018	0.461	0.202
C18:2 (LA)	4.58	4.23	4.39	4.22	3.15 B	5.56 A	0.215	0.789	0.000	0.081
C18:2 c9 t11(CLA)	0.37	0.40	0.38	0.39	0.42 A	0.34 B	0.008	0.576	0.000	0.468
C18:3 (LNA)	0.68 a	0.47 b	0.45 b	0.51 b	0.47 B	0.59 A	0.018	0.021	0.000	0.457
C20:4 (AA)	1.08 a	0.84 b	1.00 ab	0.85 b	0.53 B	1.35 A	0.078	0.030	0.000	0.075
C20:5 (EPA)	0.12	0.10	0.10	0.10	0.07 B	0.14 A	0.008	0.698	0.000	0.422
C 22:0	0.10	0.09	0.20	0.06	0.07	0.15	0.025	0.263	0.121	0.415
C22:5 (DPA)	0.33 a	0.21 b	0.27 b	0.22 b	0.16 B	0.35 A	0.018	0.037	0.000	0.088
C22:6 (DHA)	0.05	0.03	0.04	0.04	0.03 B	0.05 A	0.002	0.188	0.000	0.379

GS – grass silage; VFS – Virginia fanpetals silage; MS – maize silage; LL – *Longissimus lumborum* muscle; SM – *Semimembranosus* muscle.

SEM – standard error of the mean; TVA – trans-vaccenic acid; OA – oleic acid; LA – linoleic acid; CLA – conjugated linoleic acid; LNA – linolenic acid; AA – arachidonic acid; EPA – eicosapentaenoic acid; DPA – docosapentaenoic acid; DHA – docosahexaenoic acid.

Means followed by different letters differ within rows (within the factor): A, B ($P \leq 0.01$); a, b ($P \leq 0.05$).

Table 6. The effect of silage type and muscle type on the content of macronutrients, micronutrients and vitamins (mg/100 g of fresh meat)

Specification	Type of silage (TS)				Muscle (M)		SEM	Significance (P value)		
	GS	VFS	VFS+GS	VFS+MS	LL	SM		TS	M	TS × M
Mg	20.3	20.1	19.6	21.0	19.5 b	20.9 a	0.29	0.393	0.015	0.969
K	504.7 A	457.0 B	486.3	486.4	469.8 B	495.5A	4.62	0.000	0.002	0.302
Na	60.2	60.5	58.1	58.7	61.7 a	57.1 b	1.02	0.804	0.035	0.344
Zn	4.0	4.1	4.3	4.1	3.9	4.2	0.08	0.711	0.16	0.626
Fe	2.1	2.0	2.1	2.2	2.0 B	2.2 A	0.04	0.491	0.001	0.597
Retinol	0.064	0.056	0.067	0.061	0.077 A	0.046 B	0.003	0.574	0.000	0.115
α-tocopherol	0.245 b	0.325 ab	0.332 a	0.260 b	0.251 b	0.331 a	0.016	0.026	0.015	0.852

GS – grass silage; VFS – Virginia fanpetals silage; MS – maize silage; LL – *Longissimus lumborum* muscle; SM – *Semimembranosus* muscle.
SEM – standard error of the mean.
Means followed by different letters differ within rows (within the factor): A, B ($P \leq 0.01$); a, b ($P \leq 0.05$).

Discussion

Fatty acid profile of intramuscular fat

In the present study, bulls were fed supplemental concentrate because the quality of silage was not high. The concentrate accounted for 40% DM of the diet, which is typical of intensive fattening. A diet rich in n-6 PUFAs and poor in n-3 PUFAs, leading to a high n-6/n-3 PUFA ratio, is a risk factor for cardiovascular disease in humans (Breslow, 2006; EFSA, 2020). Attempts have been made to increase the amounts of PUFAs in beef. Dietary PUFAs are extensively biohydrogenated in the rumen, where UFAs are isomerized and hydrogenated by ruminal microbes to form SFAs, in particular C18:0 (Buccioni et al., 2012). Previous research (Nogalski et al., 2014; Momot et al., 2016) has shown that intensive fattening increases the IMF content of beef, but decreases the proportion of PUFAs and increases the n-6/n-3 PUFA ratio in IMF. In order to naturally protect PUFAs against biodegradation in the rumen, ruminants should be fed grains or cell organelles (e.g. chloroplasts in silage) where fat is enclosed and protected against ruminal microbiota (Scollan et al., 2014). On the other hand, roughage is also expected to positively affect the fatty acid profile of beef (Momot et al., 2016; Nogalski et al., 2014; Scollan et al., 2014). In a study by Lee et al. (2009), red clover silage fed to finishing cows considerably increased dietary protein levels with no adverse effect on the concentrations of health-promoting fatty acids in IMF, compared with GS. Durand et al. (2005) demonstrated the ability to markedly increase the concentrations of n-3 PUFAs in beef muscles when 18:3 n-3 (as linseed oil) was infused directly into the small intestine, thereby by-passing the rumen. This strategy increased the concentration of 18:3 n-3 in total lipids. Fortin et al. (2010) reported that abomasal infusion of fish oil increased the concentration of EPA in muscle phospholipids.

The fatty acid composition of beef can be modified primarily through the diet (Scollan et al., 2014). Grass, clover and haylage are rich sources of α-linolenic acid (C18:3 n-3), whereas cereal- and MS-based concentrates – of linoleic acid (C18:2 n-6). The fatty acid profile of IMF, determined in this study, is desirable from the perspective of beef consumers because it comprised more than 55% of UFAs, including over 7% of PUFAs, and the n-6/n-3 PUFA ratio oscillated around the value of 3. The ratio n-6/n-3 was used as an indicator of the nutritional value of fats and the value of 4.0 was set as the maximal (Valencak et al., 2015). Typically, IMF of beef consists, on average, of 45–48%, 35–45% and up to 5% of total fatty acids as SFAs, MUFAs and PUFAs, respectively and the n-6:n-3 PUFA ratio in beef is beneficially low, typically lower than 3 (Scollan et al., 2014).

In the present study, MS had a higher content of oleic acid (C18:1), compared with the other types of silage, but it did not increase the concentration of this fatty acid in the IMF of VFS+MS bulls. Higher concentrations of α-linolenic acid and lower concentrations of linoleic acid in GS and VFS, compared with MS, did significantly affect the proportions of n-6 and n-3 PUFAs or their ratio in IMF, either. The source of fatty acids influences the process of their biohydrogenation (MacKintosh et al., 2017). In general, the levels of n-3 PUFAs are higher in the muscles of cattle fed fresh grass, compared with conserved grass, and they increase with the amount

of pasture consumed and grazing time on pasture (Scollan et al., 2006). This experiment confirmed the importance of grasses for the content of n-3 fatty acids in beef. The IMF of bulls fed GS had the highest proportion of n-3 PUFAs. The consumption of beef from grass-based animals with increased n-3 fatty acid concentration can contribute to the daily human requirements for these fatty acids, especially C18:3 and C22:5.

In the current study, GS and VFS+GS had the most beneficial effect on the fatty acid composition of meat. The IMF of VFS+GS bulls had the highest proportion of UFAs, mostly oleic acid, whereas the IMF of GS bulls had the lowest n-6/n-3 PUFA ratio and the highest concentrations of n-3 acids and DPA. Bearing in mind that both silages (GS and VFS) had similarly high LNA content, the higher content of n-3 acids in the IMF of the GS group proves a less dynamic process of PUFA biohydrogenation in the rumen of GS-fed animals. In the work of Lee et al. (2009), red clover silage, with a similarly high protein content as VFS in the current study, used with a mixture of grass increased the deposition of n-3 PUFAs in the muscles of finishing cattle. This is most likely the effect of the polyphenol oxidase enzyme found in red clover. Cattle feeds containing higher amounts of UFAs contribute to improving the fatty acid profile of beef (Scollan et al., 2014). In the present study, GS and VFS had considerably higher LNA content and lower LA content, compared with MS, which however had no significant effect on the proportions of the above acids in the IMF of bulls.

In the present experiment, VFS+GS, compared with VFS+MS, also decreased IMF content and considerably increased the proportion of MUFAs, in particular oleic acid. However, the VFS+GS combination, compared with GS, adversely affected the concentrations of n-3 PUFAs. The VFS+GS combination delivered more desirable effects in the proportion of UFAs than both types of silage administered alone in groups GS and VFS. A synergistic effect of a mixture of GS and Lupins/triticale silage offered together, relative to either of the silages offered alone, was reported by Kennedy et al. (2018). Partial replacing GS with lupins/triticale silage increased total PUFAs acids in *Longissimus dorsi* muscle.

Both De Smet et al. (2000) and Hunt et al. (2016) showed that an increased fat content of bovine meat was paralleled by increased proportions of SFAs and MUFAs, and a decreased proportion of PUFAs. In the present study, the dietary treatments did not cause an increase in the share of SFAs or a decrease in the share of PUFAs with an increase in IMF content. The exception was when VFS+MS was compared to VFS+GS. The increased content of IMF in the treatment of VFS+MS increased the proportion of SFAs.

An analysis of muscle type revealed that higher IMF content was accompanied by a considerable increase in MUFA concentrations and a decrease in PUFA levels with no increase in the share of SFAs. Domaradzki et al. (2019) also demonstrated that muscle type significantly affected the concentrations of MUFAs and PUFAs in the total fatty acid pool in IMF. In the current study, the proportions of MUFAs and PUFAs were influenced by muscle type, which however had no effect on the total content of UFAs. The LL muscle accumulated 40% more IMF than the SM muscle, and IMF in the LL muscle had a lower proportion of n-3 and n-6 PUFAs and a higher

n-6/n-3 PUFA ratio. Piao et al. (2017) analyzed the concentrations of fatty acids in the loin (*Longissimus dorsi* muscle) and rump (*Semimembranosus* muscle) cuts of Korean cattle steers among five quality grades (marbling score) and found that the percentages of fatty acids were affected by muscle type rather than quality grades. Hunt et al. (2016) also noted that muscle type affected the fatty acid composition of muscles. Similarly in the current experiment, muscle type affected the fatty acid composition of muscles. IMF in the SM muscle had a higher proportion of PUFAs and, consequently, a higher PUFA/SFA ratio.

The content of most of the biologically active fatty acids, analyzed in this study, was higher in the SM muscle than in the LL muscle, which is an important consideration from the human nutritional perspective. The low fat content and, consequently, lower calorific value of the SM muscle as well as its more favorable profile of nutritionally important fatty acids should be considered attractive by consumers. However, CLA had a higher percentage in LL. Taking into account the IMF content (LL – 2.57% and SM – 1.84%), LL contained 10.22 mg CLA in 100 g of raw wet tissue, and SM only 6.0. Therefore, health-conscious consumers should eat LL beef rather (Realini et al., 2014). CLA has considerable potential benefits in human diet. Cis-9, trans-11 and trans-10, cis-12 isomers have been associated with the inhibition of carcinogenesis, the reduction of atherosclerosis, modification of the immune response, the distribution of body fat and a reduction in body-fat deposits (Williams, 2000). Turkki and Campbell (1967) found that the proportion of PUFAs was higher in the SM muscle, which has a higher number of red oxidative fibers than the LL muscle, whereas the latter had higher concentrations of SFAs and MUFAs. Saturated fatty acids have been shown to raise blood cholesterol levels. However, while it is clear that C14:0 and C16:0 are responsible for increasing total plasma and LDL cholesterol concentrations, another major SFA, C18:0, is not hypercholesterolemic and does not increase total cholesterol or LDL cholesterol levels (Williams, 2000). In the present study, the predominant SFAs in the analyzed skeletal muscles were palmitic acid (C16:0) and stearic acid (C18:0). Hunt et al. (2016) noted similar proportions of the above acids in the LL and SM muscles, but observed differences between quality classes (choice and select) and not muscle types, which could result from the fact that IMF content was more than two-fold higher in choice cuts than in select cuts.

Mineral compounds content and vitamins

In this experiment, the content of chemical elements of meat was determined mostly by muscle type. The SM muscle, which performs a greater amount of work than the LL muscle, accumulated more micronutrients and magnesium.

Higher concentrations of selected nutrients in the muscles of bulls may be related to fat content. A negative correlation between carcass fatness and nutrient content in cattle was observed by Williams et al. (1983), who found higher levels of zinc, iron, phosphorus, sodium and potassium in beef from carcasses with lower fat content. In the current study, a higher IMF content of the muscle was linked with lower concentrations of Mg, K and Fe. The SM meat was a very good source of Zn and the consumption of 100 g of cooked meat satisfies 52% and 23% of RDA (Recommended Dietary Allowance, according to IOM, 2010) for males and females

(19–50 y), respectively. Antioxidants, in particular vitamin E, stabilize high concentrations of muscle PUFAs. Muscle tissue must contain a minimum concentration of antioxidants, such as vitamin E, in order to avoid lipid oxidation and metmyoglobin formation (Scollan et al., 2014). In beef, this minimum quantity is between 3 and 4 µg of α-tocopherol/g of fresh muscle (Arnold et al., 1993). In our study, the concentration of α-tocopherol was below this minimum value in GS and VFS+MS groups. This could result from the loss of α-tocopherol during the ensiling process.

A higher concentration of α-tocopherol was noted in the SM muscle where the proportion of PUFAs was 65% higher than in the LL muscle. According to Röhrle et al. (2011), the α-tocopherol content of non-Irish beef and Irish beef falls in the range of 1.3 to 4.0 µg/g muscle, but Brazilian beef may have higher α-tocopherol concentrations, depending on season and supplementation.

Conclusions

The results of this study indicate that VFS can improve carcass and meat quality characteristics without any adverse effects on the fattening performance of bulls, when compared with a grass silage-based control diet. Grass silage had the most beneficial effect on the proportion of functional fatty acids in IMF. VFS combined with MS increased the fat content of muscles and fat cover, whereas VFS combined with GS improved the fatty acid profile and increased α-tocopherol concentration. Beef consumers should be encouraged to choose the SM muscle over the LL muscle since the former contains less IMF with a more nutritionally desirable profile, has lower calorific value, a more desirable mineral composition and a higher concentration of α-tocopherol.

Declaration of interest

The authors declare that there is no conflict of interest.

Ethics statement

The experimental procedure was approved by the Local Ethics Committee for Experiments with Animals in Olsztyn, Poland (approval no. 121/2010).

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