https://doi.org/10.17221/176/2018-CJFS

Effect of muscle anatomical location on fatty acid composition of beaver (*Castor fiber*) females

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Citation: Razmaite V., Pileckas V., Juškiene V. (2019): Effect of muscle anatomical location on fatty acid composition of beaver (*Castor fiber*) females. Czech J. Food Sci., 37: 106–111.

Abstract: Ten beaver (*Castor fiber*) females were used in the experiment. The samples were excised from a different anatomical location of each beaver carcass used in the study: *m. longissimus dorsi* (*LD*), *m. triceps brachii* (shoulder), *m. biceps femoris* (thigh). Thigh muscles were characterized by the highest percentage of free fat followed by longissimus muscle and shoulder. The total proportions of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA), including many individual fatty acids, were affected by the muscle anatomical location. The fattiest thigh had the most favourable and highest PUFA/SFA, hypocholesterolemic/hypercholesterolemic (*h*/*H*) and lowest *n*-6/*n*-3 PUFA ratios, and the lowest thrombogenic index. Despite the fact that the leanest *m. triceps brachii* had the highest proportions of total polyunsaturated fatty acids, the *n*-6/*n*-3PUFA ratio was the lowest compared with the muscles from other anatomical locations and showed lower atherogenic index compared with a more fatty longissimus muscle.

Keywords: game; human nutrition; lipids; meat

Meat has a crucial role in human evolution and continues to be an important food in the diet for many consumers, and there has been a significant increase in global meat production and consumption (Henchion *et al.* 2014). At the same time meat quality has become more significant in influencing the consumer's choice. Health conscious consumers have driven upwards the demand for low fat and turned an interest in alternatives to traditionally sourced farm meat products (Tomasevic *et al.* 2018). Such an alternative could be game meat, because it has a distinctive taste and aroma and it is characterized by a very good chemical composition, including the composition of protein and intramuscular fat (Ramanzin *et al.* 2010; Neethling *et al.* 2016). Game is considered to be healthy meat which can improve the nutrition of current consumer. The Eurasian beaver (*Castor fiber*) suffered from overexploitation, but it has seen revival and is able to modify ecosystems (Rosell *et al.* 2005; Halley *et al.* 2012), and needs to be controlled. Knowledge about the carcass and meat composition, nutritional value, properties and use of beaver meat for sausage production was gained from studies in Poland (Jankowska *et al.* 2005; Żochowska-Kujawska *et al.* 2016; Florek *et al.* 2017), Lithuania (Razmaitė *et al.* 2011) and Latvia (Strazdinia *et al.* 2015). Flavour and other characteristics of muscle foods are highly dependent on the species from which it originates and, moreover, each muscle has a distinctive anatomical location and function. As a result, the physiochemical composition of muscles differs, which influences their characteristics (Webb & O'Neill 2008; Neethling *et al.* 2016). Therefore, it is important to quantify differences in the composition of meat derived from different anatomical location or muscles. The present study aimed to investigate the fatty acid composition of beaver (*Castor fiber*) meat excised from different anatomical location.

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MATERIAL AND METHODS

Animals and samples. Ten beaver (*Castor fiber*) females with a mean weight of 23 kg were used in the experiment. Animals were hunted during March in the central parts of Lithuania in the latitude of 55°38' to 55°40' N and in the longitude of 23°41' to 23°44' E. The beavers used in this study were shot in accordance with the law on hunting of the Republic of Lithuania, law No IX-966 of 20th of June, 2002 (topical issue of 18th of May, 2010). The samples were excised from a different anatomical location of each beaver carcass used in the study: *m. longissimus dorsi* (*LD*), *m. triceps brachii* (shoulder), *m. biceps femoris* (thigh) in a special area for the dressing of hunted animals. All these samples were obtained from hunters during 24 h period after their entrapment.

Free fat content. Fat was determined by the Soxhlet extraction method (AOAC 1990, No. 960.39). The content of fat was expressed as weight percentage in wet muscle tissue.

Fatty acid profiles. Methylation of the samples was performed using sodium methoxide. The fatty acid methyl esters (FAMEs) were analysed using a gas liquid chromatograph (GC-2010 SHIMADZU). The separation of methyl esters of fatty acids was affected on the capillary column Rt 2560 (100 m × 0.25 mm × 0.2 μm; Restek, USA) by temperature programming from 160°C to 230°C. The temperatures of the injector and detector were held, respectively, at 240 and 260°C. The rate of flow of carrier gas (nitrogen) through the column was 0.79 ml/min. The peaks were identified by comparison with the retention times of the standard fatty acids methyl esters '37 Component FAME Mix', cis/trans FAME MIX, trans FAME MIX k 110, and Linoleic acid methyl ester isomer mix (Supelco, USA). The relative proportion of each fatty acid was expressed as the relative percentage of the sum of the total fatty acids using 'GC solution' software for Shimadzu gas chromatograph workstations. All samples were analysed in duplicate.

Lipid quality indices. Lipid quality indices, i.e. atherogenic index (AI) and thrombogenic index (TI), were calculated according to Ulbricht and Southgate (1991). The hypocholesterolemic/hypercholesterolemic (h/H) ratio was calculated according to Santos-Silva *et al.* (2002). The peroxidizability index (PI) was determined according to Du *et al.* (2003).

Statistical analysis. The data were subjected to the analysis of variance in general linear (GLM) procedure in SPSS 17 with Tukey tests to determine the significance of differences of means between the groups.

RESULTS AND DISCUSSION

Biceps femoris muscle in thigh was characterized by the highest percentage of free fat ($P < 0.001$) compared with *m. triceps brachii* in shoulder and longissimus muscles (Table 1). The fat contents in the present study in all muscles (1.07–4.82) of females were higher compared to the previous results (Razmaite *et al.* 2011) obtained in the thigh (0.51) from the beaver of both sexes and to the results of Florek *et al.* (2017) obtained in *m. semimembranosus* and

Table 1. Effects of muscle anatomical location on fat and saturated fatty acid (% of total *FA*) composition in intramuscular fat of beaver females (n = 10)

Fatty acids	Anatomical location			SED	*P*
	m. longissimus dorsi	*m. triceps brachii*	*m. biceps femoris*		
Fat	2.74	1.07	4.82	0.191	< 0.001
C12:0	0.01	0.00	0.00	0.006	< 0.001
C14:0	0.51^{c}	$0.34^{d,e}$	0.58^{f}	0.042	0.026
C15:0	0.80^{e}	0.58^{f}	0.84^{e}	0.044	0.003
C16:0	19.08^{a}	17.33^{b}	17.84	0.625	< 0.001
C17:0	1.71^{c}	1.76^{c}	1.18^{d}	0.169	0.399
C18:0	9.12^{ae}	$10.10^{b,e}$	7.57^{f}	0.359	0.001
C20:0	0.01	0.00	0.18	0.013	0.399
C21:0	0.02^{c}	0.01^{c}	0.11^{d}	0.025	0.001
C22:0	0.13	0.13	0.09	0.017	0.037
SFA	31.39^{c}	$30.25^{\dagger}$	$28.21^{d\dagger}$	0.862	0.003

SED – standard error of difference; SFA – sum of all detected saturated fatty acids; $^{a-b*}P < 0.05$; $^{c-d**}P < 0.01$; $^{e-f***}P < 0.001$; $^{\dagger}P \leq 0.005$

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loin of Polish male beaver. However, the fat content in *m. triceps brachii* of females (1.07) in the present study was lower than in the male shoulder (1.95) detected by Florek *et al.* (2017). The closest mean value of fat in *m.biceps femoris* (4.82) in the present study was that similar to the value (4.29) reported by Strazdina *et al.* (2015). The total proportions of SFA ($P < 0.01$), MUFA ($P < 0.01$) and PUFA ($P < 0.001$), including many individual fatty acids were affected by the muscle anatomical location and this is in agreement with the previous findings for other game species roe deer (Razmaite *et al.* 2015). As in other domestic and wild species (Ruiz-Núñez *et al.* 2016), palmitic (C16:0) and stearic (C18:0) acids were found to be dominant saturated acids in the meat (Table 1). However, meat of domestic animals has higher contents of C16:0, whereas game meat of antelope, moose (Ruiz-Núñez *et al.* 2016) and roe deer (Razmaite *et al.* 2015) have higher contents of C18:0 fatty acid. In contrast to higher proportions of C16:0 acid in the present study, Strazdina *et al.* (2015) have estimated a higher proportion of C18:0 than C16:0 in beaver *m. biceps femoris*. Although *m. biceps femoris* with the highest intramuscular fat content in the present study had lower proportion of SFA ($P < 0.01$) compared with *m. longissimus dorsi* (LD) and tended ($0.05 \leq P < 0.10$) to have lower proportion than *m. triceps brachii*, the proportion of C16:0 fatty acid in LD was higher compared to the leaner *m. triceps brachii* ($P < 0.05$) but the proportion of C18:0 in *m. triceps brachii* was higher than in LD ($P < 0.05$) and *m. biceps femoris* ($P < 0.001$). Despite the lowest proportion of total SFA in *m. biceps femoris*, it also had the highest proportions of myristic (C14:0), pentadecanoic (C15:0), arachidic (C20:0) and heneicosylic (C21:0) fatty acids. Although total SFA in this study was similar to those reported by Zalewski *et al.* (2009) but they have found significantly higher and lower, respectively, proportions of total MUFA (23.2) and PUFA (36.5) compared with the data obtained in the present study. The highest differences in the proportions of total MUFA (Table 2), including individual myristoleic (C14:1*n*-9; $P < 0.05$), palmitoleic (C16:1*n*-9 and C16:1*n*-7; $P < 0.001$) and oleic (C18:1*cis*-9; $P < 0.010$) fatty acids were between the fattiest *biceps femoris* and leanest *triceps brachii* muscles. Although *m.triceps brachii* had higher proportions of total PUFA, including individual eicosadienoic (C20:2*n*-6; $P < 0.010$) fatty acid than the longissimus muscle, the highest proportion of highly recommended for consumers α-linolenic fatty acid (C18:3*n*-3; $P < 0.05$) was found in *m.biceps femoris*. A higher proportion ($P < 0.05$) of other very important fatty acid EPA (C20:5*n*-3) compared with *m. biceps femoris* was found in *m. longissimus dorsi* (Table 4).

However, fat in *m. triceps brachii* had a lower content of palmitelaidic (C16:1*trans*-9; $P < 0.05$) than *m. longissimus dorsi* and a higher content of trans octadecanoic (C18:1*trans*-9; $P < 0.05$) acids than *m. biceps femoris*. Many studies have revealed correlations between intramuscular fat and fatty acid

Table 2. Effects of muscle anatomical location on monounsaturated fatty acid (% of total FA) composition in intramuscular fat of beaver females ($n = 10$)

Fatty acids	Anatomical location			SED	*P*
	m. longissimus dorsi	*m. triceps brachii*	*m. biceps femoris*		
C14:1*n*-9	0.14	0.08^{a}	0.17^{b}	0.031	0.018
C15:1	0.21	0.21	0.17	0.021	0.145
C16:1*trans*-9	0.26^{ac}	0.22^{d}	0.23^{b}	0.012	0.005
C16:1*n*-9	0.42^{a}	0.34^{be}	0.47^{f}	0.031	0.001
C16:1*n*-7	2.61^{ac}	2.39^{be}	2.87^{df}	0.075	< 0.001
C17:1*n*-9	0.13	0.16	0.22	0.036	0.084
C18:1*trans*-9	1.18^{a}	1.19^{a}	1.10^{b}	0.034	0.013
C18:1*cis*-9	6.61	5.23^{c}	8.38^{d}	0.911	0.006
C18:1*cis*-7	1.41	1.34	1.35	0.564	0.369
C20:1*n*-9	0.33	0.23	0.28	0.044	0.097
MUFA	13.3	11.39^{c}	15.23^{d}	0.996	0.002

SED – standard error of difference; MUFA – sum of all detected monounsaturated fatty acids; $^{a–b*}P < 0.05$; $^{c–d**}P < 0.01$; $^{e–f***}P < 0.001$

composition in the muscles of different domestic animal species. The increase in fat amount was closely associated with the increase in saturated (SFA) and monounsaturated (MUFA) and decrease in polyunsaturated (PUFA) proportions (De Smet *et al.* 2004; Hoehne *et al.* 2012; Wood *et al.* 2013). However, the highest amount of fat in beaver *m. biceps femoris* demonstrated only evident increase in MUFA while the percentage of SFA was the lowest. The lowest amount of fat in *m. triceps brachii* demonstrated decrease in MUFA and increase in PUFA but SFA was in intermediate position.

There were no significant differences (P = 0.213) between beaver muscles in the proportions of total *trans* fatty acids (Table 3). Martysiak-Żurowska *et al.* (2009) reported the same as in the present study

Table 3. Total trans fatty acids and fatty acid ratios and lipid quality indexes in intramuscular fat from beaver females (n = 10)

Variables	Anatomical location			SED	P
	m. longissimus dorsi	*m. triceps brachii*	*m. biceps femoris*		
TFA	1.73	1.67	1.61	0.625	0.213
PUFA/SFA	1.60^{c}	1.76	1.88^{d}	0.074	0.002
n-6/n-3	6.05^{a}	6.32^{c}	5.12^{bd}	0.309	0.001
AI	0.33^{a}	0.29^{b}	0.30	0.015	0.017
TI	0.58^{c}	0.55†	0.48^{d†}	0.030	0.005
h/H	3.03^{a}	3.42	3.46^{b}	0.169	0.027
PI	100.23	108.25	100.0	3.95	0.074
Unindentified FA	5.2	5.14	4.23	0.443	0.064

SED – standard error of difference; PUFA – sum of all detected saturated, monounsaturated and polyunsaturated fatty acids; TFA – total *trans* fatty acids; PUFA/SFA – ratio of total PUFA to total saturated fatty acids (SFA); n-6/n-3 – ratio of sum n-6 PUFA to sum n-3 PUFA; *AI* – atherogenic index; *TI* – thrombogenic index; h/H – hypocholesterolemic/hypercholesterolemic ratio; *PI* – peroxidizability index; $^{a–b*}P < 0.05$; $^{c–d**}P < 0.01$; $^{e–f***}P < 0.001$; $^{†}P \leq 0.005$

Table 4. Effects of muscle anatomical location on polyunsaturated fatty acid (% of total FA) composition in intramuscular fat of beaver females (n = 10)

Fatty acids	Anatomical location			SED	P
	m. longissimus dorsi	*m. triceps brachii*	*m. biceps femoris*		
C18:2n-6*trans*-9, 12	0.23	0.24	0.21	0.015	0.148
C18:2n-6*cis*-9, *trans*-12	0.35	0.01	0.49	0.021	0.152
C18:2n-6*trans*-9, *cis*-12	0.10	0.00	0.26	0.014	0.174
C18:2n-6	32.31	33.91	33.70	0.901	0.174
C18:3n-6	0.02^{ce}	0.08^{d}	0.09^{f}	0.176	< 0.001
C18:3n-3	3.45^{a}	3.30^{a}	5.47^{b}	0.733	0.010
C20:2n-6	0.43^{c}	0.59^{ad}	0.46^{b}	0.044	0.004
C20:3n-6	0.56	0.60	0.51	0.049	0.222
C20:3n-3	0.38	0.40	0.37	0.043	0.840
C20:4n-6	8.87	9.85	8.06	0.893	0.150
C20:5n-3	0.43^{a}	0.38	0.33^{b}	0.038	0.058
C22:2n-6	0.01	0.01	0.01	0.012	0.801
C22:4n-6	0.52	0.59^{c}	0.44^{d}	0.043	0.009
C22:5n-3	2.55	2.85†	2.22†	0.266	0.076
C22:6n-3	0.34	0.41	0.37	0.047	0.277
PUFA	50.13^{ae}	53.22^{f}	52.33^{b}	0.750	0.001

SED – standard error of difference; PUFA – sum of all detected polyunsaturated fatty acids; $^{a–b*}P < 0.05$; $^{c–d**}P < 0.01$; $^{e–f***}P < 0.001$; $^{†}0.005 \leq P < 0.10$

https://doi.org/10.17221/176/2018-CJFS

percentage (1.61) of *trans* fatty acids in the beaver thigh and higher amounts in the tenderloin and adipose tissues. Some studies showed that consumption of industrial *trans* fatty acids is health hazardous for consumers but there is no association between animal *trans* fatty acid intake and heart disease risk (Mozaffarian *et al.* 2006). Other analysis of resolved and unresolved questions showed that all classes of *trans* fatty acids with one or more bonds in the trans configuration raise the ratio of LDL to HDL cholesterol irrespective of their origin or structure (Brouwer *et al.* 2010, 2013). In different muscles of roe deer (Razmaite *et al.* 2015) similar and higher percentage (1.78–2.93) of trans fatty acids was found and, moreover, ruminant *trans* fatty acids are present in food at on average 4% level and sometimes up to 8% of total fatty acids in milk fat (Ferlay *et al*. 2017), therefore, the presence of trans fatty acids at a lower than 2% level in beaver muscles should not be a limiting factor for the use of beaver meat.

A higher proportion ($P < 0.05$) of other very important fatty acid EPA (C20:5*n*-3) compared with *m. biceps femoris* was found in *m. longissimus dorsi*.

The beaver females showed the PUFA/SFA ratio above the minimum (0.4) recommended for the diet (Wood *et al.* 2008) in all muscles. The muscle type appeared to affect the PUFA/SFA, *n*-6/*n*-3 PUFA ratios and such lipid quality indices as atherogenic (*AI*), thrombogenic (*TI*) indexes and hypocholesterolemic/hypercholesterolemic (*h*/*H*) ratio by a different level of significance (Table 3). The longissimus muscle had less favourable lower PUFA/SFA ($P < 0.010$) and higher *n*-6/*n*-3PUFA ($P < 0.05$) ratios than *m. biceps femoris*. Recommendations of Bellagio's report on healthy agriculture, healthy nutrition and healthy people indicated that the ratio (4:1) of *n*-6 PUFA to *n*-3 PUFA in the diet should be the goal (Simopoulos *et al.* 2013). It can be observed that *n*-6/*n*-3 ratio in all muscles was close to the recommended. Although PUFA/SFA ratio in all beaver muscles is significantly higher than the recommended, the highest PUFA/SFA and lowest *n*-6/*n*-3PUFA ratios in the *m. biceps femoris* are most favourable ratios compared with other muscles. As PUFA/SFA and *n*-6/*n*-3 ratios eliminate the effects of MUFA, the lipid quality indices were calculated. Lower thrombogenic index (*TI*) ($P < 0.01$) and higher hypocholesterolemic/hypercholesterolemic ratio (*h*/*H*) ($P < 0.01$) found in *m. biceps femoris* fat were more favourable than in the longissimus muscle and showed the highest nutritional value. *AI*, *TI* and *h*/*H* in all studied beaver muscles were significantly more favourable than adequate indices evaluated in the lamb (Sinanoglou *et al.* 2013), pork (Hanczakowska *et al.* 2015) and *AI* and *TI* in free-living and farmed red deer (Razmaite *et al.* 2017). Only muscles of roe deer demonstrated similar atherogenic (*AI*) and higher *h*/*H* ratio (Razmaite *et al.* 2015).

CONCLUSIONS

The fatty acid composition of beaver (*Castor fiber*) was affected by the muscle anatomical location and muscle fatness. Although *m. biceps femoris* with the highest amount of fat had the lowest percentage of total saturated fatty acids, it contained the highest proportions of individual C14:0, C15:0, C20:0 and C21:0, and total monounsaturated fatty acids. *M. biceps femoris* also had the most favourable and highest PUFA/SFA, *h*/*H* and lowest *n*-6/*n*-3 PUFA ratios, and the lowest thrombogenic index. Despite the fact that *m. triceps brachii* with the lowest amount of fat had the highest proportions of total polyunsaturated fatty acids, the *n*-6/*n*-3 ratio was the lowest compared with the muscles from other anatomical locations. However, *m. triceps brachii* showed lower atherogenic index compared with a more fatty *longissimus* muscle.

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Received: 2018–06–18
Accepted after corrections: 2019–04–16