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EFFECT OF DIFFERENT ROE DEER MUSCLES ON FATTY ACID COMPOSITION IN INTRAMUSCULAR FAT*

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Abstract

The aim of the work was to study the effect of different muscles on the fatty acid composition in the intramuscular fat of roe deer hunted in Lithuania. The samples were excised from the five muscles of different carcass sites: *m. longissimus dorsi* (LD), *m. deltoideus* (shoulder), *m. tensor fascia e latae* (hind quarter), *m. cleidocipitalis* (neck) and *m. intercostales interni* (brisket) of hunted animals. The data were subjected to the analysis of variance in general linear (GLM Multivariate) procedure in SPSS 17. The muscle location of roe deer males appeared to affect the fatty acid composition in the intramuscular fat. The total proportions of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA), including individual SFA, MUFA and PUFA acids were affected by the muscle. The highest levels of SFA and MUFA and the lowest levels of PUFA were found in the intramuscular fat of neck and brisket muscles and vice versa, the lowest levels of SFA and MUFA and the highest levels of PUFA were found in the intramuscular fat of LD and hind quarter muscles. The muscle type of roe deer appeared to affect the lipid quality indices. The lowest atherogenic and thrombogenic indexes, and the highest hypocholesterolemic/hypercholesterolemic ratio were in the intramuscular fat of LD and hind quarter muscles.

Key words: fatty acids, intramuscular fat, game meat, roe deer

Meat from venison is becoming increasingly popular in European markets (La Neve et al., 2008; Dannenberger et al., 2013). Game meat has a low fat content and a typical specific flavour and taste (Nuernberg et al., 2009; Daszkiewicz et al., 2009; Ramanzin et al., 2010). The high percentages of polyunsaturated fatty acids (PUFA) are characteristic of wild ruminant muscle tissue and are considerably higher than

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those found in domestic ruminants (Cordain et al., 2002). The main game meat consumed is from roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) species (La Neve et al., 2008). Roe deer is common in many European countries and their numbers during the last 20 years increased to a great extent and has a high economic significance to humans (Burbaitė and Csányi, 2009; Daszkiewicz et al., 2009; Ramanzin et al., 2010). Sensory evaluation studies have been conducted on different game species including roe deer (Rødbotten et al., 2004; Hutchison et al., 2010). Roe deer was among the species having the most distinct gamy flavour (Rødbotten et al., 2004). Recently, results have been published regarding the authentication of roe deer meat from other game and domestic species (La Neve et al., 2008), the effects of carcass weight and muscle on texture, rheological properties and myofibre characteristics of roe deer (Żochowska-Kujawska et al., 2007), the influence of gender, age on cholesterol content in roe deer meat (Cygan-Szczegielniak and Janicki, 2009), the influence of gender, age and region of Germany on macro- and micronutrient contents and fatty acid profile in the *longissimus* muscle (Dannenberger et al., 2013). However, other muscles from different carcass joints have a considerable importance, both because they represent a high percentage of the carcass (Weiner, 1973) and because they are highly valued. Despite different previous studies, information concerning the fatty acid composition in other muscles of roe deer is scarce. Moreover, the results of Dannenberger et al. (2013) showed the effect of region. Thus, the aim of the present work was to study the effect of different muscles on the fatty acid composition in the intramuscular fat of roe deer hunted in Lithuania.

Material and methods

Animals and sampling

The roe deer used in this study were shot in accordance with the law on hunting of the Republic of Lithuania (Law No IX-966 of 18th of June, 2013). Eight roebucks from 30 to 40 kg body weight hunted in the forests of the northern part of Lithuania at the latitude of 55° 33’ to 56° 18’ N and at the longitude of 22° 30’ to 24° 07’ E during summer hunting season (June-September) were used in the experiment. The samples were excised from the five muscles of different carcass sites: *m. longissimus dorsi* (LD), *m. deltoideus* (shoulder), *m. tensor fascia e latae* (hind quarter), *m. cleidocipitalis* (neck), *m. intercostales interni* (brisket) in a special area for dressing of hunted animals. All of these forty samples were provided by the local hunters in 24 h period after roebuck shooting. The muscle samples for fatty acid detection were stored at –65±2.5°C until analysis.

Chemical composition of muscles and hydroxyproline content

Intramuscular fat was determined by the Soxhlet extraction method (method No 960.39; AOAC, 1990). The content of intramuscular fat was expressed as weight

percentage of wet muscle tissue. The hydroxyproline content was determined according to the NMKL-AOAC method (Kolar, 1990).

Fatty acid profiles

The extraction of lipids for fatty acid analysis was performed with a mixture of two volumes of chloroform (Chromasolv Plus for HPLC containing 0.5–1.0% ethanol as stabilizer) and one volume of methanol (Chromasolv for HPLC≥99.9%) as described by Folch et al. (1957). The sample of 2 g meat was mixed with 40 ml chloroform/methanol mixture and homogenized using disperser T 18 (IKA®-Werke GmbH&Co.KG, Germany). After filtration, 20 ml of 0.74% KCl (Sigma-Aldrich, Reagent Plus≥99.0%) solution was added and allowed to stand for 10–12 hours. Then the bottom layer was collected by syringe into a 15 ml test-tube and evaporated under vacuum at 50ºC. Methylation of the samples was performed using sodium methoxide, 25 wt % solution in methanol (Sigma-Aldrich). 5 ml sodium methoxide was added to the sample and stirred. After 1 hour, 7 ml HCl, 6 ml hexane (Sigma-Aldrich, Chromasolv for HPLC≥97.0%) and 2 ml H_2O were added. The top layer was transferred into a new test-tube and evaporated. The FAMEs were analysed using a gas liquid chromatograph (GC – 2010 Shimadzu) fitted with flame ionization detector. The separation of methyl esters of fatty acids was effected on the capillary column Rt 2560 (100 m × 0.25 mm × 0.2 µm; Restek, Bellefonte, PA, USA) by temperature programming from 140°C to 240°C. The column was operated at 140°C for 5 min., then the temperature was increased to 240°C at 4°C/min. and held for 20 min. 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 1.06 ml/min. The peaks were identified by comparison with the retention times of the standard fatty acids methyl esters "37 Component FAME Mix" and trans FAME MIX k 110 (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.

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 Fernández et al. (2007). The peroxidizability index (PI) was determined according to Du et al. (2003).

Data analysis

The data were subjected to the analysis of variance in general linear (GLM Multivariate) procedure in SPSS 17 with Bonferroni's tests to determine the significance of differences of means between the groups. The differences were regarded as significant when $P \leq 0.05$.

Results

Roe deer neck and brisket muscles were characterized by a higher percentage (P≤0.05) of intramuscular fat (IMF) and a higher percentage of hydroxyproline in protein (P>0.05) than those of other muscles (Figure 1). The total proportions of saturated (SFA; P≤0.001), monounsaturated (MUFA; P≤0.01) and polyunsaturated (PUFA, P≤0.001) acids (Table 2), including many individual fatty acids (Table 1), were affected by the muscle. From SFA, stearic (C18:0) and palmitic (C16:0) acids were found to be dominant in the IMF of all muscles. The lowest contents of these fatty acids were detected in LD and hind quarter compared to other muscles. The lowest differences in the proportions of MUFA were between the LD and hind quarter muscles and the shoulder. Only the proportions of C16:1*n-7* in the hind quarter (P≤0.01) and C16:1*n-9* in the shoulder (P≤0.001) were significantly higher than those in LD. The highest proportions of total PUFA (37.40%) and many polyunsaturated fatty acids were found in LD. Notwithstanding higher proportions of many polyunsaturated fatty acids in LD muscle, the proportions of linolenic (C18:3*n-3*) fatty acid in LD were lower compared to the shoulder (P≤0.001) and neck (P≤0.05) muscles. The LD muscle also showed the lowest content of total *trans* fatty acid isomers compared to the other muscles (P≤0.05). The EPA+DHA sum varied in magnitude across the different muscles of the roe deer. The lowest PUFA/SFA ratios were found in the muscles of neck and brisket (Table 2). The muscle type of roe deer appeared to affect by a different level of significance the lipid quality indices (Table 2).

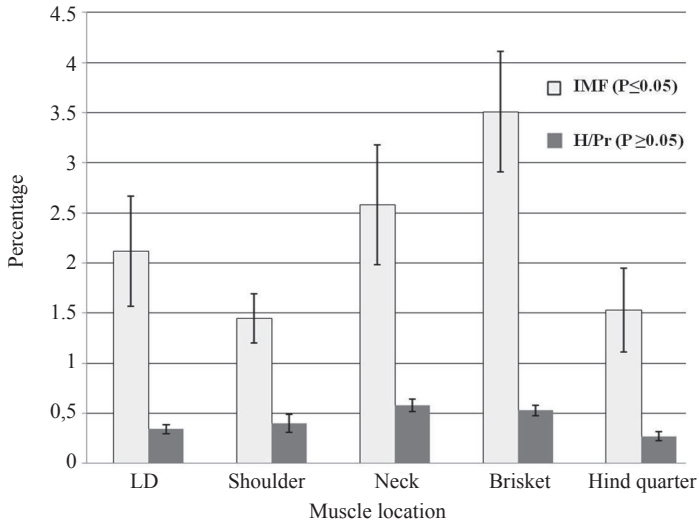

Figure 1. Percentage of intramuscular fat (IMF) and hydroxyproline in proteins (H/Pr) of muscles from different locations

Table 1. Effects of muscle location on fatty acid (% of total FA) composition in intramuscular fat of roe deer

Fatty acids	Muscle					SED	Significance
	LD n=8	shoulder (*m. deltoideus*) *n=8*	neck (*m. cleidocipitalis*) n=8	brisket (*m. intercostales interni*) n=8	hind quarter (*m. tensor fascia e latae*) n=8		
C14:0	0.61 a,c,e	1.03 d	0.93 b	1.05 f,d	0.65 c	0.10	***
C16:0	15.66	15.86	16.33	17.49	16.15	0.77	ns
C18:0	16.44 a,e	20.01 b	21.86 f,b	19.56 b	16.93 a	0.67	***
tC16:1*n-9*	0.57	0.65	0.70	0.69	0.66	0.05	ns
C16:1*n-9*	0.31 a,c,e	0.49 f	0.46 d	0.44 b	0.40	0.04	***
C16:1*n-7*	0.90 c,e	1.13	1.17	1.40 f,c	1.05 d	0.09	***
tC18:1*n-9*	0.87 a	1.35	1.48 b	1.35	0.98	0.18	**
C18:1*n-9*	14.33 a,c	16.71	19.66 b	21.60 d	16.83	1.67	**
C18:1*n-7*	1.64	1.78	1.79	1.63	1.66	0.09	ns
C20:1*n-9*	0.16	0.13	0.18	0.18	0.16	0.02	ns
tC18:2*n-6*	0.33 a,c	0.65	0.74 d	0.71 b	0.47	0.11	**
C18:3*n-6*	0.16	0.14	0.16	0.23	0.14	0.03	ns
C18:3*n-3*	3.46 e	4.52 a,c,f	4.07	3.74 d	3.83 b	0.20	***
C18:2*n-6*	16.09 a,c	14.18	12.28 b	11.47 b,d	15.00 a	1.06	***
C20:3*n-6*	0.65 e	0.39 f	0.36 f	0.37 f	0.53	0.05	***
C20:3*n-3*	0.58 a	0.25 b	0.26 b	0.25 b	0.33	0.09	**
C20:4*n-6*	9.98 a,e	6.90 b	5.34 a,f	5.74 f	8.54 b	0.93	***
C20:5*n-3*	2.31 a,e	1.38 f	1.36 a,f	1.41 f	1.95 b	0.19	***
C22:2*n-6*	0.09 a,c	0.01 b	0.00 d	0.01 b	0.04	0.02	**
C22:4*n-3*	0.37	0.23	0.25	0.15	0.40	0.10	ns
C22:5*n-3*	2.56 a,c,e	1.85 b	1.70 d	1.62 d,f	2.46 c	0.20	***
C22:6*n-3*	0.64 a	0.51	0.42 b	0.40 b	0.61 a	0.07	**

SED – standard error of difference; ns – not significant; a, b $^{*}P\leq0.05$; c, d $^{**}P\leq0.01$; e, f $^{***}P\leq0.001$.

Table 2. Total saturated, monounsaturated and polyunsaturated fatty acids, fatty acid ratios and lipid quality indexes in the intramuscular fat from roe deer males

Variables	Muscle					SED	Significance
	LD n=8	shoulder (*m. deltoideus)* n=8	neck (*m. cleidocipitalis*) n=8	brisket (*m. intercostales interni*) n=8	hind quarter (*m. tensor fascia e latae*) n=8		
SFA	34.11 a,c,e	38.68 b,c	41.03 f	39.95 b,d	35.25 a,d	1.39	***
MUFA	19.69 a,c	23.35	26.18 b	28.21 d	22.78	1.91	**
PUFA	37.40 c,e	31.18	27.10 d	26.26 a,f	34.46 b	2.42	***
PUFA/SFA	1.12 a,e	0.81 b	0.67 a,f	0.68 a,f	0.99 b	0.09	***
EPA+DHA	2.95 c,e	1.89 d	1.78 a,f	1.81 f	2.56 b	0.25	***
n-6/n-3	2.99	2.67	2.48	2.55	2.80	0.22	ns
TFA	1.78 a,c	2.65	2.93 d	2.74	2.12 b	0.30	**
Other FA	11.21e	9.68	8.33 a,f	8.37 a,f,c	10.11 b	0.40	***
AI	0.32 a	0.37	0.38	0.40 b	0.33	0.02	*
TI	0.63 a,c	0.76	0.84 b	0.85 b,d	0.65 a	0.06	***
h/H	3.43 a	3.03	2.92 b	2.82 b	3.22	0.17	**
PI	102.63 a,e	78.18 b	67.59 b,f	67.16 b,f	92.98 a	7.38	***

SED – standard error of difference; ns – not significant; a, b $^{*}P\leq0.05$; c, d $^{**}P\leq0.01$; e, f $^{***}P\leq0.001$. SFA, MUFA, PUFA = sum of all detected saturated, monounsaturated and polyunsaturated fatty acids, respectively. PUFA/SFA = ratio of ΣPUFA to ΣSFA, EPA+DHA=sum of C20:5*n-3* and C22:6*n-3*, *n-6/n-3* = ratio of Σ*n-6* PUFA to Σ*n-3* PUFA, TFA=sum of *trans* fatty acid isomers; Other FA: sum (2.41–2.88%) of excluded minor fatty acids C14:1, C15:0, C15:1, C17:0, C20:0, C22:0, C17:1*n-9* and C22:2*n-6* and sum of unindentified (5.58–8.81%) fatty acids. AI = atherogenic index, TI = thrombogenic index, h/H = hypocholesterolemic/hypercholesterolemic ratio, PI = peroxidizability index.

Discussion

There are many reports that game meat, including venison from roe deer, contains low amounts of IMF (Zomborszky et al., 1996; Hoffman and Wiklund, 2006; Ramanzin et al., 2010). Most of them presented the fat content in the muscles *longissimus* and *semitendinosus* (Ramanzin et al., 2010) or only in the *longissimus* muscle (Dannenberger et al., 2013). In the present study the contents of IMF in the *longissimus* muscle of roebucks hunted during summer hunting season (June-September) were higher than those from the roe deer hunted in Germany (Dannenberger et al., 2013). In addition, different IMF contents were measured in the roe deer hunted in different regions. The fact that these authors compared IMF contents of roe deer with farmed deer in the discussion shows that the information concerning this data of roe deer is scarce. In the present study muscle location showed higher impact on IMF than on hydroxyproline in proteins. Weiner (1973) has reported a considerable variability in the fat content of roe deer because roe deer may accumulate fat and utilize it in a period of food scarcity. The published information is available on the fatty acid composition of different muscles from the other species such as ostriches (Sales, 1998), horsemeat (Lorenzo and Pateiro, 2013) and different retail cuts of beef (Pavan and Ducket, 2013). However, there is no available information concerning the fatty acid composition in the different muscles of roe deer. There is some discrepancy between the data of this study from five different muscles of roe deer and six muscles of African Black ostriches reported by Sales (1998) who has found only the differences in individual fatty acids between the muscles, while the percentages of total SFA, MUFA and PUFA were quite similar. Unlike ostrich meat, muscle type affected most fatty acids in horsemeat (Lorenzo and Pateiro, 2013) and this is in agreement with the results obtained for roe deer in this study. With regard to SFA, palmitic acid is the predominant fatty acid in many different meats. In contrast to the fatty acid composition of pigs (Razmaitė and Švirmickas, 2012), wild boar (Razmaitė et al., 2012; Dannenberger et al., 2013), cattle (Vavrišinova et al., 2013), poultry, including chicken and goose (Pietras and Orczewska, 2013; Yanovych et al., 2013) and horses (Lorenzo and Pateiro, 2013), the intramuscular fat of all roe deer muscles has higher contents of stearic acid compared with the contents of palmitic acid. Kelly et al. (2001) and Tholstrup (2005) did not find dietary stearic acid to be more atherogenic and thrombogenic and consequently, even in respect of saturated fatty acid composition, roe deer meat in the diet is considered good for human health. The PUFA/SFA ratio minimum recommended for the human diet as a whole is 0.4 (Wood et al., 2004). The PUFA/SFA ratios in the intramuscular fat of all studied roe deer muscles were above this recommended minimum. There was a more favourable PUFA/SFA ratio in the IMF of LD compared with all other muscles. Although polyunsaturated fatty acids contribute importantly to average diets, the balance of *n-6* PUFA and *n-3* PUFA is quite different from that in the diets during human evolution (Cordain et al., 2002; Simopoulos et al., 2013). The current human diets are characterized by high *n-6* and low *n-3* PUFA intake (Givens et al., 2006; Simopoulos et al., 2013). The evidence that this imbalance contributes to diseases resulted in increased attention being devoted to increasing *n-3* PUFA in food sources (Lands and Lamoreaux, 2012).

In a review, Wood et al (2008) noticed that the nutritional advice for *n-6*/*n-3* PUFA ratio is lower than 4. Recommendations of Bellagio's report on healthy agriculture, healthy nutrition and healthy people indicated that the same ratio (4:1) of *n-6* PUFA to *n-3* PUFA in the diet should be the goal (Simopoulos et al., 2013). In the present study, in the IMF of different roe deer muscles, *n-6*/*n-3* PUFA ratios do not only meet these requirements, but also can improve the total diet. Recent reports have also demonstrated that humans hardly convert alpha linolenic acid into EPA and DHA and the conversion rate is very slow, thereby making EPA and DHA to be regarded as very essential (Hathwar et al., 2012). Although no differences were found in *n-6*/*n-3* PUFA ratios among the studied muscles, roe deer muscle affected the EPA+DHA proportion. However, the effects of dietary PUFA on regulation of lipid metabolism and prevention of cardiovascular disease (CVD) appear to be diverse. There is an opinion that the excessive intake of PUFA has undesirable effects, such as oxidative stress, because of high susceptibility to lipid peroxidation (Kang et al., 2005). The investigation of dietary PI value effects on serum lipid parameters and hepatic enzyme activities in rats showed that PUFA/SFA ratio of 1.0–1.5 and a PI value of 80–90 in the diet are within a favourable range to reduce the risk of CVD (Kang et al., 2005). In this respect, the most favourable PUFA/SFA ratio and the value of PI index could be found in the hind quarter muscles. It may be concluded that muscle location of roe deer appeared to affect the fatty acid composition and the lipid quality indices, such as atherogenic and thrombogenic indexes, and the hypocholesterolemic/hypercholesterolemic (h/H) ratio in the intramuscular fat. The highest levels of SFA and MUFA and the lowest levels of PUFA were found in the intramuscular fat of neck and brisket muscles and vice versa, the lowest levels of SFA and MUFA and the highest levels of PUFA, including EPA+DHA sum were found in the intramuscular fat of LD and hind quarter muscles. Significantly lower AI and TI indexes and higher and more favourable hypocholesterolemic/hypercholesterolemic ratio, respectively, were also found in the IMF of LD and hind quarter muscles.

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