COMPARATIVE CHARACTERIZATION OF FATTY ACID PROFILES IN INTRAMUSCULAR LIPIDS FROM DIFFERENT DOMESTIC AND WILD MONOGASTRIC ANIMAL SPECIES

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Summary. Three monogastric domestic and wild animal species: farmed Lithuanian indigenous wattle pigs (*Sus scrofa domestica*), wild boars (*Sus scrofa*) and beavers (*Castor fiber*) were tested, characterized and, compared by fatty acid composition in intramuscular lipids. Fatty Lithuanian indigenous wattle pigs had lower lipid unsaturation compared to observed wild species. Though the highest number of polyunsaturated fatty acids was identified in the intramuscular fat of wild boars, the muscle lipids of the beavers were characterized by the highest proportions of total polyunsaturated fatty acids (PUFA, 42.12% of total fatty acids) compared to 18.21% in wild boars and 7.36% in Lithuanian indigenous wattle pigs. Pigs and wild boars were found to be comparable in the relationships of the major fatty acids. Wild animals had more favourable polyunsaturated/saturated (PUFA/SFA), n-6/n-3 PUFA ratios and also atherogenic (AI) and thrombogenicity (TI) indexes. PUFA/SFA ratio in the intramuscular lipids from wild boars and beavers were higher and n-6/n-3 PUFA ratio in the beaver was lower than the reference PUFA/SFA and n-6/n-3 PUFA ratios pattern, respectively. Having predominant polyunsaturated fatty acids and favourable PUFA/SFA and n-6/n-3 PUFA ratios, wild game meat, particularly beaver meat could be the n-3 PUFA-rich food in human diets.
Keywords: fatty acids, muscles, pigs, wild boars, beavers.

ŪKINIŲ IR LAUKINIŲ RŪŠIŲ GYVŪNŲ SU VIENKAMERINIU SKRANDŽIU RAUMENŲ LIPIDŲ RIEBALŲ RŪGŠČIŲ PALYGINAMOJI CHARAKTERISTIKA

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Santrauka. Ištirta trijų rūšių gyvūnų, turinčių vienkamerinį skrandį – Lietuvos vietinių kiaulių (*Sus scrofa domestica*), šernų (*Sus scrofa*) ir bebrų (*Castor fiber*) – raumenų lipidų riebalų rūgščių sudėtis, pagal jų sudėtį charakterizuota ir palyginta šių skirtingų rūšių mėsa. Nustatyta, kad riebių Lietuvos vietinių kiaulių raumenyse sočiųjų riebalų rūgščių yra daugiau, negu laukinių gyvūnų raumenyse. Nors daugiausia įvairių polinesočiųjų riebalų rūgščių nustatyta šernienoje, didžiausia jų proporcijos dalis buvo bebrienoje – 42,12 proc. viso riebalų rūgščių kiekio. Tirtoje šernienoje polinesočiųjų riebalų rūgščių buvo 18,21 proc., o kiaulienoje – 7,36 proc. Pagrindinių riebalų rūgščių sudėtimi ir jų tarpusavio ryšiais šerniena visiškai panaši į kiaulieną, tačiau abiejų laukinių gyvūnų rūšių mėsoje nustatytas tinkamesnis vartotojų sveikatai polinesočiųjų/sočiųjų riebalų rūgščių ir n-6/n-3 polinesočiųjų riebalų rūgščių santykis, mažesni aterogeniškumo ir trombogeniškumo indeksai. Šernienoje nustatytas polinesočiųjų riebalų rūgščių ir sočiųjų riebalų santykis, o bebrienoje šis ir n-6/n-3 polinesočiųjų riebalų rūgščių santykis visiškai atitiko rekomenduojamas mitybos normas. Laukinių gyvūnų mėsa, ypač bebriena, gali papildyti žmonių maistą polinesočiosiomis riebalų rūgštimis ir vertingiausiomis iš jų n-3 polinesočiosiomis riebalų rūgštimis.
Raktažodžiai: riebalų rūgštys, raumenys, kiaulės, šernai, bebrai.

Introduction. The interaction of genetics and environment, nature and nurture is the foundation for all health and disease, and nutrition is an environmental factor of major importance. Whereas major changes have been placed in diet since the beginning of the Agricultural Revolution, human genes have not changed. Nowadays humans live in a nutritional environment that differs from that for which their genetic constitution was selected (Simopoulos, 2008).

Red meat is long established as an important dietary source of protein and essential nutrients including iron, zinc and vitamin B12, yet recent reports that its high consumption may increase the risk of cardiovascular disease (CDV) and other diseases have led to a negative perception of the role of red meat in health (McAfee et al., 2010). Despite the studies (Kontogianni et al., 2008) reporting an association between red meat and the risk of different diseases, there is evidence accumulating that meat itself is not a risk factor, but rather the risk stems from the consumption of processed meats (Micha et al., 2010), excessive fat and particularly saturated fat associated with the meat of domesticated animals (Mann, 2000).

Wild mammals have long been associated closely with human food production. Historically, some wild mammal species have provided humans with opportunities for domestication and other species, although never domesticated, can be used by humans to provide wild harvests (White and Lowe, 2008). Nowadays, consumers are increasingly concerned about game meat. Although the market for game products in recent years is very restricted, and dependent on the hunting season and the culling quotas, wild meat provides a niche in the contemporary food market (Soriano et al., 2006; Macmilan and Phillip, 2008). In recent years wild mammals represent only 2% of the herbivore biomass (Gorman and Raffaelli, 2008), therefore cognizance is also very important that among popular game species, such as wild boar, there are alternative species such as the beaver (*Castor fiber*), a semi-aquatic rodent whose meat has potential as a healthy food which complies with current healthy and dietary recommendations. In Lithuania, nowadays beaver population has increased considerably and needs to be controlled as it has become harmful to the environment (Ribikauskas, 2003). Beaver hunting and consumption should be used as a best population management tool.

The archaeological excavations and palaezoological studies have revealed that beaver bones are considered as refuse from human activity, in particular related to food consumption during Late Neolithic and confirms the importance of this species in the Narva and Bay Coast cultures (Stančikaitė et al., 2009). The subsequent decline in the size of beaver population had also resulted in the change of the consumption habits. However, hunters and consumers are increasingly interested in beaver meat. Wild boar, another species prevalent in the forests of Lithuania, is the most common terrestrial mammal since Neolithic period (Stančikaitė et al., 2009). Nowadays in Lithuania pork is the most widely produced and consumed meat. Unlike other domesticated animals, the porcine fat is not high in the muscles. Moreover, 70% of the pork's fat forms a subcutaneous layer (Bragagnolo and Rodriguez-Amaya, 2002), which can be removed before consumption.

The aim of this study was to characterize the species associated differences in the fatty acid profiles of intramuscular lipids and their quality indices.

Materials and Methods. Meat samples from three different monogastric animal species, Lithuanian indigenous wattle pigs (Sus scrofa domestica), wild boars (*Sus scrofa*) and beavers (Castor fiber) were used in the experiment. Domestic pigs were obtained from the Institute of Animal Science of the Lithuanian Veterinary Academy and reared indoors. Two genders (5 castrated males and 8 females) were represented by the domestic pigs. Each sex (entire males and females) was represented by wild boars and beavers. Wild boars were represented by 5 entire males and 3 females. The beavers were represented by 4 entire males and 4 females. All the samples of both wild species were obtained from local hunters. The samples of pigs and wild boars were excised from the *longissimus dorsi* (LD) at the 1-2 lumbar vertebra. The samples of beavers were excised from thigh muscles. All the samples were taken during winter season. The mean weight of young (6-7 months) pigs and wild boars (under 2 years of age), and adult beavers was, respectively, 89.6 kg (range 84-95 kg), 72.5 kg (range 70-80 kg) and 28.1 kg (range 21-35 kg).

The extraction of lipids for fatty acid analysis was performed with chloroform/methanol (2:1 v/v) as described by Folch et al. (1957). Fatty acid methyl esters (FAME) were prepared using the procedure of Christopherson & Glass (1969). 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 a ALLTECH capillary column AT Silar, 30 m x 0.32 mm x 0.25µm, by temperature programming from 100^{o}C to 240^{o}C. The rate of flow of carrier gas (nitrogen) through column was 0.33 ml/min. The column was operated at 100^{o}C for 4 min, then the temperature was increased to 240^{o}C at 3^{o}C/min and held for 10 min. The temperatures of the injector and detector were held, respectively, at 225^{o}C and 250^{o}C. The peaks were identified by comparison with the retention times of the standard fatty acids methyl esters FAME MIX (SUPELCO, USA). The relative proportion of each fatty acid was expressed as the relative percentage of the sum of the total fatty acids.

Lipid quality indices, i.e., atherogenic index (AI) and thrombogenenicity index (TI), were calculated according to Ulbricht and Southgate (1991).

AI=[(4×C14:0)+C16:0]/[n-6 PUFA+n-3 PUFA+MUFA];

TI=[C14:0+C16:0+C18:0]/[(0.5×MUFA)+(0.5×n-6 PUFA)+(3×n-3 PUFA)+n-3/n-6 PUFA].

The data were subjected to one-way analysis of variance (ANOVA) with Tukey's tests to determine the significance of differences of means between the groups. Differences were regarded as significant when $P<0.05$. In order to unravel the differences of fatty acid composition among species and visualize the relationships of fatty acids within species, principal component analysis was applied. The concentrations of the major fatty acids (threshold 1% of total fatty acids) were used to characterize the samples by principal component analysis (PCA). The PCA was performed on the symmetric correlation matrix. All the analyses were performed in MINITAB 15.

Results. A total number of 15, 16 and 22 fatty acids of various chain lengths and saturation levels were identified in the intramuscular fat of Lithuanian indigenous wattle pigs, wild boars and beavers, respectively (Table 1). Of the saturated fatty acids (SFA), palmitic acid (C16:0) and stearic acid (C18:0) were found to be the dominant ones in all species sampled. All the other SFA were minor components though only myristic (C14:0) comprised 1.21% in the pigs. Fatty Lithuanian indigenous wattle pigs had the highest SFA compared to the other observed species ($P<0.001$). The highest SFA levels in the pigs were attributed mainly to the highest levels of C14:0, C16:0 and C18:0 in comparison with the other species. The lowest SFA levels in the beavers were attributed to the lowest levels of C16:0 and C18:0.

The oleic acid (C18:1n-9) was the major monounsaturated fatty acid (MUFA) in all species, with C16:1n-7 comprising 2.63-3.82% of total fatty acids. All the species analysed in the present study contained low amounts of gandoic (C20:1n-9) and heptadecenoic (C17:1) acids. As far as the comparative MUFA levels among the three species are concerned, there were differences, specifically in oleic acid (C18:1n-9). The proportion of C18:1n-9 in the beavers was relatively by 70.3 % and 100.6% lower than in the wild boars and pigs, respectively (P<0.001). Also, the beavers contained the lowest levels of all identified MUFA (P<0.001).

Table 1 **Fatty acid composition** (% total fatty acids) **of intramuscular lipids in different species** (Mean±SD)

Fatty acids	Lithuanian indigenous wattle pig (n=13)	Wild boar (n=8)	Beaver (n=8)	P-value
C14:0	1.21±0.13	0.93±0.10	0.99±0.16	<0.0001
C15:0	nd	0.76±0.55	0.95±0.14	0.382
C16:0	26.39±1.26	24.03±1.25	21.56±3.28	<0.0001
C17:0	0.13±0.02	0.39±0.26	0.74±0.37	<0.0001
C18:0	11.63±0.81	10.25±0.72	6.21±1.14	<0.0001
C20:0	0.15±0.01	0.06±0.07	0.38±0.27	<0.0001
SFA	39.51±1.71	36.42±0.78	30.82±4.05	<0.0001
C15:1	nd	0.13±0.25	nd	
C16:1n-7	3.82±0.28	3.37±0.39	2.63±0.97	<0.0001
C17:1	0.16±0.03	0.30±0.14	0.13±0.19	0.017
C18:1n-9	48.36±1.34	41.07±2.88	24.11±5.17	<0.0001
C20:1n-9	0.56±0.08	0.48±0.07	0.18±0.13	<0.0001
MUFA	52.89±1.46	45.36±3.02	27.05±5.80	<0.0001
C16:2n-4	nd	0.10±0.09	1.73±0.30	<0.0001
C18:2n-6	6.16±1.45	12.04±1.73	21.04±3.17	<0.0001
C18:3n-3	0.22±0.03	0.42±0.07	16.78±4.40	<0.0001
C20:2n-6	nd	0.30±0.03	nd	
C20:3n-3	0.22±0.04	0.31±0.08	0.24±0.25	0.401
C20:4n-6	0.75±0.34	2.82±0.89	1.69±1.00	<0.0001
C20:5n-3	nd	0.38±0.20	nd	
C22:2	nd	0.36±0.75	nd	
C22:4n-6	nd	0.43±0.18	nd	
C22:5n-3	0.17±0.12	0.84±0.41	nd	
C22:6n-3	0.08±0.12	0.22±0.32	0.64±0.42	<0.0001
PUFA	7.61±1.93	18.21±3.71	42.12±4.59	<0.0001

nd, not detected

Though the highest number of polyunsaturated fatty acids was identified in the intramuscular fat of wild boars, the muscle lipids of the beavers were characterized by the highest proportions of total polyunsaturated fatty acids (PUFA, 42.12% of total fatty acids) compared to 18.21% in wild boars and 7.61% in Lithuanian indigenous wattle pigs (P<0.001). The highest PUFA levels in the beavers were attributed mainly to the highest levels of essential fatty acids linoleic (C18:2n-6), and specifically of linolenic (C18:3n-3) in comparison with the other species. The beavers had a relatively 3.4 and 1.95 times higher level of C18:2n-6, and also even 76.3 and 39.9 times higher level of C18:3n-3 compared to the pigs and wild boars, respectively (P<0.001). The beavers also had relatively 2.9 and 8 times higher level of DHA (docosahexaenoic acid C22:6n-3) in comparison with the wild boars and pigs, respectively (P<0.001). However, wild boars had relatively 3.8 times and 1.7 times higher level of a very important arachidonic fatty acid (C20:4n-6) than the pigs and the beavers. As a result, the beavers had the highest PUFA/SFA and the lowest n-6/n-3 PUFA ratios (P<0.001; Table 2).

According to the relative contents of particular groups of fatty acids, the beaver muscles showed the lowest atherogenic index (AI) and thrombogenicity index (TI) in comparison with the observed muscles of the pigs and wild boars (P<0.001; Table 2).

Table 2 **Fatty acid ratios, atherogenic index** (AI) **and thrombogenicity index** (TI) **in the muscles of different domestic and wild species** (Mean±SD)

Specification	Lithuanian indigenous wattle pig (n=13)	Wild boar (n=8)	Beaver (n=8)	P-value
PUFA/SFA	0.19±0.05	0.50±0.11	1.39±0.27	<0.0001
n-6/n-3	10.32±2.15	8.46±3.27	1.29±0.34	<0.0001
AI	0.52±0.04	0.44±0.03	0.37±0.07	<0.0001
TI	1.23±0.10	0.95±0.10	0.35±0.08	<0.0001

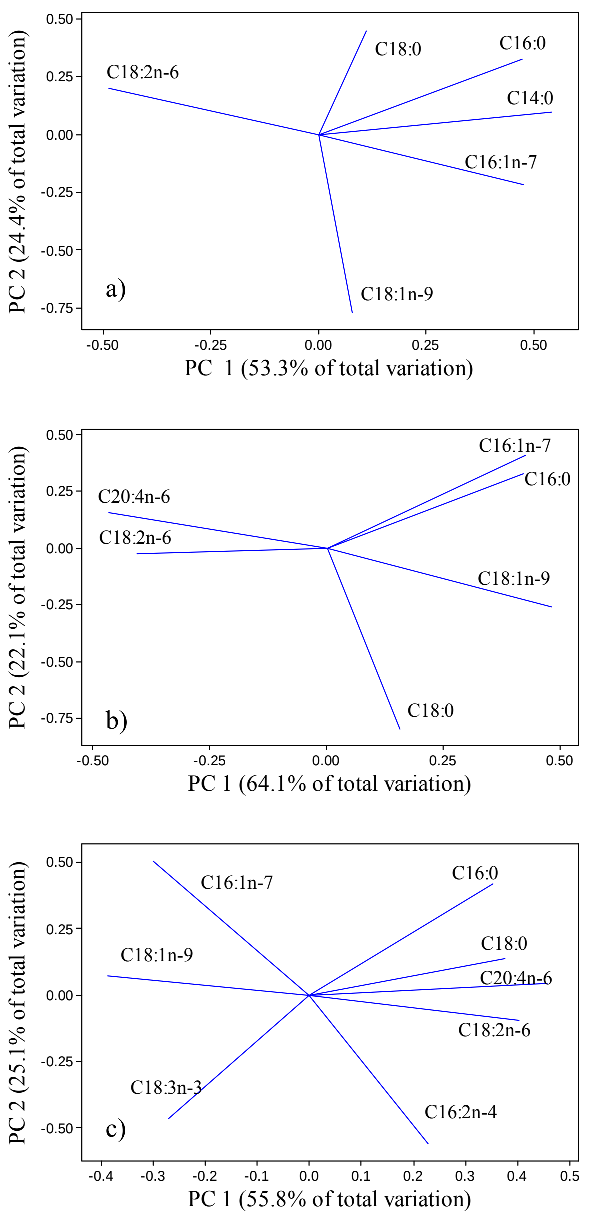

Figure 1 **Loading plots describing the relationships among fatty acids in intramuscular lipids of Lithuanian indigenous wattle pigs** (a)**, wild boars** (b) **and beavers** (c)

The principal component analysis was applied for the purpose of generating a reduced set of variates that account for the most of the variability in the original data, and that can be used in more substantial subsequent analysis. Since retention of the principal components is based on PCs with eigenvalues >1, three and two principal components (PCs) fit these criteria for domestic pigs and for wild species, respectively. Three principal components achieved for domestic pigs explained 94.8% of the total variance. Principal component 1 (PC1), with 53.3% of the total variance explained, was correlated with fatty acids C14:0 (0.541), C16:0 (0.474), palmitoleic (C16:1n-7) (0.475) and C18:2n-6 (-0.488). Principal component 2 (PC2), with 24.4% with the total variance explained, was correlated with fatty acids C18:0 (0.450) and C18:1n-9 (-0.770). Principal component 3 (PC3), with 17.1% of the total variance explained, was correlated with C18:0 (0.788).

Two principal components achieved for wild boars and beavers explained 86.1% and 81.0% of the total variance, respectively. For wild boars PC1, with 64.1% of the total variance explained, was correlated with C16:0 (0.421), C16:1n-7 (0.426), C18:1n-9 (0.482), C18:2n-6 (-0.408) and C20:4n-6 (-0.468). PC2, with 22.1% with the total variance explained, was correlated with C18:0 (-0.795) and C16:1n-7 (0.409).

For beavers PC1, with 55.8% of the total variance explained, was correlated with C18:2n-6 (0.402) and C20:0n-6 (0.457), and PC2, with 25.1% with the total variance explained, was correlated with C16:0 (0.421), C16:1n-7 (0.506), C16:2n-4 (-0.560) and C18:3n-3 (-0.467). Loading plots for PC1 versus PC2 were displayed for each species in order to visually evaluate the relationships of the major fatty acids (Fig. 1).

Discussion and Conclusions. The species is the major source of variation in meat fatty acid composition with ruminant meats being more saturated compared to the meat of monogastric animals (De Smet et al., 2004). The monogastric species are amenable to changes in the fatty acid composition of meats using different diets. Studies describing the effects of different factors on the fatty acid composition in the pork have shown that the major factors are the total amount of fat and nutrition (De Smet et al., 2004; Wood et al., 2008). Gender differences have also been examined by different authors for pork fatty acid composition (Högberg et al., 2004; Andersson et al., 2005; Razmaitė et al., 2008; Alonso et al., 2009; Razmaitė and Švirmickas, 2010). Lithuanian indigenous wattle pigs are fatty pigs (Razmaitė and Kerzienė, 2009) reared using concentrate feeds. Two genders (castrated males and females) were represented by domestic pigs. Each sex (entire males and females) was represented by wild boars and beavers. Game animals such as wild boars eat a great variety of indigenous plants, grains, seeds, roots, fruits, insects, earthworms, slugs and small mammals, and carrion (Schley and Roper, 2003) and, also, their males are entire. The nutrition basis of the Eurasian beaver could be composed of about 200 plant species (Simonavičiūtė and Ulevičius, 2007). Consequently, it is impossible to establish the influence of the diet on the lipid composition of meat in the studied species, and their characterization is based on the muscle lipid composition in their habitual environment. In this study the composition of muscle lipids of the studied monogastric animals was different from each other. Unsaturation in the muscle lipids of Lithuanian indigenous wattle pigs in this study was lower compared to modern commercial crossbreds described by Alonso et al. (2009). In the present study higher PUFA/SFA, lower n-6/n-3 PUFA ratios and lower atherogenic and thrombogenicity indexes in the muscles of wild boars was a not surprising fact as Koizumi et al. (1991) have reported that the meat of wild fed animals and wild boar is better for consumer health than the meat from pen fed animals. Meyer et al. (1998) also reported that the depot fat of wild boar showed the highest content of essential fatty acids compared with ruminant species. However, the fact that PUFA levels in the beavers comprised such high proportions of PUFA was surprising as in general fish are known to contain such high levels of PUFA, but not mammals. These results are in agreement with the findings of Käkelä and Hyvärinen (1996); Martysiak-Žurowska et al. (2009) and Zalewski et al. (2009). However, most of these studies were related to the fatty acid compositions of the adipose tissues in the extremities and metabolic lipids of the liver of beavers in comparison with the muskrat, badger and racoon dogs and aimed at clarifying the effects on peripheral heterothermy and fatty acid metabolism. However, there is no sufficient information about the nutritional value of muscle lipids from beavers in Lithuania. PUFA/SFA ratio of 0.5 in the muscle lipids of young wild boars was higher than the minimum recommended value (0.45) for the diet as a whole (Wood et al., 2004). This ratio in the muscle lipids of the beavers was even treble higher than recommended and could serve for human diet balance as well as n-6/n-3 PUFA ratio which was 6 times more favourable than in the muscles of wild boars and 11 times more favourable than in the muscles of farmed pigs. Only three of the SFA are in fact hypercholesteremic, the PUFA/SFA ratio is not a very suitable measure of the atherogenicity or thrombogenicity of the foods (Ulbricht and Southgate, 1991). Consequently, lipid quality indicators that depend on the relative contents of particular groups of fatty acids are atherogenic index (AI) and thrombogenicity index (TI), which indicate the global dietetic quality of lipids. Therefore, their potential effect on the development of coronary diseases (Ulbricht and Southgate, 1991; Jankowska et al., 2010) was calculated. In the current study these indicators showed the superiority of meats of the hunted wild species and particularly of the beavers. However, the atherogenic index of intramuscular lipids in all the species in this study is consistent with the AI observed in the perch by Jankowska et al. (2010). However, the thrombogenicity index of intramuscular lipids was higher than in the above mentioned fish and narrowly higher than of the olive oil (Ulbricht and Southgate, 1991).

Szabó et al. (2007) reported that the wild and domestic form of *Sus scrofa* was found to deposit totally identical acylglycerols in the storage lipids. In this study the chemometric data combined with the principal component analysis unravel similarities in the relationships among major fatty acids between the pigs and wild boars and differences between the beavers and *Sus scrofa* (wild boars and pigs).

On the basis of the results from this study, it can be concluded that red meat separated from fat qualitatively to food safety, is good as a source having low atherogenic index. Fatty acid composition in the muscles of wild hunted species (wild boar and beaver) showed superiority over farmed pigs. Having predominant polyunsaturated fatty acids and favourable PUFA/SFA and n-6/n-3 PUFA ratios, wild game meat, particularly beaver meat, could be the n-3 PUFA-rich food in human diets. In Lithuania, nowadays considerably increased beaver population has needs to be controlled as it has become harmful to the environment. Beaver hunting and consumption, not extirpation, as a management tool should be used for population control.

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