COMPARISON OF FATTY ACID COMPOSITION IN DIFFERENT PIG TISSUES

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Abstract. The aim of this study was to characterize the tissue-associated differences in the fatty acid profiles of intramuscular, subcutaneous and hepatic lipids and their quality indices from Lithuanian indigenous wattle pig and wild boar backcross to lean breed. The samples from hybrid pigs (1/4 Lithuanian indigenous wattle, 1/4 wild boar and 1/2 Landrace) were used in the experiment. There was little evidence that the lipids of longissimus muscle from hybrid pigs had a lower content of saturated fatty acids (SFA) and a higher content of monounsaturated fatty acids (MUFA) compared to subcutaneous fat. Hepatic lipids had the highest content of SFA and even 3.4 times higher content of polyunsaturated fatty acids (PUFA) and about treble lower content of MUFA compared to intramuscular and subcutaneous lipids. The principal component analysis (PCA) also unravelled clear differences of fatty acid composition among tissues. Having predominant polyunsaturated fatty acids and favourable PUFA/SFA ratio and superior n-6/n-3 PUFA ratio to that in muscle and subcutaneous tissues, liver could be the PUFA, including n-3 PUFA-rich food in human diets. Atherogenic index (AI) and thrombogenenicity index (TI) of liver are lower, and hypocholesterolemic/Hypercholesterolemic ratio (h/H) is desirably higher compared to muscle and subcutaneous fat. The lipid quality indices in the muscle and subcutaneous tissue showed the similar risks and benefits associated with the tissue accordingly to recommended lower fats consumption, as a part of a balanced diet.

Keywords: fatty acids, intramuscular, subcutaneous, hepatic, lipids, pigs.

SKIRTINGŲ KIAULIŲ AUDINIŲ RIEBALŲ RŪGŠČIŲ SUDĖTIES PALYGINIMAS

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Santrauka. Tyrimų tikslas buvo įvertinti hibridų (1/4 Lietuvos vietinių, 1/4 šerno ir 1/2 landrasų) skirtingų audinių (ilgiausiojo nugaros raumens, poodinių riebalų ir kepenų) lipidų riebalų rūgščių sudėtį. Hibridų raumenyse esančiuose riebaluose nustatyta mažiau sočiųjų ir daugiau mononesočiųjų riebalų rūgščių negu poodiniuose riebaluose (lašiniuose). Riebalų rūgščių skirtumus įvairiuose kiaulių audiniuose pademonstravo pagrindinių komponenčių analizė. Skirtingų audinių riebalų kokybės rodikliai, nustatyti raumeniniame ir riebaliniame audiniuose, parodė gana panašios rizikos sveikatai laipsnį vartojant rekomenduotiną mažesnį riebalų kiekį. Nors kepenų riebaluose nustatytas didžiausias sočiųjų riebalų rūgščių kiekis, jose buvo net 3,4 karto daugiau ir polinesočiųjų riebalų rūgščių negu raumenyse ir lašinių riebaluose. Kepenyse nustatytas tinkamesnis vartotojų sveikatai polinesočiųjų/sočiųjų riebalų rūgščių ir n-6/n-3 polinesočiųjų riebalų rūgščių santykis, todėl kepenys gali papildyti žmonių mitybą tiek polinesočiosiomis riebalų rūgštimis, tiek ir vertingiausiomis iš jų n-3 polinesočiosiomis riebalų rūgštimis. Kepenų riebalų kokybės rodikliai pagal atskirų riebalų rūgščių proporcijas, rodantys riebalų aterosklerozines, trombogenines savybes, hipocholesteroleminių ir hipercholesteroleminių savybių santykį, buvo geresni negu raumenų ir lašinių.

Raktažodžiai: riebalų rūgštys, raumenų, lašinių, kepenų riebalai, kiaulės.

Introduction. Meat continues to be an important food group in the diet for many consumers, particularly in the developed world. Foods derived from meat make a major contribution to intake of essential nutrients. Diet is one of the modifiable risk factors for cardiovascular disease, coronary heart disease and cancer. Despite the studies reporting an association between red meat and the risk of diseases, several methodological limitations and inconsistencies were identified. A substantial amount of evidence suggests that it is not the meat per se, but high-fat diets and an increase in saturated fatty acids that promote cardiovascular disease and carcinogenesis (Givens et al., 2006; McAfee et al., 2010; Ferguson, 2010). Pork is the most widely produced and consumed meat and has an important role in the economics of many countries. Unlike other domesticated animals, the pork fat is not high in the meat. Of the pork fat, 70% forms a subcutaneous layer, which can be removed before consumption (Bragagnolo and Rodriguez-Amaya, 2002). However, fat contributes succulence to pork cuts and many manufactured meat products, therefore total removal of carcass fat is undesirable (Darling et al., 1998). Although the consumer demand for leaner pork has increased, all parts of pig, including by-products, are edible. Most often meat and other products from slow growing, fat local pig breeds are found to be of higher eating quality compared to conventional European genotypes (Estévez et al., 2004; Bonneau and Lebret, 2010; Stimbirys and Jukna, 2010). By eating quality the authors mean the sensory attributes of pork or the main physical and biochemical parameters (Bonneau and Lebret, 2010).

The high quality of meat and lipids of wild boar and their hybrids with domestic pigs (Marchiori et al., 2003; Marsico et al., 2007; Razmaitė and Urbšienė, 2009; Razmaitė and Švirmickas, 2010; Razmaitė et al., 2011) are thought to be essential factors in the quality of meat and by-products from wild boar hybrid backcross to lean domestic pigs. Liver, one of by-products, is widely used in Lithuania and some other European countries, such as Finland, France and Spain for different liver products (Estévez et al., 2004; Estévez et al., 2005)

The aim of this study was to characterize the tissue associated differences in the fatty acid profiles of intramuscular, subcutaneous and hepatic lipids and their quality indices from Lithuanian indigenous wattle pig and wild boar backcross to lean Landrace breed.

Materials and methods. The samples of three different tissues (longissimus muscle, subcutaneous lipid and liver) from hybrid pigs (1/4 Lithuanian indigenous wattle, 1/4 wild boar and 1/2 Landrace) were used in the experiment. Hybrid pigs were reared indoors from birth to slaughter consuming twice a day the same standard concentrate feed, containing 12.28 MJ metabolisable energy and 14.49% crude protein balanced with lysine (0.80%/kg feed). The animals were slaughtered when they reached approximately 90 kg weight**.** Three genders (2 castrated, 2 entire males and 2 females) were represented by the experimental pigs. The samples of liver were taken from the dexter lateral lobe within 5 min after scalding of slaughter animals. The muscle and subcutaneous fat samples were taken from chilled carcasses after 24 hours. The muscle samples were excised from the *longissimus dorsi* (LD) at the 1-2 lumbar vertebra. Also, samples from subcutaneous fat were taken at the same lumbar vertebra. The extraction of lipids for fatty acid analysis was performed with a blend of chloroform and 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. The temperature program and operating conditions were as follows: 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 rate of flow of carrier gas (nitrogen) through column was 0.33 ml/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]. In the present study, C12:0 was not detected and was not included into calculations.

Hypocholesterolemic/Hypercholesterolemic ratio (h/H) was calculated according to Fernandez et al. (2007). h/H=(C18:1+C18:2+C18:3+C20:3+C20:4+C20:5+C22:4 +C22:5+C22:6)/(C14:0+C16:0).

The data were subjected to one-way analysis of variance (ANOVA) with Tukey's tests to determine the significance of the differences of means between the groups. The differences were regarded as significant when $P<0.05$. In order to unravel the differences of fatty acid composition among tissues, the principal component analysis (PCA) on all samples and variables was applied as a comparative evaluation. All the analyses were performed in MINITAB 15.

Results. A total number of 22, 21 and 20 fatty acids of various chain lengths and saturation levels were identified, respectively, in the intramuscular fat of the longissimus muscle, subcutaneous lipid tissue and liver of hybrid pigs (Table 1). Intramuscular fat (IMF) of the longissimus muscle had the lowest compared to the highest liver lipid content of total saturated fatty acids (SFA; $P<0.010$) Of the SFA, palmitic acid (C16:0) and stearic acid (C18:0) were found to be the dominant ones in all tissues sampled. Palmitic acid (C16:0) was found to be the dominant one in the longissimus muscle and subcutaneous lipid sampled, however, stearic acid (C18:0) was found to be the dominant one in the liver samples. The proportion of C16:0 in the liver was relatively by 51.2% and 57.1% lower than in the subcutaneous and intramuscular lipid tissues, respectively ($P<0.001$). The proportion of C18:0 in the liver was relatively 2 and 2.6 times higher than in the subcutaneous and intramuscular lipid tissues, respectively ($P<0.001$). Also, the liver tissue contained the lowest level ($P<0.001$) of myristic acid (C14:0) which was even approximately by fivefold lower compared to intramuscular and subcutaneous lipid tissues.

Oleic acid (C18:1n-9) was the major monounsaturated fatty acid (MUFA) in all studied tissues, comprising from 15.2% in liver lipids to 46.14% in IMF of total fatty acids. The proportion of total MUFA in the liver lipids was approximately three times lower than in the IMF and subcutaneous fat ($P<0.001$). Also, the liver contained the lowest levels of all identified MUFA ($P<0.001$), excluding unusual odd pentadecenoic acid (C15:1).

The liver lipids were characterized by the highest proportions of total polyunsaturated fatty acids (PUFA, 41.03% of total fatty acids) compared to 12.14% in the subcutaneous fat and 12.05% in IMF ($P<0.001$). The highest PUFA levels in the lipids of liver were attributed to the highest levels of nine fatty acids of all ten identified polyunsaturated fatty acids. The liver lipids had a relatively 77.9% and 53.7% higher level, and also 39.5% higher but 71.7% lower level of essential linoleic (C18:2n-6) and linolenic (C18:3n-3) fatty acids compared to these acid levels in IMF and subcutaneous tissue, respectively ($P<0.001$). The liver lipids were also characterized as having almost 12 times and even 63.3

times higher proportions of arachidonic acid (C20:4n-6) compared to the IMF and subcutaneous tissue, respectively (P<0.001). The liver lipids also had much higher proportions of all the other observed n-6 and n-3 PUFA. The content of EPA (C20:5n-3) in the liver lipids was relatively 10 and 18 times higher than in the IMF of the longissimus muscle and subcutaneous tissue, respectively (P<0.001). The liver lipids had a relatively 8.1 times and even 41.7 times higher level of DPA (C22:5n-3) in comparison with the IMF and subcutaneous fat, respectively (P<0.001). The level of DHA (C22:6n-3) in liver lipids was 15.1 times higher compared to IMF (P<0.001). As a result, the liver lipids had the highest PUFA/SFA and lowest n-6/n-3 PUFA ratios (P<0.001; Table 2). The highest n-6/n-3 PUFA ratio was in IMF.

Table 1 **Fatty acid composition** (% total fatty acids) **in different tissues from hybrid pigs**

Fatty acids	Longissimus muscle	Subcutaneous tissue	Liver	P-value
C14:0	1.08±0.17	1.10±0.14	0.22±0.07	<0.0001
C15:0	0.18±0.28	0.02±0.04	nd	0.166
C16:0	25.48±2.15	24.53±2.38	16.22±1.29	<0.0001
C17:0	0.38±0.29	0.35±0.12	1.02±0.13	<0.0001
C18:0	9.67±0.80	12.54±1.63	25.10±1.00	<0.0001
C20:0	0.06±0.07	0.17±0.03	nd	<0.0001
SFA	36.84±1.76	38.72±3.84	42.56±0.89	0.004
C15:1	0.38±0.30	0.02±0.04	0.08±0.06	0.006
C16:1n-7	3.69±0.57	2.31±0.35	0.71±0.08	<0.0001
C17:1	0.28±0.29	0.36±0.15	0.20±0.02	0.379
C18:1n-9	46.14±3.61	45.51±2.02	15.20±1.84	<0.0001
C20:1n-9	0.62±0.13	0.94±0.07	0.22±0.05	<0.0001
MUFA	51.11±3.51	49.14±2.41	16.41±1.91	<0.0001
C16:2n-4	0.15±0.13	0.01±0.03	nd	0.007
C18:2n-6	8.75±2.87	10.13±1.65	15.57±1.94	<0.0001
C18:3n-3	0.43±0.14	1.03±0.30	0.60±0.12	<0.0001
C20:2n-6	0.25±0.05	0.46±0.07	0.57±0.04	<0.0001
C20:3n-3	0.19±0.05	0.09±0.02	0.70±0.13	<0.0001
C20:4n-6	1.48±0.66	0.28±0.10	17.72±1.15	<0.0001
C20:5n-3	0.09±0.10	0.05±0.08	0.90±0.12	<0.0001
C22:2n-6	0.06±0.09	0.01±0.03	0.10±0.12	0.281
C22:4n-6	0.23±0.15	0.02±0.04	0.88±0.19	<0.0001
C22:5n-3	0.36±0.26	0.07±0.03	2.92±0.26	<0.0001
C22:6n-3	0.07±0.11	nd	1.06±0.19	<0.0001
PUFA	12.05±4.36	12.14±2.02	41.03±1.63	<0.0001

nd, not detected (<0.01)

Table 2 **Fatty acid and hypocholesterolemic/Hypercholesterolemic** (h/H) **ratios, atherogenic index** (AI) **and thrombogenicity index** (TI) **in different tissues from hybrid pigs**

Specification	Longissimus muscle	Subcutaneous tissue	Liver	P-value
PUFA/SFA	0.33±0.13	0.32±0.09	0.96±0.04	<0.0001
Σn-6/Σn-3	10.24±2.71	8.97±1.16	5.66±0.57	0.001
AI	0.47±0.06	0.48±0.07	0.30±0.03	<0.0001
TI	1.06±0.13	1.14±0.20	0.94±0.04	0.063
h/H	2.19±0.27	2.26±0.37	3.40±0.32	<0.0001

According to the relative contents of the particular groups of fatty acids, the liver lipids showed the lowest atherogenic (AI; P<0.001) and thrombogenic (TI; P=0.063) indexes in comparison with the observed IMF of the longissimus muscle and subcutaneous fat (Table 2). Also, the highest h/H ratio was at a advantage compared to the IMF of the longissimus muscle and subcutaneous fat.

Since retention of the principal components is based on PCs with eigenvalues >1, four principal components (PCs) fit these criteria for the fatty acids (Fig. 1). The first two principal components for 78.5% of the total variation and a plot of the scores is shown in Figure 2. In the PCA plot for fatty acids in different tissues, the scores for these tissues were grouped separately. The scores for the subcutaneous tissue and muscle were grouped to the left, while the scores for liver were grouped to the right along principal component PC 1, which explained 63.1% of the accounted variation. Along principal component PC 2, which explained 15.4% of the accounted variation, the scores for the subcutaneous tissue were grouped on the positive side, while muscle IMF were grouped on the negative side.

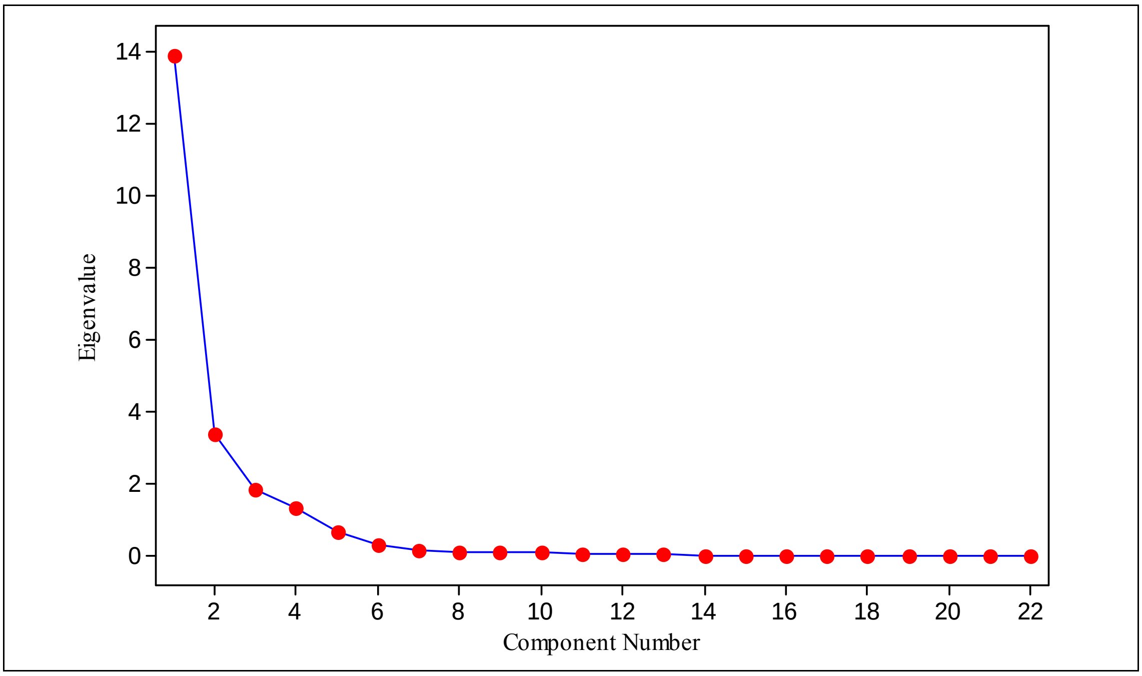

Fig. 1. **PCA screen plot of fatty acids on all samples and variables from different tissues**

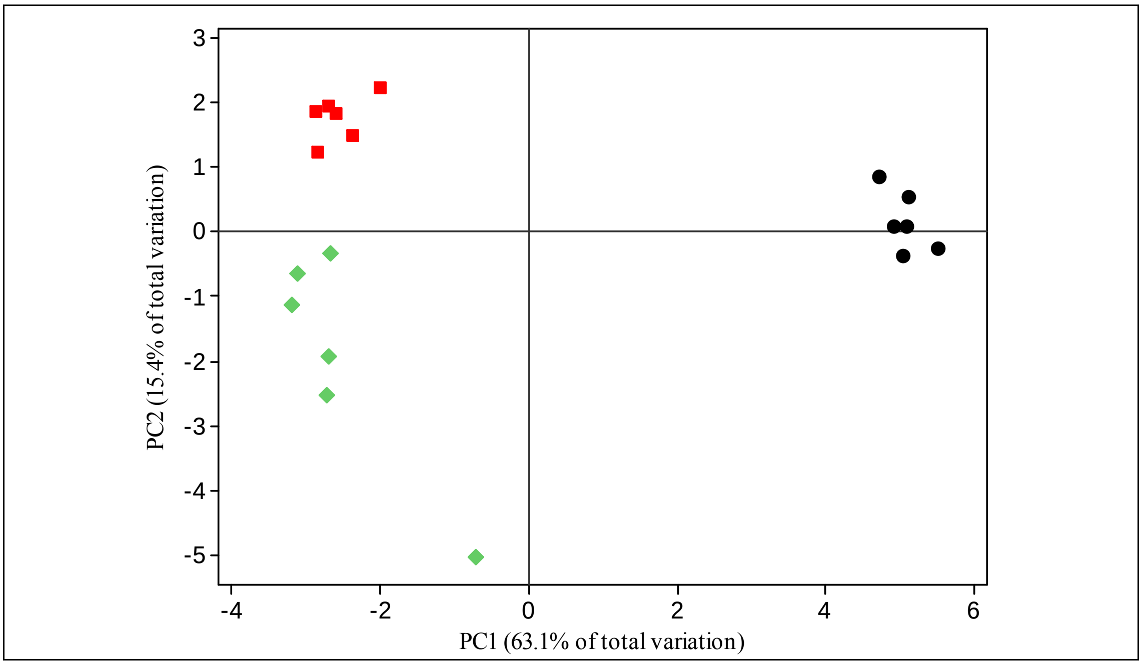

Fig. 2. **PCA plot of scores in lipids illustrating tissue differences between longissimus muscle** (green rhombus) **and subcutaneous fat** (red squares) **and liver** (filled dots)

Discussion and conclusions. Different studies have shown that feeding of unsaturated fat and use of probiotics in the diet of pigs (Enser et al., 2000; Matthews et al., 2000; Estévez et al., 2004; Martin et al., 2008) or extensive rearing (Estévez et al., 2004) result in increase of PUFA content and decrease of n-6/n-3 ratios in different tissues. The present study was designed to compare fatty acid profiles in different tissues of 1/4 wild boar hybrid pigs reared indoors and consuming the same standard concentrate feed. In this study higher levels of C18:2n-6 and C18:3n-3 in the subcutaneous tissue than in the muscle is consistent with the results of Enser et al.

(2000). However, these authors reported almost similar levels of EPA and DHA. In the present study DHA was not detected in the subcutaneous tissue and the level of EPA in the subcutaneous tissue was lower compared to the muscle IMF. Much higher levels of PUFA and lower levels of MUFA in hepatic lipids compared to the muscle and subcutaneous tissue are in agreement with other authors (Estévez et al., 2004; Martin et al., 2008; Parra et al., 2010). However, in the present study differences between the proportions of MUFA and PUFA in hepatic lipids were higher than in the above mentioned studies on Iberian pigs. Higher proportion of C18:0 and lower proportion of C16:0 in hepatic lipids in the present study is in agreement with the data of other authors (Enser et al., 2000; Estévez et al., 2004). Stearic acid (C18:0) has been found to be controversial as associated with CHD risk, although others argue that a distinction from SFA should be made for stearic acid which has been found to have only little cholesterol raising effects in humans (McAfee et al., 2010).

Increased consumption of animal products has occurred with a consequent increase in fat content. Due to the composition of many animal fats, their increased consumption has been associated with an increase in dietary ratio of n-6/n-3 PUFA. The concerns about excess saturated fat and a deficiency of n-3 fatty acids in the human diet has led to the recommendations that the ratio of polyunsaturated fatty acids were increased to 0.4 or higher and that the ratio of n-6/n-3 PUFA in the diet were lowered to the ratio of less than 4 (Enser et al., 2000; Wood et al., 2004). The most obvious difference in fatty acid composition was that C18:2n-6 and the total PUFA were highest in the liver, causing superior hepatic PUFA/SFA and n-6/n-3 PUFA ratios. However, in the present study PUFA/SFA ratio in the muscle was lower than recommended and lower compared to extensively and intensively reared Iberian pigs (Estévez et al., 2004). Although PUFA/SFA ratio in the subcutaneous tissue also was lower than recommended, it was higher compared to extensively reared Iberian pigs. PUFA/SFA ratio in the liver was 2.4 times higher than the recommended minimal ratio and higher compared to Iberian pigs (Estévez et al., 2004). In this study, the n-6/n-3 PUFA ratios in the muscle and subcutaneous tissue remained high. However, the n-6/n-3 PUFA ratio in the liver was close to the recommended level. Lipid quality indices, AI and TI calculated from the fatty acid proportions in the liver showed the lowest and highest h/H values what is most desirable from the consumer health perspective. Moreover, high nutritional value of the liver shows relatively high contents of C20:4n-6, C22:5n-3 and C22:6n-3 which is of particular importance for human nutrition (Wood et al., 2008; Hoffman et al., 2009).

It can be concluded, that there is little evidence that lipids of the longissimus muscle from hybrid pigs had a lower content of SFA and a higher content of MUFA compared to the subcutaneous fat. Hepatic lipids had the highest content of SFA and even 3.4 time higher content of PUFA and about treble lower content of MUFA compared to intramuscular and subcutaneous lipids. The principal component analysis (PCA) also unraveled clear differences of fatty acid composition among tissues. Having predominant polyunsaturated fatty acids and favourable PUFA/SFA ratio and superior n-6/n-3 PUFA ratio to that in muscle and subcutaneous tissues, liver could be the PUFA-rich food in human diets. The lipid quality indices in the muscle and subcutaneous tissue show similar risks and benefits associated with the tissue accordingly to recommended lower consumption of fats, as a part of a balanced diet. Atherogenic index (AI) and thrombogenenicity index (TI) are lower, and hypocholesterolemic/Hypercholesterolemic ratio (h/H) of the liver is higher compared to the muscle and subcutaneous fat values what is most desirable from the consumer health perspective.

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