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The Fatty Acid Profile of Muscle Tissue of Ram Lambs with Diverse Genotypes*

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The aim of the study was to determine the fatty acid profile of intramuscular fat for genetically diverse sheep breeds kept in the same environmental and feeding conditions. The study was conducted on 30 (15 in each breed) wrzosówka and żelazneńska ram lambs slaughtered at 23-25 kg of life weight. The meat samples for analysis were taken from *longissimus lumborum* muscle. The meat of wrzosówka ram lambs contained almost twofold less ($P \leq 0.01$) intramuscular fat compared to żelazneńska sheep. Lower ($P \leq 0.05$) total of SFA and higher ($P \leq 0.05$) MUFA content in muscle tissue of wrzosówka sheep was shown. The predominant monounsaturated acid was oleic acid which amounted to almost 90% of all MUFA. The activity of enzyme Δ9 desaturase evidenced by the higher values of C14:1/C14:0, C18:1/C18:0 ($P \leq 0.01$) and C16:1/C16:0 ($P \leq 0.05$) indices was also recorded for wrzosówka ram lambs. The UFA/SFA ($P \leq 0.05$) and MUFA/SFA ($P \leq 0.05$) ratios as well as the value of the trombogenic index (TI) ($P \leq 0.05$) were more favourable in muscle tissue for wrzosówka than żelazneńska rams lambs. Therefore, the meat from leaner animals is more beneficial for human health.

Key words: sheep, genotype, muscle tissue, fatty acids.

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Animal fat, in addition to being a source of energy, has a positive effect on the technological and culinary value of meat. On the other hand, a large share in the human diet is considered to be the cause of cardiovascular diseases (LIN *et al*. 2004). This opinion is not fully advisable, because intramuscular fat, having greater importance to the consumer, contains a significant part of phospholipids, rich in polyunsaturated fatty acids (PUFA) for which bioactive properties are well recognized (WOOD *et al*. 2008).

The content of PUFA in the human diet, especially n-3, not only reduces the risk of vascular disease but also prevents the development of cancer, stimulates the immune system and affects the development of the nervous system (WIJENDRAM & HAYES 2004; PALMQUIST 2009). Also, palmitic and stearic acids, predominant in animal fat, are not hypercholesterolaemic and C18: 0 even effectively reduces blood cholesterol, as well as oleic acid, dominant in the monounsaturated fatty acids group (MUFA) (THOLSTRUP *et al*. 1994). Other bioactive components of animal fat are conjugated linoleic acid isomers (CLA), found mainly in ruminants, among which lamb meat is the richest source (SCHMID *et al*. 2006).

The share and proportion of fatty acids in animal fat are dependent on many factors. The most important is animal nutrition. Dietary fatty acids could modify both intramuscular and subcutaneous fat in ruminants (STASINIEWICZ *et al*. 2000; WOOD *et al*. 2008). Other factors affecting fat quality are body weight and age at slaughter (MARINO *et al*. 2008; RADZIK-RANT *et al*. 2012). Nonetheless the effect of breed on fatty acids profile may be important. Ruminants deposit PUFA in phospholipids, so lean breeds could have relatively high proportions of PUFA compared to fat genotypes in which the phospholipid effect is diluted by higher levels of neutral storage lipid (FISHER *et al*. 2000).

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Therefore, the aim of this study was to determine if different sheep breeds, genetically diverse in terms of fat content, maintained at the same environmental and feeding conditions differ in the fatty acids profile of intramuscular fat.

Material and Methods

The study was carried out according to the guidelines of the Third Local Ethics Committee in Warszawa (No. 54/2010 from the 14th July 2010).

Animals and treatment

The study was conducted on wrzosówka and żelazneńska ram lambs kept in one flock on the Experimental Farm of the Warsaw University of Life Sciences-SGGW. Wrzosówka is an indigenous breed, characterized by a thin, small and proportionally built figure, while Żelazneńska is a typical meat-and-wool purpose sheep.

The lambs were nursed by ewes until 100 days of life. After weaning, 30 ram lambs (15 animals in each breed) were chosen for slaughter. The average body weight at weaning was 15.09 kg for wrzosówka and 17.14 kg for żelazneńska ram lambs. The animals were fed a grain mix containing oat meal (30%), barley meal (40%), triticale meal (30%) and grass hay according to standards (OSIKOWSKI *et al*. 1998). The chemical composition and fodder nutritional value are presented in Table 1.

The lambs were slaughtered at 23-25 kg of life weight. Before slaughter ram lambs were fasted for 12 h and weighed. Then, they were taken to an abattoir and slaughtered according to standard commercial procedures used in Poland (rule no. 2013.856). Carcasses were chilled at 4°C for 24 h. From the right halves of the carcasses, samples of *longissimus lumborum* muscle were taken and stored in vacuum pack at -20°C until used for analysis.

Chemical analysis

Chemical composition of fodder was analysed according to AOAC standard methods (1990).

The content of water, protein and fat in muscle samples was determined according to PN-73/A-82110, PN-75/A-04018 and PN-73/A-82111, respectively.

Total lipids in lamb muscle were extracted according to FOLCH *et al.* (1957). Saponification of fat was carried out in 0.5 M KOH in methanol and estrification in 10 – percent BF_3 in methanol. Fatty acid methyl esters were extracted in hexane. Fatty acid profile of lipids was assessed by gas- chromatograph analysis using an Agilent Technologies GC 6890 N instrument equipped with capillary column BPx70 (length 60 m, internal diameter 0.22 mm, film thickness 0.25 μm). Operation conditions were: helium gas (41 psi); a FID detector at 240°C. The temperature programme was: 3 min at 130°C, an increase to 235°C by +2 C/min; 4 min at 235°C.

The fatty acids were identified via reference material BCR 163 (Beef/Pig Fat Blend) and isomer linoleic acid (CLA) by standard *cis*-9, *trans*-11 octadecadienoic acid-Larodon AB, Sweden.

Statistical analysis

Statistical analysis of the data of chemical composition of meat and fatty acid composition of intramuscular fat was performed using SPSS 14.0 software (2005), based on a linear model that included the effect of breed. The effect was tested against residual mean-squares to determine the level of significance.

Table 1

The chemical composition and nutritional value of fodder given to ram lambs

Item	Grain mix	Grass hay
Dry matter (%)	88.85	91.68
Crude protein (%/kgDM)	12.10	11.91
Ether extract (%/kgDM)	1.68	2.48
Crude fiber (%/kgDM)	4.11	27.93
UFV/kgDM	1.01	0.69
PDIN (g/kgDM)	76.00	81.00
PDIE (g/kgDM)	93.00	85.00
SFU/kgDM	–	1.32

UFV – unit of energy for meat production; PDIN – protein digested in the small intestine depending on rumen degraded protein; PDIE – protein digested in small intestine depending on rumen-fermented organic matter; SFU – fill unit for sheep; DM – dry matter

Results and Discussion

The chemical composition of muscle tissue is shown in Table 2. The tested genotypes differed only in the content of intramuscular fat. As expected, in wrzosówka ram lambs the fat content was almost twofold lessless ($P \leq 0.01$) compared to żelazneńska sheep, due to large diversity between native and more improved breeds. Similarly, lower intramuscular fat content in breeds of poor meat performance like Finn and Friesian sheep compared to typical meat breeds was recorded by BORYS *et al.* (2005). However, MARTINEZ-CERESO *et al*. (2005) compared less diverse genotypes and did not obtain differences in fat content in m.l.d. muscle.

The fatty acids profile of intramuscular fat of the investigated breeds is presented in Table 3. Among saturated fatty acids (SFA) higher content of C15:0, C17:0 and C18:0 ($P \leq 0.01$) was recorded in meat of Żelazneńska ram lambs. Within this acid group palmitic and stearic acids showed the highest proportion which in wrzosówka and Żelazneńska rams were 51%; 48% and 37%; 38% respectively. The total SFA content was lower ($P \leq 0.05$) for the wrzosówka breed compared to żelazneńska rams (Table 4). A higher amount of SFA (47.69 vs. 42.96 g/100g fat) was found in muscle tissue of the wrzosówka breed, but in adult animals with greater intramuscular fat content (RADZIK-RANT (1996). SALVATORI *et al*. (2004) also observed a higher share of SFA in native Ital-

Table 2

The chemical composition of muscle tissue of investigated genotypes

Item	Wrzosówka	Żelazneńska	SE	P value
Water (%)	76.54	76.17	0.43	0.56
Protein (%)	21.75	20.28	0.48	0.07
Fat (%)	2.10	3.94	0.29	0.00

SE – standard error; $P \leq 0.05$ – significant effect; $P > 0.05$ – not significant effect.

Table 3

The content of individual fatty acids in intramuscular fat of investigated genotypes (g/100 g fat)

Fatty acid	Wrzosówka	Żelazneńska	SE	P value
C10:0	0.16	0.19	0.01	0.19
C:12:0	0.20	0.16	0.04	0.48
C:14:0	2.92	2.43	0.24	0.21
C:15:0	0.47	0.60	0.02	0.01
C:16:0	21.81	23.11	0.76	0.27
C:17:0	1.46	2.69	0.12	0.00
C:18:0	15.78	18.34	0.52	0.01
C:20:0	0.17	0.14	0.01	0.20
C:14:1 c9	0.17	0.07	0.02	0.01
C:16:1 c9	2.01	1.54	0.09	0.01
C:17:1 c10	0.78	1.18	0.05	0.00
C:18:1 c9	37.08	32.82	0.98	0.02
C:18:1 t11 TVA	1.31	1.50	0.06	0.07
C:20:1	0.10	0.11	0.00	0.42
C:18:2 n-6	3.56	3.58	0.27	0.96
C:18:2 *c*9 *t*11 CLA	0.58	0.55	0.03	0.59
C:18:3 n-3 LNA	1.12	1.07	0.07	0.58
C:20:3 n-3	0.11	0.09	0.02	0.36
C:20:4 n-6	1.12	0.79	0.12	0.09
C:20:5 n-3 EPA	0.47	0.38	0.02	0.01
C:22:6 DHA	0.08	0.07	0.01	0.63

SE – standard error; $P \leq 0.05$ – significant effect; $P > 0.05$ – not significant effect; TVA – *trans* vaccenic acid; CLA – conjugated linoleic acid; LNA – linoleic acid; EPA – eicosapentaenoic acid; DHA – docosahexaenoic acid.

Table 4

The content of fatty acid groups and values of atherogenic and trombogenic indices in intramuscular fat of investigated genotypes

Item	Wrzosówka	Żelazneńska	SE	P value
∑SFA	42.96	47.66	1.22	0.05
∑MUFA	41.59	37.39	1.01	0.03
∑PUFA	7.04	6.53	0.42	0.42
UFA/SFA	1.13	0.92	0.05	0.02
MUFA/SFA	0.96	0.79	0.04	0.02
PUFA/SFA	0.16	0.14	0.01	0.19
∑ n-6	4.69	4.37	0.36	0.56
∑ n-3	1.78	1.60	0.08	0.16
n-6/n-3	2.64	2.72	0.16	0.72
AI	0.70	0.76	0.04	0.34
TI	1.40	1.69	0.08	0.03

SE – standard error; P≤0.05 – significant effect; P>0.05 – not significant effect; TI = thrombogenic index (C14:0 + C16:0 + C18:0/ 0.5 ∑MUFA + 0.5 ∑PUFA n-6 + 3 ∑PUFA n-3 + n-3/n-6) (ULBRICHT & SOUTHGATE 1991); AI = atherogenic index (C12:0 + 4 x C14:0 + C16:0/ ∑MUFA + ∑PUFA n-6 and n-3) (ULBRICHT & SOUTHGATE 1991); SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; UFA – unsaturated fatty acids.

ian sheep crossbreeds which are characterized by higher content of intramuscular fat in muscle tissue (2.94%) in comparison to wrzosówka.

In the monounsaturated acid group (MUFA) a higher content of C14:1 (P≤0.01), C16:1 and C18:1 (P≤0.05) in intramuscular fat for wrzosówka ram lambs was recorded, while the amount of C17:1 was higher (P≤0.01) for the żelazneńska breed (Table 3).

The monounsaturated fatty acids are formed in adipocytes as a result of saturated acids destauration by the enzyme Δ9 desaturase, otherwise called stearoyl Co-A desaturase (SCD) (NTAMBI & MIYAZAKI 2004). The higher content (P≤0.05) of total MUFA and lower amount (P≤0.05) of SFA in muscle tissue of wrzosówka ram lambs (Table 4) may be caused by enhanced activity of this enzyme as evidenced by the higher values of C14:1/C14:0, C18:1/C18:0 (P≤0.01) and C16:1/C16:0 (P≤0.05) indices (Table 5).

In both studied breeds the predominant monounsaturated acid was oleic acid which amounted to almost 90% of all MUFA. A similar value of C18:1c9 obtained for żelazneńska rams in the present study was recorded in earlier research by RADZIK-RANT *et al*. (2012) in intramuscular fat of lambs of this breed slaughtered at 3 months of age. A higher content of this acid for another meat-and-wool purpose breed was registered by HOROSZKIEWICZ *et al.* (2011). PRIOLO *et al*. (2003) reported less (33.14 vs. 37.08 g/100 g fat) content of oleic acid in muscle tissue for indigenous Barbaresca sheep characterized by the same intramuscular fat content as the studied wrzosówka breed.

In the polyunsaturated fatty acid group (PUFA) a greater amount of C20:5 n-3 fatty acid (P≤0.01) was found in intramuscular fat of wrzosówka in comparison with żelazneńska ram lambs (Table 3). The differences in total PUFA content between the

Table 5

The Δ9 desaturase activity in intramuscular fat of investigated genotypes

The Item	Wrzosówka	Żelazneńska	SE	P value
C14:1/C14:0	0.06	0.03	0.00	0.00
C16:1/C16:0	0.09	0.06	0.01	0.02
C18:1/C18:0	2.35	1.80	0.10	0.01
CLA/TVA	0.44	0.37	0.02	0.04

SE – standard error; P≤0.05 – significant effect; P>0.05 – not significant effect; TVA – *trans* vaccenic acid; CLA – conjugated linoleic acid.

studied breeds were not confirmed statistically (Table 4).

In intramuscular fat, fatty acids are found in phospholipids and triacyloglicerols. In the latter C18:1c9 is predominant. Long chain polyunsaturated fatty acids (PUFA n-6 and n-3) are mainly incorporated into the structure of phospholipids (DEMIREL *et al*. 2004). Phospholipids are essential components of cell membranes and their amount remains fairly constant (WOOD *et al*. 2008). In animals with less fat due to the ratio between phospholipids and neutral lipids, the proportion of oleic acid in intramuscular fat should be smaller and greater C18:2 n-6 acid occurring in the phospholipids (KOUBA *et al*. 2003; WARREN *et al*. 2008; WOOD *et al*. 2008). This was confirmed by FISHER *et al*. (2000) in a comparative study on less fat Soay breed and Welsh mountain sheep, slaughtered at the same life weight. Contrary to the results of the above-mentioned authors, the breed with less content of intramuscular fat in comparison to the fatter breed was characterized by a higher ($P \leq 0.05$) amount of oleic acid and almost the same value of linoleic acid (Table 3).

The monounsaturated and polyunsaturated fatty acids in contrast to saturated acids may perform similar functions, particularly in dealing with the competitive position at sn-2 of glycerol in phospholipid molecules (EDWARDS-WEBB & GURR 1998). Therefore, monounsaturated fatty acids, especially oleic acid predominant in the intramuscular fat of wrzosówka, may be incorporated into phospholipids instead of long chain PUFAs. The dominance of MUFAs resulting from high activity of $\Delta 9$ desaturase in the milk fat fraction for indigenous wrzosówka breed in comparative studies of lowland sheep has also been observed (ROZBICKA-WIECZOREK 2001).

In both studied genotypes a difference in content of the C18:2*c*9,*t*11 (CLA) isomer was not recorded (Table 3). However, $\Delta 9$ desaturase activity in the conversion of C18:1 t11 in C18:2 *c*9,*t*11 was higher ($P \leq 0.05$) for wrzosówka rams (Table 5). Differences in content of CLA between the Suffolk meat breed and Scottish black face were also absent (DEMIREL *et al*. 2004).

Due to the higher content of MUFA, the UFA/SFA ($P \leq 0.05$) and MUFA/SFA ($P \leq 0.05$) ratios as well as the value of the trombogenic index (TI) ($P \leq 0.05$) were more beneficial for human health in muscle tissue of wrzosówka rams.

In conclusion it is possible to ascertain that the differences of fatty acids profile in intramuscular fat of muscle tissue of diverse sheep genotypes mainly concerned the monounsaturated fatty acids content, but not polyunsaturated acids as expected. This was due to increased activity of $\Delta 9$ desaturase enzyme in the more primitive wrzosówka breed. In general, it can be stated that lean sheep are characterized by a more health favourable fatty acid profile than more fatty genotypes.

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