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# FATTY ACID COMPOSITION OF SUBCUTANEOUS AND INTRAMUSCULAR ADIPOSE TISSUE IN EAST BALKAN PIGS

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**Abstract:** The aim of this study was to provide information on the fatty acid profile of different adipose depots – subcutaneous (upper and inner backfat layers) and intramuscular (*m. Longissimus dorsi*) in East Balkan pigs. The animals were reared in free-range conditions and slaughtered at an average live weight of 107±1.65kg. The results of the study showed that the various adipose tissues in pigs have different lipid metabolism and hence differ in their fatty acid composition. Intramuscular fat had significantly higher content of the saturated C16:0 and C18:0 ($P<0.001$), as well as the C16:1 ($P<0.001$) than the subcutaneous fat. In regards to the content of the polyunsaturated fatty acids, the latter displayed considerably higher content of both C18:2 and C18:3 ($P<0.001$) in comparison to the intramuscular fat in *m. Longissimus dorsi*. The differences between the subcutaneous and intramuscular adipose tissue in the individual fatty acids determined the similar trend of change in the total content of saturated and polyunsaturated fatty acids. Significant differences between the backfat layers were detected for C16:1, C18:0 and C18:3 ($P<0.001$). Stearic acid (C18:0) displayed higher content of the inner, while both C16:1 and C18:3 had higher proportion in the outer backfat layer in the East Balkan pigs. Except for C20:2, the long chain polyunsaturated n-6 and n-3 fatty acids had significantly higher proportions in the intramuscular fat, however no differences were determined between the two backfat layers.

**Key words:** East Balkan pigs, adipose tissue, fatty acid profile

## Introduction

Adipose tissue in pigs is one of the main organs involved in the regulation of lipid metabolism, particularly in the overall fatty acid synthesis with consequences in other lipid-target organs such as muscles and the liver *(Corominas et al., 2013).*

Porcine carcass fat is deposited in four depots, with different anatomical locations: visceral, subcutaneous, inter- and intramuscular. A number of studies have reported considerable anatomical variation in fatty acid composition in the pig adipose tissues, since they are not similar but each shows specific development and metabolism *(Mourot et al., 1995, 1996; Monziols et al., 2007; Daza et al., 2007).* Most of these experiments have been undertaken on the subcutaneous adipose tissue because a high proportion of fat is subcutaneous and it is very obvious at retail or in cuts. East Balkan pig is an indigenous slow-growing breed mainly in the eastern parts of Bulgaria. The animals are exclusively reared in free-range conditions, have considerably higher body fat content than other conventional lean pig breeds, possess unique sensory characteristics of meat and are used for manufacturing of high quality meat products. The extensive conditions of rearing of the pigs of the East Balkan breed suggest higher deposition of polyunsaturated fatty acids in their adipose tissues, however profound studies on the lipid profile of this breed have been scarce in Bulgaria. Hence the aim of this study is to provide information on the fatty acid profile of different adipose tissues in East Balkan pigs.

## Materials and Methods

The study was carried out with 10 (5 male + 5 female) pigs of East Balkan breed. The animals were slaughtered at an average live weight of 107±1.65kg in a standard slaughterhouse. The carcasses were split in half, kept for 24 h at 4°C, after which samples of the inner and outer backfat layer as well as *m. Longissimus dorsi* were taken at the last rib.

Total lipids of the tissues were extracted according to the method of *Bligh and Dyer (1959).* Methyl esters of the total lipids, isolated by preparative TLC were obtained using 0.01 % solution of sulphuric acid in dry methanol for 14 h, as described by *Christie (1973).* The fatty acid composition of total lipids was determined by GLC analysis using chromatograph C Si 200 equipped with capillary column (TR-FAME - 60 m x 0.25 mm x 0.25 μm) and hydrogen as a carrier gas. The oven temperature was first set at 160 °C for 0.2 min, then raised until 220 °C at a rate of 5 °C/min and hold for 5 minutes. The temperatures of the detector and injector were 230 °C. Methyl esters are identified comparing to the retention times of the standards. Fatty acids are presented as percentages of the total amount of the methyl esters identified *(Christie, 1973).*

Data were statistically analysed by one way ANOVA procedure using JMP v.7 software package. Protected Fisher LSD test was performed as post-hoc test to compare means, as differences with a level of significance below 0.05 were considered significant.

# Results and Discussion

The type of the adipose tissue - subcutaneous or intramuscular induced significant differences in the contents of the individual fatty acids of the studied depots (Table1). The content of C16:0 was lower in the backfat than in *m. Longissimus dorsi* ($P<0.001$) but no differences were observed between the inner and outer backfat layer. Subcutaneous adipose tissue displayed considerably lower content of C18:0 when compared to intramuscular fat ($P<0.001$) however further reduction of this fatty acid was observed in the outer layer. Significant difference between the tissues was detected in regards to the levels of C18:1 and it tended to be lower in the intramuscular fat. The differences in the content of C18:0 and C18:1 between the two backfat layers are in agreement with those reported by *Daza et al. (2007)* in Iberian pigs. On the other hand the content of C16:1 differed between the backfat layers and *m. Longissimus dorsi*, as the latter displayed the highest level of C16:1, while the lowest was observed in the inner backfat layer. Desaturation indices are indirect indicator of desaturase activity *(Attie et al., 2002)*. In this study we used the product-to-precursor ratio (C16:1/C16:0 as well as C18:1/C18:0) calculated from the content of respective fatty acids in lipids extracted from the inner, outer backfat layer and *m. Longissimus dorsi*. The ratios are well related to the stearoyl-CoA (SCD) activity *(Klingenberg et al., 1995; Kouba et al., 1997)*. Differences in the indices were observed between the two backfat layers, as they were lower in the inner one. This corresponded to the lower content of C16:1 on one hand and on the other- the higher content of C18:0 in this backfat layer. Surprisingly the desaturase indices determined for the intramuscular fat were not uniform, although significantly different from the ones of the adipose tissue. They suggest different activity of the enzyme toward its substrates, showing higher value of SCD16, corresponding to the considerable amount of C16:1 in *m. Longissimus dorsi* (3.58% vs.1.81 % and 1.22% for the outer and inner layers respectively). On the other hand, the highest content of C18:0 and respectively the lowest of C18:1 determined the lowest index of SCD18. This together with the considerable amount of C16:0 contributed to the higher ($P<0.001$) total saturation of the intramuscular fat, compared to the subcutaneous adipose layers. In this study we did not find changes in the saturation of the two backfat layers. This contradicted to a previous study *(Popova, 2014)*, showing greater unsaturation in the outer backfat layer in Youna x Pietrain pigs. *Migdal et al. (2001)* and *Monziols et al. (2007)* reported negative gradient of the degree of unsaturation from outside inwards in selected pigs.

Pork tissues are characterised by high content of linoleic fatty acid (C18:2). The type of fat depot influenced the content of C18:2 ($P<0.001$) showing lower levels in the intramuscular fat compared to the subcutaneous adipose tissue. The same was observed in regards to the contents of the linolenic (C18:3) fatty acid.

Our study demonstrated greater deposition of these polyunsaturated fatty acids in the backfat as significant differences between layers were detected for C18:3 incorporated in the outer backfat layer. Our results contradict to those of *Monziols et al. (2007)* who reported higher levels of C18:2 in the outer layer of the subcutaneous adipose tissue, instead of C18:3 as observed in our study. However, in the experiment of *Daza et al. (2005)* no differences in the proportions of C18:2 n-6 and C18:3 n-3 between the outer and the inner layers from free-range Iberian pigs were observed. Both C18:2 and C18:3 are essential for pigs and come exclusively from feed. The preferential deposition of C18:3 in our study could be explained by the extensive rearing of the East Balkan pigs and their access to grass, which is known to have much higher content of C18:3 that the compound feeds used in pig nutrition. This is confirmed by the values of C18:3 determined in this study, being higher than the ones observed the industrially reared pigs *(Šolević Knudsen and Stanišić, 2015).*

**Table. 1 Fatty acid composition in the backfat layers and intramuscular fat in East Balkan pigs**

| Fatty acids,% | Subcutaneous (backfat) | | Intramuscular (m.LD) | SEM | Significance |
|---|---|---|---|---|---|
| | Outer layer | Inner layer | | | |
| C14:0 | 1.20 | 0.95 | 1.08 | 0.087 | NS |
| C16:0 | 17.47$^{a}$ | 16.84$^{a}$ | 20.35$^{b}$ | 0.47 | *** |
| C16:1 | 1.81$^{a}$ | 1.22$^{b}$ | 3.58$^{c}$ | 0.17 | *** |
| C18:0 | 3.25$^{a}$ | 4.41$^{b}$ | 5.79$^{c}$ | 0.26 | *** |
| C18:1 | 52.79$^{a}$ | 53.66$^{a}$ | 49.81$^{b}$ | 0.79 | ** |
| C18:2 | 20.27$^{a}$ | 20.13$^{a}$ | 14.36$^{b}$ | 0.61 | *** |
| C18:3 | 2.28$^{a}$ | 1.84$^{b}$ | 1.14$^{c}$ | 0.08 | *** |
| C20:2 | 0.44$^{a}$ | 0.42$^{a}$ | 0.34$^{b}$ | 0.02 | ** |
| C20:3 | 0.10$^{a}$ | 0.08$^{a}$ | 0.17$^{b}$ | 0.01 | *** |
| C20:4 | 0.09$^{a}$ | 0.20$^{a}$ | 2.77$^{b}$ | 0.23 | *** |
| C20:5 | 0.07$^{a}$ | 0.07$^{a}$ | 0.23$^{b}$ | 0.02 | *** |
| C22:5 | 0.20$^{a}$ | 0.15$^{a}$ | 0.37$^{b}$ | 0.02 | *** |
| SFA[1] | 21.93$^{a}$ | 22.20$^{a}$ | 27.22$^{b}$ | 0.55 | *** |
| MUFA[2] | 54.61 | 54.89 | 53.39 | 0.82 | NS |
| PUFA[3] | 23.46$^{a}$ | 22.90$^{a}$ | 19.38$^{b}$ | 0.81 | ** |
| P/S | 1.08$^{a}$ | 1.04$^{a}$ | 0.72$^{b}$ | 0.04 | *** |
| n-6/n-3 | 8.31$^{a}$ | 10.16$^{b}$ | 10.21$^{b}$ | 0.38 | *** |
| C16:1/C16:0 | 0.10$^{a}$ | 0.07$^{b}$ | 0.17$^{c}$ | 0.008 | *** |
| C18:1/C18:0 | 16.61$^{a}$ | 12.56$^{b}$ | 8.89 | 0.73 | *** |

**P<0.01;***P<0.001; Within a row, values connected with different letters are significant (P<0.05)
[1]SFA- Saturated fatty acids
[2]MUFA- monounsaturated fatty acids
[3]PUFA- polyunsaturated fatty acids

The contents of C20:2 displayed significantly lower content in the intramuscular fat compared to the backfat (P<0.01), while both C20:3 and C20:4

had higher proportions in the muscle (P<0.001). The same was observed for the contents of C20:5 and C22:5 (P<0.001). The levels of these fatty acids did not differ between the two backfat layers.

The ratio n-6/n-3 exceeds the recommended limit of 4 in the backfat and intramuscular fat, showing the relatively unbalanced fatty acid profile of the pork tissues. However significant difference between the backfat layers and the intramuscular fat existed corresponding to the discrepancies in the contents of C18:3.

The total contents of SFA and PUFA (Table 1) showed significant difference between the subcutaneous and intramuscular adipose tissue (P<0.001) and corresponded to the already reported changes in the content of C16:0 and C18:2. No difference was observed in the total MUFA content.

In regards to P/S ratio, in all three tissues the values were within the range 0.57-1.03, higher than the minimal recommended value of 0.4.

Significant correlation coefficients (Table 2) were found for the proportions of C16:0 and C18:1 between the inner and outer layers of the subcutaneous adipose tissue, as well as for the content of C18:2 between the inner layer and the intramuscular fat. The correlation coefficients for the levels of the other major fatty acids between the tissues were not significant. These results are indicative for the different fatty acid metabolism in the various adipose depots in pigs.

**Table 2. Correlation coefficients between the major fatty acids proportion of the inner and outer layers (I/O), inner layer and intramuscular fat (I/M), and outer layer and intramuscular fat (O/M)**

| Fat depot | C14:0 | C16:0 | C16:1 | C18:0 | C18:1 | C18:2 | C18:3 | C20:4 |
|---|---|---|---|---|---|---|---|---|
| I/O | 0.309 | 0.766* | 0.115 | 0.105 | 0.656* | 0.583 | -0.106 | 0.496 |
| I/M | -0.295 | -0.001 | 0.110 | -0.144 | 0.262 | 0.719* | -0.051 | 0.438 |
| O/M | 0.248 | 0.348 | 0.092 | 0.403 | 0.229 | 0.081 | -0.043 | 0.414 |

*P<0.05.

# Conclusion

The data presented confirm that the various porcine adipose tissues differ in their metabolism and fatty acid profile. Intramuscular fat displayed significantly higher content of the saturated C16:0 and C18:0, as well as the C16:1 than the subcutaneous fat. In regards to the content of the polyunsaturated fatty acid, the latter had higher content of both C18:2 and C18:3 in comparison to the fat in *m. Longissimus dorsi*. The differences in these individual fatty acids between the subcutaneous and intramuscular fat contributed to the similar pattern in their total content. Significant differences between the backfat layers were detected for C16:1, C18:0, and C18:3. C18:0 displayed higher content of the inner, while both C16:1 and C18:3 had higher proportion in the outer backfat layer in the East

Balkan pigs. With exception of C20:2, the of the long chain polyunsaturated n-6 and n-3 fatty acids displayed significantly higher proportions in the intramuscular fat, however no differences were determined between the two backfat layers.

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## Sastav masnih kiselina potkožnog i intramuskularnog masnog tkiva istočno-balkanske svinje

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## Rezime

Cilj ove studije je da pruži informacije o profilu masnih kiselina različitih masnih depoa - potkožni (gornji i unutrašnji slojevi leđne slanine) i intramuskularni (*m. longissimus dorsi*) istočno-balkanske rase svinja. Životinje su gajene u uslovima slobodnog držanja i zaklane u proseku žive mase od 107 ± 1,65kg. Rezultati studije su pokazali da različiti depoi masnog tkiva kod svinja imaju različit metabolizam lipida i stoga se razlikuju u sastavu masnih kiselina. Intramuskularna mast je imala značajno veći sadržaj zasićenih C16: 0 i C18: 0 ($P<0,001$), kao i C16:1 ($P<0.001$) nego potkožno tkiva. Što se tiče sadržaja polinezasićenih masnih kiselina, potkožno tkivo prikazuje znatno veći sadržaj i C18:2 i C18:3 ($P<0,001$) u odnosu na intramuskularnu masti u *m. longissimus dorsi*. Razlike između potkožnog masnog tkiva i intramuskularne masti u pojedinim masnim kiselinama određuju i sličan trend u ukupnom sadržaju zasićenih i polinezasićenih masnih kiselina. Značajne razlike između slojeva leđne slanine su otkriveni za C16:1, C18:0 i C18:3 ($P<0,001$). Stearinska kiselina (C18: 0) prikazuje veći sadržaj u unutrašnjem, a C16:1 i C18:3 su imali veći udeo u spoljašnjem sloju leđne slanine istočno-balkanskih svinja. Osim C 20:2, polinezasićene masne kiselina dugog lanca n-6 i n-3 su imale značajno veće razmere u intramuskularnoj masti, međutim, statistički signifikantne razlike nisu utvrđene između dva sloja leđne slanine.

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