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ANALYSIS OF FATTY ACIDS IN SUBCUTANEOUS FAT OF BERRICHON DU CHER AND SUFFOLK HEAVY LAMBS IN SEMI-INTENSIVE PRODUCTION SYSTEMS IN SLOVAKIA

Marta ORAVCOVÁ[1]*, Milan MARGETÍN[1,2], Jaroslav BLAŠKO[3], Andrzej JUNKUSZEW[4]

[1]NPPC – Research Institute for Animal Production Nitra, Lužianky, Slovak Republic
[2]Slovak University of Agriculture in Nitra, Department of Animal Husbandry, Faculty of Agrobiology and Food Resources, Nitra, Slovak Republic
[3]Comenius University, Institute of Chemistry, Faculty of Natural Sciences, Bratislava, Slovak Republic
[4]University of Life Sciences, Institute of Animal Breeding & Biodiversity Conservation, Lublin, Poland

ABSTRACT

The objective of the study was to determine the content of fatty acids (FAs) in subcutaneous fat from tailhead area of heavy lambs of Berrichon du Cher (BE) and Suffolk (SF) breeds. Lambs of both sexes were kept in semi-intensive production systems (SI1 and SI2). The production systems differed in nutrition during period of two weeks before slaughter: BE/SI1 lambs were fed with hay and concentrates, SF/SI2 lambs were on pasture and dam milk. The analysis of variance with two factors – breed/production system (BE/SI1, SF/SI2) and lamb sex (males, females) was applied to investigate differences between lamb groups. The content of essential FAs (sum of linoleic and α-linolenic acids), determined by a gas chromatography method, was higher ($P < 0.001$) in subcutaneous fat of BE/SI1 lambs (2.90 g.100 g^{-1} FAME) when compared to SF/SI2 lambs (2.53 g.100 g^{-1} FAME). The contents of arachidonic and docosapentaeonic acids were higher ($P < 0.05$) in BE/SI1 lambs (0.08 and 0.15 g.100 g^{-1} FAME) than in SF/SI2 lambs (0.05 and 0.12 g.100 g^{-1} FAME), whereas the contents of eicosapentaeonic and docosahexaenoic acids did not differ between groups. The content of conjugated linoleic acid (CLA) was higher ($P < 0.001$) in SF/SI2 lambs than in BE/SI1 lambs (1.84 and 1.09 g.100 g^{-1} FAME). In relation to sex, the content of CLA in ewe lambs (1.60 g.100 g^{-1} FAME) was higher ($P < 0.05$) than in ram lambs (1.33 g.100 g^{-1} FAME). The content of docosapentaeonic acid was also higher ($P < 0.05$) in ewe lambs (0.16 vs. 0.12 g.100 g^{-1} FAME). These findings contribute to expanding knowledge about sheep breeds in Slovakia.

Key words: sheep; production system; sex; tail fat; fatty acids

INTRODUCTION

Red meat (also lamb meat) is considered to be a less desirable component of the human diet due to relatively high amounts of cholesterol; contrariwise, meat fat (intramuscular, intermuscular and subcutaneous) is considered to be a concentrated source of energy and bioactive components and an important determinant of culinary qualities and texture parameters of meat, which are searched by consumers (Kaczor *et al.*, 2010). Fatty acids (FAs) play a significant role in appropriate cellular function and human health, for instance, they are essential for fat-soluble vitamin A, D, E and K intake (Maleki *et al.*, 2015). In this respect, research was focused on revealing the potencial benefits of lamb consumption (Swanson *et al.*, 2012; Ponnampalam *et al.*, 2014). Many studies aimed at assessing lamb quality on the basis of essential fatty acids (FAs), e.g. linoleic acid, α-linolenic acid and other

***Correspondence:** E-mail: marta.oravcova@nppc.sk,
Marta Oravcová, NPPC – Research Institute for Animal Production Nitra,
Hlohovecká 2, 951 41 Lužianky, Slovak Republic
Tel.: +421 376546328

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health promoting polyunsaturated FAs (PUFA) in intramuscular (Mortimer *et al.* 2014; Ponnampalam *et al.* 2014) and subcutaneous (Maleki *et al.*, 2015; Momen *et al.*, 2019) fat were done. Linoleic and α-linolenic acids serve as precursors for other important PUFA compounds. Linoleic acid is the precursor for n-6 PUFA, whereas α-linolenic acid is the precursor of n-3 PUFA (Maleki *et al.*, 2015). Other polyunsaturated FAs, such as eicosapentaenoic acid and docosahexaenoic acid, are valuable for their anti-inflammatory effects (Simopoulos, 2002, McAfee *et al.*, 2010). Conjugated linoleic acid is valuable for its anticarcinogenic, antiatherosclerotic and anti-diabetic effects (Raes *et al.*, 2004; Serra *et al.* 2009). Saturated FAs (SFA), mainly when consumed at high amounts, are believed to be of undesirable effects on human health. For instance, myristic and palmitic acids are assumed to increase the risk of cardiovascular diseases (Howes *et al.*, 2015). The exception is stearic acid (also SFA), which is believed to be of neutral effect on cholesterol amount (Howes *et al.*, 2015). Besides the importance of proportions of individual FAs to be known, knowledge about various ratios and indexes is also important in relation to human health. For instance, Wood and Enser (1997), Williams (2000), Simopoulos (2002), Wood *et al.* (2003), Raes *et al.* (2004), Ulbricht and Southgate (1991) and Sinanoglou *et al.* (2013) focused on this area and proposed values, which are considered appropriate for ratios of n-6/n-3 PUFA, PUFA/SFA, atherogenic and thrombogenic indexes.

Various factors, such as breed, live weight, fatness, gender, production system and diet may affect proportion of individual FAs in lamb fat (Momen *et al.*, 2016). Many studies revealed that FAs depend mainly on production system and diet i.e., whether lambs are pastured or their diet consists of hay/silage and concentrate (Fisher *et al.*, 2000; Díaz *et al.*, 2005; Aurousseau *et al.*, 2007 and Nuernberg *et al.*, 2008). The effect of breed, which, according to Sanudo *et al.* (2000), includes degree of fatness, live weight or slaughter age, was also found. However, this was of lesser influence (Santos-Silva *et al.*, 2002; Ponnampalam *et al.*, 2014).

Only few studies, aimed at determination of FAs content in intramuscular or subcutaneous fat of lambs in Slovakia, were done (Margetín *et al.* 2014; 2018; Janíček *et al.*, 2019; 2020). These focused on traditional and intensive production systems. Nevertheless, the importance of semi-intensive production system in lamb breeding has been increasing in recent years and needs for its investigations are, therefore, of rising awareness. The content of FAs in intramuscular fat (samples taken from *Musculus longissimus dorsi*) of heavy lambs of Berrichon du Cher (BE) and Suffolk (SF) breeds in semi-intensive production systems (SI1 and SI2) was analysed by Margetín *et al.* (2019). Since samples of subcutaneous fat from tailhead area of the same individuals were taken, the objective of this study was to evaluate the content of FAs in this lamb body part. Two-way analysis of variance, which included overlapping breed/production system (BE/SI1, SF/SI2) and lamb sex (males, females) factors, was applied. The overlapping factor was considered due to the fact that flocks with identical semi-intensive production systems for different breeds cannot be found in Slovakia.

MATERIAL AND METHODS

Animals and production systems

The trial was carried out on Berrichon du Cher (BE) and Suffolk (SF) heavy lambs from two flocks reared in semi-intensive production system with different feeding (SI1 and SI2). Twenty lambs (13 males and 7 females) per each breed/production system were included. This design was chosen due to fact that flocks with the identical semi-intensive system for different breeds cannot be found in Slovakia. The flocks were situated in close distance (about 15 km) and were placed not only on territory with similar height above sea, but also with similar amount of annual rainfall and average temperature.

The group 1 consisted of BE lambs (referenced as BE/SI1). Ewes lambed indoors, mainly in April; their diet consisted of 2 kg hay (mixture of alfalfa and grass hay), 3 kg of alfalfa and 200 g of oat per head per day. Lambs were housed with ewes in stable (maternity pens and nurseries) and suckled dam milk. Since two/three weeks after birth, in addition to milk, lambs were fed with on-farm grained oat and barley (ratio 1:1) per head per day. When pasture was available, lambs and ewes were allowed to graze. Lambs were offered on-farm grained oat and barley (100 to 200 g per head per day). Two weeks before slaughter, lambs were separated from ewes and allowed neither to graze

nor suckle milk. They were fed with 200 g of grained oat and barley (ratio 1:1) per head per day.

The group 2 consisted of SF lambs (referenced as SF/SI2). Ewes lambed indoor, mainly in April; their diet consisted of 2 kg hay (mixture of alfalfa and grass hay), 3 kg of alfalfa silage and 400 g of concentrates per head per day. Lambs were housed with ewes in stable (maternity pens and nurseries) and suckled dam milk. In addition, lambs were fed with commercial starter PURINA (Agribrands Europe, Hungary) up to three weeks of age. This consisted of dry matter (88 %), NL (16 %), fat (2.2 %), fiber (11 %), Ca (1.3 %), P (0.4 %) and Na (0.3 %) and supplements. When pasture was available, ewes and lambs were allowed to graze. Instead of PURINA, lambs were offered on-farm grained oat and barley (100 to 200 g per head per day). Two weeks before slaughter, lambs continued to graze and suckle milk. No concentrates were included in their diet.

Lambs were slaughtered in the slaughter house of the National Agricultural and Food Centre (Research Institute for Animal Production Nitra). The average weigth before slaughter was 31.8 ± 3.4 kg (BE/SI1 lambs) and 36.1 ± 5.0 kg (SF/SI2 lambs). The average age of lambs was 86 ± 2.9 days (BE/SI1 lambs) and 93 ± 6.8 days (SF/SI2 lambs). The average daily gain of lambs was 290 ± 40 g (BE/SI1 lambs) and 330 ± 50 g (SF/SI2 lambs).

Analysis of fatty acids

Twenty-four hours after slaughter, subcutaneous fat samples (2 g) were taken from a tailhead area of refrigerated carcasses. The analysis of the content of fatty acids (FAs) was done in the laboratory of the Institute of Chemistry (Faculty of Natural Sciences at Comenius University in Bratislava), according to the procedure described by Janíček *et al.* (2020). Capillary gas chromatography (GC) was used for analysis of proportion of the individual FAs and FAs isomers. For preparation of fatty acid methyl esters (FAME), the base-catalysed methylation procedure with a sodium methoxide in methanol was used. N-hexane (1mL) was added to the dried chloroform extract in a 2mL autosampler vial and mixing was resumed. Next, 100 µL of sodium methoxide (0.5 M solution in methanol) was added,

Table 1. Fatty acids (least squares means) in subcutanous fat (g.100 g^{-1} fatty acid methyl esters) of lambs

Fatty acids	Breed/Production system		Sex		RMSE
	BE/SI1	SF/SI2	Male	Female	
C12:0 (lauric)	0.99	0.96	0.98	0.98	0.296
C14:0 (myristic)	7.36	7.71	7.36	7.71	1.335
C16:0 (palmitic)	23.40	22.93	23.14	23.19	1.261
C17:0 (margaric)	1.19	1.10	1.14	1.15	0.138
C18:0 (stearic)	17.14	15.71	18.01^{a}	14.85^{b}	3.662
C16:1 *cis9* (palmitoleic)	0.62^{A}	0.72^{B}	0.66	0.68	0.056
C 18:1 *trans9* (elaidic)	0.25^{A}	0.31^{B}	0.28	0.29	0.017
C18:1 *cis9* (oleic)	32.54	31.75	31.24^{a}	33.05^{b}	2.280
C18:1 *trans11* (TVA)	2.07^{A}	3.91^{B}	2.90^{a}	3.08^{b}	0.256
C18:2 *n-6* (linoleic)	2.35^{A}	1.93^{B}	2.14	2.14	0.200
C18:3 *n-6* (GLA)	0.02^{A}	0.01^{B}	0.02	0.02	0.005
C18:3 *n-3* (ALA)	0.55	0.61	0.58	0.59	0.082
C18:2 *cis9 trans11* (RA)	1.03^{A}	1.70^{B}	1.24^{a}	1.49^{b}	0.321
C20:4 *n-6* (arachidonic)	0.08^{a}	0.05^{b}	0.06	0.07	0.025
C20:5 *n-3* (EPA)	0.02	0.02	0.01	0.02	0.009
C22:5 *n-3* (DPA)	0.15^{a}	0.12^{b}	0.12^{a}	0.16^{b}	0.040
C22:6 *n-3* (DHA)	0.03	0.03	0.02	0.03	0.013

BE/SI1: Berrichon du Cher in semi-intensive system 1; SF/SI2: Suffolk in semi-intensive system 2; RMSE: Root mean square error. TVA: *trans*-vaccenic acid; ALA: α-linolenic acid; GLA: γ-linolenic acid; RA: rumenic acid; EPA: eicosapentaeonic acid; DPA: docosapentaeonic acid; DHA: docosahexaeonic acid.
$^{A, B}$: differences between individual levels of factors at $P < 0.001$; $^{a, b}$: differences between individual levels of factors at $P < 0.05$.

mixed, and the vial contents were allowed to react for 15 min at 40 °C with occasional mixing. The vial was cooled at -20 °C for 10 min, before 60 µL of oxalic acid (0.5 g in 15 mL diethyl ether) was added and mixed thoroughly. The vial was centrifuged to settle the sodium oxalate precipitate, and the FAME solution was used directly for GC analyses. Gas chromatography analyses were performed by gas chromatograph (Agilent Technologies 6890N) with flame ionization detector (Agilent, Waldbronn, Germany) and 5973 Network mass-selective detector. Fatty acid methyl esters were separated in a capillary column 100 m x 0.25 mm i.d. x 0.2 µm film thickness of HP-88 stationary phase (J & W Scientific, Agilent Technologies, California, USA). Separated FAs were identified by reference materials (Supelco 37 Component FAME Mix; PUFA No. 3 from menhaden oil, Sigma Aldrich, Germany), published retention data and mass spectrometric measurements. The content of FAs was expressed in grams of each individual FAME per 100 grams of sum detected FAME. The average relative standard deviation of analysed FAME with proportion above 0.5 g.100 g^{-1} was 1.1 %, for the whole analytical procedure and 5 replicate samples.

A total of 70 FA, were identified. The following ratios/indexes were calculated: hypocholesterolaemic FA/hypercholesterolaemic FA ratio (h/H ratio) according to Santos-Silva *et al.* (2002) and Sinanoglou *et al.* (2013), the atherogenic index (AI) and thrombogenic index (TI) according to Ulbricht and Southgate (1991) and Sinanoglou *et al.* (2013), respectively.

Statistical analysis

General Linear Model procedure as implemented in a SAS software (2009) was applied. The model included: (A) the overlapping breed/production system factor (BE/SI1 and SF/SI2 lambs) due to fact that variance of breed was hardly possible to be distinguished from variance of production system, and (B) factor of lamb sex (males and females). The analysis focusing on breed/production system*sex interaction was of non-significant influence, therefore, only effects of simple fixed factors were investigated. Statistical significance of differences $P < 0.05$) between least squares means was determined using Scheffe's test.

RESULTS AND DISCUSSION

Analysis of individual fatty acids

Effect of breed/production system

The content of FAs, as affected by breed/production system, is shown in Table 1. Regarding individual saturated FAs (SFA) in subcutantaneous fat, these (palmitic, lauric, myristic, margaric and stearic acids) were almost the same, regardless lamb group. The contents of palmitic acid, which seems to be individual SFA of the highest content in many sheep breeds (Díaz *et al.*, 2005; Fiori *et al.*, 2013), were – 23.40 g.100 g^{-1} FAME (BE/SI1 lamb group) and 22.93 g.100 g^{-1} FAME (SF/SI2 lambs). Both lamb groups had lower content of palmitic acid than that reported by Janíček *et al.* (2019) for subcutaneous fat of Ile de France lambs in intensive production system (28.45 g.100 g^{-1} FAME). The contents of lauric acid were 0.99 g.100 g^{-1} FAME (BE/SI1 lambs) and 0.96 g.100 g^{-1} FAME (SF/SI2 lambs). These values were about two times higher than reported by Margetín *et al.* (2019) for intramuscular fat of investigated lamb breeds. The contents of margaric acid were also close to 1, but only slightly higher than reported by Margetín *et al.* (2019) for intramuscular fat of investigated lamb groups. The contents of stearic acid (SA) were also similar (between 14 and 17 g.100 g^{-1} FAME) regarless lamb group.

Regarding individual monounsaturated FAs (MUFA), palmitoleic acid, elaidic acid and trans-vaccenic acid (TVA) were found to differ ($P < 0.001$) between investigated lamb groups with higher values for subcutaneous fat in SF/SI2 lambs (0.62 and 0.72 g.100 g^{-1} FAME as far as palmitoleic oleic acid is related). With exception of elaidic acid, the pattern of MUFA in subcutaneous fat was similar (with values slightly higher) to that found for intramuscular fat in BE/SI1 and SF/SI2 lambs (Margetín *et al.*, 2019). The most common MUFA was oleic acid (OA) with similar content in subcutaneous fat of both lamb groups (32.54 and 31.75 g.100 g^{-1} FAME in BE/SI1 and SF/SI2 lambs). This was lower than reported by Maleki *et al.* (2015) for OA in subcutaneous fat of Mehraban (37.9 g.100 g^{-1} FAME) and Sanjabi fat-tailed lambs (37.8 g.100 g^{-1} FAME) fed only with forage and concentrate (ratio 60:40). The higher content of OA was reported by Kaczor *et al.* (2010) for subcutaneous fat of Koluda and Ile de France x Koluda male lambs in intensive fattening

(38.67 and 40.48 g.100 g^{-1} FAME). Contrariwise, Janíček *et al.* (2019) reported lower content of OA (25.52 g.100 g^{-1} FAME) for subcutaneous fat of pastured Ile de France lambs. According to Aurousseau *et al.* (2007), it may be expected that the more concentrates in diet, the more OA absorbed. When comparing the content of TVA in subcutaneous fat of investigated lamb groups with that reported by Janíček *et al.*, (2019) for pastured Ile de France lambs (4.04 g.100 g^{-1} FAME), it was lower in subcutaneous fat of BE/SI1 and SF/SI2 lambs (2.07 and 3.91 g.100 g^{-1} FAME). These findings agree with those of Nuernberg *et al.* (2005) and Aurousseau *et al.* (2007), who reported that TVA was of highest content in grazed lambs. The content of elaidic acid in subcutaneous fat was 0.25 g.100 g^{-1} FAME (BE/SI1 lambs) and 0.31 g.100 g^{-1} FAME (SF/SI2 lambs) i.e. higher ($P < 0.001$) in lambs those diet consisted of grass (although for limited period). This finding is in agreement with Li *et al.* (2017), who reported up to 3-time higher content of elaidic acid for subcutaneous fat of Altay lambs on pasture.

Regarding individual polyunsaturated FAs (PUFA), γ-linolenic acid (GLA) was PUFA of the lowest

Table 2. Fatty acids (least squares means) in subcutanous fat summed by type (g.100 g^{-1} fatty acid methyl esters), ratios and indexes

Fatty acids	Breed/Production system		Sex		RMSE
	BE/SI1	SF/SI2	Male	Female	
SFA[1]	54.16	52.32	54.70[a]	51.79[b]	2.693
MUFA[2]	40.23	41.50	39.64[a]	42.09[b]	2.718
PUFA[3]	5.60[a]	6.18[b]	5.66[a]	6.12[b]	0.610
*Trans*UFA[4]	4.28[A]	6.29[B]	5.15[a]	5.41[b]	0.363
*Cis*UFA[5]	36.73	36.78	35.58[a]	37.93[b]	2.628
BCFA (*iso, anteiso*)[6]	2.47[a]	2.11[b]	2.32	2.26	0.213
Essential FA (LA+ALA)	2.90[A]	2.53[B]	2.71	2.73	0.248
n-6 PUFA[7]	2.47[A]	2.02[B]	2.24	2.25	0.205
n-3 PUFA[8]	0.78	0.80	0.76	0.82	0.122
CLA[9]	1.09[A]	1.84[B]	1.33[a]	1.60[b]	0.337
PUFA/SFA	0.10[a]	0.12[b]	0.10[a]	0.12[b]	0.015
∑*n-6* PUFA/∑*n-3* PUFA	3.23[A]	2.56[B]	2.98	2.81	0.449
LA/ALA	4.30[A]	3.24[B]	3.78	3.76	0.509
LC*n-6*PUFA/LC*n-3* PUFA[10]	0.57[A]	0.45[B]	0.55[a]	0.47[b]	0.090
AI (atherogenic index)	1.24	1.24	1.26	1.22	0.171
TI (thrombogenic index)	2.01	1.09	2.08[a]	1.84[b]	0.251
h/H[11] index	1.17	1.13	1.13	1.17	0.134

[1]SFA is the sum of saturated fatty acids: C8:0 + C10:0 + C11:0 + C12:0 + C13:0 + *iso*C14:0 + C14:0 + *iso*C15:0 + *anteiso*C15:0 + C15:0 + *iso*C16:0 + C16:0 + *iso*C17:0 + *anteiso*C17:0 + C17:0 + *iso*C18:0 + C18:0 + C19:0 + C20:0 + C21:0 + C22:0; [2]MUFA, sum of monounsaturated FA: C12:1 + C14:1 + *t*C16:1 + *c*C16:1 + 9*c*C16:1 + C17:1 + 6-8*t*C18:1 + 9*t*C18:1 + 10*t*C18:1 + 11*t*C18:1 + 12*t*C18:1 + 9*c*C18:1 + (15*t*+11*c*C18:1) + 12*c*C18:1 + 13*c*C18:1 + (14*c*C18:1+9*t*12*t*18:2 / 2) + 15*c*C18:1 + (C18:2+C19:1 / 2) + C20:1; [3]PUFA is the sum of polyunsaturated FA: (14*c*C18:1+ 9*t*12*t*18:2 / 2) + 9*c*13*t*C18:2 + (8*t*13*c*+9*c*12*t*C18:2) + (9*t*12*c* + 11*t*15*c*C18:2) + C18:2*n-6* + 9*c*15*c*C18:2 + 12*c*15*c*C18:2 + *cc*C18:2 + *cc*C18:2 + (C18:3 *n-6* GLA) + (C18:2+C19:1 / 2) + cyklo + 9*t*12*c*15*c*C18:3 + C18:3 *n-3* + (9*c*11*t*C18:2 CLA) + *ct*CLA + *cc*CLA + *tc*CLA + *tt*CLA + C18:3 + C20:2 + C20:3 *n-9* + C20:3 *n-6* + C20:4 *n-6* + C20:3 *n-3* + C20:4 *n-3* + C20:5 *n-3* + furyl C22 + C22:4 *n-3* + C22:5 *n-3* + C22:6 *n-3*; [4]*Trans*UFA is the sum of *trans*UFA: *t*C16:1 + 6-8*t*C18:1+ 9*t*C18:1+ 10*t*C18:1 + 11*t*C18:1 + 12*t*C18:1 +(15*t*+11*c*C18:1/3)*2) + (14*c*C18:1+ 9*t*12*t*18:2 / 2) + 9*c*13*t*C18:2 + (8*t*13*c*+9*c*12*t*C18:2) + 9*t*12*c*+11*t*15*c*C18:2 + *tt*CLA; [5]*Cis*UFA is the sum of *cis*UFA: *c*C16:1 + 9*c*C16:1 + 9*c*C18:1 + (15*t*+11*c*C18:1 / 3) + 12*c*C18:1 + 13*c*C18:1 + (14*c*C18:1+ 9*t*12*t*C18:2 / 2) + 15*c*C18:1 + 9*c*15*c*C18:2 + 12*c*15*c*18:2 + *cc*C18:2 +*cc*C18:2 + 9*c*11*t*C18:2 CLA + *ct*CLA + *cc*CLA + *tc*CLA; [6]BCFA is the sum of *iso* and *anteiso* FA: *iso*C14:0 + *iso*C15:0 + *anteiso*C15:0 + *iso*C16:0 + *iso*C17:0 + *anteiso*C17:0 + *iso*C18:0; [7]*n-6* PUFA is the sum of *n-6* PUFA: C18:2 *n-6* + C18:3 *n-6* GLA + C20:3 *n-6* + C20:4 *n-6*; [8]*n-3* PUFA is the sum of *n-3* PUFA: C18:3 *n-3* + C20:3 *n-3* + C20:4 *n-3* + C20:5 *n-3* + C22:4 *n-3* + C22:5 *n-3* + C22:6 *n-3*; [9]CLA = 9*c*11*t*C18:2 CLA + *ct*CLA + *cc*CLA + *tc*CLA + *tt*CLA; [10]LC *n-6* PUFA = *n-6* PUFA – LA and [10]LC *n-3* PUFA = *n-3* PUFA – ALA; [11]h/H = hypocholesterolaemic FA/hypercholesterolaemic FA. For remaining explanations see Table1.

content in subcutaneous fat of investigated lamb groups (0.02 g.100 g^{-1} FAME and 0.01 g.100 g^{-1} FAME in BE/SI1 and SF/SI2 lambs, $P < 0.001$). The low contents of GLA agree with Margetín *et al.* (2019), who reported similar values of GLA for intramuscular fat of these lamb groups. The contents of essential α-linolenic acid (ALA) in subcutaneous fat of BE/SI1 and SF/SI2 lambs were found not to differ significantly ($P > 0.05$) and were 0.55 g.100 g^{-1} FAME and 0.61 g.100 g^{-1} FAME i.e. lower than reported by Margetín *et al.* (2019) for intramuscular fat. The remaning individual PUFA significantly differed ($P < 0.001$ or $P < 0.05$) between investigated lamb groups. The essential linoleic acid (LA) was PUFA of the hightest content: 2.35 g.100 g^{-1} FAME (BE/SI1 lambs) and 1.93 g.100 g^{-1} FAME (SF/SI2 lambs). The content of rumen acid (RA) was 1.03 g.100 g^{-1} FAME (BE/SI1 lambs) and 1.70 g.100 g^{-1} FAME (SF/SI2 lambs). The content of LA was lower, whereas the content of RA was higher in subcutaneous than that in intramuscular fat (Margetín *et al.*, 2019). Health beneficial PUFA – arachidonic acid (AA), eicosap-entaeonic acid (EPA), docosapentaeonic (DPA) and docosahexaeonic acid (DHA) were of almost zero content (less than 0.15 g.100 g^{-1} FAME) and several times lower than those in intramuscular fat (Margetín *et al.*, 2019).

Effect of lamb sex

The content of FAs, as affected by lamb sex, is shown in Table 1. Comparisons within individual SFA, MUFA and PUFA revealed significant differences ($P < 0.05$) between sexes in SA, OA, TVA, RA and DPA. SA was found of higher content for subcutaneous fat in males (18.01 g.100 g^{-1} FAME) as compared to females (14.85 g.100 g^{-1} FAME). The opposite pattern was found for contents of OA (33.05 g.100 g^{-1} FAME in females and 31.24 g.100 g^{-1} FAME in males), TVA (3.08 g.100 g^{-1} FAME in females and 2.90 g.100 g^{-1} FAME in males), RA (1.49 g.100 g^{-1} FAME in females and 1.24 g.100 g^{-1} FAME in males) and DPA (0.16 g.100 g^{-1} FAME in females and 0.12 g.100 g^{-1} FAME in males). According to Margetín *et al.* (2019), significant differences between sexes were found only in SA and RA content in intramuscular fat (SA was found higher for ram lambs, whereas RA was found higher for ewe lambs). Regarding remaining individual FAs, no differences between sexes were reported by Margetín *et al.* (2019). As a difference, Horcada-Ibánez *et al.* (2009) reported no differences between sexes in SA and OA contents for subcutaneous fat of Aragonesa lambs.

Analysis of defined partial sums of fatty acids, ratios and indexes

Effect of breed/production system

The defined partial sums of FAs (SFA, MUFA, PUFA, etc.), ratios and indexes, as affected by breed/production system, are shown in Table 2. The partial sums of SFA (54.16 and 52.32 g.100 g^{-1} FAME, $P > 0.05$) and MUFA (40.23 and 41.50 g.100 g^{-1} FAME, $P > 0.05$) in subcutaneous fat between BE/SI1 and SF/SI2 lambs were similar. The partial sums of PUFA between BE/SI1 and SF/SI2 lambs were 5.60 and 6.18 g.100 g^{-1} FAME ($P < 0.05$). The partial sums of SFA and PUFA were found by 10 to 13 % higher (SFA) or up to two times lower (PUFA) for intramuscular fat of the same lamb groups (Margetín *et al.*, 2019). The partial sums of SFA were similar to that for subcutaneous fat of pastured Ile de France lambs (55.93 g.100 g^{-1} FAME), whereas the partial sums of PUFA were similar to that for subcutaneous fat of intensively reared Ile de France lambs (5.55 g.100 g^{-1} FAME), as reported by Janíček *et al.* (2019). In addition, the partial sums of PUFA were higher than those reported by Kaczor *et al.* (2010) for subcutaneous fat of purebred Koluda and crossbred Koluda x Ile de France ram lambs in intensive fattening (4.34 and 4.43 g.100 g^{-1} FAME). Regarding contents of *cis*-UFA in subcutaneous fat of BE/SI1 and SF/SI2 lambs (36.73 and 36.78 g.100 g^{-1} FAME, $P > 0.05$) and *trans*-UFA (4.28 and 6.29 g.100 g^{-1} FAME, $P < 0.001$), these were similar to those for intramuscular fat of the same lamb groups (Margetín *et al.*, 2019). Regarding contents of BCFA (2.47 and 2.11 g.100 g^{-1} FAME in subcutaneous fat of BE/SI1 and SF/SI2 lambs, $P < 0.05$), these were found to be higher by 21 and 15 % than those for intramuscular fat of the same lamb groups (Margetín *et al.*, 2019).

The contents of essential FAs (summed LA and ALA) in subcutaneous fat were 2.90 g.100 g^{-1} FAME (BE/SI1 lambs) and 2.53 g.100 g^{-1} FAME (SF/SI2 lambs); $P < 0.001$. The contents of CLA in subcutaneous fat were 1.09 g.100 g^{-1} FAME (BE/SI1 lambs) and 1.84 g.100 g^{-1} FAME (SF/SI2 lambs); $P < 0.001$. When the contents of summarized LA and ALA in subcutaneous fat were compared with those in intermuscular fat of the same lamb groups (4.64 and 6.26 g.100 g^{-1} FAME), the values were by 37 and 60 % higher in favour of intramuscular fat (Margetín *et al.*, 2019). However, the contents of CLA in subcutaneous and intramuscular fat of BE/SI1 lambs and SF/SI2 lambs were either

the same or by 11 % higher in favour of subcutaneous fat, since these values were 1.09 and 1.64 g.100 g^{-1} FAME in intramuscular fat (Margetín *et al.*, 2019). The contents of CLA reported by Kaczor *et al.* (2010) for subcutaneous fat of Koluda and Koluda x Ile de France ram lambs (0.48 and 0.49 g.100 g^{-1} FAME) were by 2 – 3 times lower than those in subcutaneous fat of investigated lamb groups. The contents of CLA in subcutaneous fat of Mehraban and Sanjabi breeds (1.35 and 1.64 g.100 g^{-1} FAME), as reported by Maleki *et al.* (2015), were either higher by 19 % or lower by 11 % than those measured in investigated breeds. The contents of CLA in subcutaneous fat of purebred and crossbred lamb rams of Zandi and Chal sheep (all finished on the same concentrate diet) were reported by Momen *et al.* (2016) to be either the same or lower than those found for investigated lamb groups.

The ratios and indexes of FA groups are shown in Table 2. The ratios of *n-6/n-3* PUFA were 3.23 (BE/SI1 lambs) and 2.56 (SF/SI2 lambs); $P < 0.001$. Both fell below the recommended value (less than 4), as proposed by Simpoulos (2002) and Wood *et al.* (2003), however, these were slightly higher than reported for intramuscular fat of BE/SI1 and BE/SI2 lambs (2.94 and 2.40) (Margetín *et al.*, 2019). Contrariwise, the ratios determined in this study, were lower than reported for subcutaneous fat of Mehraban and Sanjabi breeds kept in intensive production system (4.19 and 3.75) (Maleki *et al.*, 2015). The ratios of LC *n-6*/LC *n-3* PUFA for subcutaneous fat were found about two-times lower (0.57 and 0.45) than reported by Margetín *et al.* (2019) for intramuscular fat of these breeds (1.32 and 1.18). The ratios of PUFA/SFA for subcutaneous fat were 0.10 (BE/SI1 lambs) and 0.12 (SF/SI2 lambs), i.e. lower than those for intramuscular fat of the same lamb groups and did not agree with the recommended values (more than 0.7 or more than 0.45) and (more than 0.4), as proposed by Raes *et al.* (2004), Williams (2000) and Wood and Enser (1997). The atherogenic index (AI) in subcutaneous fat exceeded the value (less or equal to 1) recommended by Sinanoglu *et al.* (2013). In both lamb groups, AI was 1.24, i.e. by 25 and 33 % higher than those for intramuscular fat (0.93 and 0.82 in BE/SI1 and SF/SI2 lambs), as reported by Margetín *et al.* (2019). The thrombogenic index (TI) in subcutaneous fat was 2.01(BE/SI1 lambs). In subcutaneous fat of SF/SI2 lambs it was equal to 1.09 (similar to the recommended value of 1). The ratios of h/H (hypocholesterolaemic FA/hypercholesterolaemic FA) in subcutaneous fat were lower (1.17 and 1.13) than in intramuscular fat (1.44 and 1.58) of BE/SI1 and SF/S2 lambs, as reported by Margetín *et al.* (2019). In accordance with Hosseini-Vashan *et al.* (2016), not only value of TI found for subcutaneous fat of BE/SI2 but also values of h/H and AI found for subcutaneous fat of both lamb groups indicate that consumption of lamb fat may not have unfavourable effect on human health. This opinion is confirmed by Mahachi *et al.* (2020), who reported that addition of fat from tail improved TI of finished cabanossi product almost to the recommended value (0.99), whereas addition of pork backfat was without effect.

Effect of lamb sex

The defined partial sums of FAs, ratios and indexes, as affected by lamb sex, are shown in Table 2. The differences between males and females were significant ($P < 0.05$) in partial sums of SFA (54.7 and 51.79 g.100 g^{-1} FAME), MUFA (39.64 and 42.9 g.100 g^{-1} FAME), PUFA (5.66 and 6.12 g.100 g^{-1} FAME), *trans*-UFA (5.15 and 5.41 g.100 g^{-1} FAME), *cis*-UFA (35.58 and 37.93 g.100 g^{-1} FAME) and CLA (1.33 and 1.60 g.100 g^{-1} FAME). Similar differences in partial sums of SFA (59.43 and 56.70 g.100 g^{-1} FAME i.e. lower in ewe lambs) and PUFA (6.89 and 7.82 g.100 g^{-1} FAME i.e. higher in ewe lambs) were reported by Janíček *et al.* (2019) for Ile de France breed. Contrariwise, Horcada-Ibánez *et al.* (2009) reported no difference in partial sums of SFA for subcutaneous fat of Aragonesa ram and ewe lambs.

Regarding ratios and indexes, significant differences ($P < 0.05$) between males and females were found for ratios of PUFA/SFA (0.10 and 0.12), LC *n-6*/LC *n-3* PUFA (0.55 and 0.47) and for TI (2.08 and 1.84). With exception of *trans*-UFA and CLA, no differences between sexes for intramuscular fat were reported by Margetín *et al.* (2019).

CONCLUSION

Some differences in compostion of FAs were found in relation to breed/production system and lamb sex. The contents of elaidic acid and TVA were higher in subcutaneous fat of SF/SI2 lambs,

as compared to subcutaneous fat of BE/SI1 lambs. Regarding health beneficial FAs, only arachidonic acid and DPA differed between investigated lamb groups and were higher in BE/SI1 lambs. The ratio of PUFA/SFA was relatively close to the recommended value, whereas the ratio *n-6*/*n-3* PUFA was far away from the recommended value, regardless lamb group. The indexes, primarily AI and h/H, were also close to the recommended value, indicating that consumption of subcutaneous fat may not have unfavourable effect on human health. The differences between sexes were primarily in SFA, MUFA and PUFA partial sums; more favourable values were found for ewe lambs. This is one of the first studies investigated FAs composition in subcutaneous fat of lambs kept in Slovakia, which expands existing knowledge about sheep breeds.

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