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Effect of production system on fatty acid composition in subcutaneous adipose tissue of Ile de France lambs

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A study was conducted on the effect of two different lamb production systems on the fatty acid (FA) composition of subcutaneous fat of 40 purebred Ile de France lambs. In the first production system, ewes and lambs grazed on pasture without concentrate (GS), whereas in the second production system, ewes and lambs were housed indoor and fed silage/hay and concentrate (IS). Twenty lambs (7 females and 13 males) were included in each group. Lambs were slaughtered at 28.75 ± 2.76 kg. The FA were determined by gas chromatography and analysed through ANOVA by considering the following fixed effects: production system, sex and the interaction between production system and sex. Subcutaneous fat of GS lambs had greater proportion of C18:3n-3 (P <0.001), C22:5n-3 (P <0.05) and C22:6n-3 (P <0.05) than IS lambs, which resulted in a higher sum of n-3 polyunsaturated FA in GS compared to IS lambs (2.00 vs. 1.15 g/100 g FAME, P <0.001). Moreover, subcutaneous fat of GS lambs had greater proportion of *c*9,*t*11-C18:2 (P <0.001) and sum of detected conjugated linoleic acid isomers than IS lambs (2.21 vs. 0.67 g/100 g FAME, P <0.001). Females had significantly greater proportion of C18:2n-6 (P <0.05) and C18:3n-6 (P <0.001) than males. We can conclude that the GS system where lambs are raised under grazing conditions may provide carcasses with a more acceptable subcutaneous fat, as far as a human health and nutrition perspective is concerned.

Keywords: lamb, production system, subcutaneous fat, fatty acid composition

1 Introduction

The quality of food, especially in regard to its benefits for human health, plays an important role in affecting customer purchase decision. In recent years, a discussion has emerged surrounding both the positive and negative effects of red meat consumption, including lamb meat consumption (McAfee et al., 2010, Corpet, 2011). The average consumption of lean red meat, which is considered part of a balanced diet, evidently does not increase the risk of cardiovascular disease and colon cancer. It presents a positive effect on the intake of essential nutrients and a spectrum of beneficial fatty acids (FA; Nudda et al., 2009, Nudda et al., 2011, Binnie et al., 2014).

The proportion of FA may differ depending on the location of adipose tissue in the lamb's carcass. Potkanski et al. (2002) and Wood et al. (2008) reported that intramuscular fat has a more beneficial proportion of healthy FA than subcutaneous fat. As reported by Díaz et al. (2005), the proportion of FA in sheep meat is affected primarily by diet, as well as factors such as age, sex, breed and production system. Vasta et al. (2012) reported that pasture grazing is the best feeding system for lambs because the meat is much juicier and tastier, whilst having a higher proportion of beneficial FA for human health. Previous studies indicated higher proportion of n-3 polyunsaturated FA (PUFA) in the tissue of lambs from pasture than from concentrate-fed (Santos-Silva et al., 2002, Cividini et al., 2008, Araba et al., 2009, Popova, 2014, Karaca et al., 2016).

The demand for light lambs in the Mediterranean countries is largely affecting Slovak lamb meat production. The last decade has been characterized by light lamb's price fall. A large number of

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stakeholders have transitioned from the dairy to the meat production system, due to lower production costs. There are two semi-intensive systems that are preferable for heavy carcass lamb production; the grazing system with ewes not receiving concentrate and the indoor system with ewes fed meadow hay or alfalfa silage, with unrestricted access to concentrate for lambs. The grazing system is more convenient in the foothill pastures and the indoor system in the areas rich in arable land. There is an opportunity for heavy lambs in Slovakia, due to growing demands from cultural minorities in Europe that tend to prefer lambs of a higher weight.

For this reason, it is necessary to study the quality of heavy lambs from Slovak production systems. The aim of this study was to investigate the effect of grazing and indoor systems on the FA composition of subcutaneous fat in Ile de France heavy lambs.

2 Material and methods

2.1 Experimental design and animal management

Forty Ile de France heavy lambs were included in the trial. Two experimental groups, namely the indoor system (IS) and the grazing system (GS) were formed, each consisting of 7 female and 13 male lambs. The IS consisted of lambs housed with ewes and allowed to suckle milk *ad libitum*. Lambs received an access to the separate area two weeks after birth, where lamb starter (crude protein: min 19.0%; crude fiber: max 9.0%; crude fat: max 6.0%; ash: max 10.0%; calcium: min 1.12%; phosphorus: min 0.61%; sodium: min 0.2%) and hay were offered *ad libitum*. Moreover, until 60 days of age, lambs were fed 0.2 kg mixture of lucerne and maize silage (ratio 2:1) per capita per day. The ewes were fed 1 kg maize silage, 1 kg lucerne silage, 2 kg meadow hay and 0.2 kg of concentrate per capita per day. Lambs in the GS were born indoor and shortly after parturition they were moved with ewes to pasture. The pasture was natural and rich in *Lolium perenne*, *Lolium multiflorum*, *Trifolium repens*, *Trifolium pretense*, *Poa pratensis* and *Festuca rubra*.

The slaughter of lambs was carried out in the authorised slaughterhouse of Slovak University of Agriculture in Nitra. The average age at slaughter was 107 ± 8 days, and the average live weight was 29.23 ± 8.20 kg for GS and 32.2 ± 2.62 kg for IS. The average daily gain was 0.242 ± 0.034 kg for GS and 0.264 ± 0.029 kg for IS. Animal care, management and slaughter procedures followed the EU Directive 2010/63/EU on the protection of animals used in scientific experiments.

2.2 Fatty acid analysis

Two g of subcutaneous fat were taken from the tailhead area of every lamb to determine the FA profile of subcutaneous fat. Samples were collected 24 h after slaughter and carcass chilling. Capillary gas chromatography was used to determine the proportion of the individual FA and FA isomers. The fatty acid methyl esters (FAME) preparation and gas chromatography determination are described in Margetín et al. (2018).

The FA profile of subcutaneous fat was detected in grams of individual FAME per 100 g of total detected FAME. The average relative standard deviation of analysed FAME with content >0.5 g/100 g was 1.1% for the entire analytical procedure and five replicated samples.

2.3 Statistical analysis

The experimental data was analysed by ANOVA using the GLM procedure of the software SAS (SAS Institute Inc., Cary, NC, USA). The linear model included the fixed effects of production system, sex and the interaction between production system and sex. Least squares means were compared by applying Scheffe's test at the significance limits: $P<0.05$, $P<0.01$ and $P<0.001$.

3 Results and discussion

The composition of important FA identified in subcutaneous fat which are believed to affect human health, are reported in Table 1. Saturated FA (SFA) are formed from hydrolysed unsaturated FA (UFA) and this process depends on the biohydrogenation activity in rumen (Zervas and Tsiplakou, 2011). The ewes' maternal milk is rich in lauric acid (C12:0) and myristic acid (C14:0); these FA are mostly provided through the diet used to fed lambs, including dietary fat (Juárez et al., 2008). As previously reported by Santos-Silva et al. (2002), lambs raised under a pasture system showed higher C14:0 in intramuscular fat in relation to concentrate-fed lambs. The authors interpreted this result as consequence of the greater C14:0 proportion in maternal milk compared to concentrate-fed diet. In the current study, lambs from both groups had unlimited access to maternal milk and no effect on the proportion of C12:0 and C14:0 was observed. The effect of different production systems significantly affected the proportion of palmitic acid (C16:0; 28.45 vs. 22.67 g/100 g FAME, *P* <0.001) and margaric acid (C17:0; 1.47 vs. 1.28 g/100 g FAME, *P* <0.01) and the total SFA (60.20 vs. 55.93 g/100 g FAME, *P* <0.001). The proportion of FA groups and their ratios are shown in Table 2. Mora et al. (2016) and Leão et al. (2011) found a similar concentration of C16:0 in subcutaneous fat of lambs finished by concentrates. Díaz et al. (2002) reported similar results concerning the proportion of C16:0 in subcutaneous loin fat, yet the concentration of the total SFA was higher in pastured lambs (59.37 vs. 57.16% of FA, *P* <0.01). A potential explanation could be that the energy intake of IS lambs was higher because the IS lambs' feed was richer in energy. Thus, the *de novo* synthesis of C16:0 was also higher. The GS lambs had higher proportion of SFA only for stearic acid (C18:0; 17.86 vs. 14.96 g/100 g FAME, *P* <0.01), which is one of the primary FA in meat of ruminants. It does not have an effect on cholesterol concentration in the bloodstream because it is desaturated and converted to oleic acid (*c*9-C18:1; Willians, 2000). The results of C18:0 were similar to some earlier investigations (Díaz et al., 2002, Guler et al., 2011, Karaca et al., 2016). It is likely that the ewes' milk fat consumed by GS lambs had a higher proportion of C18:1, which is biohydogenated to C18:0 by rumen microbiota. That could be affected by the nature of dietary lipids, as well as ewes' rumen FA metabolism and FA synthesis in the intestinal, adipose tissue and mammary gland of ewes.

Proportion of palmitoleic acid (*c*9-C16:1), n-7 monounsaturated FA (MUFA), was higher in GS than IS group (0.64 vs. 0.48 g/100 g FAME, *P* <0.001). Karaca et al. (2016) reported different findings, i.e. pastured lambs had a lower proportion of *c*9-C16:1 than intensively fattened lambs (3.58 vs. 5.24% of FA, *P* <0.001). Proportion of *c*9-C18:1 was significantly higher in IS than GS (27.00 vs. 25.52 g/100 g FAME, P <0.05). The GS group also had a higher proportion of elaidic acid (*t*9-C18:1) than the IS group (0.33 vs. 0.22 g/100 g FAME, *P* <0.001).

Table 1 Fatty acid composition (g/100 g FAME) of subcutaneous fat of lambs according to the production system (GS – grazing system, IS – indoor system) and sex (male and female)

Fatty acid	Production system (PS)		P-value	Sex		P-value	P-value
	GS	IS		Male	Female		PS x Sex
C12:0	1.22	1.19	0.833	1.35	1.05	0.027	0.194
C14:0	8.38	9.38	0.101	9.39	8.37	0.091	0.096
C16:0	22.67	28.45	<0.001	26.20	24.92	0.032	0.226
*c*9-C16:1	0.64	0.48	<0.001	0.57	0.55	0.166	0.196
C17:0	1.28	1.47	0.001	1.34	1.41	0.180	0.627
C18:0	17.86	14.96	0.002	16.40	16.43	0.976	0.730
*c*9-C18:1	25.52	27.00	0.030	25.62	26.91	0.055	0.017
*t*9-C18:1	0.33	0.22	<0.001	0.27	0.28	0.595	0.105
*t*11-C18:1	4.04	1.18	<0.001	2.60	2.62	0.777	0.341
C18:2n-6	2.84	2.26	<0.001	2.41	2.68	0.040	0.931
*c*9,*t*11-C18:2	1.96	0.60	<0.001	1.23	1.33	0.374	0.793
C18:3n-6	0.02	0.03	<0.001	0.01	0.03	<0.001	0.402
C18:3n-3	1.38	0.84	<0.001	1.15	1.07	0.243	0.962
C20:4n-6	0.29	0.10	0.176	0.08	0.30	0.104	0.143
C20:5n-3	0.08	0.04	0.117	0.03	0.09	0.090	0.088
C22:5n-3	0.36	0.19	0.051	0.20	0.35	0.103	0.092
C22:6n-3	0.09	0.04	0.050	0.05	0.09	0.090	0.070

The proportion of vaccenic acid (*t*11-C18:1) and rumenic acid (*c*9,*t*11-C18:2), the latter being the important isomer of conjugated linoleic acid (CLA), were significantly higher in GS than IS (4.04 vs. 1.18 and 1.96 vs. 0.60 g/100 g FAME, respectively, *P* <0.001). Nuernberg et al. (2008) did not find any differences in the proportion of *t*11-C18:1 and *c*9,*t*11-C18:2 in subcutaneous fat of pastured and intensive reared lambs. Di Memmo (2014) reported a high proportion of total CLA in the meat of lambs which were fed their mother's milk at pasture. We found a similar effect on the total CLA, when the proportion was more than threefold higher in GS than IS (2.21 vs. 0.67 g/100 g FAME, *P* <0.001, Table 2). The higher proportion of CLA in subcutaneous fat of GS than IS is in agreement with studies focused on differences in intramuscular fat (Aurosseau et al., 2004, Scerra et al., 2011, Popova, 2014). The difference of MUFA between GS and IS was not significant. Pastured lambs exhibited higher proportion of both essential PUFA, linoleic acid (C18:2n-6) and *α*-linolenic acid (C18:3n-3) in subcutaneous fat compared to the IS lambs (2.84 vs. 2.26 and 1.38 vs. 0.84 g/100 g FAME, respectively, *P* <0.001). In agreement with results of Karaca et al. (2016), the proportion of C18:2n-6 was higher in the IS lambs.

The second important n-6 FA, i.e. the *γ*-linolenic acid (C18:3n-6), was higher in IS than GS lambs (0.03 vs. 0.02 g/100 g FAME, *P* <0.001). However, these values are relatively low. The essential C18:3n-3 plays an important role in the production of the n-3 long chain PUFA, resulting in a positive effect on higher proportions of docosapentaenoic acid (C22:5 n-3) and docosahexaenoic acid (C22:6n-3) in the GS than IS lambs (0.36 vs 0.19 and 0.09 and 0.04 g/100 g FAME, respectively, *P* <0.05). The results of n-6 and n-3 acids obtained in this study were similar to those reported by Karaca et al. (2016). The proportion of total n-6 PUFA and n-3 PUFA was higher in the GS than in the IS lambs (3.18 vs. 2.42 and 2.00 vs. 1.15 g/100 g FAME, respectively, *P* <0.001). This affected total PUFA, which was higher for the GS than the IS lambs (9.17 vs. 5.55 g/100 g FAME, *P* <0.001). Value of the important n-6/n-3 ratio was more suitable in subcutaneous fat of the GS lambs, even if in both groups the values did not exceed the recommended standard 4:1 (1.61 vs. 2.15, *P* <0.001). According to Simopoulos (2016), increasing both the proportion of n-6 FA and n-6/n-3 ratio in phospholipid membrane of red blood cells, can cause an increased risk of obesity. On the other hand, a high proportion of n-3 FA in the phospholipid membrane of red blood cells can reduce this risk.

Table 2 Groups of and ratios between fatty acids (g/100 g FAME) of subcutaneous fat of lambs according to the production system (GS – grazing system, IS – indoor system) and sex (male and female)

Fatty acid	Production system (PS)		P-value	Sex		P-value	P-value
	GS	IS		Male	Female		PS x Sex
SFA[a]	55.93	60.20	<0.001	59.43	56.70	0.006	0.071
MUFA[b]	34.90	34.08	0.337	33.74	35.25	0.085	0.027
PUFA[c]	9.17	5.55	<0.001	6.89	7.82	0.010	0.152
CLA[d]	2.21	0.67	<0.001	1.38	1.49	0.361	0.848
n-6 PUFA[e]	3.18	2.42	0.001	2.54	3.06	0.025	0.320
n-3 PUFA[f]	2.00	1.15	<0.001	1.48	1.67	0.200	0.063
PUFA/SFA	0.17	0.09	<0.001	0.12	0.14	0.007	0.319
n-6/n-3	1.61	2.15	<0.001	1.76	2.00	0.041	0.063
LA[g]/ALA[h]	2.15	2.77	0.003	2.17	2.75	0.006	0.627

[a] 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

[b] sum of monounsaturated fatty acids: C12:1 + C14:1 + *t*-C16:1 + *c*-C16:1 + *c*9-C16:1 + C17:1 + *t*6/*t*7/*t*8-C18:1 + *t*9-C18:1 + *t*10-C18:1 + *t*11-C18:1 + *t*12-C18:1 + *c*9-C18:1 + (*t*15-C18:1 + *c*11-C18:1) + *c*12-C18:1 + *c*13-C18:1 + (*c*14-C18:1 + *t*9, *t*12-C18:2/2) + *c*15-C18:1 + (C18:2/2 + C19:1) + C20:1

[c] sum of polyunsaturated fatty acids: (*c*14-C18:1 + *t*9,*t*12-C18:2/2) + *c*9,*t*13-C18:2 + (*t*8,*c*13-C18:2 + *c*9,*t*12-C18:2) + (*t*9,*c*12-C18:2 + *t*11,*c*15-C18:2) + C18:2n-6 + *c*9,*c*15-C18:2 + *c*12,*c*15-C18:2 + *cc*C18:2 + C18:3 n-6 + (C18:2 + C19:1/2) + cyklo + *t*9,*c*12,*c*15-C18:3 + C18:3n-3 + *c*9,*t*11-C18:2 + *ct*CLA + *cc*CLA + *tc*CLA + *tt*CLA + C18:3 + C20:2 + C20:3n-9 + C20:3n-6 + C20:4n-6 + C20:3n-3 + C20:4n-3 + C20:5n-3 + furyl C22 + C22:4n-3 + C22:5n-3 + C22:6n-3

[d] sum of n-6 PUFA: C18:2n-6 + C18:3n-6 + C20:3n-6 + C20:4n-6

[e] sum of n-3 PUFA: C18:3n-3 + C20:3n-3 + C20:4n-3 + C20:5n-3 + C22:4n-3 + C22:5n-3 + C22:6n-3

[f] sum of conjugated linoleic acid isomers: *c*9,*t*11-C18:2 + *ct*CLA + *cc*CLA + *tc*CLA + *tt*CLA

[g] linoleic acid

[h] α-linolenic acid

The sex effect significantly explained the variation of C12:0 and C16:0; indeed, the proportion of both FA was higher in subcutaneous fat of males than females (1.35 vs. 1.05 and 26.20 vs. 24.92 g/100 g FAME, *P* <0.001), whereas females had significantly higher proportion of C18:2n-6 (2.68 vs. 2.41 g/100 g FAME, *P* <0.05) and C18:3n-6 (0.03 vs. 0.01 g/100 g FAME, *P* <0.001) than males (Table 1). Males had a lower n-6/n-3 ratio than females (1.76 vs. 2.00, *P* <0.05, Table 2). The production system by sex interaction was significant for *c*9-C18:1 (*P* =0.017), which possibly affected the sum of MUFA (*P* =0.027). Interaction effect in proportion of healthy FA was not significant. Because of that, our sample data did not provide enough evidence to conclude that the relationship between production system and sex has positive effect on FA composition. To the best of authors' knowledge, investigations on the interaction effect between grazing/indoor system and sex have not been published yet. Therefore, appropriate comparison with the literature could not be carried out.

4 Conclusions

Results of this study indicated that the production system affected the FA profile of subcutaneous fat in Ile de France heavy lambs. The muscular lipids of lambs raised on pasture exhibited higher proportions of n-3 FA, in particular α-linolenic acid, docosapentaenoic acid, docosahexaenoic acid and CLA, alongside a preferable n-6/n-3 ratio, which are beneficial for human health. Therefore, the pasture grazed lambs may be considered as a better nutritional source for humans than lambs raised in indoor systems.

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