EFFECT OF PHYSIOLOGICAL FACTORS (AGE AND SEX) ON THE FATTY ACID PROFILE OF MUSCLE TISSUE IN SHEEP

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Abstract

The aim of this study was to investigate the effect of age (young fattened intensively vs. reformed adult sheep) and sex (male vs. females) on fatty acid profile (FA) of intramuscular fat (Longissimus dorsi, LD), with particular reference the PUFA n-3 and CLA (mainly isomer, cis-9 trans-11 C18: 2). 8 heads were used intensively fattened young sheep heads and 8 adult sheep breed reformed pan (4 males and 4 females). The results reveal a significant influence of age and sex on FA profile or nutritional quality of fat in muscle tissue. Highest proportion of n-3 FA and CLA (isomer cis-9, trans-11 C18: 2) in LD muscle fat was found in intensively fattened males compared to females and that fattened in the intensive sheep adult reconditioned. Mainly young lambs fattened intensively and male sex in intramuscular fat were significantly higher proportions of PUFA n-3 (p <0.005), especially C18: 3 n-3, whose weight was increased by 52.0% and 30.8% in young males compared to adult animals and that sex female lambs. The proportion of cis-9, trans-11 CLA in intramuscular fat was higher in young males gained intensive and compared with females and adult animals.

Key words: PUFA n-3, *cis*-9, *trans*-11 CLA, age and sex, meat, sheep

INTRODUCTION

Intramuscular fatty acid profile of fat storage and can be affected by several factors, such as diet, breed, sex, age, and weight at slaughter (Aharoni et al., 1995, Rule et al., 1995, Wood and Enser, 1997, Nürnberg et al., 2005; Guler and Aktumsek, 2011). The most important factor role in manipulating fatty acid profile of lambs, is the food.

Metabolic studies have shown that the total amount of fat in human nutrition determine serum cholesterol levels, but importance is the type of fat (Sanders, 2003). Additionally controlled trials have shown that replacing saturated fat and high in trans fats with unsaturated fats and especially n-3 fatty acids, is more effective in preventing heart disease than reducing fat (Renaud and Lanzmann-Petithory, 2002, Hu and Willett, 2002; Sanders, 2003). Animal feeding strategies may lower proportion of saturated fat and increase weight polyunsaturated fatty acids n-3 and CLA (conjugated linoleic acid) in intramuscular fat, which would improve the quality of sheep meat.

Lipids from ruminants are the richest sources of CLA, especially the brown acid (C18: 2 *cis*-9, *trans*-11) which is the most important isomer of CLA. This isomer, which represents over 80% of CLA in foods from ruminant (Ha et al., 1990), proved very important for human health because it inhibits the proliferation of cancer cells (Schultz et al., 1992; Belury et al.,

1995), inhibits the accumulation of body fat (Park et al., 1997) and antioxidant effect antidiabetogenic (Ip et al., 1994).

Isomer cis-9, trans-11 CLA, are formed in the rumen by incomplete biohidrogenation fatty acids in the diet, especially C18: 2 n-6, but a substantial fraction of the amount of CLA in tissues derived from desaturation C18: 1 trans-11 Δ^9-desaturase enzyme in action acting on adipose tissue (Santora et al., 2000, Bauman et al., 2001).

In terms of nutrition, fat from lambs fattened is more appropriate than those from adult sheep, because it contains a higher proportion of n-3 polyunsaturated fatty acids (PUFA n-3) and CLA and report n-6/n-3 lower (Santos et al., 2002; Nuernberg et al., 2008; Guler and Aktumsek, 2011).

However there is little information on the influence of physiological factors on nutritional quality of mutton fat. The aim of this study was to investigate the effect of age and sex on the fatty acid profile of intramuscular fat (LD), with particular reference to PUFA n-3 and CLA (mainly isomer *cis* -9, *trans*-11 C18: 2).

MATERIAL AND METHODS

The study was conducted at the University of Oradea mainly aimed profiling fatty acids (FA) of intramuscular fat (*longissimus dorsi*) in relation to age (young fattened intensively vs. reformed adult sheep) and sex (males vs. females).

For sampling analysis (muscle tissue) were used 8 young sheep fattened heads and 8 heads intensively reformed adult sheep breed pans, distributed as follows: 4 males and 4 females. Biological material derived from commercial farms in Bihor County.

With 60 days before sampling analysis, respectively slaughter for food rations were used as fodder uniform structures are based on alfalfa hay, green fodder and feed hay combined with specific nutritional requirements. Alfalfa hay and green fodder were provided *ad libitum* and premixtures were administered twice a day for 200 g / head for adults and 300 g / head youth. In this way food was removed influence the fatty acid profile of housing. Mixed fodders used both youth nutrition and adult sheep consisted of: corn-45,5%; triticale-20,0%; meal sunflower-10,0% and vitamin-mineral premix-4,5%. Mixed fodder provided for 1 kg DM: 1.41 UNC (meat nutrition units = 1481 kcal EN; 6.2 MJ), 127.3 g PDIN (protein digestible in the intestine in the diet levels rumen degradable azot) and 116.7 g PDIE (protein digestible in the intestine in the diet levels rumen degradable energy).

Slaughtering control were preceded by a 12-hour dietary animal and sampling LD muscle analysis was done in 24 hours postmortem (after drying carcass). Samples for analysis were collected from the lower back

after the 13th thoracic vertebra to a length of 10 cm. Samples were stored at -20^{0}C until fat isolation and identification of their profile.

For extraction of total lipids from muscle tissue samples was used classical method described by Folch et al. (1987). Weighed sample (about 1 g) was triturated with an electric homogenizer and treated with 10 ml of methanol and BHT. After mixing for 1-2 minutes, were added 20 ml of chloroform and stirred again for 2 minutes. The mixture was filtered and the waste is treated again with a mixture of chloroform : methanol in the ratio of 2 : 1 (v / v). Total extract obtained was added to a volume of 88% KCl solution so that the ratio of chloroform : methanol : potassium chloride to be 8 : 4 : 3 (v / v). After phase separation by centrifugation, recovered hipofaza chloroform containing total lipids. Chloroform extract was passed over anhydrous sodium sulphate to remove traces of water, was evaporated to dryness and restart in a known volume of chloroform (1 ml). Total lipid extract was stored in glass bottles with ground glass stopper, in darkness and temperatures of - 20^{0}C until use for further analysis.

For determination of fatty acids, total lipid extract was transesterificat with methanol saturated with hydrochloric acid for 2 hours at 80^{0}C in Pyrex tubes. Methyl esters were extracted in petroleum ether : benzene (8 : 1), purified, separated and isothermal identified as methyl esters by gas chromatography with an HP 5890 gas chromatography coupled with II/5972 GC-MSD mass spectrometer. Grout was used to identify PUFA No. 2 (Animal Source). Fatty acids were expressed as a percentage of total methyl esters identified.

ANOVA was used to assess the effect of age and sex on FA profile of intramuscular fat. Comparison of the means was performed using Duncan test. Differences between means were considered for $p < 0.05$.

RESULTS AND DISCUSSION

The results presented in tables 1 and 2 reveals a significant influence of age and sex on nutritional quality of fat in muscle tissue, that the fatty acid profile. Highest proportion of n-3 FA and CLA (isomer *cis*-9, *trans*-11 C18: 2) in LD muscle fat was found in intensively fattened males compared to females in system ingrasatate intensive (table 1), and reconditioned or adult sheep (table 2). Share saturated FA (SFA) and monounsaturated FA (MUFA) in intramuscular fat was higher by 10.0% and 18.2% in adult sheep compared with youth reconditioned gained intensive, while the share of polyunsaturated FA (PUFA) was lower by 32.5%. Similar issues were found and about the influence of sex, meaning that the largest proportion of SFA and MUFA in intramuscular fat and the lowest proportion of PUFA were found in females compared with males (Fig. 1). These results are in

agreement with previous findings established in studies in fattening calves (Steen et al., 2010).

Mainly young lambs fattened intensively and male sex in intramuscular fat were significantly higher proportions of PUFA n-3 ($p < 0.005$), especially C18: 3 n-3 (Fig. 2), whose weight was increased by 52.0% and 30.8% in young males compared to adult animals and that sex female lambs.

Table 1

Influence of age on fatty acid profile of muscle tissue (% of FAME)

	Age		SEM[1]	p
	Youth intensive fattening	Mature reconditioned		
Total lipids	6,25	8,45	0,54	***
C 12:0	0,02	0,03	0,01	*
C 14:0	4,60	5,97	0,10	**
C 16:0	18,87	21,48	0,41	***
C 18:0	12,29	11,94	0,37	NS
C 20:0	0,12	0,13	0,10	NS
C 18:1	28,30	33,46	0,71	***
C 18:2 n-6	9,74	6,45	0,69	**
C 18:3 n-3	3,71	2,44	0,15	***
CLA *(cis*-9, *trans*-11 C18:2)	2,64	1,31	0,20	***
C 20:2 n-6	0,75	0,82	0,07	NS
C 20:4 n-6	3,80	3,48	0,23	NS
C 20:5 n-3, EPA	2,69	1,45	0,18	***
C 22:3 n-3	0,74	0,32	0,07	***
C 22:5 n-3 DPA	0,58	0,41	0,02	**
C 22:6 n-3 DHA	2,64	1,73	0,11	***
SFA	35,95	39,55	0,65	**
MUFA	28,30	33,46	0,71	***
PUFA	27,29	18,41	1,16	***
FUFA n-3	10,36	6,35	0,29	***
PUFA n-6	14,29	10,75	0,97	*
n-6/ n-3	1,38	1,69	0,21	*
SFA/MUFA	1,27	1,18	0,10	*
MUFA/PUFA	1,03	1,82	0,12	**
PUFA/SFA	0,76	0,46	0,08	***

FAME = methyl esters of fatty acids; n=4; SEM = standard error of least sqare means;
SFA = fatty acids saturated, MUFA = fatty acids monounsaturated, PUFA = fatty acids polyunsaturated
PUFA n-6 = C 18:2 n-6 + C 20:2 n-6 + C 20:4 n-6
PUFA n-3 = C 18:3 n-3 + EPA + C 22:3 n-3 + DPA + DHA
CLA = conjugated linoleic acid, isomer c9, t11 C18:2 (acid rumenic)
DPA = acid docosapentaenoic, EPA = acid eicosapentaenoic, DHA = acid docosahexaenoic,
NS = insignificant, * = $p \leq 0,05$; ** = $p \leq 0,01$; *** = $p \leq 0,001$

Table 2

The influence of sex on fatty acid profile of muscle tissue (% of FAME)

	Sex		SEM	p
	Males	Females		
Total lipids	5,41	7,39	0,35	**
C 12:0	0,02	0,02	0,01	NS
C 14:0	3,72	5,02	0,12	**
C 16:0	18,25	19,30	0,51	**
C 18:0	12,49	13,74	0,45	NS
C 20:0	0,10	0,14	0,01	*
C 18:1	26,53	29,19	0,86	**
C 18:2 n-6	9,50	7,87	0,85	**
C 18:3 n-3	4,08	3,12	0,18	**
CLA *(cis*-9, *trans*-11 C18:2)	2,97	2,18	0,13	**
C 20:2 n-6	1,28	0,85	0,08	*
C 20:4 n-6	4,17	3,43	0,19	**
C 20:5 n-3, EPA	2,58	1,74	0,08	***
C 22:3 n-3	0,95	0,67	0,03	*
C 22:5 n-3 DPA	0,50	0,37	0,01	*
C 22:6 n-3 DHA	2,18	1,95	0,05	NS
SFA	34,58	38,22	0,80	**
MUFA	26,53	29,19	0,86	**
PUFA	28,31	20,72	1,12	***
FUFA n-3	10,29	7,85	1,19	**
PUFA n-6	15,05	10,69	0,69	**
n-6/ n-3	1,46	1,36	0,30	NS
SFA/MUFA	1,30	1,31	0,24	NS
MUFA/PUFA	0,94	1,41	0,10	**
PUFA/SFA	0,81	0,54	0.09	***

FAME = methyl esters of fatty acids; n=4; SEM = standard error of least sqare means;
SFA = fatty acids saturated, MUFA = fatty acids monounsaturated, PUFA = fatty acids polyunsaturated
PUFA n-6 = C 18:2 n-6 + C 20:2 n-6 + C 20:4 n-6
PUFA n-3 = C 18:3 n-3 + EPA + C 22:3 n-3 + DPA + DHA
CLA = conjugated linoleic acid, isomer c9, t11 C18:2 (acid rumenic)
DPA = acid docosapentaenoic, EPA = acid eicosapentaenoic, DHA = acid docosahexaenoic,
NS = insignificant, * = $p \leq 0,05$; ** = $p \leq 0,01$; *** = $p \leq 0,001$

Since all animals were fed the same diet, we assume that differences in the concentration of C18: 3 n-3 in intramuscular fat is mainly due to the peculiarities of rumen fermentation processes which alter the rate of linoleic acid in the rumen biohidrogenare food reducing its level in intramuscular fat (Chilliard et al., 2001; Nuernberg et al., 2005). Increase of C18: 3 n-3 in intramuscular fat is considered beneficial to consumer health (Gill et al., 1995; Warnants et al., 1996).

High content of intramuscular fat in C20: 5 n-3 (EPA), C22: 5 n-3 (DPA) and C22: 6 n-3 (DHA) in young intensive fattening versus adultele and males versus females that suggests higher bioavailability of C18: 3

which favored the synthesis of these long chain polyunsaturated fatty acids of carbon atoms.

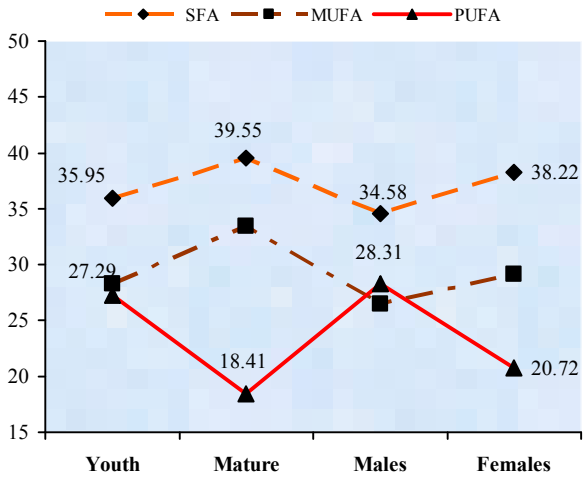

Fig. 1. Influence of age and sex on FA profile of muscle tissue

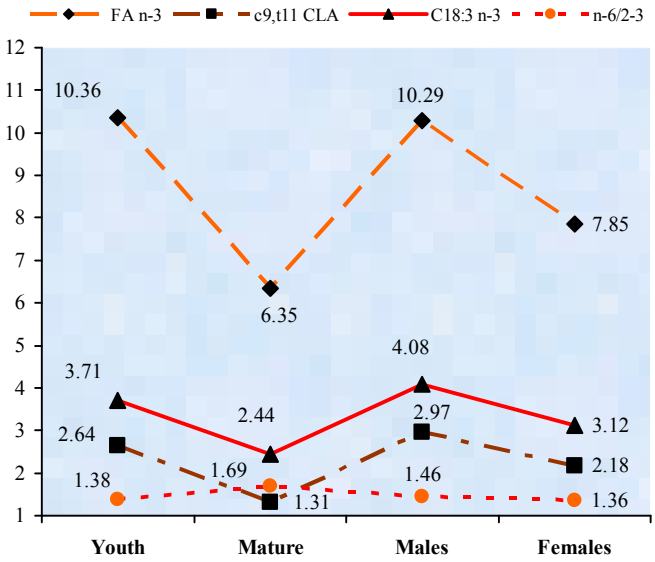

Fig. 2. Influence of age and sex on the content of FA n-3 and CLA muscle tissue

The proportion of *cis*-9, *trans*-11 CLA in intramuscular fat was higher in young males gained intensive and compared with females. The content of cis-9, trans-11 CLA may be due to biohidrogenarea linoleic acid (C18: 2 n-6) in the rumen, and increased production of C18: 1 *trans*-11, of which C18:

2 c9, t11 can be produced in tissues by enzymatic desaturation processes (Griinari et al., 2000; Santora et al., 2000, Cooper et al., 2004).

Report PUFA / SFA in intramuscular fat ranged between 0.81 and 0.46 which are much higher than those mentioned in similar studies aimed at enabling improved fatty acid profile of intramuscular fat. The best PUFA / SFA, in terms of impact on human health, intramuscular fat was obtained coming from intensively fattened rams.

CONCLUSION

The results demonstrate the opportunity of using sustainable local animal genetic resources in order to improve the fatty acid profile of muscle tissue, analyzed in terms of impact on consumer health. Young male breed fattened intensively pans recorded in intramuscular fat increased levels of n-3 polyunsaturated FA (especially C18: 3 n-3), *cis*-9, *trans*-11 CLA and best relationships PUFA / SFA. High content of intramuscular fat in C20: 5 n-3 (EPA), C22: 5 n-3 (DPA) and C22:6 n-3 (DHA) and intensively fattened young, especially in males suggests a higher bioavailability of C18: 3 out of food that favored the synthesis of these FA.

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