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Fatty acid composition of *Longissimus dorsi* and *Semimembranosus* muscles during storage in lambs reared indoors and on pasture

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Abstract

The changes of the fatty acid profile during frozen storage of *Longissimus dorsi* (LD) and *Semimembranosus* (SM) muscles were studied in ram lambs according to the rearing systems (indoors and on pasture) and breed (Northeastern Bulgarian Fine Wool Breed and cross of this breed with Ile de France). Both muscles in the pastured lambs had higher content of C18:3 and total CLA while the content of C20:5 was significantly higher in the SM ($P<0.05$). The crossbred lambs displayed higher content of oleic acid and total monounsaturated fatty acids (MUFA) in the LD ($P<0.05$) as well as higher content of C16:0 in the SM ($P<0.05$). Both muscles of the lambs of the cross had lower content of C18:2 and polyunsaturated fatty acids (PUFA). The duration of frozen storage led to lower total content of saturated fatty acids ($P<0.05$) and higher amount of MUFA and PUFA through the course of the storage as the influence was stronger in the SM.

Key words: Fatty acids, Lambs, Muscles, Rearing system, Storage

Introduction

Ruminant meat is considered to be less healthy due to its relatively high content of saturated fatty acids which stands behind several diseases such as cancer, obesity, cardiovascular diseases (Jimenez-Colmenero et al., 2001). Although the possibilities for changing the fatty acid composition in ruminants are much lower compared to the monogastric animals, numerous investigations focus on the ways of dietary manipulation of the lipid profile through various feeding strategies. Pasture rearing appeared to be an effective way to change the lipid composition in ruminants and several studies reported that grazing animals have higher content of n-3 polyunsaturated fatty acids in their tissues (Cividini et al., 2008; Araba et al., 2009). Besides feeding the other major factor affecting various aspects of meat quality including its fatty acid composition is the genotype of the animals and significant influence of the crossbreeding on the lipid profile as well has been confirmed by several studies (Salvatory et al., 2004; Borys et al., 2007). In order to meet the requirements for a healthy human diet it is important not only to acquire meat with desirable fatty acid composition but also to preserve it the best. Freezing is considered to be an excellent mean for maintaining acceptable meat quality for long periods and frozen storage has been regarded as a useful technological aid (Mateo-Oyague and Perez-Chabela, 2004). However, during frozen storage various changes in meat lipids occur, and their fatty acid profile becomes more or less altered (Zymon et al., 2007). These changes depend not only on the fatty acid composition of the meat itself or the degree of the saturation but also on the temperature and the duration of the storage.

The aim of the present work is to study the effect of rearing (indoors vs. pasture) and storage time on the fatty acid composition of *Longissimus* dorsi (LD) and *Semimembranosus* (SM) muscles in lambs.

Material and Methods

Experimental animals and rearing systems

The experiment was carried out with 28 male lambs of Northeastern Bulgarian Fine Wool Breed (NBFWB) and lambs crosses of this breed with Ile de France breed (♀NBFWB x ♂IDF) in the Institute of Animal Science – Kostinbrod. The animals were divided in two groups (each containing 14 lambs) according to the breed and each of the groups was subsequently divided in 2 subgroups (7 animals each) – one reared indoors

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and the other reared on pasture. The mean age and live weight of the animals at the beginning of the trial were 95 days ±5 and 19,5 kg ±0.5. Before the onset of the experiment, one group of lambs of both NBFWB and the cross received concentrate for 10 days. Hay and water were ad libitum. The other two groups received hay which was gradually replaced by fresh grass and the lambs were adapted to pasture. During the experiment, the groups received 620g/d/animal and 420g/d/animal concentrate respectively for the groups reared indoors and on pasture. The composition of the diet was as follows: maize 29.5%, wheat -36%, sunflower meal -32%, vitamin premix -0.5%, lime -2%. The concentrate, hay and grass had 18.72%, 6.8% and 15.34% CP content and 7.44, 2.88 and 0.84 MJ/kg. The fatty acid profile of the diet is presented on Table 1. The experiment continued 73 d and the animals reached the following live weight NBFWB: indoors -31.13 kg, pasture -31.80 kg; NBFWB x IDF: indoor - 34.25 kg, pasture-32.32 kg.

Slaughtering and sampling

At the end of the trial, 5 animals of each group were slaughtered. The carcasses was split along the vertebral column, mid line into two halves and stored at 4°C for 24 h. The LD and SM muscles were carefully dissected from each left half of the carcasses. A part of each muscle was minced and samples for fatty acid composition were taken. The rest was stored at -20°C for 3 and 6 months for subsequent analysis of fatty acids after frozen storage.

Fatty acids analysis

Total lipids of the muscles were extracted according to the method of Bligh and Dyer (1959). Methyl esters of the 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 the lipids was determined by GLC analysis using chromatograph C Si 200 equipped with capillary column (TR-FAME - 60 m x 0.25 mm x 0.25μm) 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 min. 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 (Christie, 1973).

Statistical analysis

Data was analyzed using ANOVA. The mathematical model included fixed effects ascribed to rearing system (indoors and pasture), breed (NBFWB and NBFWB x IDF) and storage time on the fatty acid composition of the muscles. The interactions were included only if significant. Whenever necessary, the means were compared through the Student t-test. Differences with a level of significance below 0.05 were considered significant. Statistical analyses were performed using JMP version 7 software (SAS Institute Inc. 2007).

Results and Discussion

Effect of the rearing system on the fatty acid composition

Rearing affected the fatty acid profile in the lambs and its influence concerned mainly the PUFA. In LD muscle (Table 2) of the lambs, we observed significant effect of rearing on the content of C18:3 and CLA ($P<0.001$), while in SM (Table 3) rearing affected significantly the content of C18:3 ($P<0.001$), CLA ($P<0.01$) and C20:5 ($P<0.05$). The percentages of C18:3 in both muscles were higher in the pastured lambs. They resembled the values reported by Scerra et al. (2007) (0.78% in pastured lambs vs.0.51% in concentrate fed lambs) while Cividini et al. (2008) and Bas and Morrand-Fehr (2000) reported much higher values in the pastured lambs - respectively 3.3% and 1.7% in the intramuscular fat.

Table 1. Fatty acid composition (% of total fatty acids) of the diet.

Fatty acids	Concentrate	Hay	Grass
C14:0	0.20	4.16	1.38
C16:0	11.10	31.82	18.30
C16:1	0.30	1.50	1.18
C18:0	3.60	4.89	3.70
C18:1	26.00	10.09	6.12
C18:2	57.80	20.98	23.21
C18:3	1.00	26.56	46.11

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Table 2. Effect of rearing system, breed and storage time on the fatty acid composition (% of total fatty acids) in *Longissimus dorsi* in lambs (values least square means).

Fatty acids	Rearing system		Breed		Storage time			SE[1]	Significance		
	Indoors	Pasture	NBFWB	NBFWB xIDF	0 months	3 months	6 months		Rearing system	Breed	Storage time
C14:0	2.71	2.72	2.83	2.60	2.86	2.71	2.57	0.93	NS	NS	NS
C16:0	24.46	24.92	24.13	25.24	24.94	24.77	24.35	2.28	NS	NS	NS
C16:1	1.03	0.89	0.89	1.04	0.99	0.93	0.97	0.40	NS	NS	NS
C18:0	20.83	21.40	21.37	20.86	20.72	21.85	20.77	1.91	NS	NS	NS
C18:1[2]	33.31	32.3	31.92[b]	33.69[a]	32.05	32.3	34.06	3.24	NS	*	NS
C18:2	11.78	11.68	12.85[a]	10.61[b]	11.95	11.75	11.49	3.10	NS	**	NS
C18:3	0.53[b]	0.89[a]	0.73	0.70	0.78	0.65	0.71	0.21	***	NS	NS
CLA[3]	0.94[b]	1.15[a]	1.06	1.04	1.07	1.01	1.06	0.17	***	NS	NS
C20:4	4.19	4.13	4.35	3.97	4.50	4.01	3.98	1.36	NS	NS	NS
C20:5	0.17	0.27	0.24	0.21	0.33[a]	0.15[b]	0.19[b]	0.20	NS	NS	*
SFA[4]	48.00	49.04	48.33	48.72	48.54	49.34	47.7	2.62	NS	NS	NS
MUFA[2,5]	34.34	33.2	32.81[b]	34.73[a]	33.05	33.23	35.04	3.42	NS	*	NS
PUFA[6]	17.64	18.14	19.24[b]	16.54[a]	18.64	17.59	17.44	4.65	NS	*	NS
PUFA/SFA	0.37	0.38	0.40	0.34	0.38	0.36	0.36	0.11	NS	NS	NS
AI[7]	0.68	0.70	0.68	0.70	0.71	0.71	0.66	0.15	NS	NS	NS

Values connected with different superscripts are significantly different (P<0.05)
Significance effects : *P<0.05; ** P<0.01; *** P<0.001; NS- non significant
[1]SE : standard error
[2]Significant interaction Rearing system x Breed at P< 0.05
[3]CLA : total conjugated linoleic acid
[4]SFA : total saturated fatty acids = Σ C14:0, C16:0, C18:0
[5]MUFA : total monounsaturated fatty acids = Σ C16:1, C18:1
[6]PUFA : polyunsaturated fatty acids = ΣC18:2, C18:3, CLA, C20:4, C20:5
[7]AI : atherogenic index = [(4xC14:0)+C16:0)]/(MUFA+PUFA)

Table 3. Effect of rearing, breed and storage time on the fatty acid composition (% of total fatty acids) in *Semimbranosus* muscle in lambs (values least square means).

Fatty acids	Rearing system		Breed		Storage time			SE[1]	Significance		
	Indoors	Pasture	NBFWB	NBFWB x IDF	0 months	3 months	6 months		Rearing system	Breed	Storage time
C14:0	2.94	2.92	2.91	2.95	3.01	2.74	3.02	0.71	NS	NS	NS
C16:0	24.61	24.43	23.71^{b}	25.34^{a}	25.48^{a}	24.41^{ab}	23.68^{b}	2.64	NS	*	NS
C16:1	1.17	1.05	1.05	1.23	1.03	1.02^{b}	1.36^{a}	0.44	NS	NS	*
C18:0	20.24	19.78	20.44	19.58	20.84^{a}	19.86^{ab}	19.32^{b}	1.87	NS	NS	*
C18:1	34.35	34.26	33.85	34.76	34.09	33.41	35.42	3.14	NS	NS	NS
C18:2	11.47	12.17	12.73^{a}	10.91^{b}	11.07	12.55	11.85	3.12	NS	*	NS
C18:3	0.40^{b}	0.72^{a}	0.59	0.54	0.42^{b}	0.71^{a}	0.56^{ab}	0.23	***	NS	**
CLA[2]	0.89^{b}	0.99^{a}	0.97	0.92	0.89	0.95	0.99	0.19	*	NS	NS
C20:4	3.75	3.78	3.95	3.57	3.13^{b}	4.38^{a}	3.78^{ab}	1.37	NS	NS	*
C20:5	0.12^{b}	0.19^{a}	0.15	0.16	0.12^{b}	0.21^{a}	0.14^{ab}	0.12	*	NS	*
SFA[3]	47.8	47.13	47.08	47.87	49.35^{a}	47.02^{b}	46.04^{b}	3.46	NS	NS	*
MUFA[4]	35.53	35.36	34.90	36.00	35.12^{ab}	34.43^{b}	36.78^{a}	3.31	NS	NS	*
PUFA[5]	16.65	17.87	18.41	16.11	15.64^{b}	18.82^{a}	17.33^{ab}	4.65	NS	NS	*
PUFA/SFA	0.35	0.38	0.39	0.34	0.32^{b}	0.40^{a}	0.38^{ab}	0.11	NS	NS	*
AI[6]	0.7	0.69	0.66	0.72	0.75	0.67	0.66	0.15	NS	NS	NS

Values connected with different superscripts are significantly different (P<0.05)
Significance effects : *P<0.05; ** P<0.01; *** P<0.001; NS- non significant
[1]SE- standard error
[2]CLA : total conjugated linoleic acid
[3]SFA : total saturated fatty acids = ΣC14:0, C16:0, C18:0
[4]MUFA : total monounsaturated fatty acids = ΣC16:1, C18:1
[5] PUFA : polyunsaturated fatty acids = ΣC18:2, C18:3, CLA, C20:4, C20:5
[6]AI : atherogenic index = [(4 x C14:0) + C16:0)]/(MUFA + PUFA)

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Conjugated fatty acids are considered very important for the human health. In this study we found that the contents of CLA in the muscles of the pastured animals were higher compared to the indoors fed which is in agreement with the findings of Aurosseau et al. (2004), Daley et al. (2010) and Scerra et al. (2011). On the other hand, Kaczor et al. (2010) did not observe any significant differences in the content of CLA in pastured and stall fed lambs. The percentages of CLA in the intramuscular fat in the lambs that we reported are similar to those of other studies (Nurnberg et al., 2001; Aurosseau et al., 2004).

The increase of the content of C20:5 in the pastured lambs could be associated to the higher content of C18:3 which is a predecessor for the synthesis of the other long chain n-3 PUFA.

A major part of the PUFA in the intramuscular fat is formed by C18:2. In our experiment we did not observe any significant changes in the content of C18:2 due to the rearing system which is in agreement with Scerra et al. (2007). On the other hand Cividini et al. (2008) reported higher percentage of C18:2 in the intramuscular fat of pastured lambs while Aurosseau et al. (2004) observed lower contents of this fatty acid in the muscles of lambs reared on pasture. The lack of significant difference in the content of C18:2 in our study could be due to the presence of concentrate in the diet of the pastured lambs.

The content of PUFA in the intramuscular fat of the grazing lambs tended to increase. This shows that despite the biohydrogenation in the rumen a certain amount of the unsaturated fatty acids escapes this process and remains unaltered.

The greatest part of the fatty acids in the lamb meat consists of the saturated C16:0 and C18:0 and the monounsaturated C18:1. Some studies report significant increase of the total amount of the saturated fatty acids in the lambs reared on pasture (Kaczor et al., 2010) while the result of other experiments show lower amount of C16:0 and higher content of C18:0 in pastured ruminants (Daley et al., 2010). Cividini et al. (2008), observed lower amount of C18:1 and MUFA in pastured lambs. In our experiment we did not observe any significant effect of the rearing system on the content of these major fatty acids.

The values of the ratio PUFA/saturated fatty acids (SFA) should be at least 0.4 (Simopoulos, 2004). In this study rearing indoors and on pasture did not induce any significant changes in the ratio and its values remained close to the recommended. The values we observed in LD muscle were within the range of 0.37- 0.38 and in SM muscle -0.35-0.38, respectively for the indoors and pasture reared lambs, while Costa et al. (2009) reported values of 0.2.

Atherogenic index did not differ significantly between the rearing strategies and the values that we reported were in the range of 0.68 and 0.70. Similar values were reported by Zapletal et al. (2010) and Costa et al. (2009).

Effect of the breed on the fatty acid composition

In both muscles we observed changes in some individual fatty acids due to the breed of the animals. In LD muscle breed affected the content of C18:1 and the total amount of MUFA ($P<0.05$) though for these two significant interactions between the effects of breed and rearing system existed. The lambs of NBFWB x IDF showed higher amounts of C18:1 and MUFA which is in agreement with the results of Maia et al. (2012). Breed displayed significant effect on the content of C16:0 in SM muscle ($P<0.05$) which was higher in the crossbred lambs but no differences in the total amount of the saturated fatty acids were observed. Contrary to us, Salvatori et al. (2004) reported lower content of SFA in lambs that were crosses of IDF. We observed significant changes in C18:2 as well in LD ($P<0.01$) and SM ($P<0.05$) muscles. In both muscles C18:2 was significantly lower in the crossbred lambs. There was also a tendency toward lower total amount of PUFA in both muscles in the lambs of NBFWB x IDF. This is in agreement with Kaszor et al. (2010) who observed lower content of PUFA in lambs IDF crosses. The lower content of PUFA in the muscles of the crossbred lambs corresponds to the higher intramuscular fat content in the same animals that we observed (unpublished data). Ile de France breed is known to deposit more fat in earlier age (Maia et al., 2012) and the difference in the fatty acid composition depends on the fatness. According to Wood et al. (2008), the muscles of breeds or genotypes that are characterized with higher content of lipids, have lower part of phospholipids and are poorer in PUFA.

In regards to the ratio PUFA/SFA, no significant changes were observed between the lambs of both breed and cross though its values tended to be lower in the crossbred lambs. According to Scollan et al. (2003) a strong negative exponential relationship exists between the

intramuscular fat content and the PUFA/SFA ratio. Atherogenic index showed no significant differences between the breed and the cross though in both muscles it tended to be higher for the crossbred lambs. This corresponded to the lower amount of PUFA in the latter. Contrary to our results, Salvatori et al. (2004) observed effect of the genotype on the AI and the values reported in their study were higher than those observed by us.

Effect of the storage on the fatty acid composition

The duration of the storage had more pronounced influence on the fatty acid composition in SM muscle. It affected significantly the content of C18:0 ($P<0.05$) and the total amount of SFA ($P<0.05$) which decreased through storage, while the content of C16:1 ($P<0.05$) increased at the 6th month. Zymon et al. (2007) reported higher content of total fatty acids and lower content of C16:1 in veal stored for 3 months under freezing conditions. Frozen storage affected the amount of the polyunsaturated C18:3 ($P<0.01$) and C20:4 ($P<0.05$) of SM muscle and their content increased significantly until the 3d month of the storage. The contents of C20:5 in both LD and SM muscles were affected by the duration of the storage as well. In *Longissimus* muscle it decreased in the course of the storage while in SM it increased until the 3d month of the storage. The changes in the content of the individual PUFA in SM through the frozen storage led to the relative changes of the total amount of PUFA and the ratio between PUFA and SFA which increased until the third month of the frozen storage. The changes of the PUFA that we observed are in agreement with the results reported by Zymon et al. (2007), while Samouris et al. (2011) found no significant changes in the content of the SFA, MUFA and PUFA in lamb meat during frozen storage. The lower extent of oxidation could probably be due to the fact that muscle samples were stored in sealed bags and were not exposed to oxygen. The ratio between PUFA and SFA remained unchanged during the storage in LD while in SM it followed the same trend as the amount of PUFA. In both muscles AI was not significantly influenced in the course of storage though it tended to become lower at the 6th month. The results obtained suggest no negative effect of the frozen storage on the fatty acid profile and the nutritional value of lamb meat.

Conclusions

Rearing system affected the fatty acid profile of the intramuscular fat in lambs as its influence concerned mainly the content of PUFA in LD and SM. The lipids in both muscles of the pastured lambs displayed higher content of linolenic acid and CLA while the content of C20:5 was higher in SM. The changes in the fatty acid composition due to the breed of the lambs included higher content of oleic and MUFA in LD and higher content of C16:0 in SM in the crossbred lambs. Both muscles of the crossbred lambs were characterized by lower content of linoleic fatty acid and PUFA. The frozen storage affected mostly the fatty acid profile in m. SM leading to lower content of SFA and higher amount of MUFA and PUFA through the course of the storage.

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