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Lipid Oxidation in *M. longissimus dorsi* and *M. semimembranosus* in Lambs Reared Indoors and on Pasture

Research Article

T. Popova[1*] and P. Marinova[1]

[1] Institute of Animal Science, 2232, Kostinbrod, Bulgaria

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*Correspondence E-mail: tlpopova@yahoo.com
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ABSTRACT

Lipid oxidation was studied in *M. longissimus dorsi* and *M. semimembranosus* in male lambs of Northeastern Bulgarian Fine Wool Breed and cross of this breed with Ile de France, reared indoors and on pasture. The oxidation of the lipids was determined by quantification of thiobarbituric acid reactive substances (TBARS), formed during the storage of the muscles at 4 ˚C until 6th day and -20 ˚C until 90th day. The way of the rearing had significant influence (P<0.01; 0.001) on the lipid oxidation in the muscles during the storage in both lambs of Northeastern Bulgarian Fine Wool Breed and the cross. The lipid oxidation was lower in the animals reared on pasture. The muscle type also had significant effect on the amounts of TBARS, as they were higher in *M. semimembranosus*. The dynamic of changes in TBARS contents on the muscles in both groups of animals from the breed and the cross showed influence of the duration of the storage on the lipid oxidation, as the highest values were achieved on the 90th day of samples storage.

KEY WORDS lambs, lipids, oxidation, storage.

INTRODUCTION

Oxidative changes are common in the metabolism of living organisms. Due to imbalance between the production of reactive oxygen and nitrogen species and the defense mechanisms of the organism against the oxidative stress, they might have negative influence on vital components of the biological systems (Smet *et al.* 2008).

Oxidation affects lipids, proteins, pigments, DNA and vitamins. In the muscle and adipose tissue, the oxidation continues *post mortem* as it might influence the shelf life of meat in animals after slaughter.

Lipid oxidation is considered to be the main reason for the negative changes in meat and meat products after slaughter (Morrissey *et al.* 1998), and it is affected by the tempera

ture and the duration of the storage as well as the balance between pro- and antioxidant substances in meat (Bertelsen *et al.* 2000).

The antioxidant activity in the muscle tissue may be modified by the way of the feeding and rearing of the animals. Rearing on pasture is of great importance not only because the fresh grass, consumed by the animals is rich in polyunsaturated fatty acids, is beneficial for the health (Enser *et al.* 1998), but it is also a good source of vitamin E and other natural antioxidants (Warren *et al.* 2008), which accumulate in the organism and help to reduce the intensity of the lipid oxidation in the tissues *post mortem*. The aim of the study was to determine the changes in the lipid oxidation during the storage in muscle (*M. longissimus dorsi* and *M. semimembranosus*) in lambs, reared indoors and on pasture.

MATERIALS AND METHODS

Experimental animals and rearing

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 (NBFWB × IDF) in the experimental farm at the Institute of Animal Science Kostinbrod. The animals were divided in two groups (containing 14 lambs) according to the breed and each of the groups was subsequently divided in 2 subgroups (of 7 animals) one reared indoor and the other reared on pasture. The mean age and live weight of the animals at the beginning of the experiment were 95 (±5) days and 19.47 kg (±0.5). Before the onset of the experiment two groups of lambs from the NBFWB and the cross received concentrate for 10 days. The hay and water for the lambs 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 two groups reared indoors received 620 g concentrate and the pastured lambs received 420 g concentrate. The composition of the concentrate was as follows: maize -29.5%, wheat -36%, sunflower meal -32%, vitamin premix -0.5%, lime -2%. The experiment continued 73 d. The mean live weight at the slaughter of the lambs in the NBFWB group was 31.13 kg and 31.80 kg for the indoors and pastured lambs, respectively. The lambs of NBFWB × IDF, reared indoors were slaughtered at 34.25 kg, while the pastured ones at 32.32 kg.

Sampling and lipid oxidation measurements

After slaughtering the animals 24 h *post mortem*, *M. longissimus dorsi* (*M. LD*) and *M. semimembranosus* (*M. SM*) were carefully dissected from the left side of the carcasses. For lipid oxidation measurements, samples of both muscles were taken, wrapped in foil and stored for a period of 6 days at 4 °C, after which the storage continued at -20 °C till the 90th day. Lipid oxidation was measured on 24 h, 4 8 h, 4th, 6th and 90th day of the storage determining the content of thiobarbituric acid reactive substances (TBARS) according to the method of Lynch and Frei (1993), modified by Mercier *et al*. (1998). Muscle samples (1 g) were homogenized in 10 mL KCl 0.15 M + 0.1 m*M* butylhydroxytoluene by ULTRATURRAX (Type T-25, Janke and Kunkel, Staufen, Germany). Homogenate samples of 0.5 mL were incubated with 1% (w/v) 2-thiobarbituric acid in 50 m*M* NaOH (0.25 mL) and 2.8% (w/v) trichloroacetic acid (0.25 mL) in a boiling water bath (100 °C) for 10 min. After cooling at room temperature for 20 min, the pink chromogen was extracted with n-butanol (2 mL) and the absorbance was measured at 535 nm against a blank of n-butanol. TBARS concentrations were calculated using 1, 1, 3, 3 tetraethoxypropane (0-0.8 µM) as standard. Results were expressed as mg malondialdehyde per kg of meat or TBA units.

Statistical evaluation

Data were analyzed by two-ways ANOVA (JMP, 2007). The model included fixed effects ascribed to rearing (indoors and pasture), muscle type (*M. longissimus dorsi* and *M. semimembranosus*) and feeding x muscle interaction on the TBARS formation.

For evaluation the influence of the storage time on the lipid oxidation one-way ANOVA was applied. Post-test comparisons were made, using *t*–criterion of Student. In all cases differences with a level of significance below 0.05 were considered significant.

RESULTS AND DISCUSSION

Influence of the rearing and the muscle type on the lipid oxidation

The results presented in Table 1 showed the influence of the way how rearing was made on animals of NBFWB on the 24 h, 4th and 90th day (P<0.01), as well as on the 6th day (P<0.001) of the storage. In the lambs of the cross (Table 2) rearing affected significantly oxidation on all of the intervals 24 h, 48 h , 4th day (P<0.001), 6th day (P<0.01) and 90th day (P<0.05).

The values of TBARS measured on the separate intervals were at average two times lower in both pure and crossbred lambs, reared on pasture, thus showing higher oxidative stability in the muscles of these lambs compared to the indoors reared.

This could be attributed to the additional amount of natural antioxidants coming from the plants that are more abundant in the diet of the pastured groups. Such a hypothesis is partly based on experiments mainly carried out with cattle. In studies with calves (Farouk *et al*. 2003; Realini *et al*. 2004) higher oxidative stability of the lipids in the meat of the animals reared on pasture was found. Results of experiment with a model system (Mercier *et al*. 2004) showed that in pastured calves there has been reduced intensity of lipid oxidation in the meat as well as higher activity of the catalase and superoxidismutase, which is related to lower oxidation.

Muscle type also affected the lipid oxidative stability. In the lambs of NBFWB we observed significant influence of the muscle type on the contents of TBARS on the 4th day (P<0.01) whereas in the crossbred animals it was on the 4th (P<0.05), 6th (P<0.01) and 90th day (P<0.01). In both lambs from NBFWB and NBFWB × IDF reared indoors and on pasture the TBARS contents were higher in *M. SM* compared to *M. LD*. The differences are due to the metabolic type of the muscles (Marinova, 2000).

Table 1 Influence of the way of the rearing and muscle type on TBARS contents (mg malondialdehyde per kg of meat) in lambs of Northeastern Bulgarian Fine Wool Breed (least square means)

Duration of storage	Rearing		Muscle		SE	Significance		
	Indoor	Pasture	*M. LD*	*M. SM*		Rearing	Muscle type	Interaction
24 h	0.89^{a}	0.48^{b}	0.59	0.78	0.23	**	NS	NS
48 h	0.96	0.67	0.76	0.87	0.35	NS	NS	NS
4 d	0.75^{a}	0.40^{b}	0.40^{a}	0.75^{b}	0.21	**	**	NS
6 d	1.23^{A}	0.69^{B}	0.89	1.03	0.26	***	NS	NS
90 d	2.11^{a}	1.31^{b}	1.51	1.91	0.52	**	NS	NS

M. LD: *M. longissimus dorsi* and *M. SM*: *M. semimembranosus*.
* P<0.05; ** P<0.01 and *** P<0.001.
NS: non significant and SE: standard error.
^{ab}: the means within the same row with different letter, are significantly different (P<0.01).
^{AB}: the means within the same row with different letter, are significantly different (P<0.001).

Table 2 Influence of the way of the rearing and muscle type on TBARS contents (mg malondialdehyde per kg of meat) in lambs of Northeastern Bulgarian Fine Wool Breed × Ile de France (least square means)

Duration of storage	Rearing		Muscle		SE	Significance		
	Indoor	Pasture	*M. LD*	*M. SM*		Rearing	Muscle type	Interaction
24 h	1.71^{A}	0.53^{B}	1.04	1.19	0.44	***	NS	NS
48 h	2.14^{A}	0.46^{B}	1.38	1.22	0.66	***	NS	NS
4 d	1.18^{A}	0.44^{B}	0.61$^{\alpha}$	1.01$^{\beta}$	0.31	***	*	NS
6 d	1.37^{a}	0.89^{b}	0.94^{a}	1.32^{b}	0.26	**	**	NS
90 d	2.23$^{\alpha}$	1.71$^{\beta}$	1.59^{a}	2.34^{b}	0.4	*	**	NS

M. LD: *M. longissimus dorsi* and *M. SM*: *M. semimembranosus*.
* P<0.05; ** P<0.01 and *** P<0.001.
NS: non significant and SE: standard error.
$^{\alpha\beta}$: the means within the same row with different letter, are significantly different (P<0.05).
^{ab}: the means within the same row with different letter, are significantly different (P<0.01).
^{AB}: the means within the same row with different letter, are significantly different (P<0.001).

Usually the muscles with more pronounced oxidative metabolism have higher phospholipid content which is strongly altered during the storage (Hernandez *et al.* 1998) and it could explain the higher TBARS contents in *M. SM*. Apart from the lipid profile, the oxidation is determined by the availability of antioxidants in the medium. Some studies (Guidera *et al.* 1997) show that there are differences in the content of α-tocopherol between the muscles as in lambs it is lower in *M. SM*, than in *M. LD*. Though the effect of breed was not examined here, as it could be seen from the results, the values of TBARS are higher in the crossbred animals.

This could be explained by the higher fat content in the muscles of the lambs of NBFWB x IDF that we observed (unpublished data).

Influence of the duration of storage on the lipid oxidation

Time also affected significantly the formation of TBARS during storage (Figure 1 A). In both indoor and pasture reared lambs of NBFWB the differences in the amount of TBARS in *M. LD* were most pronounced between the 90th day and all the rest of the intervals. Significant differences were also observed between 48 h and the 4th day and between the 4th and the 6th day in the indoors and pastured lambs.

Similar to *M. LD* there was also influence of the duration of the storage on the TBARS formation in *M. SM*, as the differences were significant between the all intervals and the 90th day of storage (Figure 1 B). As in the animals of NBFWB, time influenced the TBARS formation in *M. LD* the lambs of NBFWB × IDF. In the indoors reared animals significant differences were found between the 48 h and the 4th and 48 h and the 6th day, whereas in the pastured animals there were significant differences between all of the intervals and the 90th day, as well as on the 24 h, 48 h and the 4th day with the 6th day (Figure 2 A). The dynamic of TBARS formation in *M. SM* of the lambs (both indoors and pasture reared) showed that the differences between the intervals of the measurement and the 90th day were significant (Figure 2 B). In the course of the storage of *M. LD* samples, we observed similar tendencies for the development of the lipid oxidation in the groups of NBFWB and NBFWB × IDF but in *M. SM* the oxidative changes were diverse. In both muscles we found a decrease of the oxidation until the 4th day of the storage when the measured TBARS content was minimal. After the 4th day TBARS increased and this also continued during the storage at -20 ˚C. The observed tendency in the dynamic of TBARS formation towards decrease of its contents at certain stage of the storage coincides with results of other experiments with lambs (Popova *et al.* 2005).

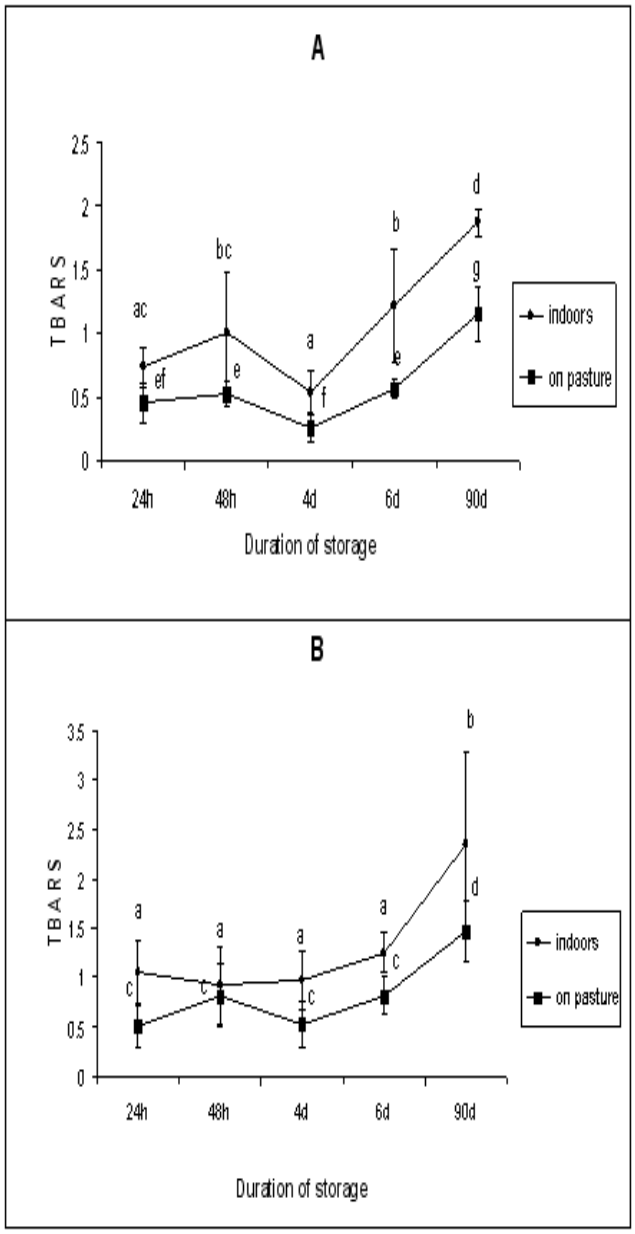

Figure 1 Effect of the duration of storage on the TBARS formation (mg malondialdehyde per kg meat) in *M. longissimus dorsi* (A) *and M. semimembranosus* (B) in lambs of Northeastern Bulgarian Fine Wool Breed

The intervals within group connected with different letters are statistically different (P<0.05)

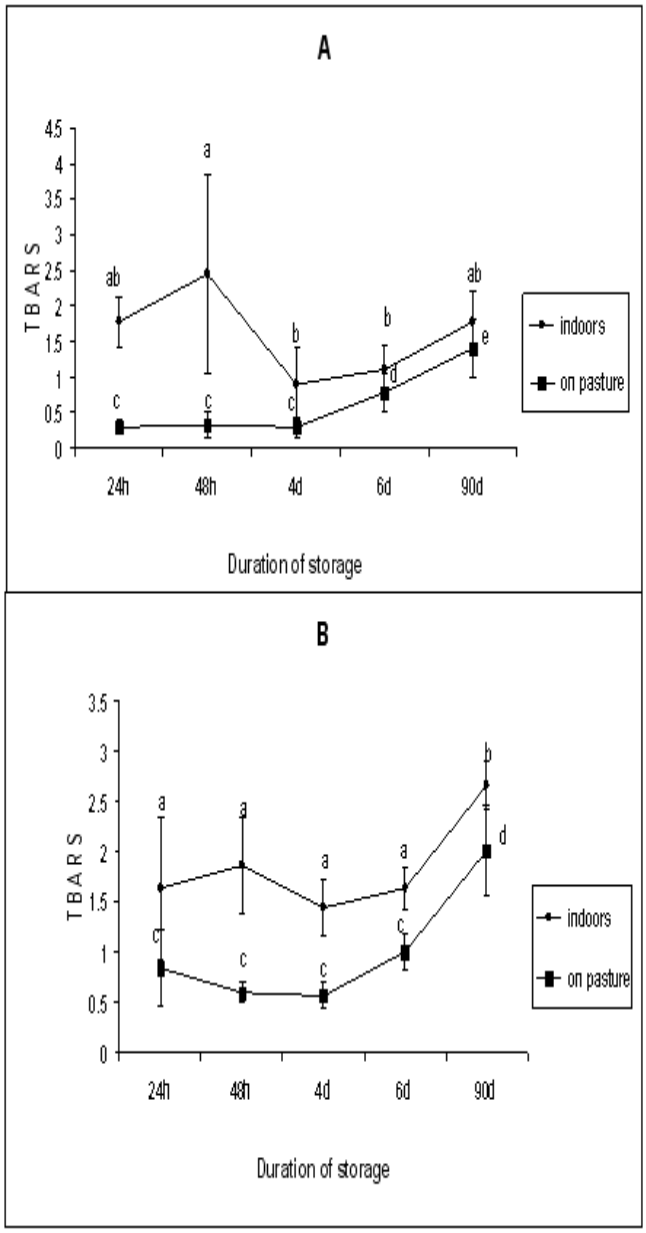

Figure 2 Effect of the duration of storage on the TBARS formation (mg malondialdehyde per kg meat) in *M. longissimus dorsi* (A) *and M. semimembranosus* (B) in lambs of Northeastern Bulgarian Fine Wool Breed × Ile de France

The intervals within group connected with different letters are statistically different (P<0.05)

Perhaps the decrease in the amounts of TBARS in the course of the storage is due to its reaction with other secondary products of lipid oxidation or aminoacids of the myosin fraction. On the other hand the new increase of the TBARS content after the 4th day until the 90th day of storage shows that the lipid oxidation does not stop at low temperatures, since the free radicals that provoke it remain stable (Zarzycky and Swiniarska, 1993). The secondary products of lipid oxidation (malondialdehyde among them) are linked to negative changes in the sensory characteristics of the meat and also with the appearance of rancid odour.

Experiments with ruminants (Verme and Sahoo, 2000) report a threshold of 2 mg malondialdehyde/kg meat, the same as the method applied by us, beyond which rancid odour could be detected. In our study at the end of the storage of the samples the TBARS contents are increased and in the indoor reared animals they are around or beyond the threshold compared to the pastured animals. This could be accepted as an indicator of the positive influence of the pasture rearing and at the same time it shows the reduced possibilities for long time storage of the meat of indoor reared animals.

CONCLUSION

This study shows advantages of the pasture over indoors rearing in regards to lipid oxidation in lamb meat. During storage the lipid oxidation in *M. longissimus dorsi* and *M. semimembranosus* of the lambs from Northeastern Bulgarian Fine Wool Breed and its cross with Ile de France had lower intensity in the pastured animals. The formation of lipid oxidation products is significantly affected by the muscle type, showing higher content in *M. semimembranosus*. In both studied muscles from the lambs of groups reared indoors and on pasture the formation of TBARS was most pronounced on the 90th day of storage and the contents are higher, compared to the other intervals, thus indicating certain negative effect of the longtime frozen storage on meat quality.

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