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

Research Article

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ABSTRACT

Protein oxidation *M. longissimus dorsi* and *M. semimembranosus* was studied in ram lambs of Northeastern Bulgarian Fine Wool Breed and cross of this breed with Ile de France, reared and in doors and on pasture. The degree of protein oxidation is determined by measuring the content of carbonyls, formed during the storage of the investigated muscles at low temperatures (4 ˚C till 6th day and -20 ˚C till 90th day). Protein oxidation was lower in pastured animals. Differences between the groups were significant at 4th ($P<0.05$) and 90th day ($P<0.001$) for the animals of Northeastern Bulgarian Fine Wool Breed and at 24 h ($P<0.05$), 6th and 90th day ($P<0.01$) in crossbred lambs. Differences were reported in protein oxidation in the muscles at 48 h ($P<0.01$) and the 4th day ($P<0.05$) as well as at 48 h ($P<0.05$) and the 90th day ($P<0.001$), for the lambs of Northeastern Bulgarian Fine Wool Breed and the cross, respectively. The dynamic of changes of the carbonyls in the muscles shows that storage duration influences protein oxidation. The carbonyl contents in *M. longissimus dorsi* were significantly higher the 90th day of the storage, compared to the other intervals in both indoor reared lambs of Northeastern Bulgarian Fine Wool Breed and the crossbred animals, as well as in pastured crossbred lambs. In *M. semimembranosus* differences between the contents of the carbonyls in the intervals of measurement and the 90th day of the storage were significant in both animals of Northeastern Bulgarian Fine Wool Breed and the cross, reared indoors.

KEY WORDS carbonyls, meat, rearing, storage.

INTRODUCTION

The major part of muscle tissue is formed by proteins and they play important role in meat and meat products with regard to sensory, nutritional and technological quality. Proteins have been the subject of research focused mainly on the modifications that occur during *post mortem* changes, processing and storage of meat such as denaturation and hydrolytic degradation. In contrast, little attention has been payed to the processes of oxidation in the protein fraction. They are caused by different initiators such as oxidizing lipids, metal ions and other pro-oxidants (Estevez *et al*. 2008; Xiong and Decker, 1995) and lead to degradation of aminoacid side chains, formation ofcross-links and breakage of peptide bonds (Stadman and Levine, 2000). The oxidation of proteins influences the nutritional value of meat since it causes loss of essential aminoacids and decreases protein digestibility. The development of protein oxidation in meat has also been related to colour and texture alteration (Estevez *et al*. 2005). One of the most remarkable measurable changes in proteins induced by the oxidation is the formation of carbonyl compounds (Lund *et*

al. 2011; Requena*et al.* 2003) and their quantification has been commonly used as indicator of proteinoxidation in both biological and food systems. The aim of this experiment was to study the protein oxidation during storage in *Longissimus dorsi* and *Semimembranosus* muscles in lambs reared indoors and on pasture.

MATERIALS AND METHODS

Experimental animals and feeding regimes

The experiment was carried out with 28 ram lambs of Northeastern Bulgarian Fine Wool Breed (NBFWB) and lambs crosses of this breed with Ile de France (NBFWB × IDF) in the Institute of Animal Science-Kostinbrod. The animals were divided in two groups (14 lambs each) according to the breed and each one of the groups was subsequently divided in 2 groups of 7 animals each one reared indoors and the other reared on pasture. The average age and live weight of the animals at the beginning of the experiment were 95 (±5) days and 19.47 kg (±0.5), respectively. Before the onset of the trial, one group of NBFWB and NBFWB × IDF lambs received concentrate for 10 days, hay and water were *ad libitum*. The other two groups received hay which was gradually replaced by fresh grass; in order to adapt the lambs to pasture. During the experiment the two groups reared indoors received 620 g concentrate, whereasthe groups on pasture received 420 g concentrate. The concentrate consisted of: maize -29.5%, wheat -36%, sunflower meal -32%, vitamin premix -0.5%, lime -2%. The trial continued for 73 days. The average live weight of the animals at slaughter was as follows: NBFWB: indoors 31.13 kg and pasture 31.80 kg; NBFWB × IDF: indoors 34.25 kg and pasture -32.32 kg.

Sampling and storage

After slaughtering the animals, at 24 h *post mortem*, *M. longissimus dorsi* (*M. LD*) and *M. semimembranosus* (*M. SM*) were dissected from the left side of the carcasses. 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 ˚Cuntil the 90th day.

Protein oxidation measurements

Protein oxidation was measured at 24 h, 48 h, and then the 4th, 6th and 90th day of storage, determining the content of carbonyl substances (Olivier *et al.* 1987). Muscle samples (1 g) were homogenized in 10 mL KCl 0.15 M using ULTRATURRAX (Type T-25, Janke and Kunkel, Staufen, Germany). Each sample of the homogenate was divided into two equal aliquots of 0.5 mL. Proteins in both aliquots were precipitated by 10% trichloroacetic acid (w/v, final concentration) and centrifuged at 2000 g for 10 min.

One pellet was treated with 1ml of 2 NHCl and the other with an equal volume of 0.2% solution of 2,4-dinitrophenylhydrazine (DNPH) in 2 NHCl. Both samples were incubated for 1 h at room temperature and stirred regularly. The samples were again precipitated with 10% trichloroacetic acid (w/v final concentration) and centrifuged at 2000 g for 10 min. The pellets were then washed twice with 1 mL of ethanol: ethyl acetate (1:1) to eliminate the traces of DNPH and to dissolve the residual lipids. Proteins were finally dissolved in 2 mL of 6 M guanidine with 20 m*M* sodium phosphate buffer pH 6.5. To remove insoluble fragments, samples were centrifuged 10 min at 2000 g. Protein concentration was calculated at 280 nm in the HCl control using BSA in 6 M guanidine as standard. Carbonyl concentration was measured on the treated sample by measuring DNPH incorporated on the basis of absorption at 370 nm for protein hydrazones. The results were expressed as nanomoles of DNPH fixed per milligram of protein.

Statistical evaluation

The data of the study was analysed by two-ways ANOVA (JMP version 7 software). The model included fixed effects ascribed to rearing (indoors and pasture), muscle type (*M. longissimus dorsi* and *M. semimembranosus*) and rearing × muscle interaction on the carbonyl formation. For evaluation of the influence of the storage time on the oxidation of proteins in muscles,one-way ANOVA was applied. Post-test comparisons were made, using Student test. Differences with a level of significance below 0.05 were considered significant.

RESULTS AND DISCUSSION

Influence of the rearing and muscle type on the protein oxidation

Type of Animal rearing influenced significantly the content of carbonyls in both NBFWB and crossbred lambs. Differences between indoors and pasture reared NBFWB lambs were significant the 4th ($P<0.05$) and 90thday of the storage ($P<0.001$) (Table1), while in the crossbred animals the influence of the type of rearing was significant at 24 h ($P<0.05$), and then the 6th and 90th day ($P<0.01$) (Table2).

Carbonyl content remained lower in the animals reared on pasture. This is in agreement with the results of Sante–Lhoutellier *et al.* (2008) and corresponds to the lower degree of lipid oxidation found in the same experiment (Popovaand Marinova, 2013).

When developing rearing strategies in order to achieve high nutritional quality and to minimize the oxidative processes in the meat *post mortem* usually the goal is to increase the content of the polyunsaturated fatty acidsand that of the antioxidants such as α-tocopherol (Estevez *et al.* 2011).

Table 1 Effect of the rearing (indoors and pasture) and muscle type, on carbonyl content (nmol/mg protein) in Northeastern Bulgarian Fine Wool Breed lambs (values least square means)

Duration of storage	Rearing		Muscle		SE	Effect		
	Indoors	Pasture	*M. LD*	*M. SM*		Rearing	Muscle type	Interaction
24 h	6.56	6.18	6.18	6.57	0.69	NS	NS	NS
48 h	6.75	6.30	7.76^{A}	5.29^{B}	1.27	NS	**	NS
4 d	7.28^{a}	5.27^{b}	7.52^{a}	5.03^{b}	1.92	*	*	NS
6 d	8.19	6.57	6.98	7.78	1.67	NS	NS	NS
90 d	12.79^{α}	7.51^{β}	9.73	10.57	2.05	***	NS	NS

* P<0.05; ** P<0.01 and *** P<0.001.
Values with different superscript are statistically different ab: P<0.05; AB: P<0.01 and αβ: P<0.001.
NS: non significant and SE: standard error.

Table 2 Effect of the rearing (indoors and pasture) and muscle type, on carbonyl content (nmol/mg protein) in Northeastern Bulgarian Fine Wool Breed x Ile de France lambs (values least square means)

Duration of storage	Rearing		Muscle		SE	Effect		
	Indoors	Pasture	*M. LD*	*M. SM*		Rearing	Muscle type	Interaction
24 h	7.25^{a}	5.21^{b}	6.20	6.26	1.54	*	NS	NS
48 h	7.53	5.92	8.66^{a}	7.78^{b}	2.96	NS	*	NS
4 d	6.66	5.05	6.41	5.30	1.59	NS	NS	NS
6 d	8.98^{A}	6.38^{B}	8.40	6.95	1.61	**	NS	NS
90 d	13.86^{A}	10.08^{B}	14.44^{α}	9.50^{β}	1.78	**	***	NS

* P<0.05; ** P<0.01 and *** P<0.001.
Values with different superscript are statistically different ab: P<0.05; AB: P<0.01 and αβ: P<0.001.
NS: non significant and SE: standard error.

The supplementation of the diet with α-tocopherol and carotenoids has been proven to be effective in diminishing the protein oxidation and carbonyl formation, while the effect of the modification of fatty acid composition has not been conclusively determined. Pasture rearing is a good strategy to increase natural antioxidants in animals due to the relatively high content of such components in the plants and consequently to reduce the extent of the oxidative processes (Ventanas *et al*. 2006; Estevez and Cava, 2006). The muscle type affected significantly the carbonylation of the proteins in both NBFWB and crossbred lambs. The differences between the two muscles were significant at 48 h and the 4th day (P<0.01; 0.05) in NBFWB animals, while in crossbred lambs such difference was found significant at 48 h (P<0.05) and the 90th day (P<0.001). At the initial stages in NBFWB lambs and during the whole period of storage in crossbred animals, the content of carbonyls was lower in M. SM compared to M. LD. This is in agreement with the observations of Estevez *et al*. (2011) in pigs. The different susceptibility of M. LD and M. SM to oxidative changes during storage could be ascribed to their metabolic fiber type which largely determines the *post mortem* biochemical changes and the susceptibility of muscle proteins to denaturation (Klont *et al.* 1998). According to Klont *et al*. (1998) and Lawrie, (1998) the muscles rich in glycolytic fibers, such as M. LD, are more prone to undergo a faster pH decline and hence more intense denaturation than muscles rich in oxidative fibers. According to Aristoy and Toldra (1998) the proteolysis *post mortem* is much more severe in glycolytic muscles than in the oxidative ones.

Although the connection between protein denaturation, proteolysis and protein oxidation is not fully understood, processes affecting the solubility and integrity of muscle proteins could enhance the oxidative instability of such proteins and promote carbonyl formation.

Influence of storage durationon protein oxidation

The dynamic of carbonyl formation in M. LD in NBFWB lambs and the cross (Figure 1 A and B), showed that storage duration exerted a significant effect on carbonyl contents. In indoors NBFWB reared animals (Figure 1 A), the content of carbonyls increased until the 90th day with significant difference between this and all of the remaining intervals, except the 4th day. No significant differences between the intervals were observed in pastured lambs.

Similarly to purebred animals, crossbred (Figure 1 B) reared indoors showed increase of the carbonyl content the 90th day of storage and significant differences with all of the remaining intervals. The same tendency in the dynamic of protein oxidation was observed in pastured animals.

Protein oxidation development in NBFWB and NBFWB × IDF lambs, reared indoors and on pasture showed similar tendencies in M. SM (Figure 2 A and B). In both groups reared indoors we observed the highest amount of carbonyls the 90th day of storage, which differed significantly with the content measured on the remaining intervals. Pastured lambs also showed the tendency to increase carbonyl formation until day 90th, but the differences highlighted with the other intervals of measurement were not so pronounced as in the indoors reared lambs.

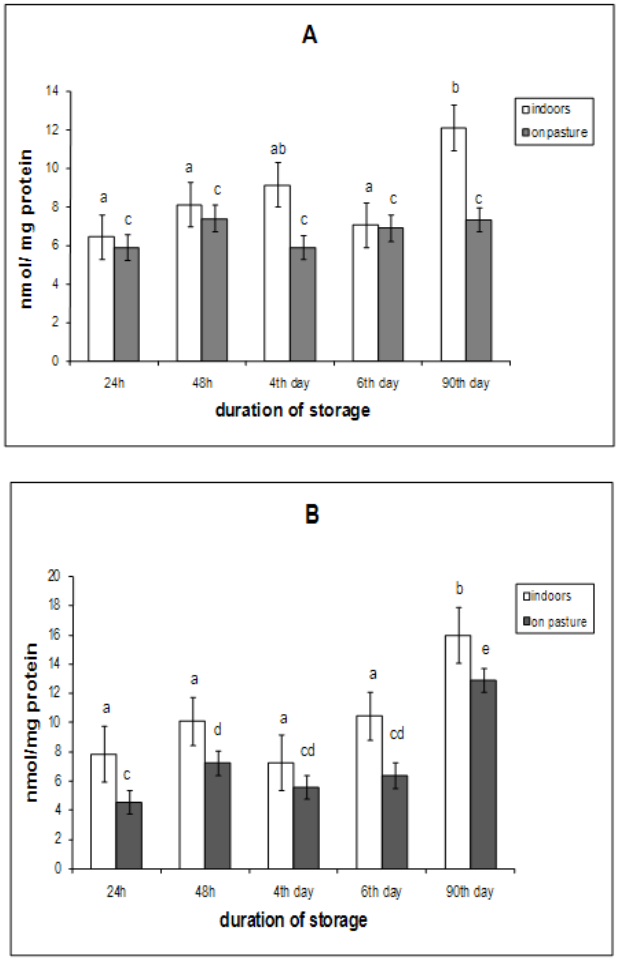

Figure 1 Dynamic of change of carbonyl formation during storage in *M. longissimus dorsi* in Northeastern Bulgarian Fine Wool Breed lambs (A) and Northeastern Bulgarian Fine Wool Breed × Ile de France lambs (B)
Intervals within group with different letters are statistically significant (P<0.05)

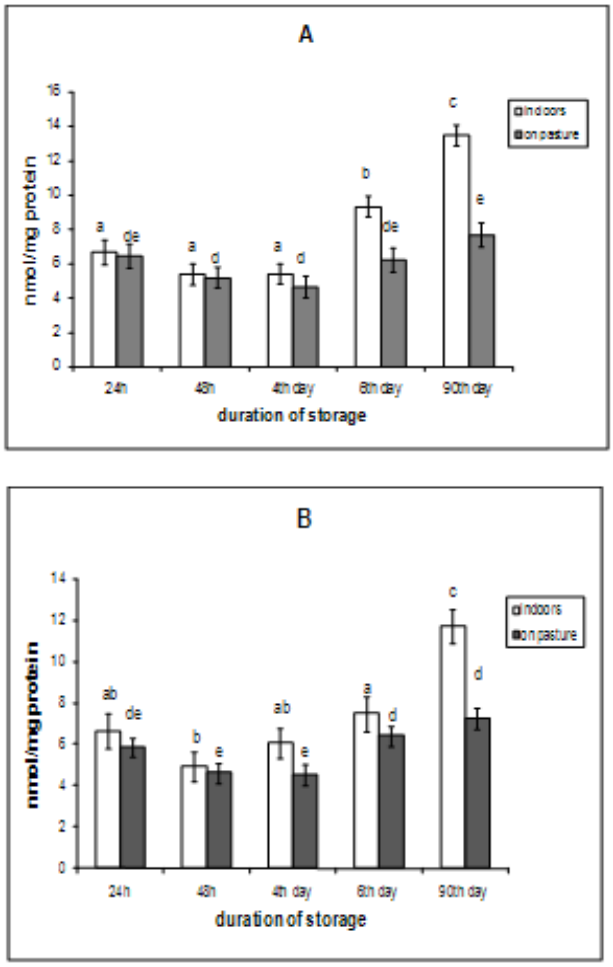

Figure 2 Dynamic of change of carbonyl formation during storage in *M. semimembranosus* in Northeastern Bulgarian Fine Wool Breedlambs (A) and Northeastern Bulgarian Fine Wool Breed × Ile de France lambs (B)
Intervals within groups with different letters are statistically significant (P<0.05)

In both muscles of NBFWB and NBFWB × IDF lambs, reared indoors and on pasture, there was a decrease of carbonyl content the 4th day of storage at its minimal values. Highest level of protein oxidation was observed the 90th day of samples storage.

The development of protein oxidation corresponds to the dynamic of lipid oxidation, determined by the content of TBARS in the same animals (Popova and Marinova, 2013).

This is confirmed by the results of previous studies, showing connection between lipid and protein oxidation (Mercier *et al*. 1995; Mercier *et al*. 1997; Srinivasan and Hultin, 1995).

Throughout the storage of muscle samples in both NBFWB and NBFWB × IDF lambs, carbonyl formation remains lower in pastured compared to indoors reared lambs. It is known that protein carbonylation is connected to changes in the texture, and to a decrease of nutritional value of the meat.

Hence, the lower content of carbonyls in the muscles of animals reared on pasture could be considered a good indicator, highlighting the advantages of this type of rearing with regard to the possibility of longer storage and better-meat quality.

CONCLUSION

The typeofrearing (indoors *vs*. pasture) influenced significantly the oxidation of proteins in *M. longissimus dorsi* and *M. semimembranosus* of the lambs of Northeastern Bulgarian Fine Wool Breed (on the 4th and 90th day of storage) and its cross with Ile de France (at the 24 h, 6th and 90th day of storage). Oxidative process had lower intensity in pastured animals and gives indication of some advantages of this type of rearing with regard to meat quality during storage. Muscle type had significant effect on carbonyl content as it was lower in *M. semimembranosus* in both indoors and

pastured lambs, and most pronounced at 48h and the 4th day as well as at 48 h and the 90th day, for Northeastern Bulgaria Fine Wool Breed and the cross, respectively. Storage duration affected significantly the formation of carbonyls in both type of muscles in both in doors and pastured lambs, and the differences in carbonyl content were most pronounced the 90th day of storage.

REFERENCES

Aristoy M.C. and Toldra F. (1998).Concentration of free amino acids and dipeptides in porcine skeletal muscles with different oxidative patterns. *Meat Sci*. **50,** 327-332.

Estevez M. and Cava R. (2006). Effectiveness of rosemary essential oil as an inhibitor of lipid and protein oxidation: contradictory effects in different types of frankfurters. *Meat Sci*. **72,** 348-355.

Estevez M., Kylli P., Puolanne E., Kivikari R. and Heinonen M. (2008). Fluorescence spectroscopy as a novel approach for the assessment of myofibrillar proteinoxidation in oil-in-water emulsions. *Meat Sci*. **80,** 1290-1296.

Estevez M., Ventanas S. and Cava R. (2005). Protein oxidation in frankfurters with increasing levels of added rosemary essential oil: effect on colour and texture deterioration. *J. Food Sci*. **70,** 427-432.

Estevez M., Ventanas S., Heinonen M. and Puolane E. (2011). Protein carbonylation and water holding capacity of pork subjected to frozen storage: effect of muscle type, premincing, and packaging. *J. Agric. Food Chem*. **59,** 5435-5443.

SAS Institute. (2007). JMP. V.7, SAS Institute, Cary, NC.

Klont R.E., Brocks L. and Eikelenboom G.(1998). Muscle fibre type and meat quality. *Meat Sci*. **49,** 219-229.

Lawrie R.A. (1998). Meat Science, 6th Ed. Woodhead Publishing: Cambridge, U.K.

Lund M.N., Heinonen M., Baron C.P. and Estevez M. (2011). Protein oxidation inmuscle foods: a review. *Mol. Nutr. Food Res*. **55,** 83-95.

Mercier Y., Gatellier P. and Renerre M. (1995). Relationships between lipid and protein oxidation in different beef muscles. Pp. 562-563 in Proc. 41th Int. Cong. Meat Sci. Technol., San-Antonio, TX, USA.

Mercier Y., Gatellier P., Viau M., Remignon H. and Renerre M. (1997). Effect of dietary fat and vitamin E on colour stability and on lipid and protein oxidation in turkey meat during storage. *Meat Sci.* **48,** 301-318.

Olivier C.N., Ahn B.W., Moerman E.J., Goldstein S. And Stadtman E.R. (1987). Aged-relatedchanges in oxidized proteins. *J. Biol. Chem.* **262,** 5488-5491.

Popova T. and Marinova P. (2013). Lipid oxidation in *M. longissimus dorsi* and *M. semimembranosus* in lambs reared indoors and on pasture. *Iranian J. Appl. Anim. Sci.* **3(3),** 533-537.

Requena J.R., Levine R.L. and Stadtman E.R. (2003). Recent advances in the analysis of oxidized proteins. *Amino Acids*. **25**, 221-226.

Santé-Lhoutellier V., Engel E., Aubry L. and Gatellier P. (2008). Effect of animal (lamb) diet and meat storage on myofibrillar protein oxidation and in vitro digestibility. *Meat Sci*. **79,** 777-783.

Srinivasan S. and Hultin H.O. (1995). Hydroxyl radical modification of fish muscle proteins. *J. Food Biochem.* **18,** 405-425.

Stadman E.R. and Levine R.L. (2000). Protein oxidation. *Annals New York Acad. Sci.* **899,** 191-208.

Ventanas S., Estevez M., Tejeda J.F. and Ruiz J. (2006). Protein and lipid oxidation in *longissimus dorsi* and dry cured loin from Iberian pigs as affected by crossbreedingand diet. *Meat Sci*. **72,** 647-655.

Xiong Y.L. and Decker E.A. (1995). Alterations in muscle protein functionality byoxidative and antioxidative processes. *J. Muscle Foods*. **6,** 139-160.