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LIPID AND PROTEIN OXIDATION DURING COOKING IN MEAT OF LAMBS REARED INDOORS AND ON PASTURE

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Abstract

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Lipid and protein oxidation during cooking in *m. Longissimus dorsi* was studied in male lambs of Northeastern Bulgarian Fine Wool Breed and cross of this breed with Ile de France, reared indoors and on pasture. Cooking was carried out at 100ºC for 45 and 60 minutes.

The oxidation of the lipids was determined by quantification of 2-thiobarbituric acid reactive substances and the degree of protein oxidation was determined by measuring the amount of carbonyls, formed before and after different duration of thermal heating. Rearing had significant influence ($P<0.001$) on formation of the 2-thiobarbituric acid reactive substances in *m. Longissimus dorsi* in the fresh samples and after 45th minute of cooking while the carbonyl formation was influenced by the rearing before heat treatment ($P<0.01$) and after 60 min ($P<0.05$). The duration of the cooking affected significantly the dynamics of lipid and protein oxidation showing increased contents of both 2-thiobarbituric acid reactive substances and carbonyls in the cooked meat.

Key words: meat, TBARS, carbonyls, cooking, rearing

Introduction

Oxidative processes lead to the degradation of lipids and proteins and are one of the primary mechanisms of quality deterioration in meat and meat products. Meat is consumed most often after thermal heating, which accelerates lipid and protein oxidation (Han et al., 1995; Byrne et al., 2002; Beltran et al., 2003) by disrupting muscle cell structure, inactivating antioxidant enzymes and other antioxidant compounds, and releasing iron from heme pigments (Kanner, 1994; Mei et al., 1994). High temperature causes reduction of activation energy for lipid oxidation and decomposes preformed hydroperoxides to free radicals, which stimulates autoxidation processes and off-flavor development (Min and Ahn, 2005). Thermal heating induces modifications of the aminoacids in proteins, that are related to the formation of carbonyl compounds, thyols and aromatic hydroxylation (Morzel et al., 2006), enhancing aggregation and hence reduced digestibility of the proteins in the gastrointestinal tract (Santé – Lhoutellier et al., 2008a).

To delay lipid oxidation, synthetic antioxidants have been applied extensively in food products (Ahmad, 1996) but the consumer preferences for natural ingredients over synthetic compounds (Ahn et al., 2002) increase. Rearing strategies are alternative way to increase the antioxidant content in meat and hence the pasture rearing is of great importance because the fresh grass, consumed by the animals is a good source of vitamin E and other 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.*

Despite rearing, the other major factor affecting various aspects of meat quality including its oxidative stability is the breed specific of the animals. Since oxidation in meat relates to its fatty acid composition breed of the animals is known to have effect on the fatty acids in their meat. Significant influence of crossbreeding on the lipid profile has been recently confirmed as well (Salvatory et al., 2004; Borys et al., 2007). On the other hand, according to Hernandez et al. (2004) breed might influence the antioxidant, lipolytic and proteolytic enzyme activities in meat.

The aim of the study was to determine the changes in the lipid and protein oxidation after thermal heating in *m. Longissimus dorsi* in lambs of a local Bulgarian breed and a cross of this breed, reared indoors and on pasture.

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Material and Methods

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 x ♂IDF) in the experimental base of 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 19,47kg (±0.5). Before the onset of the experiment, two groups of lambs of 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 620g/d per animal concentrate and the pastured lambs received 420g/d per animal concentrate. The composition of the diet was as follows: maize - 29.5 %, wheat - 36 %, sunflower meal - 32 %, vitamin premix - 0.5 %, lime - 2%. The experiment continued 73 d. After finishing the experiment, 5 animals of each group were slaughtered at the following mean live weight: NBFWB: indoor –31.13 kg; pasture -31.80 kg; NBFWB x IDF: indoor - 34.25 kg; pasture –32.32 kg.

After slaughtering the animals 24 h *post mortem*, *m. Longissimus dorsi* (*m.LD*) was dissected from the left side of the carcasses of each group and samples for lipid and protein oxidation determination were taken. They were cut in small size pieces in parallel with muscle fibers, put in glass tubes and cooked in water bath at 100 ºC for 45 and 60 minutes.

The oxidation of lipids was measured determining the amount of the 2-thiobarbituric acid reactive substances (TBARS), expressed as mg malondialdehyde per kg of muscle or TBA units (Lynch and Frei, 1993; modified by Mercier et al., 1998) and the protein oxidation by measurement of the content of carbonyls as nmol DNPH/ mg protein (Olivier et al., 1987) with slight modifications.

Data was analyzed by two-ways ANOVA (JMP version 7 software). The model included fixed effects ascribed to rearing (indoors and pasture), breed (NBFWB and NBFWB x IDF) and rearing x breed interaction on the TBARS and carbonyl formation.

For evaluation of the influence of the thermal heating on the lipid and protein 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

Lipid oxidation

Rearing had significant influence (P<0.001) on the TBARS formation in *m. Longissimus dorsi* before heating (Table 1). When examining the influence of breed on the lipid oxidation we observed significant effect of this factor (P<0.001) in the raw meat, however there is a strong interaction between breed and rearing (P<0.001). Rearing affected significantly the contents of TBARS after 45 min of cooking (P<0.05). After 60 minutes of heating the amounts of TBARS increased as in the indoors reared animals and in the group of NBFWB the values were above 2 mg malondialdehyde/kg muscle which is the threshold for detecting of rancid odour.

The dynamics of changes in the TBARS formation showed influence of the duration of the thermal treatment in both lamb breeds, reared indoors and on pasture.

In the lambs of NBFWB (Figure 1A), both reared indoors and on pasture the content of TBARS differed significantly between the raw meat and after 60 minutes of cooking. In the crossbred lambs (Figure 1B) reared indoors no significant difference in the TBARS formation was observed in the course of thermal treatment whereas in the pastured lambs the content of TBARS differed significantly from those after 45 and 60 minutes of cooking.

The values of TBARS measured after the cooking resembled those determined after 90 days of frozen storage of the samples from the same animals (Popova and Marinova, in

Table 1
Effect of rearing and breed on the TBARS (mg malondialdehyde/ kg muscle) during cooking in lambs (values least square means)

Duration of cooking	Rearing		Breed		S.E.	Significance		
	Indoors	Pasture	NBFWB	NBFWB xIDF		Rearing	Breed	Interaction
0 min	1.19[a]	0.38[b]	0.59[a]	0.98[b]	0.17	***	***	***
45 min	1.67[a]	1.17[b]	1.36	1.47	0.48	*	NS	NS
60 min	2.08	1.85	2.33	1.59	0.86	NS	NS	NS

Values connected with different letters are statistically different (P<0.05)
Significance effects:* P<0.05; ***P<0.001; NS- non significant; S.E. – standard error

press). It seems that high cooking temperatures increase the oxidation processes in meat. The increase of lipid oxidation could be explained by the loss of antioxidant activity such as glutathione or catalase, which could drop by up to 80% after heat treatment (Hoac et al., 2006).

In both lambs of NBFWB and the crossbred ones, the amounts of TBARS remained lower before and after thermal heating in the pastured groups compared to the indoors reared lambs.

Our findings are in agreement with the results of several other studies (Descalzo et al., 2005; Gatellier et al., 2005; Santé-Lhoutellier et al., 2008a; Soon Rhee et al., 2003; Warren et al., 2008) showing the protective effect of grass-based rearing system against oxidative processes in meat. According to Descalzo et al. (2005), Insani et al. (2008), Yang et al. (2002) antioxidants, such as vitamin E, ascorbic acid and carotenoids, can be found at higher concentrations in muscles from animals fed grass than in muscles from animals raised on concentrates. This antioxidant pool can, thus, counteract the increased susceptibility of meat to oxidation due to the high concentrations of PUFA usually associated with a grass-based diet. Realini et al. (2004) found improved lipid stability in *Longissimus dorsi* steaks from steers, raised on pasture compared to steaks from concentrate-fed animals.

Protein oxidation

The carbonyl formation was significantly influenced by the way of rearing the animals before cooking ($P<0.01$) and after the 60th minute ($P<0.05$) (Table 2). No breed effect was observed.

Similar to lipids, in proteins the carbonyl formation was also influenced by the duration of the cooking. In the lambs of NBFWB (Figure 2A) reared indoors we observed significant difference in the content of carbonyls between all the measurement points while in the lambs reared on pasture the differences were found between the raw samples and those treated for 45 and 60 minutes. In the crossbred lambs (Figure 2B) protein oxidation exhibited similar dynamics and significant differences existed only between the raw and the cooked muscle samples.

Similarly, to the TBARS reported above, the values of the carbonyl content were high and could be compared to that measured after long term frozen storage (Popova, Marinova, in press b). In lamb meat, Santé-Lhoutellier et al. (2008b)

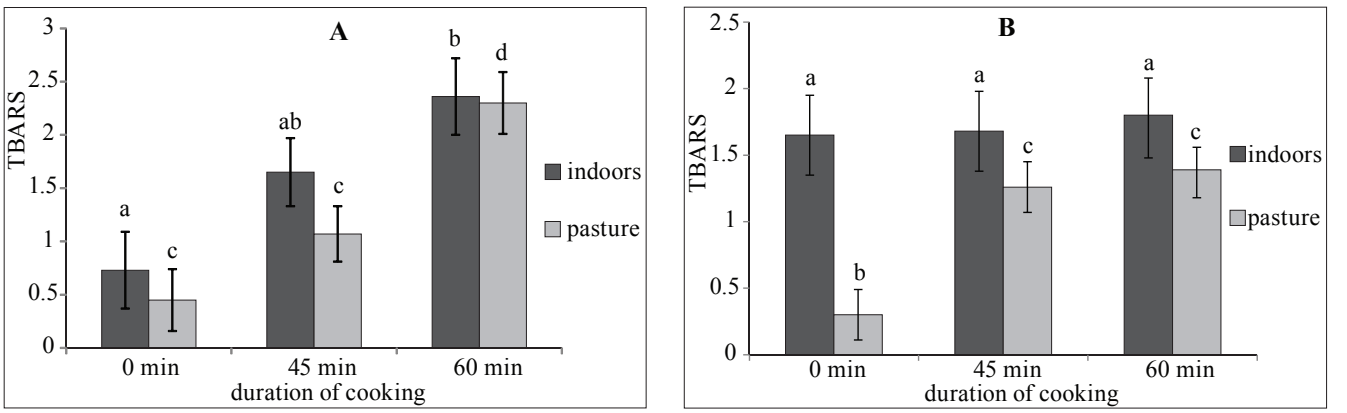

Fig. 1. Dynamics of change in TBARS content (mg malondialdehyde/ kg muscle) in lambs of NBFWB (A) and NBFWB x IDF (B)

Intervals within groups connected with different letters are statistically different ($P<0.05$)

Table 2

Effect of rearing and breed on the carbonyls (nmol DNPH/mg protein) during cooking in lambs (values least square means)

Duration of cooking	Rearing		Breed		S.E.	Significance		
	Indoors	Pasture	NBFWB	NBFWB x IDF		Rearing	Breed	Interaction
0min	7.15^{a}	5.23^{b}	6.18	6.20	1.23	**	NS	NS
45 min	11.93	10.59	11.03	11.5	2	NS	NS	NS
60 min	13.77^{a}	10.48^{b}	12.77	11.48	2.51	*	NS	NS

Values connected with different letters are statistically different ($P<0.05$)

Significance effects: * $P<0.05$; ** $P<0.01$; NS- non significant S.E. – standard error

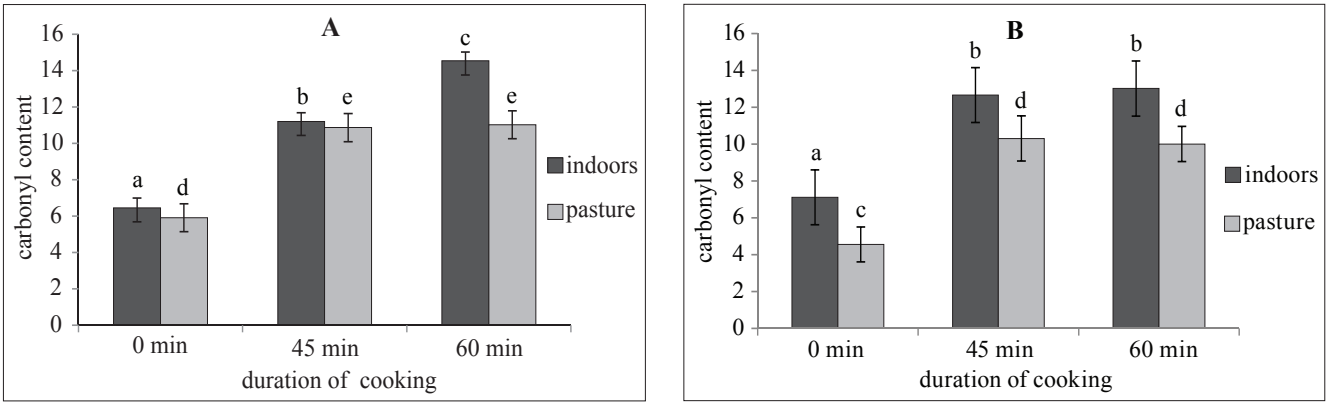

Fig. 2. Dynamics of change in carbonyl content (nmol DNPH/mg protein) in lambs of NBFWB (A) and NBFWB x IDF (B)

Intervals within groups connected with different letters are statistically different (P<0.05)

showed that carbonyl content increased 13% in *Longissimus dorsi* muscle from pasture-fed animals and 31% in *LD* muscle from concentrate-fed animals after 7 days long storage. Oxidation levels do increase during storage in refrigerated conditions, but not greatly. It is widely known that refrigerated meat oxidizes less than cooked meat. Astruc et al. (2007) and Santé-Lhoutellier et al. (2008a) also observed a significant 3-fold increase in carbonyl content from bovine proteins after cooking (100°C, 45 min). In beef meat heated for a short time (60 s) at high temperature (270°C), Astruc et al. (2007) found a three-fold increase in carbonyl levels, while Gatellier et al. (2010) heating the same material for 300 s at 207°C found a five-fold increase. The time/temperature couple used for cooking the meat could explain these high values. The increase in oxidation after cooking can be partly attributed to the fact that the muscle loses antioxidant protection (Hoac et al., 2006). Moreover, cooking leads to myoglobin denaturation and the oxidative cleavage of hematin pigment would lead to the release of iron from the heme molecules. The iron released is known to promote the formation of free radicals involved in lipid and protein oxidation (Purchas et al., 2006).

Conclusions

The type of rearing influenced significantly the oxidation of muscle lipids and proteins in both lambs of NBFWB and the cross, as those reared on pasture had lower amounts of TBARS and carbonyls after cooking, thus indicating the advantage of the pasture rearing in regards to oxidative changes in meat and its quality. Cooking and its duration affected significantly the dynamics of lipid and protein oxidation showing dramatically increase in the contents of TBARS and carbonyls in the cooked meat. However, additional experiments are needed in order to examine different durations and temperatures in regards to preserve nutritional and healthy quality of meat during cooking.

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