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EFFECT OF PASTURE BOTANICAL DIVERSITY ON THE OXIDATIVE STABILITY OF LAMB MEAT

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Introduction

Nowadays, increasing attention is paid to the quality of the animal's diet in relation to meat quality. In this context, a lot of studies aimed at increasing the polyunsaturated fatty acid content (PUFA), particularly the n-3PUFA, in animal products. This can be achieved by selecting an appropriate dietary fat source such as forages, fish oil, linseed(oil) (see review e.g. Raes et al., 2004). As PUFA are more prone to oxidation, these dietary strategies could compromise the oxidative stability of the product. Oxidation is a complex process, involving pro- and antioxidants, and can damage lipids as well as proteins and DNA. The effect of antioxidants, of which α-tocopherol(acetate) is the most frequently studied one, has mainly been focused on limiting lipid and pigment oxidation. However, besides tocopherols, plants contain numerous other compounds with antioxidative characteristics, e.g. flavonoids, polyphenols, carotenoids. The effects of this complex of plant antioxidative compounds to prevent oxidation of lipids, pigments and proteins *post mortem* is not well studied yet. In addition, besides the possible contribution from the supply of dietary antioxidants, muscle contains an endogenous antioxidative system of which the enzymes glutathione peroxidase (GSH-Px), catalase (Cat) and superoxide dismutase (SOD) are considered the most important. The effect of diet on the activity of these enzymes has not been investigated thoroughly.

Objectives

The aim of this study was to investigate the effect of botanical diversity in pastures (intensive ryegrass, herbage rich grass and leguminosa rich grass) on the oxidative stability of lamb meat.

Methodology

Experimental set-up

A group of 21 lambs (mean (sd) live weight: 22.3 (3.06) kg) was bought from an organic farm (Berendrecht, Belgium). Prior to the experiment, lambs had been exclusively grazing with their mother. Lambs were divided in three groups (n=7) for grazing on three different pastures from 01/07/2004 till 22/09/2004, i.e. an intensive ryegrass pasture (IR) (Melle, Belgium), a herbage rich pasture (HBG) and a leguminosa rich pasture (LG). The botanical composition of the pastures was determined weekly. The intensive ryegrass consisted mainly of *Lolium perenne*, while the herbage rich grass consisted for 80 to 90% of *Agrostis stolonifera* and *Bromus hordeaceus*. The leguminosa rich pasture contained as predominant plants red clover (*Trifolium pratense*) and lucerne (*Medicago sativa*). After 3 months grazing, animals (mean (sd) live weight: 32.3 (6.53) kg) were slaughtered in a local slaughterhouse (Ronse, Belgium). Carcasses were cooled for 24 h at 2°C. The *longissimus thoracis* was sampled for oxidative measurements (color, lipid and protein stability). Samples for measuring the α-tocopherol content and antioxidative enzymes were vacuum-packed and stored at -18°C.

Meat oxidative stability analyses

Color stability Steaks (2.5 cm thickness) were over-wrapped in a O_2 permeable PVC film and stored at 4°C for 8 days under constant illumination with white fluorescent lights (900 lux). Color and color stability measurements were performed using a HunterLab Miniscan spectrocolorimeter (D65 light source, 10° standard observer, 45°/0° geometry, 1 in. light surface, white standard). The color coordinates, expressed as CIE L*a*b* values and the reflectance values, to calculate % metmyoglobin (Krzywicki, 1979), were measured daily.

Lipid oxidation was measured as thiobarbituric acid reacting substances (TBARS) at day 4 and 8 of display and is expressed as µg malonaldehyde (MDA)/g muscle (Tarladgis et al., 1960).

Protein oxidation was measured by following a decrease in the amount of thiol groups and expressed as nmol free SH-groups/mg protein (Batifoulier et al., 2002). Protein oxidation was determined on the same samples used for lipid oxidation measurements.

α-tocopherol content (µg α-tocopherol/g meat) was determined after saponification and extraction by HPLC on a Supelcosil LC18 column (25mm x 4.6mm x 5µm) with UV-detection (λ = 292 nm) (Desai, 1984).

Endogenous antioxidative enzyme activities *GSH-Px* activity was determined by measuring spectrophotometrically (340 nm) the oxidation of NADPH at 22 °C (DeVore and Greene, 1982). One unit of GSH-Px was defined as one mole NADPH oxidised per min and per g meat. *Cat* activity was measured as described by Aebi (1983). The reaction (H_2O_2 loss) was monitored by measuring the absorbance at 240 nm at 22°C. One unit (U) of catalase was defined as one mole H_2O_2 decomposed per min and per g meat. *Total* SOD activity was determined according to the procedure of Marklund & Marklund

(1974) using inhibition of pyrogallol autoxidation by measuring an increase in absorbane at 340 nm at 22°C. One unit was taken as the activity that inhibits the reaction by 50%.

Statistical analysis

Oxidative stability measurements were analysed by one-way analysis of variance using pasture type as fixed factor. Comparison of means was performed using Tukey test ($P < 0.05$) (SPSS for Windows, version 11.0).

Results & Discussion

The intramuscular α-tocopherol content showed a trend towards a higher content in meat from lambs fed intensive ryegrass compared to those fed herbage rich grass or leguminosa (1.72, 1.24 and 1.09 µg α-tocopherol/g muscle for IR, HRG and LG respectively) ($P = 0.091$). No effect of forage type was observed on the color parameters (data not shown) and color stability measurements (Table 1). Also lipid oxidation, measured as TBARS-values, was not influenced by the pasture type (Table 1). Although the TBARS-values increased during time of display, the lipid oxidation was still limited after 8 days (mean (sd) 0.61 (0.30) µg MDA/g muscle). However, a significant effect of the pasture type was observed on protein oxidation, measured by the amount of free thiol groups after 8 days of display (Table 1). The data suggest a more pronounced protein oxidation in the meat from the lambs on the herbage rich pasture. It is not clear why an effect of diet on protein oxidation was observed, while colour and lipid oxidation remained unaffected. Sista et al. (2000) suggested, using a chicken muscle model system, that sulfhydryls were utilized to stabilise primary oxidation products. However, measuring thiol groups is only one method to evaluate protein oxidation.

Oxidation of muscle post mortem can also be limited by the endogenous antioxidative enzymes, which seem to be relatively stable during refrigerated storage (Renerre et al., 1996). The activities of the endogenous antioxidative enzymes, depending on the feeding group, are presented in Table 2. No effect of the pasture type was observed on the SOD activity, while a trend for a higher Cat activity was observed for the intensive ryegrass group compared to the other groups. A significantly higher GSH-Px activity was found for the leguminosa rich pasture group compared to the other groups. This finding is in line with studies on bovine meat showing a strong dependence of the activity of GSH-Px on the finishing mode (i.e. concentrate or grass-fed) (Gatellier et al., 2004). The effect of diet on SOD and Cat seems to be less consistent. An elevation of GSH-Px activity is commonly associated with oxidative stress (Frank and Messano, 1980), which would suggest a higher oxidative stress for the animals on the leguminosa rich pasture. Factors that may be responsible for this dietary effect include differences in the deposition of n-3PUFA (e.g. red clover was shown to limit biohydrogenation of n-3PUFA by Lee et al., 2003), but other antioxidants, minerals and especially Se could also influence the activity of GSH-Px. However, differential effects of diet on protein oxidation and GSH-Px activity were observed, whereas no effect of diet on colour and lipid oxidation was seen.

Conclusions

The oxidative stability of lamb meat was not clearly affected by the type of pasture. Only effects on protein oxidation and activity of GSH-Px were observed.

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Tables and Figures

Table 1. Effect of pasture botanical diversity on color, lipid and protein oxidation in *longissimus thoracis* of lambs (n = 7)

	Intensive Ryegrass	Herbage rich pasture	Leguminosa rich pasture	SEM	P
Color oxidation: % Metmyoglobin					
Day 4	33.4	40.4	38.6	1.63	0.196
Day 8	38.2	41.6	42.9	1.75	0.552
Lipid oxidation: TBARS (µgMDA/g muscle)					
Day 4	0.32	0.41	0.31	0.06	0.359
Day 8	0.68	0.63	0.52	0.12	0.631
Protein oxidation: nmol free SH-groups/mg protein					
Day 4	62.8	62.8	70.4	2.71	0.106
Day 8	61.0[a]	48.6[b]	64.4[a]	1.97	0.000

[ab] Means within a row with different superscripts are significantly different (P < 0.05)

Table 2. Effect of pasture botanical diversity on the activity of endogenous antioxidative enzymes in *longissimus thoracis* of lambs (n = 7)

	Intensive Ryegrass	Herbage rich pasture	Leguminosa rich pasture	SEM	P
GSH-Px	0.09[a]	0.08[a]	0.18[b]	0.07	0.006
Cat	40.8	30.8	30.1	9.96	0.074
SOD	64.4	68.5	72.4	8.99	0.259

[ab] Means within a row with different superscripts are significantly different (P < 0.05)