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Effect of grazing pastures of different botanical composition on antioxidant enzyme activities and oxidative stability of lamb meat

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Abstract

The aim of this work was to study the influence of different pastures (*Intensive ryegrass*, *Botanically diverse* and *Leguminosa rich* pastures) on the antioxidant status and oxidative stability of meat from lambs that had been exclusively grazing for three months. Lipid, colour and protein oxidation, α-tocopherol content and activity of antioxidant enzymes (superoxide dismutase (SOD), catalase (Cat) and glutathione peroxidase (GSH-Px)) were measured in *Longisimus thoracis et lumborum* muscle samples taken 1 day after slaughter. Pasture type significantly affected protein oxidation and the activity of GSH-Px, but no significant differences were found for the α-tocopherol content, colour and lipid oxidation, and the activities of SOD and Cat. Grazing a *Botanically diverse* pasture induced significantly higher protein oxidation in meat, as measured by the free thiol and carbonyl contents, compared to a *Leguminosa rich* or *Intensive ryegrass* pasture ($P < 0.05$). The GSH-Px activity was significantly higher in meat from lambs on the *Leguminosa rich* pasture compared to the other pasture groups ($P < 0.01$).

Keywords: Pasture; Lamb meat; Superoxide dismutase; Glutathione peroxidase; Catalase; Protein oxidation; Lipid oxidation; Colour oxidation

1. Introduction

Oxidative processes in meat are the most important factors responsible for quality deterioration including flavour, colour and nutritive value. The oxidative stability of meat depends on the balance between antioxidants and pro-oxidants and the content of oxidation substrates including polyunsaturated fatty acids (PUFA), cholesterol, proteins and pigments (Bertelsen et al., 2000; Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998).

Living cells have several mechanisms of protection against oxidative processes which include the endogenous enzymes superoxide dismutase (SOD), catalase (Cat) and glutathione peroxidase (GSH-Px). SOD and Cat are coupled enzymes. SOD scavenges superoxide anions by forming hydrogen peroxide and catalase safely decomposes hydrogen peroxide to H_2O and O_2^-. GSH-Px can decompose both hydrogen peroxide and lipoperoxides formed during lipid oxidation. It has been postulated that the activity of these antioxidant enzymes might be induced in animals exposed to oxidative stress, reflected in an increased production of free radicals (De Haan, Newman, & Kola, 1992). It has also been shown that these enzymes exhibit residual activity in muscle postmortem (De Vore & Greene, 1982; Renerre, Dumont, & Gatellier, 1996).

Besides the contribution of endogenous enzymes, the oxidative stability of meat is determined by the presence of antioxidants of dietary origin, e.g., the role of vitamin E in retarding lipid oxidation and improving colour stability (Liu, Lanari, & Schaefer, 1995; Morrisey, Buckley, Sheehy, & Monahan, 1994; Morrissey et al., 1998) is well

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recognized. The effects of other dietary compounds with antioxidative properties, such as carotenoids or flavonoids, and the contribution of endogenous enzymes on the oxidative status of meat is less well known but is being increasingly investigated.

In addition, the endogenous antioxidant enzyme activity system may be modulated by nutritional factors (Huang, Chen, Osio, & Cohen, 1994), e.g., some trace elements are essential for the normal function of endogenous antioxidant systems. The minerals Cu, Mn, Zn, Se and Fe are important cofactors of the antioxidant enzyme activities (Papas, 1999). Also other antioxidants could interfere, e.g., the regeneration of α-tocopherol by the oxidation/reduction of glutathione (Budowski & Sklan, 1989).

Apart from the many studies that have examined the supplementation of diets with antioxidants on the antioxidant status of meat, few studies have been performed on the effects of the basal diet, e.g., the effect of pasture varying in botanical composition and hence in the supply of minor compounds. The objective of the present study was to examine the effect of grazing pastures with different botanical composition by lambs on the oxidative stability of their meat.

2. Material and methods

2.1. Animals and experimental setup

A total of 21 male lambs (mean age 86 ± 9 days, mean live weight 22.3 ± 3.1 kg) of similar genetic background ('Vlaams Kuddeschaap', a 'herding' sheep breed), all born from yearling ewes, and originating from an organic farm were used for this experiment. At weaning, they were assigned to one of three pasture types, i.e. an *Intensive ryegrass (IR)* pasture, a *Leguminosa rich (L)* pasture and a *Botanically diverse (BD)* pasture. Lambs had been exclusively grazing with their mother before the trial. The L and BD pastures were located on the farm of origin (Berendrecht; near Antwerp), whereas the IR pasture was located on the experimental farm of Ghent University (Melle; near Ghent). No supplementary feeding was provided, except for a licking mineral block (Timac Potasco, Belgium) with the following composition: sodium (270 g/kg), calcium (60 g/kg), phosphor (2 g/kg), magnesium (1 g/kg), zinc (18 000 mg/kg), manganese (2000 mg/kg), iodine (100 mg/kg), cobalt (40 mg/kg) and selenium (10 mg/kg). The trial lasted for 83 days from 1 July 2004 until 22 September 2004, when the lambs were slaughtered (mean live weight at slaughter 32.3 ± 6.5 kg) and sampled.

The lambs were pastured in a larger group, but the experimental subgroups were matched for similar average live weight at the onset of the trial. On the IR pasture, some heifers were intermittently introduced to manage the sward height. The stocking density was low and on average less than 850 kg live weight ha^{-1} for the BD pasture and less than 1200 kg live weight ha^{-1} for the IR and L pasture. The L and BD pasture were not fertilized at all. The IR pasture received 59 kg N ha^{-1} and 3 kg P_2O_5 ha^{-1} on 4 May and an additional 35 kg N ha^{-1} on 18 August. All pastures were mowed once in the first week of August.

After slaughter, carcasses were cooled for 24 h at 2 °C. One day after slaughter, the *Longissimus thoracis et lumborum* (LTL) muscle was dissected. Sub-samples were used immediately for measuring colour, lipid and protein oxidation after chilled illuminated storage (until 8 days postmortem), whereas other sub-samples were vacuum packaged and stored immediately at −18 °C until analysis (α-tocopherol content and enzyme activities).

2.2. Pasture botanical and chemical composition

The botanical composition of the pastures was assessed according to the method of De Vries (1933) on nine occasions during the trial (three times per month). At these times, samples were also taken for chemical analyses, dried at 50 °C for 48 h, finely (0.5–1 mm) ground (Grindomix GM 200, Retsch, Germany) and pooled per month. Crude protein was determined according to the Kjeldhal method (European Community, 1993), Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) using the Van Soest method (Van Soest, Robertson, & Lewis, 1991), lignin according to the method described by Van Soest and Wine (1968) and crude fat by the Soxhlet method (International Standards Organisation, ISO-1444, 1973). The following mineral and trace elements were determined by plasma emission spectrometry (ICP-AES Iris Intrepid II XSP, Thermo Electron corporation) following dry incineration at 500 °C for 4 h and dissolving in 6 N HCl: B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S. The content of Se was determined by ICP-AES with hydride generation, but with a different preparation. The sample is first destroyed with HNO_3 and H_2O_2 in a microwave, pre-reduced with HCl to convert Se^{6+} to Se^{4+} and further reduced and converted to H_2Se using $NaBH_4$.

2.3. Colour stability

Steaks (1.5 cm thickness) were over-wrapped in an O_2-permeable PVC film and stored at 4 °C for 8 days under constant illumination with white fluorescent lights (900 lux). Colour and colour stability measurements were performed using a HunterLab Miniscan spectrocolorimeter (D65 light source, 10° standard, 45°/0° geometry, 1 in. light surface, white standard). The colour coordinates, expressed as CIE L^*, a^*, b^* values and the reflectance values to calculate metmyoglobin (MetMb%) (Krzywicki, 1979) were measured daily for 8 days.

2.4. Lipid and protein oxidation

Lipid and protein oxidation were measured on day 4 and 8 for the muscle samples. Lipid oxidation was measured by the TBARS (2-thiobarbituric acid reactive sub-