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Effect of animal (lamb) diet and meat storage on myofibrillar protein oxidation and in vitro digestibility

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Abstract

Effect of pasture- or concentrate-diet on myofibrillar protein oxidation and in vitro digestibility was measured in lamb meat (M. *longissimus dorsi*) during a refrigerated storage of 7 days under gas permeable film. Protein oxidation was measured by the carbonyl content determined chemically using 2,4-dinitrophenylhydrazine (DNPH) and specific targets of oxidation were identified by immunoblotting. Carbonyl content significantly increased during storage and diet affected protein oxidation where animals fed concentrate showed higher carbonyl group levels than animals fed pasture. To evaluate effect of diet and storage time on protein digestibility, myofibrillar proteins were exposed to proteases of the digestive tract (pepsin, and a mixture of trypsin and α-chymotrypsin) in conditions of pH and temperature which mimic digestive process. The myofibrillar protein digestibility was not influenced by the diet. Storage time had no significant effect on myofibrillar protein susceptibility to pepsin while an important increase in digestibility by trypsin and α-chymotrypsin was detected during storage.

Keywords: Lamb meat; Myofibrillar proteins; Storage; Diet; Protein oxidation; Immunoblotting; Digestibility

1. Introduction

In post-mortem muscle tissue, release of transition metals from carrier proteins (Kanner & Doll, 1991; Kanner, Hazan, & Doll, 1988) and decrease in antioxidant defense systems (Renerre, Dumont, & Gatellier, 1996; Renerre, Poncet, Mercier, Gatellier, & Metro, 1999) lead to formation of reactive oxygen species. These oxidative species which include hydroxyl, superoxide, peroxyl, and nitric oxide radicals can interact with lipids and proteins. Lipid oxidation in meat has been extensively described and its impact on meat quality through the formation of rancid odours and deterioration of flavour is well known (Asghar, Gray, Buckley, Pearson, & Boren, 1988). Less attention has been given to protein oxidation in meat. Nevertheless protein oxidation is responsible for many biological modifications such as protein fragmentation or aggregation, changes in hydrophobicity, and protein solubility, affecting technological properties such as gelation (Srinivasan & Xiong, 1996) and emulsification (Srinivasan & Hultin, 1997). Protein oxidation might also play a role in meat tenderness (Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004a) by controlling protease activity (Kristensen, Moller, & Andersen, 1997; Morzel, Gatellier, Sayd, Renerre, & Laville, 2006; Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004b).

Meat proteins serve as an important source of energy and essential amino acids for humans. Nutritional quality of meat is largely dependent on protein digestibility because, to pass through the small intestine wall and to enter the bloodstream, proteins must be broken down into amino acids or small peptides. Little is known about the effect of oxidation on meat protein digestibility. Limited studies have been performed in model systems to link chemical oxidation of meat proteins with digestibility but results have been inconsistent and contradictory. Kamin-Belsky, Brillon, Arav, and Shaklai (1996) and Liu and Xiong

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(2000a) have demonstrated that chemical oxidation of myosin can affect its proteolytic susceptibility to enzymes of the digestive tract with increased or decreased digestibility depending of level of oxidation and presence or not of reducing agents. Moreover myosin is not representative of the whole meat proteins. The myofibrillar structure is a more complex system than myosin with many protein–protein interactions (Morzel et al., 2006) and even protein–lipid interactions (Chelh, Gatellier, & Santé-Lhoutellier, 2007) which can affect its susceptibility to proteolysis (Morzel et al., 2006). So, in studies relating to digestibility, model systems using myofibrils are more suitable than those with purified myosin. Recently, we have demonstrated that chemical oxidation of myofibrillar proteins by hydroxyl radicals decreased their digestibility by the enzymes of the digestive tract (Santé-Lhoutellier, Aubry, & Gatellier, 2007) and that loss of digestibility was correlated with oxidative parameters of proteins as hydrophobicity change, aggregation and carbonylation. Nevertheless, levels of oxidation induced by these chemical oxidations are considerably higher than those observed in aged meat (Martinaud et al., 1997) and so effects on protein digestibility may be overestimated. Thus, it would be of great interest to evaluate protein digestibility under lower oxidative conditions such as those generally observed during meat storage.

Currently, no study has been performed about the effect of animal diet and meat ageing on myofibrillar protein digestibility. Therefore, our objective was to examine the impact of pasture- and concentrate-diet, which has been described to generate different levels of meat oxidation (Gatellier, Mercier, Juin, & Renerre, 2004; O'Sullivan et al., 2003), and a refrigerated storage of 7 days on both oxidation and in vitro digestibility of myofibrillar proteins of lamb meat. With this aim, myofibrils were exposed to proteases of the digestive tract (pepsin, and a mixture of trypsin and α-chymotrypsin) in conditions of pH and temperature which mimic gastric and intestinal fluids.

2. Materials and methods

2.1. Animals and diet

We used 16 lambs (castrated males). After birth, animals were reared in a sheepfold with their dams for 51 days. After weaning, 8 animals remained in the sheepfold and were fed with high energy concentrate and 8 animals were reared on pasture. Concentrate was purchased from Guyomarc'h Nutrition Animale (France) and its composition provided by the retailer is given in Table 1.The pasture diet consisted essentially (more than 90%) of the graminae *dactylis glomerata*. Animals of each group were slaughtered at 220 days. Mean weights at slaughter were 28.1 kg (24.7–32.2) for animals fed pasture and 33.6 kg (29.3–37.2) for animals fed concentrates. They were processed and eviscerated according to standard commercial procedures at the INRA experimental abattoir. Muscle *longissimus dorsi* of each animal was immediately taken and placed on a fibre board tray, wrapped in air-permeable film and stored during 7 days in darkness at 4 °C to mimic commercial conditions of meat storage.

Table 1
Composition of concentrate used for finishing lamb

Ingredients	Content
Beet pulp	Non-specified
Barley	NS
Corn	NS
Maize	NS
Soya cattle cake	NS
Colza cattle cake	NS
Beet treacle	NS
Calcium carbonate	NS
Sodium bicarbonate	NS
Calcium phosphate	NS
Ammonium chloride	NS
Sodium chloride	NS
Magnesia	NS
Choline chloride	NS
Pam oil	NS
Analytic constituents	
Proteins	15.00%
Fats	2.50%
Cellulose	9.50%
Ashes (inorganic matter)	9.00%
Vitamins	
Vitamin A	6.00 IU/kg
Vitamin D3	1.80 IU/kg
Vitamin E (alphatocopherol)	20 IU/kg
Vitamin B1 (thiamin)	10 mg/kg

2.2. Determination of muscle antioxidant status

Vitamin E content was determined according to the method of Buttriss and Diplock (1984). Activity of antioxidant enzymes was measured on a meat extract prepared on day 0 as previously described (Renerre et al., 1996). Total superoxide dismutase activity (Cu–Zn SOD and Mn SOD) was measured according to the procedures of Marklund and Marklund (1974) using inhibition of pyrogallol autoxidation. Catalase activity was measured by the rate of disappearance of H_2O_2 following the method of Aebi (1974). Glutathione peroxidase (GPx) activity was assayed with GSH reduction coupled to NADPH oxidation by glutathione reductase (Agergaard & Thode Jensen, 1982).

2.3. Isolation of myofibrils

Myofibrils were prepared according to the method of Ouali and Talmant (1990) with some modifications as outlined by Martinaud et al. (1997). Myofibrils were prepared after 0, 2, 4 and 7 days of refrigerated storage.

2.4. Determination of carbonyl content

Carbonyl groups were estimated using the method of Oliver, Alin, Moerman, Goldstein, and Stadtman (1987)