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Assessment of the influence of diet on lamb meat oxidation

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Abstract

The effect of pasture- or concentrate-diet on colour stability, lipid oxidation and protein oxidation was measured in lamb meat (M. *longissimus dorsi*) during refrigerated storage of 7 days under gas permeable film. Lipid and protein oxidation increased rapidly with storage time while evolution of colour parameters exhibited a biphasic curve. Diet had an important effect on lipid oxidation where animals fed concentrate showed higher thiobarbituric reactive substance (TBARS) levels than animals fed pasture-diet. However the nature of diet did not affect protein oxidation or colour parameters of meat. In parallel anti-oxidant status of meat was estimated by measurement of vitamin E content and anti-oxidant enzyme activities while pro-oxidant status was evaluated by haeminic iron, polyunsaturated fatty acid (PUFAs) and glycogen content of muscle. Statistical analysis was performed in order to relate oxidation parameters to pro- and anti-oxidant status of muscle.

Keywords: Lamb; Meat; Diet; Colour; Lipid oxidation; Protein oxidation

1. Introduction

Meat storage or meat processing generates free oxygenated radicals while the intrinsic anti-oxidant defence systems decrease (Renerre, Dumont, & Gatellier, 1996). This combination leads to the accumulation of oxidative damage both in lipids and in proteins (Mercier, Gatellier, Viau, Remignon, & Renerre, 1998). These oxidative processes are known to be the major cause of meat quality deterioration affecting colour, flavour and nutritional value.

Among meat qualities, colour of meat is one of the most important factors influencing consumer purchase (Dransfield, 1985). In lamb, many studies have analysed the effect of age, sex, slaughter weight or specific breeds on meat colour (Sanudo, Alfonso, Sanchez, Delfa, & Teixeira, 2000; Teixeira, Batista, Delfa, & Cadavez, 2005). However, apart from studies that have examined vitamin E supplementation of diet, few studies have been performed on the effect of basal diet and storage time on lamb meat colour.

Lipid oxidation in meat is implicated in the formation of rancid odours and deterioration of flavour (Asghar, Gray, Buckley, Pearson, & Boren, 1988). It has been well established that animal diet affects the flavour of meat. In beef, off-flavours have been noticed with forage-diet or with pasture-diet when compared with grain-diet (Sapp, Williams, & McCann, 1998), but the dietary effect can be confounded by differences in animal age, carcass weight at slaughter and back fat thickness which have all been shown to alter meat quality with respect to tenderness and flavour (Spanier, McMillian, & Miller, 1990).

Proteins are the target of free radical attack as well as lipids. Even if less attention has been given to such phenomenon, protein oxidation is responsible for many biological modifications affecting meat quality and meat technology. Different groups of amino acid are sensitive to oxidation (Davies, 1987). Basic amino acids are oxidized into carbonyls which can interact with amino groups to form amide links, oxidation of thiol groups from cysteine leads to the formation of disulfide bridges, and tyrosine can oxidize to give dityrosine bridges. These examples of oxidative cross linking can induce polymerisation and aggregation of proteins. This aggregation has been

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reported to decrease protein susceptibility to proteases and thus oxidation can decrease digestibility of proteins and therefore could affect the nutritional value of meat (Santé-Lhoutellier, Aubry, & Gatellier, 2007). In spite of that, protein oxidation is poorly documented in meat, especially in lamb meat where only one study reports the effect of diet (nature of the grass) and storage time on protein oxidation (Petron et al., 2007).

The aim of this study was to investigate the effect of pasture- and concentrate-diet on colour stability, lipid and protein oxidation of M. *longissimus dorsi* from lamb during storage, and to propose a mathematical model that describes these oxidations with respect to pro- and anti-oxidant status of muscle.

2. Materials and methods

2.1. Animals and diet

We analysed 16 lambs (castrated males). After lambing, animals were reared in sheepfold with their dams during 51 days. After weaning, 8 animals remained in sheepfold and were fed with high energy concentrate and 8 animals were reared in pasture. Concentrate was purchased from Guyomarc'h Nutrition Animale (France). It was mainly composed of cereals (Corn, maize, barley), soya, colza, and beet. Pasture diet was essentially composed (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.

2.2. Colour measurement

Colour measurements were performed after 2 h, 2, 4 and 7 days of storage. Visible reflectance spectra (from 360 to 760 nm) were determined with an Uvikon 933 (Kontron) spectrophotometer equipped with an integrating sphere of Teflon. Reflectance measurements were collected from a 2°-viewing angle and with illuminant D65 (Daylight) lighting conditions. Colour coordinates were calculated in the *CIE-L*a*b** (1976) system where L^* is lightness, a^* redness and b^* yellowness.

2.3. Lipid oxidation measurement

Lipid oxidation was measured by the ThioBarbituric Acid Reactive Substances (TBARS) method according to the method of Lynch and Frei (1993) modified by Mercier et al. (1998). TBARS measurements were performed as colour measurements. The results were expressed as mg of malondialdehyde (MDA) versus kilogram of meat (TBA units).

2.4. Protein oxidation measurement

Protein oxidation was measured by an estimation of the carbonyl groups using the method of Oliver, Alin, Moerman, Goldstein, and Stadtman (1987) with slight modifications (Mercier et al., 1998). Carbonyl measurements were performed at the same time than colour measurements. The results were expressed as nanomoles of DNPH fixed per milligram of protein.

2.5. Determination of muscle anti-oxidant status

2.5.1. Vitamin E content

Vitamin E content was determined on fresh meat (day 0) according to the method of Buttriss and Diplock (1984). The results were expressed in milligrams of vitamin E per gram of tissue.

2.5.2. Anti-oxidant enzyme activity

Activity of anti-oxidant enzymes was measured on a meat extract prepared at 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. One SOD unit was taken as the activity that inhibits the pyrogallol autoxidation by 50%. Catalase activity was measured by the rate of disappearance of H_2O_2 (nmol of decomposed H_2O_2 min^{-1} mg^{-1} protein) following the method of Aebi (1974). Glutathione peroxidase (GPx) activity was assayed with a GSH reduction coupled to a NADPH oxidation by glutathione reductase (Agergaard & Thode Jensen, 1982). GPx activity was expressed as nmol of oxidized NADPH min^{-1} mg^{-1} protein.

2.6. Determination of muscle pro-oxidant status

2.6.1. Haeminic iron determination

Haeminic iron was determined at day 0 on 1 g of each muscle samples using the method of Hornsey (1956). Values were expressed in milligram of iron per gram of meat (ppm).

2.6.2. Lipid composition

Intramuscular lipids were extracted at day 0 from muscle according to the method of Folch, Lees, and Sloane Stanley (1957) and their amount was expressed in g/100 g of fresh tissue. Fatty acids were analysed by gaseous phase chromatography (protocol under INRA licence) and results were expressed in percentage of the whole fatty acids.

2.6.3. Glycogen content

In order to obtain values for residual glycogen level samples were collected at 24 h post-mortem. Muscle sam-

ples were frozen in liquid nitrogen prior to analysis. About 1 g of muscle tissue was homogenised in 10 ml of 0.5 M perchloric acid. Glycogen was determined on the homogenate according to Dalrymple and Hamm (1973), after hydrolysis of glycogen with amyloglucosidase. Concentrations were expressed as μmol/g fresh tissue.

2.7. Statistical analysis

All values are reported as the mean ± SEM for each animal group. The unpaired Student *t*-test was used to determine the levels of statistical significance between groups, with $p > 0.05$, NS; $p < 0.05$, $*$; $p < 0.01$, $**$; $p < 0.001$, $***$. To assess the effect of diet and storage time and their interactions, data were also analysed by a two-way analysis of variance (ANOVA). The mixed procedure with time repetition was used. A principal component analysis (PCA) was also performed. In order to correlate oxidation parameters with characteristic components of the meat, single variable regression analyses and multiple variable regression analyses using a step by step ascending method were performed on the statistical system.

3. Results and discussion

3.1. Effect of diet on meat colour

The two-way analysis of variance showed an important effect of storage time on L^*, a^* and b^* ($p < 0.001$) but did not reveal any diet effect. Table 1 shows the effect of storage and diet on colour parameters L^*, a^*, b^*. Between 0 and 2 days of storage all parameters increased due to myoglobin oxygenation. After 2 days storage, myoglobin oxidation induced an important decrease in colour parameters especially a^*.

Effect of animal diet on colour has been largely described in bovine meat. Results generally described in the literature show that pasture- or forage-finished cattle had darker coloured meat when compared with grain-fed cattle (Vestergaard, Oksbjerg, & Henckel, 2000). In lamb, effect of diet on meat colour is not so well documented. Nevertheless, our results are in good accordance with two studies showing no effect of diet on meat colour. Ponnampalam, Trout, Sinclair, Egan, and Leury (2001) showed that colour stability of meat was not affected during refrigerated display, by enhanced levels of omega-3 and omega-6 fatty acids due to dietary treatments. More recently, Petron et al. (2007) did not show any effect of the nature of pasture on meat colour stability.

Table 1
Colour parameters according to the diet source (c: concentrate and p: pasture) and storage time

Day	L^* c	L^* p	a^* c	a^* p	b^* c	b^* p
0	35.7 ± 2.2	36.1 ± 1.6	11.5 ± 0.9	10.0 ± 0.5	4.2 ± 1.2	3.9 ± 0.6
2	43.4 ± 1.9	42.2 ± 1.5	16.1 ± 0.7	13.9 ± 1.2	10.9 ± 0.9	10.5 ± 0.6
4	42.5 ± 2.3	43.2 ± 1.9	12.9 ± 1.2	11.0 ± 1.2	8.2 ± 1.1	6.9 ± 1.3
7	42.7 ± 2.6	41.0 ± 0.8	8.1 ± 1.1	6.8 ± 0.8	6.6 ± 1.6	4.9 ± 0.8

Values are means ± SEM of 8 determinations.

3.2. Effect of diet on lipid oxidation

The two-way analysis of variance showed time ($p < 0.001$) and diet ($p < 0.001$) effects with an important interaction ($p < 0.001$). This interaction occurred because the TBARS value of concentrate group was initially lower than that of pasture group and increased more rapidly during storage (Fig. 1). After 2 days storage TBARS values were already significantly higher ($p < 0.05$) in the concentrate group and after 7 days storage TBARS values were approximately six folds higher in the concentrate group when compared with the pasture group. As lipid oxidation leads to warmed-over flavour, pasture-diet seems to be more appropriate to limit this quality loss during refrigerated storage. The threshold for detection of off-flavour being 0.5 mg MDA/kg meat (Lanari, Schaefer, & Scheller, 1995), in our study, the meat of pasture group remained acceptable during the 7 days of storage, whereas, for meat of concentrate group off-flavours appeared after 2 days of storage.

In the literature, the effect of diet on lipid oxidation of meat has been described essentially on beef. Our results are in good accordance with those of Descalzo et al. (2000). These authors described a three fold increase of TBARS levels in beef from grain-fed steers when compared with beef from pasture fed steers and these authors concluded that natural anti-oxidants in pasture compensate for the high level of polyunsaturated fatty acids (PUFAs) with a protecting effect on lipids against oxidation. Again in beef we have recently demonstrated that meat from pasture fed animals as more resistant to lipid oxidation than meat from mixed-diet fed animals (Gatellier, Mercier, Juin, & Renerre, 2004). However, our results contradict two earlier studies showing no effect of diet on lipid oxidation.

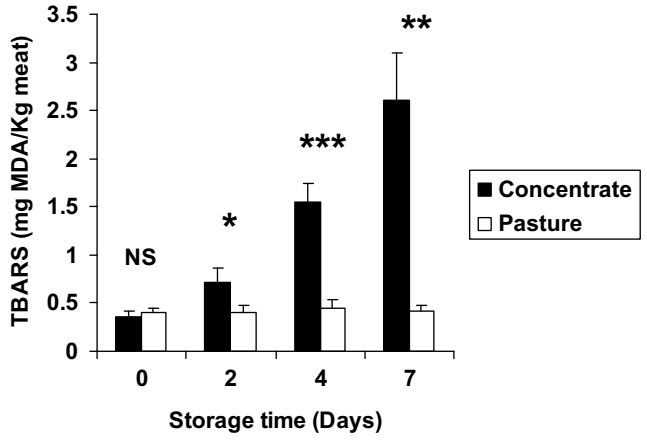

Fig. 1. Effect of diet and storage time on lipid oxidation of lamb meat measured by TBARS. Values are means ± SEM of 8 determinations. Significance (S) is noticed as NS: not significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

In lamb meat, Ponnampalam et al. (2001) demonstrated that lipid oxidation was not affected by supplementation with polyunsaturated fatty-acids and Petron et al. (2007) showed that, as with colour stability, lipid oxidation was not significantly affected by nature of the grass.

3.3. Effect of diet on protein oxidation

The two-way analysis of variance showed an effect of storage time on protein oxidation ($p < 0.05$) but did not reveal any diet effect. Fig. 2 shows that the protein oxidation appeared more rapidly than the lipid oxidation and reached its maximum after 2 days storage for the concentrate group and 4 days for the pasture group. The carbonyl levels tended to be higher in concentrate group ($p = 0.09$) only at the end of storage. Our results contradict those of Petron et al. (2007) showing, in lamb, an effect of the diet (different pastures) on protein oxidation without any effect on lipid oxidation.

3.4. Effect of diet on anti-oxidant status of muscle

The oxidative stability of meat is mainly determined by the presence of anti-oxidants of dietary origin. Effect of vitamin E on lipid oxidation and colour stability has been well documented (Lanari et al., 1995; Mercier et al., 1998). Other anti-oxidants of the diet such carotenoids and flavonoids can also protect meat against oxidation (Wood & Enser, 1997). In addition, the activity of the endogenous anti-oxidant enzyme system may be modulated by trace elements such as Cu, Mn, Zn, Se, and Fe which must be provided by diet. Apart from studies that have examined vitamin E supplementation of diet, no study has been performed on the effect of basal diet (as pasture or concentrate diet) on anti-oxidant status of lamb muscle.

The muscle anti-oxidant status was estimated in this study by measurement of individual anti-oxidant enzyme activities and vitamin E content. Due to financial and time constraints the muscles were not assayed for carotenoids and flavonoids. No diet effect was observed on the activity of anti-oxidant enzymes (Catalase, Superoxide dismutase, and Glutathione peroxidase), while an important difference was measured on vitamin E level (Table 2). We can notice that vitamin E levels measured in muscle of animal fed pasture were considerably higher than those measured by Petron et al. (2007) in the same muscle of lambs fed varied grazing pasture.

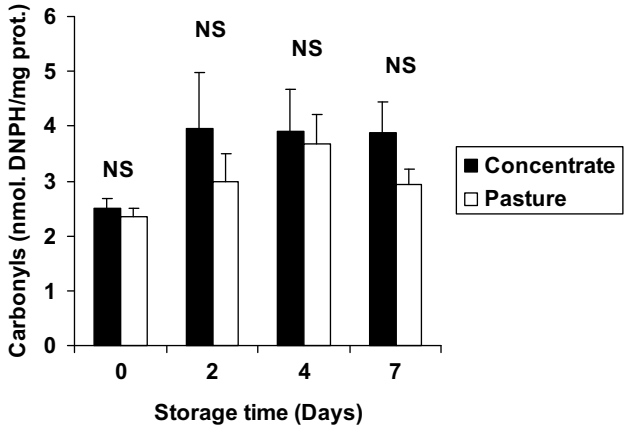

Fig. 2. Effect of diet and storage time on protein oxidation of lamb meat measured by carbonyl group content. Values are means ± SEM of 8 determinations. Significance (S) is noticed as NS: not significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

3.5. Effect of diet on pro-oxidant status of muscle

Haeminic pigment can be categorized as a pro-oxidant substance because its oxidation has been described to be a major catalyst of free radical formation such as superoxide radical (Kanner, Harel, & Granit, 1992) and peroxyl radical (Kanner & Harel, 1985). No significant difference ($p > 0.05$) was observed in haeminic iron content between concentrate and pasture group (Table 2). Values measured in these two groups agree with those found by Sanudo et al. (2000) in Mediterranean lambs.

By generating free radicals during the oxidative process, polyunsaturated fatty acids can act as pro-oxidant substances. In a previous study, radical production occurring during oxidation of lipids in turkey meat has been identified by ESR spectroscopy (Gatellier, Mercier, Rock, & Renerre, 2000), and was mostly composed of alkyl radicals: ethyl and pentyl radicals. Table 2 shows the level of total lipids and the distribution of the different classes of fatty acids. No diet effect was measured on the percentage of saturated fatty acid while a significant effect was measured on monounsaturated fatty acids with a higher percentage in the concentrate group. Percentage of polyunsaturated fatty acid was two fold higher in animals fed pasture than in animals fed concentrate. This difference was due to a higher percentage of $n-3$ PUFAs and conjugated linoleic acid (CLA) in animals fed pasture.

The role of glucose in lipid and protein oxidation has been studied in diabetes mellitus and ageing related diseases (Delattre, Bonnefont-Rousselot, Bordas-Fonfrèdre, & Jaudon, 1999). Glucose in its ene-diol form can react with transition metals to yield superoxide anions ($O_2^{\cdot -}$) which can form hydrogen peroxide in a reducing medium. Transition metals can then produce hydroxyl radicals ($OH^{\cdot}$) by reaction with hydrogen peroxide. By its capacity to release glucose during meat ageing, glycogen can also be assimilated to a pro-oxidant compound, especially as in the same time, due to pH fall; iron is released from ferritin under its catalytic form. In this study a significant higher level of glycogen was measured in animals fed concentrates (Table 2).

3.6. Principal component analysis and predictive models of oxidations

The principal component analysis (Fig. 3) was applied to investigate the relationship between the oxidation

Table 2
Effect of diet on characteristic components of lamb meat

	Concentrate	Pasture	Significance
Anti-oxidant compounds			
Vitamin E	1.61 ± 0.27	6.42 ± 0.47	***
Catalase	0.0018 ± 0.0004	0.0016 ± 0.0002	NS
SOD	0.53 ± 0.03	0.58 ± 0.02	NS
GPx	340 ± 26	352 ± 23	NS
Pro-oxidant compounds			
Haeminic iron	8.05 ± 0.47	8.25 ± 0.66	NS
Total lipids	2.80 ± 0.30	2.91 ± 0.28	NS
SFAs	47.22 ± 0.45	46.67 ± 0.55	NS
MUFAs	44.49 ± 0.61	36.34 ± 0.31	***
PUFAs	7.99 ± 0.81	16.1 ± 0.70	***
n−6 PUFAs	7.11 ± 0.69	7.8 ± 0.40	NS
n−3 PUFAs	0.71 ± 0.12	6.10 ± 0.28	***
CLA	0.19 ± 0.02	1.05 ± 0.07	***
Glycogen	56.19 ± 3.00	44.80 ± 3.18	*

Units are given in Section 2.
Values are means ±SEM of 8 determinations.
Significance (S) is noticed as NS: not significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

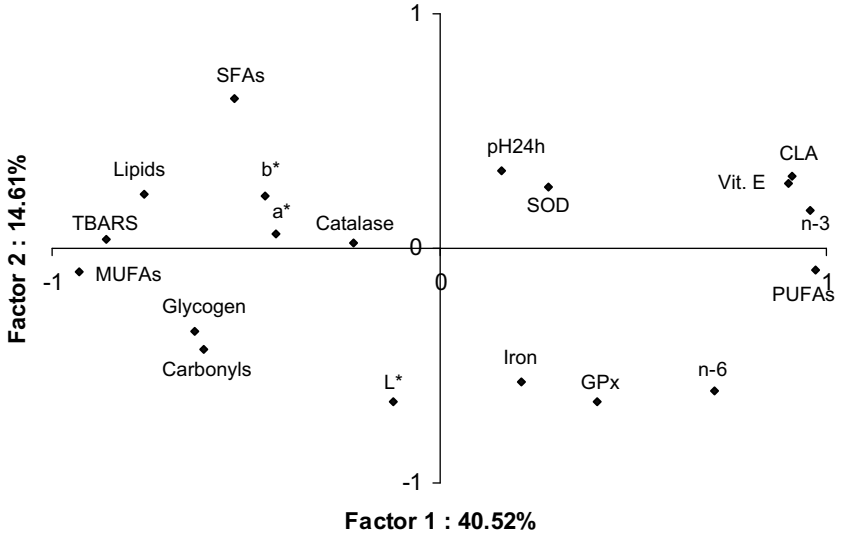

Fig. 3. Loading plot of the variables in the principal component analysis including lipid content, fatty acid groups, iron, pH at 24 h, glycogen, vitamin E content, anti-oxidant activities and oxidation parameters measured at day 7.

parameters at the end of storage (D7) and the characteristic components of meat. On account of its importance in many biochemical processes affecting meat quality, pH measured 24 h post-mortem was also plotted.

The first factor accounted for 40.52% of the variance explained; the second one scored 14.61%. Factor 1 was highly positively related to PUFAs (total, *n*−3, *n*−6 and CLA) as well as vitamin E content of muscle. On the contrary lipid oxidation, MUFAs, total lipids content and, to a lesser extent, glycogen and protein oxidation were negatively associated to this axis. The second factor was positively related to SFAs and negatively related to L^*, GPx, *n*−6 PUFAs and to a less extent to haeminic iron content. Colour parameters (a^* and b^*), catalase, SOD and pH 24 h were not significantly correlated to these two factors.

In Fig. 4, animal representation on the two factors obtained in the principal component analysis is shown. Pasture and concentrate fed groups were well differentiated. The pasture fed group was related to PUFAs and vitamin E content while the concentrate fed group was related to total lipids, saturated and monounsaturated fatty acids, glycogen and protein and lipid oxidation. The high dispersion on factor 2 observed in the concentrate group was mainly due to one animal exhibiting characteristics very different from the rest of the group especially with

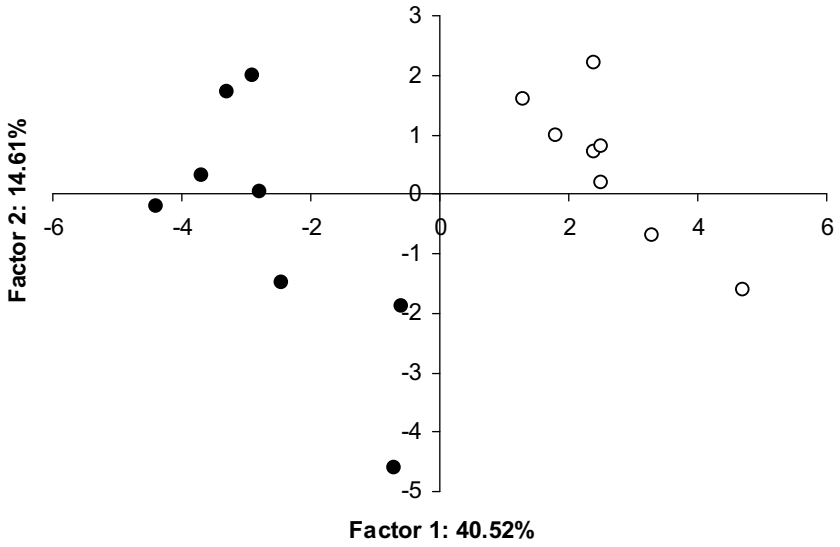

Fig. 4. Plot of animals in the principal component analysis. Black circles: animals from concentrate fed group, white circles: Animals from pasture fed group.

the highest GPx activity, carbonyl and glycogen content. Its pH 24 h post-mortem was also the lowest of the group.

To establish a mathematical model for prediction of oxidation parameters from pro- and anti-oxidant status of muscle, only correlated variables were extracted and analysed at first by a linear regression using a single variable.

No satisfactory prediction of colour parameters by the pro- or anti-oxidant status of meat has been gained in this study. This result is not surprising because of the low variability in meat colour parameters and also by the fact that colour development during meat storage is complex and is largely dependent on reduction processes catalysed by metmyoglobin reductase (Ledward, 1972) which was not estimated in this study.

Lipid oxidation measured by TBARS (at day 7) was significantly correlated with vitamin E content and it can be estimated as: $TBARS = 2.45-0.30[Vitamin\ E]$, $R^2 = 0.46$, $p < 0.01$. A slight and positive correlation was observed between TBARS and glycogen ($TBARS = -1.86 + 0.06[glycogen]$, $R^2 = 0.28$, $p < 0.05$) which can reinforce the hypothesis of the pro-oxidant effect of hexoses in ageing meat. However, we can not rule out the hypothesis that glucose, in its reduced form, could react directly with TBA independently of any oxidative process. A negative correlation was measured between TBARS and total polyunsaturated fatty acid content: $TBARS = 3.50-0.19[PUFAs]$, $R^2 = 0.68$, $p < 0.001$. These results are rather surprising because of the high oxidability of PUFAs, but can be explained by the high and positive correlation measured between PUFAs and vitamin E ($R^2 = 0.85$) and especially between $n-3$ PUFAs and vitamin E ($R^2 = 0.93$). These results confirm that, from the viewpoint of oxidation, the high level of polyunsaturated fatty acids measured in animals fed pasture is compensated by the large amount of natural anti-oxidants, especially vitamin E, present in pasture feeding.

Other pro- and anti-oxidant components of muscle were not significantly correlated to lipid oxidation.

Protein oxidation was also negatively correlated to vitamin E ($Carbonyl = 4.4-0.24[Vitamin\ E]$, $R^2 = 0.43$, $p < 0.01$) and in a less extent to PUFAs content ($Carbonyl = 3.1-0.13[n-3\ PUFAs]$, $R^2 = 0.31$, $p < 0.05$). These results call for the same comments as before on TBARS.

Then the multiple variable linear regression analysis using a step by step ascending method was used to simulate the joint effect of vitamin E, glycogen and PUFAs on TBARS on one hand and of vitamin E and $n-3$ PUFAs on carbonyl on the other hand. Unfortunately no valid model was found to explain the level of oxidation at day 7 by the combination of multiple variables.

4. Conclusions

This study clearly shows that the oxidative stability of lamb meat depends on diet. The pasture-diet presents some advantages over concentrate-diet in term of lipid oxidation and to a lesser extent on protein oxidation, while meat colour was not affected by diet. Moreover, pasture feeding increases polyunsaturated fatty acids (especially $n-3$ PUFAs) and conjugated linoleic acid content of the meat, which are beneficial for human health. So considering the meat quality and the dietetic aspect we could recommend this practice.

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different physicochemical tools and on correlation between instrumental measurements of meat characterisation during storage.

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