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Oxidation and antioxidant status: effects on shelf-life of meat from Limousine cattle fed with supplements of α-tocopherol

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ABSTRACT

The purpose of this study was to evaluate the antioxidant status of meat from cattle fed diets supplemented with vitamin E (α-tocopherol acetate) during the finishing period and to evaluate the effect of this treatment on meat shelf-life. Twenty purebred Limousine calves reared in the same farm, were randomly selected, divided into control group (n=10) and treated group (n=10) and fed a total mixed ration: treated group received a supplementation of vitamin E (900 mg/kg of CMF) for a period of 150 days before slaughter.

Meat quality was evaluated by the following analyses: pH, water holding capacity (drip loss), colour (L*, a*, b*, C*, H*), chemical forms of myoglobin, substances reactive to thiobarbituric acid (MDA) and enzymatic antioxidant activity (superoxide dismutase, catalase and glutation peroxidase). Dietary vitamin E supplementation had a positive effect on water holding capacity; in the control group a considerable increase in drip loss from the 2nd to 6th day of conservation was observed (2.83% *vs* 7.54%), while in the treated group during the same time period this increase appeared to be much more gradual and occurred to a lesser degree (2.31% vs 4.15%). Moreover, administration of vitamin E led to greater stability of colorimetric coordinates and reduced discoloration of the longissimus dorsi muscle during conservation as indicated by the redness a* (control: 23.85 and 23.87 *vs* 19.34 at 2h, 2 and 6 days, respectively; treated: 24.88, 23.91 and 24.01 at 2h, 2 days and 6 days, respectively) and in the Chroma* (control: 26.89 and 26.77 *vs* 21.90 at 2h, 2 days and 6 days, respectively; treated: 27.67, 26.57 and 26.77 at 2h, 2 and 6 days, respectively). Superoxide dismutase activity was significantly greater in the meat from cattle treated with vitamin E compared to that observed in the meat from controls (0.204 *vs* 0.167). The study showed that vitamin E supplementation in the finishing diets of calves caused only slight modifications in the antioxidant status of the meat; however, it positively influenced several qualitative characteristics which appeared to be more stable over time, thus extending the shelf-life of the meat.

Key words: Beef, Vitamin E, Meat colour, Water holding capacity, Antioxidant status.

RIASSUNTO

OSSIDAZIONE E STATUS ANTIOSSIDANTE: EFFETTI SULLA SHELF-LIFE DELLA CARNE DI BOVINI LIMOUSINE ALIMENTATI CON INTEGRAZIONE DI A-TOCOFEROLO

Lo scopo di questo lavoro è stato quello di studiare lo status antiossidante della carne prodotta da bovini alimentati nel periodo di finissaggio con diete integrate con vitamina E (α-tocoferolo acetato) e di valutarne l'effetto sulla shelf-life. Venti vitelli di razza Limousine allevati nella stessa azienda ed alimentati con la stessa razione unifeed, sono stati selezionati at random e ripartiti in due gruppi: controllo (n=10) e trattato (n=10); 150 giorni prima della macellazione il gruppo trattato ha ricevuto un'integrazione di vitamina E (900 mg/kg di mangime commerciale). La qualità della carne è stata valutata con le seguenti analisi: pH, potere di ritenzione idrica (drip loss), colore (L, a*, b*, C*, H*), forme chimiche della mioglobina, sostanze reattive all'acido tiobarbiturico (MDA) ed attività enzimatica antiossidante (superossido dismutasi, catalasi e glutatione perossidasi). L'integrazione di vitamina E nella dieta ha avuto un effetto positivo sul potere di ritenzione idrica: nel gruppo di controllo si osserva un brusco aumento delle perdite di liquidi da 2 a 6 giorni di conservazione (2,83 %* vs *7,54 %), mentre nel gruppo trattato, nello stesso intervallo di tempo, tale aumento appare molto più graduale e di entità inferiore (2,31% vs 4,15%). La somministrazione di vitamina E ha indotto inoltre maggiore stabilità delle coordinate colorimetriche ed ha ridotto lo scolorimento del muscolo* longissimus dorsi *durante la conservazione, come evidenziato dall'indice del rosso a* (controllo: 23,85 e 23,87* vs *19,34 a 2h, 2 e 6 giorni rispettivamente; trattato: 24,88, 23,91 e 24,01 a 2h, 2 e 6 giorni rispettivamente) e dal Croma* (controllo: 26,89 e 26,77* vs *21,90 a 2h, 2 e 6 giorni rispettivamente; trattato: 27,67, 26,57 e 26,77 a 2h, 2 e 6 giorni rispettivamente). L'attività delle superossido dismutasi (SOD) è risultata significativamente maggiore nella carne dei bovini trattati con vitamina E rispetto a quella rilevata nelle carni di quelli di controllo (0,204* vs *0,167).*

Lo studio ha evidenziato che l'integrazione di vitamina E in diete di finissaggio di vitelloni ha indotto solo lievi modificazioni dello status antiossidante della carne, influendo comunque positivamente su alcune caratteristiche qualitative, che sono risultate più stabili nel tempo, estendendo così la shelf-life della carne.

Parole chiave: *Carne bovina, Vitamina E, Colore, Potere di ritenzione idrica, Status antiossidante.*

Introduction

The main sensory properties by which consumers judge meat quality at purchase are colour and flavour (Liu *et al.*, 1995). Colour is one of the most important qualitative characteristics and depends mainly on the haeminic pigment concentration, myoglobin, and the related chemical forms it can take (Renerre, 1990). A bright-red colour is more attractive, indicates a long shelf-life and is generally associated with good organoleptic quality of the meat, while meat of a brownish colour is not appreciated by the consumer and indicates an oxidative state caused by the transformation of oxymyoglobin in meta-myoglobin (Faustman and Cassens, 1989; Morrissey *et al.*, 1994; Renerre *et al.*, 1996). A decline in the meat's colour during display also depends on the oxidation of membrane phospholipids; in fact, some authors have pointed out that the formation of meta-myoglobin is positively correlated to lipid oxidation and seems dependent on meat antioxidant status (Yin *et al.*, 1993; Sherbeck *et al.*, 1995).

Flavour influences the consumer as well when judging meat quality; this depends on the composition and on lipid oxidation induced by free radicals, which cause the formation of the rancid odour and deterioration of the flavour (Anton *et al.*, 1993).

Oxidation could thus be considered an important non-microbial factor responsible for meat deterioration, and the degree of myoglobin and lipids oxidation is the main parameter to consider when predicting preservation; these parameters are closely connected

and dependent on the equilibrium between pro-oxidant (free radicals) and anti-oxidants substances (Descalzo *et al.*, 2005).

The primary antioxidants affecting antioxidant status are represented by an enzymatic complex (superoxide dismutase, catalase and glutation peroxidase), that neutralizes free radicals, delaying the initial phase of meat oxidation (Nakano *et al.*, 1992; Mei *et al.*, 1994; Renerre *et al.*, 1996). Each of these enzymatic systems has a specific role; in particular, the activity of glutation peroxidase in muscles is directly related to selenium levels and is the most important defence against meat deterioration, due to its high inhibitory capacity regarding lipid oxidation (De Vore and Greene, 1982; De Vore *et al.*, 1983).

The second line of defence against attack by free radicals consists of several natural antioxidants that interrupt the chain of free radicals production (Kerry *et al.*, 2000; Lanari *et al.*, 2002); those which are most active in protecting meat from oxidation are glutation, vitamin A, C and E, carotenoids and flavonoids. These antioxidants cannot be synthesized by the animal organism and between feeds, green fodder is a good source compared to cereals, which contain fewer of these antioxidants (Daly *et al.*, 1999). Antioxidant supplements in the diet can be a very efficient tool against oxidative damage caused by free radicals; for example, being stored in biological membranes and in fat, vitamin E in the diet protects polyunsaturated fatty acids from peroxidation by reactive oxygen species (ROS), improving colour and lipids stability in meat (Faustman and Wang, 2000; Gatellier *et al.*, 2001).

The purpose of this study was to investigate the antioxidant status of the meat from cattle fed a vitamin E supplemented diet during the finishing period and to evaluate the effect of this treatment on meat shelf-life.

Material and methods

Animals and diet

The trial was carried out on 20 purebred female Limousine cattle from the same farm, randomly selected and divided into control group (n=10) and treated group (n=10), both fed with 8 kg/d of a total mixed ration consisting of 40% commercial mixed feed (CMF), 40% corn meal and 20% wheat straw; vitamin E (90% α-tocopherol acetate) was included in the commercial feedstuff at the dose of 100 mg/kg in the control group diet. The treated group received a supplementation of vitamin E of 900 mg/kg of CMF for a period of 150 days before slaughter, receiving approximately 2500 UI/head/day of Vitamin E. Ingredients and chemical composition of total mixed ration are given in Table 1.

Meat samples and preservation

At the average age of 14 months, the animals were slaughtered at an EU licensed abattoir. Twenty-four hours after slaughter, from each right half-carcass a steak between the 6th and 8th thoracic vertebra was taken; the longissimus dorsi muscle was isolated and divided into two 5-cm-thick slices (A, B) for the following analysis:

- slice A was frozen at -80°C until analysis of the antioxidant status: superoxide dismutase (SOD), catalase (CAT), glutation peroxidase (GPx) and lipid peroxydation (malondialdehyde) (Time 0d).
- slice B was vacuum-packaged and aged for 7 days at +4°C; for meat quality assessment it was subdivided into two samples: the first sample was weighed, placed in a plastic container with a double bottom, covered with polyethylene film and kept in standardized conditions at +4°C; pH and colour were measured at 2h, 2 and 6 days of storage in order to determine their evolution; drip loss was evaluated at 24h, 2 and 6 days

Table 1. Ingredients and chemical composition of total mixed ration.

Ingredients:		
Commercial mixed feed[1]	%	40
Corn meal	"	40
Wheat straw	"	20
Chemical composition:		
Dry matter (DM)	% as fed	92.39
Crude protein	% DM	13.94
Ether extract	"	2.61
Crude fibre	"	13.59
Ash	"	6.43
NDF	"	41.03
ADF	"	19.29
ADL	"	3.41

[1]Commercial mixed feed composition (% as fed): wheat bran 22, sunflower meal 21, soybean meal 19, wheat flour middlings 15, wheat middlings 11, sugar cane molasses 5, yeasts 3.5, $CaCO_3$ 1.2, mineral and vitamin supplement[2] 1, $Ca(HPO_4)_2$ hydr. 0.8, NaCl 0.3, $NaHCO_3$ 0.2.

[2]Vitamin E content for diet 1=100 mg/kg of commercial mixed feed; Vitamin E content for diet 2=900 mg/kg of commercial mixed feed.

of storage. On the 6th day the sample was frozen at -80°C for determination of its content in malondialdehyde (MDA), as an indicator of the evolution of lipid oxidation process (Time 6d). The second sample was divided into 6 sub-samples which, placed in a plastic container with a double bottom, covered with polyethylene film and kept in standardized conditions at +4°C, were utilized to evaluate the chemical forms of the myoglobin at 2h, 2 and 6 days of storage.

Determination of pH and water holding capacity

The pH was determined using a pH-meter Hanna pH 211 provided with a Hanna FC 200B electrode and an automatic temperature compensator (Hanna Instruments, Padova, Italy).

The water holding capacity, expressed as drip loss, was measured as a percentage of weight loss of a meat sample of known weight, kept in standardized conditions at +4°C for 24h, 2 and 6 days (Lundström and Malmfors, 1985).

Determination of colour and chemical forms of myoglobin

The colour was measured on the surface of the meat by spectrocolorimeter Minolta 2500 (Minolta Camera Co. Ltd, Osaka, Japan), (observation angle of 2° and Illuminant D65), which measures the values of coordinates Lightness (L*), redness (a*), yellowness (b*), Chroma (C*) and Hue (H*) (Renerre, 1990).

For the direct evaluation of the percentage of oxymyoglobin, the AMSA formula (1991) was applied:

$$\text{Oxymyoglobin} = \frac{\left(\frac{\text{K/S 610 nm of 100\% MMb}}{\text{K/S 525 nm of 100\% MMb}} - \frac{\text{K/S 610 nm of sample}}{\text{K/S 525 nm of sample}}\right)}{\left(\frac{\text{K/S 610 nm of 100\% MMb}}{\text{K/S 525 nm of 100\% MMb}} - \frac{\text{K/S 610 nm of 100\% OMb}}{\text{K/S 525 nm of 100\% OMb}}\right)} \times 100$$

where: MMb=meta-myoglobin; OMb=oxymyoglobin; K=absorption coefficient; S=scattering coefficient.

The 100% of meta-myoglobin (MMb) was determined with the Krzywicki method (1979), placing the meat sample in a solution of potassium ferrocyanide at 1% for 1 minute, keeping it at +2°C for 12h in a plastic container covered with polyethylene film and measuring the spectral colour with the Minolta 2500 spectrocolorimeter. The parameter 100% of oxymyoglobin (OMb) was obtained by measuring the spectral colour of a meat sample subjected to a flow of oxygen at 100% for 10 minutes at a temperature between 0° and +2°C. The spectrocolorimeter Minolta 2500, equipped with an integrated sphere and the appropriate software (Spectramagic, 2002), performed the immediate conversion of the visible spectra of reflectivity (from 360 to 760 nm) in values of K/S, rendering the data more linear for the expression of absorption capacity and properties of dispersion, utilizing the Kubelka-Munk equation:

$$K/S=(1-R)^2/2R$$

where: R is the factor of spectral reflection (Hunter, 1987; Mancini and Hunt, 2005).

Preparation of samples for evaluation of antioxidant status and lipid oxidation

After defrosting, 1g of meat was taken from each sample: it was then finely minced and homogenized per 3 minutes in 5 ml of potassium phosphate (50 mM; pH 7.4); 20 µl of benzotriazol were then added to block the oxidative processes. The test tubes for measuring the malondialdehyde and CAT activity were centrifuged at 700 revolutions per 20 minutes while those intended for determining the activity of SOD and GPx were centrifuged at 20,000 revolution per 30 minutes (Şahin and Gümüşlü, 2004). Colorimetric determination of the proteins was carried out on the supernatant (Lowry *et al.*, 1951).

Quantification of malondialdehyde (MDA)

To evaluate meat lipid oxidation, MDA was measured using the test of reactivity to thiobarbituric acid (TBARS) (Raharjo and Sofos, 1993; Dirinck *et al.*, 1996). The levels of substances reactive to the acid were measured by the fluorimetric method (Lynch and Frei, 1993) and results were expressed in nmoles MDA/mg protein.

Evaluation of the enzymatic antioxidant complex

The SOD activity was evaluated by determining the capacity of the antioxidant system to inhibit auto-oxidation of adrenaline into adenochrome at an alkaline pH; levels were calculated using a sigmoid curve and expressed as mg SOD/g protein at +37°C (Misra and Fridovich, 1972).

The CAT activity was evaluated using the disappearance of hydrogen phosphate method; the reading was carried out at 240 nm and levels were calculated by means of a linear curve and expressed as mg CAT/g protein (Aebi, 1987).

The GPx activity was measured using the method of Paglia and Valentine (1967),

consisting of measuring the oxidation rate of glutation by H_2O_2 catalyzed by the GPx in the supernatant. The reading was carried out at 340 nm at +20°C and levels were calculated using a linear curve and expressed as mg GPx/g protein.

Statistical analysis

Results regarding pH, drip loss, colorimetric characteristics, percentage of oxymyoglobin and malondialdehyde were subjected to a two-way analysis of variance, considering the effects of the treatment, storage time and the relative interaction, using the formula:

$$Y_{ijk}=\mu + \alpha_i+\beta_j+(\alpha\beta)_{ij}+\varepsilon_{ijk}$$

where: Y=dependent variable; μ = overall mean; α =treatment effect; β =storage time effect; αβ=effect of the interaction (treatment, storage time); ε=residual error (SAS, 1995).

Data regarding the enzymatic activity were analyzed using one-way analysis of variance, considering the effects of the treatment, with the following formula:

$$Y_{ij}=\mu + \alpha_i+\varepsilon_{ij}$$

where: Y=dependent variable; μ = overall mean; a =treatment effect; ε=residual error (SAS, 1995).

Results and discussion

Table 2 reports the results of pH and drip loss measured on the *longissimus dorsi* muscle. The pH showed significant differences for the main effects, although it remained within the normal range.

Regarding the water holding capacity, it was observed that drip loss increased over time ($P \leq 0.01$): in particular, for both experimental groups statistically significant differences were found between the drip loss observed at 24h and 2 days compared to that observed at 6 days. This increase was particularly marked in the control group at 6 days, when the greatest loss of liquids (7.54%) was observed. This result, in agreement with other findings claiming the positive effect of vitamin E administration in the finishing period on drip loss, is very interesting since water holding capacity greatly affects meat acceptability at retail and during domestic storage (Cannon *et al.*, 1996; Dirinck *et al.*, 1996; Den Hertog-Meiscke *et al.*, 1997; Dufrasne *et al.*, 2000; Macit *et al.*, 2003).

Regarding the colorimetric parameters reported in Table 3, it can be observed that the lightness (L*) of the meat from the group treated with vitamin E remained more stable over time compared to that from the control group, which instead tended to diminish; this result highlights the effect of vitamin E on maintaining some meat colour parame-

Table 2. Effect of vitamin E supplementation and storage time on pH and drip loss.

	Control Group			Treated Group			Significance			MSE
	2h	2d	6d	2h	2d	6d	Treatment	Time	Interaction	
pH	5.55	5.61	5.58	5.58	5.63	5.65	0.019	0.008	ns	0.01
	24h	2d	6d	24h	2d	6d				
drip loss %	2.01^{c}	2.83^{bc}	7.54^{a}	1.58^{c}	2.31^{c}	4.15^{b}	0.001	0.001	0.007	2.07

MSE=mean square error.
ns=not significant.

Table 3. Effect of vitamin E supplementation and storage time on colour and oxymyoglobin percentage (OMb).

	Control Group			Treated Group			Significance			MSE
	2h	2d	6d	2h	2d	6d	Treat.	Time	Inter.	
L*	46.19	45.74	43.61	41.51	41.43	42.12	0.002	ns	ns	13.23
a*	23.85[a]	23.87[a]	19.34[b]	24.88[a]	23.91[a]	24.01[a]	0.012	0.010	0.031	6.55
b*	12.43	12.42	10.26	12.13	11.58	11.80	ns	ns	ns	2.74
C*	26.89[a]	26.77[a]	21.90[b]	27.67[a]	26.57[a]	26.77[a]	0.038	0.017	0.043	8.85
H*	27.41	27.43	27.92	25.84	25.59	26.06	0.000	ns	ns	2.25
OMb %	92.80	91.80	76.29	100.56	94.68	106.72	ns	ns	ns	971.26

Treat.=treatment; Inter.=interaction; MSE=mean square error.
ns=not significant.

ters, as shown in previous studies. (Renerre, 1990; Lynch *et al.*, 1999; Eikelenboom *et al.*, 2000; Renerre, 2000).

The redness (a*) in the control group remained stable until the 2nd day of conservation, then decreased significantly between days 2 and 6, with considerable fading of the colour of the meat; in fact, it was observed that the transformation of oxymyoglobin in meta-myoglobin led to a decreased redness (a*), rendering the colour of the meat less pleasing to consumers (Renerre, 2000). In the group treated with vitamin E, the redness remained stable over time, as seen in the values measured at 2h, 2 and 6 days, in analogy with values recorded in the control group until 2 days of conservation. In fact, statistical analysis showed significant differences between the two experimental groups regarding the redness (a*) measured at 6 days, reflecting the positive effect of vitamin E in keeping the value of the redness closer to initial values during the conservation of the meat (Mancini *et al.*, 2003; Dunne *et al.*, 2005).

Concerning the yellowness (b*), although no significant differences were seen between the groups, it may be observed that in the control samples this parameter tended to decrease between the 2nd and 6th days of conservation, while the meat from the treated group did not undergo alterations, confirming the greater stability conferred by supplementation with vitamin E. The tone of the meat colour expressed by the Hue (H*) remained constant over time in both of the groups considered; instead the intensity of the colour, Chroma (C*), was altered considerably during conservation in the control group, going from values of 26.77 measured at 2 days to values of 21.90 measured at 6 days, while in the treated group this parameter remained stable. After 6 days of conservation, the Chroma (C*) was significantly lower in the control group, reflecting the faded colour of the meat.

In the control group the percentage of oxymyoglobin remained stable for 2 days, then decreased on the 6th day. Since a decrease in the percentage of oxymyoglobin corresponds to an increase in the oxydized form meta-myoglobin (Mancini and Hunt, 2005), this result, although it did not have statistical significance, confirms the alteration in colour seen in the meat after 6 days of storage. In the group treated with

vitamin E, the percentage of oxymyoglobin tended to remain unvaried, with values that tended to be higher than those recorded in the control group, confirming that the antioxidants supplementation in the last phase of rearing leads to greater stability in the meat colour, which appeared brilliant even after 6 days of storage (Lynch *et al.*, 1999; Faustman and Wang, 2000; Renerre, 2000; Gatellier *et al.*, 2001).

Regarding lipid oxidation (Table 4), it was observed that the malondialdehyde (MDA) content was significantly influenced by the storage time ($P \leq 0.01$); in fact, independently of the treatment, the highest content of MDA was found at 6 days (control group: 0.34 – treated group: 0.39). This result does not agree with data previously reported in the literature, regarding the positive effects of antioxidants in limiting the oxidative process during conservation of meat (Yang *et al.*, 2002; Dal Bosco *et al.*, 2004).

Table 5 reports the results regarding the antioxidant complex of the meat, analyzed only for the main effect of treatment. The SOD activity was significantly greater in meat from cattle treated with vitamin E than in the meat from controls; this result was in agreement with several studies that showed a correlation between the diet given to the animals during the finishing period and SOD activity. In particular, Gatellier *et al.* (2004) reported an important effect of a diet based on green fodder containing vitamin E, in the increasing activity of that antioxidant enzymatic system.

Regarding CAT, Renerre *et al.* (1996; 1999) observed greater activity of that enzyme system in the meat of animals fed diets supplemented with vitamin E. Since CAT is an enzymatic system acting in cooperation with SOD, in this study an analogous effect of the diet on the activity of the two enzymatic systems was predictable, and thus it was expected that CAT activity would be greater in meat from the treated group. Results failed to fulfil these expectations, in fact showing significantly decreased CAT

Table 4. Effect of vitamin E supplementation and storage time on lipid oxidation (MDA).

	Control Group		Treated Group		Significance			MSE
	Time 0d	Time 6d	Time 0d	Time 6d	Treatment	Time	Interaction	
MDA (nm/mg protein)	0.18	0.34	0.21	0.39	ns	0.001	ns	0.01

MSE=mean square error.
ns=not significant.

Table 5. Effect of vitamin E supplementation on antioxidant complex.

		Control Group	Treated Group	Significance	MSE
SOD	mg/g protein	0.167^{b}	0.204^{a}	0.023	0.01
CAT	" "	5.771^{a}	3.424^{b}	0.043	4.62
GPx	" "	1.798	1.681	ns	0.16

MSE=mean square error.
ns=not significant.

activity in the group treated with vitamin E; nevertheless, similar results have been obtained by other authors. Gatellier *et al.* (2004) even encountering greater SOD activity in calves fed at pasture during the finishing period, found less CAT activity. It was hypothesized that the supplementation of vitamin E induces an adaptive response of the endogenous antioxidant defences by means of a negative feedback reaction.

Regarding GPx, studies concerning the effect that antioxidant administration could have on this enzymatic system activity are contradictory. Some authors indicated that GPx activity is independent of the dietary concentration of vitamin E (Rojas *et al.*, 1994; Cadenas *et al.*, 1995; Renerre *et al.*, 1999), while others observed increased activity of this enzyme following the increase of vitamin E in finishing ration (Conti *et al.*, 1993). In our study, the GPx activity was not influenced by the supplementation of vitamin E; this could be explained considering that GPx activity is selenium-dependent and that the feed administered to the animals consisted mainly of straw which usually is somewhat poor in selenium (Gatellier *et al.*, 2004; Mercier *et al.*, 2004).

Conclusions

The study showed only slight modifications of the antioxidant status of the meat from cattle fed diets supplemented with vitamin E during the finishing period, although an improved shelf-life of the meat was observed. In fact, the treatment led to a greater water holding capacity, greater stability of colorimetric characteristics and less pigment oxidation, confirming the favourable effects of dietary supplementation with vitamin E on meat quality.

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