Vitamin E and plant extracts rich in antioxidants given to bovine efficiently protect beef against lipoperoxidation during processing.

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Abstract

The bioefficiency of dietary vitamin E associated with a mixture of plant extracts (PE) rich in antioxidants against lipoperoxidation before and after meat processing was investigated in cows given unsaturated lipid supplemented diets. Twelve Normand cull cows were given for 100 d. a concentrate/straw based diet (70/30) (C group) or supplemented with extruded linseeds (40 g oil/kg diet DM; L group), or with extruded linseeds plus vitamin E (155 IU/kg diet DM) and PE mixture (0.7 g/kg diet DM; LEP group). *Semintendinosus* muscle was removed just after slaughter and matured for 12 d.. It was then processed and stored at +4°C for 4 d. in a tray overwrapped under air, or for 7 d. under modified atmosphere packaging (70:30, O_2/CO_2) (MAP), or for 14 d. under vacuum. Compared to that in meat just at slaughtering, malondialdehyde (MDA), a marker of lipoperoxidation, was 8 and 17 fold higher ($P<0.05$) in overwrapped meat and in meat under MAP respectively from C and L groups. Nevertheless, in animals given the antioxidant supplements, beef MDA was 3 fold lower than that of C and L groups ($P<0.01$), even in packaging that mostly favoured lipoperoxidation (MAP). These results showed for the first time an efficient protection of processed beef against lipoperoxidation based on the incorporation in diets of an original mixture of dietary antioxidants for the finishing period.

Introduction

Addition of extruded linseeds rich in polyunsaturated fatty acids in diets of ruminants can reduce the atherogenic FA content of meats and favour their content in beneficial FA such as n-3 PUFA. However, PUFA were known to favour the oxidative process in meat of which final products are considered to be responsible for developing rancidity in stored meats. Vitamin E played a role of chain-breaking antioxidant, limiting propagation of the peroxidation reaction by captation of radical electron. But, a high concentration of vitamin E in the diet would not be efficient, because the excess of vitamin E would be catabolised or excreted. In this context, new dietary antioxidant molecules provided together with vitamin E could be more efficient for preventing lipoperoxidation. Thus, a mixture of vitamin E (as α-tocopherol acetate) associated with plant extracts rich in polyphenols would preserve the animal health against lipoperoxidation as shown in rats (Gladine et al. 2007a), sheep (Gladine et al. 2007b) and dairy cows (Gobert et al., 2008) given lipid supplements rich in n-3 PUFA. The objective of the present study was to investigate; in ruminants in production such as cull cows in the finishing period, the bioefficiency of this dietary antioxidant mixture to protect meats against lipoperoxidation, especially during meat processing.

Materials and methods

Animals and Diets. The experiment was performed with 12 Normand cull cows [48-60 months old, mean live weight 642 kg] selected for their live weight, age and body fat score for a 100d. finishing period. Animals were assigned at random to three isoenergetic and isonitrogenous rations (n=4 for each diet). All rations were straw (30%) and concentrate (70%)-based. Animals were given the basal diet without any supplements (C group), or the same diet supplemented with extruded linseeds (40 g oil/kg diet DM; L group), or supplemented with extruded linseeds plus vitamin E (155 IU/kg diet DM) and plant extracts (PE) rich in polyphenols from rosemary, grape, citrus and marigold (0.7 g/kg diet DM; LEP group).

Meat treatments. Samples of *Semitendinosus* muscle were collected just at slaughtering (D_0), matured for 12 d. on cold room (+4°C) (D_{12}), and then cuted into pieces as steaks on the market. They were stored at +4°C and illuminated with a standard supermarket fluorescent light i) for 4d. in a tray under air packaging (UAP), ii) for 7d. under modified atmosphere packaging (MAP) (70:30, O_2/CO_2), iii) for 14d. under vacuum packaging (UVP).

Peroxidizability index (PI). PI was calculated from the FA composition of total meat lipids according to the equation reported by Hu et al. (1989) as follows: IP = (% dienoic x 1) + (% trienoic x 2) + (% tetraenoic x 3)

+ (% pentaenoic x 4) + (% hexaenoic x 5). This index estimated the concentration of bis-allylic hydrogen atoms in PUFA and therefore their susceptibility to peroxidation.

Malondialdehyde (MDA). As marker of lipoperoxidation intensity, MDA was extracted by hexane from muscle powder prepared by breaking meat in N_2 liquid. Meat MDA was separated by HPLC and quantified by fluorescence (excitation at 515 nm, emission at 553 nm) using a tetraethoxipropane calibration curve.

Statistical analysis. All data were subjected to analysis by ANOVA using the general linear model procedure of Statistical Analysis System software (SAS Institute, Cary, USA, 2000). A P-value lower to 0.05 was considered to be significant and a P-value lower to 0.1 was considered as a trend. "Diet", "meat treatment" effects and "diet x meat treatment" interaction were analysed. When the interaction "diet x meat treatment" was statistically significant (P<0.05), meat treatments were compared within each diet. Within each meat treatment, the different diets were compared by using the Student's-test.

Results and discussion

Effect of n-3 PUFA rich diets on FA composition. Addition of extruded linseeds rich in n-3 PUFA (4% oil/kg diet DM) led to an increase of n-3 PUFA (+23.9%, P=0.1) to the detriment of n-6 PUFA content (-26.6%, P<0.01) leading to a lower n-6/n-3 (3.3 *vs* 3.9 in the C group, P<0.005) in ST muscle. Due to the exchange between n-6 and n-3 PUFA, PI did not vary in meat of the L group, which differed to that observed in steers (+9%) given the same level of lipids by extruded linseeds (Scislowski et al. 2005). This lack of effect was probably due to the age of animals, since our cows were 4 years old so lipid supplement should be higher to impact tissues as shown in De la Torre et al. (2005) study increasing PI of old cows (+9%) with 8% of lipids in diet.

Effect of processing on meat lipoperoxidation. From MDA values (Table 1), ANOVA analysis showed diet (P=0.05) and meat treatment (P<0.0001) effects as well an interaction (P=0.03) between diet and meat treatment effect. This validated the comparison of means within each diet and within each meat treatment.

At slaughter (D_0), MDA values in C cows were in accordance with values on bovine fed concentrate based diet. A 12 d. ageing (D_{12}) did not favour apparently lipoperoxidation in ST compared to that measured at slaughter. However, studies on meat ageing under vacuum had shown an increase of lipoperoxidation This could be attributed to the oxygen availability when muscles were removed just before vacuum-packaging,leading to the initiation of lipoperoxidation by oxygen. ST is an internal muscle not directly exposed to oxygen when matured on the entire carcass. After ageing, intensity of lipoperoxidation varied with conditions of meat storage (P<0.0001). MDA value was similar with UVP storage for 14d. in C group (P<0.05). But, lipoperoxidation was 6.7 fold higher with UAP storage for 4d. (P<0.05) and, more markedly, was 13.4 fold higher with storage under MAP for 7d. (P<0.05) when compared to values determined in muscles just after slaughter. These results confirmed previous observations in Charolais beef (Gattelier et al. 2001).

Table 1. Malondialdehyde (MDA) concentration (µg/g of tissue) in *Semitendinosus* muscle of cows given C, L and LEP diets. Meat MDA was determined at slaughter (D_0), after meat ageing (D_{12}), and after storages for 4d. in a tray under air packaging (UAP), for 7d. under modified atmosphere packaging (MAP) (70:30, O2/CO2) and for 14d under vacuum packaging (UVP)

	Meat treatments				
MDA (µg/g tissue)	D_0	D_{12}	UVP	UAP	MAP
C	0.163 ^{a} ± 0.052	0.145 ^{a} ± 0.010	0.164 ^{ab} ± 0.043	1.095 ^{b} ± 0.376	2.193 ^{c} ± 1.878
L	0.136 ^{a} ± 0.040	0.193 ^{a} ± 0.091	0.190 ^{a} ± 0.085	1.412 ^{b} ± 0.724	2.964 ^{c} ± 1.361
LEP	0.164 ^{a} ± 0.054	0.136 ^{a} ± 0.033	0.142 ^{a} ± 0.018	0.650 ^{a} ± 0.486	0.927 ^{a} ± 0.816
Diet effect					
C *vs* L	0.9	0.9	0.9	0.5	0.1
C *vs* LEP	0.6	0.7	0.8	0.3	0.01
L *vs* LEP	0.6	0.8	0.8	0.09	<0.0001

Within each treatment, means with different superscripts (a, b) in each row differ significantly (P<0.05).

Effect of dietary n-3 rich on meat lipoperoxidation. At slaughter, meat MDA values were similar in animals given the control and the n-3 PUFA rich diet, since PI value was unchanged by the linseed supplemented in diet (L group). As in C group, due to the high oxygen concentration, lipoperoxidation tended to be higher in meats of L group after UAP and under MAP storage (x21.8, P<0.05) when compared

to that at slaughter. Even if PI was unchanged, lipoperoxidation tended to be higher in meats packaged under MAP of the L group (2.96 *vs* 2.19 in the C group, P=0.1). This could be the result of a higher sensitivity of meats (from the L group) to n-3 PUFA, confirming previous results on meats from steers given PUFA-rich diets (Campo et al. 2006).

Effect of dietary antioxidants. Vitamin E and plant extracts rich in polyphenols efficiently protected beef against lipoperoxidation as shown by MDA values systematicaly lower than 1µg/g tissue in all treated meats ($P<0.05$). Antioxidants were efficient to protect meats produced from animals of the C group in the packaging condition that mostly favoured lipoperoxidation (MAP) ($P<0.01$) but the variations were not significant in UAP storage, whereas dietary antioxidants had protected meats enriched in n-3 PUFA from the two most deleterious packaging conditions, that were UAP ($P<0.09$) and MAP, ($P<0.0001$).

As shown by Gatellier et al. (2000), vitamin E alone was not sufficiently efficient when lipoperoxidation was increased by a higher incorporation of PUFA in meat lipids. Moreover, natural plant extracts, used for their antioxidant properties, could be added directly into the meat packaging, thus providing a direct antioxidant action by captation of reactive oxygen species on the meat surface. As described in rats, sheep and cows study (Gladine et al. 2007a,b; Gobert et al. 2008), antioxidant mixture showed a synergistic mechanism of action due to the respective lipophilic and hydrophilic properties of vitamin E and PE. Vitamin E was known to act as a chain breaking antioxidant and PE as a radical trapper. Therefore, vitamin E associated to PE given to bovine in the finishing period would act efficiently by complementary mechanisms of action to protect beef against lipoperoxidation, even in the most deleterious storage conditions.

Conclusion

For the first time, these results clearly showed that a combination of vitamin E with plant extracts rich in polyphenols given oraly to ruminants was an efficient mean to protect meats enriched in n-3 PUFA against lipoperoxidation. The protective effect was particularly efficient when meat lipoperoxidation was favoured by packaging conditions such under the air or under the modified atmosphere. In a pratical view supplementation of diets of bovines during the finishing period with such original dietary antioxidants would secure meat lipids from lipoperoxidation whatever diets or meat processings.

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