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Plant polyphenols associated with vitamin E can reduce plasma lipoperoxidation in dairy cows given n-3 polyunsaturated fatty acids

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ABSTRACT

Diets rich in n-3 polyunsaturated fatty acids (PUFA) improve the nutritional value of ruminant products but also increase the risk of lipoperoxidation in plasma and tissues. The relative effectiveness of dietary antioxidants such as vitamin E (vit E) given alone or with plant extracts rich in polyphenols (PERP) containing rosemary, grape, citrus, and marigold was investigated in the plasma of mid-lactation dairy cows given diets enriched in 18:3 n-3. For a 30-d period, the animals were given a maize silage-based diet (control group C, n = 6) or the same basal diet supplemented with extruded linseed rich in 18:3 n-3 [50 g of oil/kg of diet dry matter (DM); group L, n = 6], extruded linseed + vit E (375 international units/kg of diet DM; 7,500 IU/cow per day; group LE, n = 6), or extruded linseed + vit E + PERP (10 g/kg of diet DM; group LEP, n = 5). Plasma susceptibility to lipoperoxidation was evaluated using in vitro parameters of conjugated diene formation (lag phase and maximum oxidation rate). Plasma indicators of lipoperoxidation and antioxidant status were analyzed in the 4 experimental groups as well as the fatty acid (FA) composition of total plasma lipids. At d 30, group L significantly increased plasma cholesterol esters (+57%) and phospholipids (+35%) compared with group C. It also increased plasma n-3 PUFA (4.7-fold increase) to the detriment of n-6 PUFA (−30%), leading to a higher peroxidizability index (+20%). Plasma in vitro lipoperoxidation was higher in group L, rich in 18:3 n-3, than in group C. Vitamin E alone had no effect on lipoperoxidation, whereas vit E in association with PERP lowered lipoperoxidation by increasing the resistance time against peroxidation (+47%) and by decreasing the oxidation rate (−48%) compared with group L at d 30. Surprisingly, in vivo plasma lipoperoxidation estimated by the plasma level of the major lipoperoxidation product (malondialdehyde) was not significantly increased in group L. This study shows, for the first time, that PERP supplied in association with vit E were able to reduce lipoperoxidation in lactating cows given a diet rich in 18:3 n-3, thereby helping to protect cows against the deleterious consequences of lipoperoxidation and potentially ensuring antioxidant potential for 18:3 n-3–enriched dairy products.
Key words: 18:3 n-3, lipoperoxidation, antioxidant, plasma

INTRODUCTION

To improve the nutritional quality of dairy products for consumers, lipid supplementation of ruminant diets with n-3 polyunsaturated fatty acids (**PUFA**)-rich oilseeds is a rapid and effective feed strategy (Chilliard et al., 2007). However, this strategy could increase the risk of plasma lipoperoxidation, as reported in steers (Scislowski et al., 2005a), rats (Gladine et al., 2007) and sheep [C. Gladine, E. Rock, C. Morand (all of INRA, UMR1019, St-Genès-Champanelle, France), D. Bauchart, D. Durand (both of INRA, UR1213, St-Genès-Champanelle, France); unpublished data]. This could not only be detrimental to animal health (Miller et al., 1993) but also decrease the nutritional value of their products, thus prompting recommendations to add antioxidants to lipid-enriched diets. Vitamin E (**vit E**) is commonly added to animal and human diets because of its ability to inhibit lipoperoxidation (Cuvelier et al., 2003). This lipophilic antioxidant inactivates the lipid peroxyl radicals and thus acts as a chain-breaking antioxidant limiting the propagation of the oxidation reaction by capturing radical electrons (Brigelius-Flohe and Traber, 1999; Goupy et al., 2007). However, in rats and sheep given 18:3 n-3–enriched diets, vit E supplementation (200 IU/kg of diet DM; 300 IU/sheep per day) did not effectively prevent lipoperoxidation chain reactions [Gladine et al., 2007; C. Gladine, E. Rock, C. Morand (all of INRA, UMR1019, St-Genès-Champanelle, France), D. Bauchart, D. Durand (both of INRA, UR1213, St-Genès-Champanelle, France); unpublished data]. One option for improving vit E's protective effect against lipoperoxidation could be to increase the dietary vit E level, but high dietary concentrations of

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vit E would not be efficient because the excess vit E would be catabolized or excreted (Aurousseau, 2002). A study on rats and guinea pigs showed that tocopherol excretion in the feces increased significantly according to vitamin E intake (0.03 to 10 g/kg of diet), and tocopherol absorption rate was inversely proportional to level of supply (Elmadfa and Walter, 1981). Moreover, high-dosage vit E supplements may be deleterious to animals: a meta-analysis on humans had shown that high-dosage vit E supplements (≥400 IU/d) increased all-cause mortality and should be avoided (Miller et al., 2005). In this context, new dietary antioxidant molecules given in combination with vitamin E could be more effective for preventing plasma lipoperoxidation. Plant polyphenols are known to play an important protective role in the lipoperoxidation process. Human studies have demonstrated better protection against oxidative damage and related diseases when a variety of fruit and vegetable-based antioxidants are consumed (Jacob and Burri, 1996) and have shown inhibition of carcinogenesis by dietary polyphenolic compounds (Yang et al., 2001). In a ruminant husbandry context, polyphenolic compounds abundant in fresh grass were partially recovered as active molecules in the ruminant milk (King et al., 1998). Recent studies in rats (Gladine et al., 2007) and sheep [C. Gladine, E. Rock, C. Morand (all of INRA, UMR1019, St-Genès-Champanelle, France), D. Bauchart, D. Durand (both of INRA, UR1213, St-Genès-Champanelle, France); unpublished data] given 18:3 n-3–supplemented diets proved the effectiveness and complementary effect of a mixture of plant extracts rich in polyphenol (**PERP**) and vit E against plasma and tissue lipoperoxidation. This effect was probably because of the hydrophilic properties of PERP exhibiting affinity for different parts of cells complementary to the lipophilic vit E (Yeum et al., 2004). The present study aimed to analyze 1) the putative stimulatory effect of 18:3 n-3 from linseed (50 g of oil/kg of diet DM) on plasma lipoperoxidation in mid-lactation dairy cows and 2) the effectiveness of dietary vit E (375 IU/kg of diet DM per day) provided alone or with PERP (10 g/kg of diet DM per day) to optimize protection against plasma lipoperoxidation (Miller et al., 1993).

MATERIALS AND METHODS

Animals and Treatments

The experiment was performed with 24 cows (12 Holstein, 12 Montbéliarde) calving between November 2006 and January 2007, and selected on similar weight, lactation stage, milk production, and milk chemical composition, in the INRA experimental herd of Marcenat (France). During a 3-wk preexperimental period, animals were given a daily diet based on hay and maize silage ad libitum with a cereal mixture (70% barley and 30% maize) and soybean meal individually adapted according to the daily milk yield (**DMY**). At the end of the preexperimental period (d 0), mean BW was 647 ± 47 kg, lactation stage was 67 ± 16 d, DMY was 31.7 ± 5.7 kg, and milk fat and protein contents were 39.2 ± 3.3 and 31.6 ± 1.7 g/kg, respectively. The animals were assigned at random to 4 rations for 30 d (3 Holstein and 3 Montbéliarde cows per group) after a 3-d transition period. Chemical composition of the experimental diets, as detailed by Ferlay et al. (2009), are summarized in Table 1. Animals of the control group (**C**) were given maize silage ad libitum at 0930 h and grassland hay (7.9% of DMI) at 1500 h supplemented with cereal mixture (70% barley and 30% maize; 14.2% of DMI) and 12.7% of DMI of soybean meal. A second group was given the same basal diet supplemented with 10.1% of DMI of soybean meal and 17.9% of DMI of extruded linseed providing 50 g of oil/kg of DM (group **L**). A third group was given diet L supplemented with DL-α-tocopherol acetate (375 IU/kg of diet DM; 7,500 IU/cow per day; group **LE**). Animals in the final group were given diet LE supplemented with a mixture of 4 PERP (10 g/kg of diet DM; group **LEP**). The PERP were prepared from rosemary (*Rosemarinus officinalis*), grape (*Vinis vitifera*), citrus (*Citrus paradisi*), and marigold (*Calendula officinalis*) by Phytosynthèse (Riom, France); this mixture was patented (patent #P170-B-23.495 FR) by INRA on September 19, 2006. In the 4 experimental groups, rations were adjusted to individual animal requirements to cover energy and protein needs (INRA, 1988). The linseed, vitamin E, and PERP were mixed with maize silage and offered in the morning meal. The cows were housed in a tie-stall barn and milked at 0600 and 1600 h.

Blood Samples

Blood samples (60 mL) were collected just before the morning meal on d 0 and d 30 of the experimental period. Samples were taken from the jugular vein into tubes containing various anticoagulants: K_3-EDTA (0.47 mol/L) tubes were used to determine plasma lipids (only at d 30), fatty acid (**FA**) composition, α-tocopherol, and malondialdehyde (**MDA**); lithium heparin (5 IU /mL) tubes were used to determine plasma total antioxidant status (**TAS**; expressed as Trolox-equivalent antioxidant capacity, **TEAC**) and liver enzyme activities; and sodium citrate (18 m*M*) tubes were used to determine the kinetics of in vitro conjugated diene (**CD**) production. All plasma samples were isolated from blood by centrifugation at 1,600 × *g* for 10 min at 4°C and stored at −80°C until vit E,