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Tocopherols and tocotrienols in serum and liver of dairy cows receiving conjugated linoleic acids or a control fat supplement during early lactation

H. Sadri,*† S. Dänicke,‡ Ulrich Meyer,‡ J. Rehage,§ J. Frank,# and H. Sauerwein*
*Institute of Animal Science, Physiology and Hygiene Unit, University of Bonn, 53115 Bonn, Germany
†Department of Clinical Science, Faculty of Veterinary Medicine, University of Tabriz, 5166616471 Tabriz, Iran
‡Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), 38116 Braunschweig, Germany
§University for Veterinary Medicine, Foundation, 30173 Hannover, Germany
#University of Hohenheim, Institute of Biological Chemistry and Nutrition, 70599 Stuttgart, Germany

ABSTRACT

The fat-soluble vitamin E comprises the 8 structurally related compounds (congeners) α-, β-, γ-, and δ-tocopherol (with a saturated side chain) and α-, β-, γ-, and δ-tocotrienol (with a 3-fold unsaturated side chain). Little is known regarding the blood and liver concentrations of the 8 vitamin E congeners during the transition from pregnancy to lactation in dairy cows. We thus quantified tocopherols (T) and tocotrienols (T3) in serum and liver and hepatic expression of genes involved in vitamin E metabolism in pluriparous German Holstein cows during late gestation and early lactation and investigated whether dietary supplementation (from d 1 in milk) with conjugated linoleic acids (CLA; 100 g/d; each 12% of *trans*-10,*cis*-12 and *cis*-9,*trans*-11 CLA; n = 11) altered these compared with control-fat supplemented cows (CTR; n = 10). Blood samples and liver biopsies were collected on d −21, 1, 21, 70, and 105 (liver only) relative to calving. In both groups, the serum concentrations of αT, γT, βT3, and δT3 increased from d −21 to d 21 and remained unchanged between d 21 and 70, but were unaffected by CLA. The concentrations of the different congeners of vitamin E in liver did not differ between the CTR and the CLA groups. In both groups, the concentrations of the vitamin E forms in liver changed during the course of the study. The hepatic mRNA abundance of genes controlling vitamin E status did not differ between groups, but α-tocopherol transfer protein and tocopherol-associated protein mRNA increased with time of lactation in both. In conclusion, the concentrations of vitamin E congeners and the expression of genes related to vitamin E status follow characteristic time-related changes during the transition from late gestation to early lactation but are unaffected by CLA supplementation at the dosage used.

Key words: tocopherol, tocotrienol, conjugated linoleic acid, dairy cow

INTRODUCTION

The term "vitamin E" comprises 8 different natural compounds: α-, β-, γ-, and δ-tocopherol (**T**) and α-, β-, γ-, and δ-tocotrienol (**T3**), of which αT is the most abundant form in mammals (Frank et al., 2012). Tocopherols have a saturated phytyl side chain and T3 a 3-fold unsaturated isoprenoid side chain. In plasma and tissues of humans and animals, T, and particularly αT, are the predominant congeners and T3 are usually found at very low concentrations, if at all (Frank et al., 2012). The selective retention of αT over all other congeners and the particularly low concentrations of T3 are a consequence of the preferential metabolism (initiated by cytochrome P450 monooxygenases) of the non-αT forms to side-chain shortened water-soluble carboxyethyl hydroxychromanol (**CEHC**) metabolites in combination with the activity of the hepatic α-tocopherol transfer protein (**TTP**; Grebenstein et al., 2014).

α-Tocopherol is the only congener presently used as vitamin E supplement for dairy cows. Plasma vitamin E concentrations decrease gradually throughout the antepartum period, with nadir concentrations around calving and a gradual increase thereafter (Weiss, 1998). The depletion of serum vitamin E concentrations during early lactation may be related to increased incidences of postpartum diseases, such as left displaced abomasum (Qu et al., 2013). Supplementation of dairy cows with vitamin E during the dry period can partially attenuate the decline in plasma vitamin E concentrations around calving (Politis, 2012). Furthermore, beneficial effects of vitamin E supplementation during the dry period have also been reported; for example, enhancing immune function (Politis et al., 1995, 1996) and

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[1]Corresponding author: sauerwein@uni-bonn.de

decreasing the incidence of clinical mastitis in dairy cows (Smith et al., 1984; Weiss et al., 1997). Such effects seem to depend on the plasma αT concentration achieved and on the infection pressure present in the herds studied (Politis, 2012).

Supplementation with *trans*-10,*cis*-12 CLA is used to reduce milk fat content in dairy cows as a dietary strategy aimed to improve energy balance, especially during early lactation (Sippel et al., 2009; Schlegel et al., 2012). The observation that CLA-fed laboratory animals have improved vitamin E status attracted interest in the interaction between vitamin E and CLA (Liao et al., 2008; Chao et al., 2010). Liver vitamin E concentrations were 15-fold higher in CLA-fed mice than in control animals (Liao et al., 2008). The improved vitamin E status of CLA-treated animals might be due to the induction of TTP expression in the liver (Chen et al., 2012). An increase in longissimus muscle αT concentration as a result of feeding rumen-protected CLA was also recently reported in young Simmental heifers (Schlegel et al., 2012).

To the best of our knowledge, the effect of dietary CLA on blood and liver concentrations of all vitamin E congeners and on the hepatic expression of genes involved in the regulation of vitamin E concentrations has not yet been investigated in dairy cows. The current study was designed to characterize potential effects of CLA supplementation on vitamin E status during the transition from pregnancy to lactation in dairy cows.

MATERIALS AND METHODS

Animals, Treatment, and Experimental Design

The experiment was conducted at the experimental station of the Friedrich-Loeffler-Institute (Braunschweig, Germany). The experimental procedures performed in this study were in accordance with European Community regulations concerning the protection of experimental animals and the guidelines of the LAVES (Lower Saxony State office for Consumer Protection and Food Safety, Germany, File Number 33.14.42502-04-071/07). Cows, experimental design, and treatments were described in detail by Pappritz et al. (2011). A subset of animals and samples from that study; that is, only pluriparous cows, was considered for the present study. Briefly, 21 German Holstein cows in late gestation were housed in freestall barns and fed ad libitum with a partial mixed ration (6.8 MJ of NE_L/kg of DM) consisting of 37.8% corn silage, 25.2% grass silage, and 37% concentrate (DM basis) during the study period. The concentrate portion of the ration contained 2% mineral feed (per kg of mineral feed: 140 g of Ca, 120 g of Na, 70 g of P, 40 g of Mg, 6 g of Zn, 5.4 g of Mn, 1 g of Cu, 100 mg of I, 40 mg of Se, 5 mg of Co, 1,000,000 IU of vitamin A, 100,000 IU of vitamin D_3, and 1,500 mg of vitamin E). Diets were formulated according to the recommendation of the German Society of Nutrition Physiology (GfE, 2001). At 1 DIM, cows were assigned to either the CLA group (n = 11) or control group (**CTR**; n = 10) and fed 100 g of the respective fat supplements daily until 182 DIM. The fat supplements were incorporated into 4 kg of additional concentrate (8.8 MJ of NE_L/kg DM) consisting of the same components as the concentrate used in the partial mixed ration. Cows in the CLA group received 100 g/d of rumen-protected CLA fat (Lutrell Pure, BASF SE, Ludwigshafen, Germany) supplying 7.6 g of *cis*-9,*trans*-11 CLA and 7.6 g of *trans*-10,*cis*-12 CLA per day. The CLA supplement contained (% of total FAME): $C_{16:0}$ (10.9%), $C_{18:0}$ (50.3%), $C_{18:1}$ *cis*-9 (10.7%); $C_{18:2}$ *cis*-9,*trans*-11 (12.0%), $C_{18:2}$ *trans*-10,cis-12 (11.9%), other CLA (0.95%), and other FA (3.3%). The cows in the CTR group received 100 g/d of rumen-protected control fat (Silafat, BASF SE) in which CLA was substituted by stearic acid. The control fat supplement contained (% of total FAME): $C_{16:0}$ (10.9%), $C_{18:0}$ (87.3%), $C_{18:1}$ *cis*-9 (<0.01%); $C_{18:2}$ *cis*-9,*trans*-11 (0.06%), $C_{18:2}$ *trans*-10,cis-12 (0.02%), other CLA (0.15%), and other FA (1.58%).

Blood and Liver Tissue Sampling

Blood samples were collected via the jugular vein using evacuated tubes. Samples collected on d −21, 1, 21, and 70 relative to parturition were used. All tubes were centrifuged at 4°C and 3,000 × *g* for 10 min and the serum obtained was kept frozen (−80°C) until analyzed. Liver tissue samples were obtained on d −21, 1, 21, 70, and 105 relative to calving by transcutaneous biopsy using an automatic device (BARD, Tempe, AZ) under ultrasound control. Tissue samples were snap-frozen in liquid nitrogen and stored at −80°C.

Quantification of Vitamin E Congeners in Serum Samples

Tocopherols and T3 were quantified by a validated method described elsewhere (Grebenstein and Frank, 2012). Briefly, serum was thawed on ice and 100 μL was transferred to a glass tube. Two milliliters of ethanol containing 1% ascorbic acid (wt/vol), 900 μL of deionized water, and 25 μL of butylated hydroxytoluene (BHT; 1 mg/mL dissolved in ethanol) were added. n-Hexane (2 mL) was added, hand-mixed by inversion for 1 min, and centrifuged (5 min, ambient temperature, 800 × *g*). A 1.5-mL volume of the supernatant was transferred to a fresh test tube and the extraction repeated. The