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Short communication: Effect of conjugated linoleic acid on concentrations of fat-soluble vitamins in milk of lactating ewes

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ABSTRACT

Conjugated linoleic acids (CLA) are well known as milk fat-reducing feed supplements in diets for lactating ruminants. However, their effects on milk concentrations of fat-soluble vitamins are unknown. This study was performed to investigate the hypothesis that CLA affect the concentrations of retinol and tocopherol in ewe milk. For that purpose, group-housed Merino ewes (101 ± 13.7 kg) nursing twin lambs and fed with a hay:concentrate diet were supplemented with either 45 g of a rumen-protected CLA supplement containing 3.4 g of *cis*-9,*trans*-11-CLA and 3.4 g of *trans*-10,*cis*-12-CLA (CLA group, n = 11) or with 45 g of a hydrogenated vegetable fat (control group, n = 12) per ewe per day during the first 6 wk of lactation. Feed intake was recorded daily (concentrate) or weekly (hay) per group. Milk spot samples were collected at the beginning of the experiment (5 ± 2.4 d postpartum) and then weekly after lambs had been separated for 2 h from their mothers. The milk fat content was determined and feed and milk were analyzed for concentrations of α-, γ-, and δ-tocopherol and for retinol by HPLC. Dietary intake of tocopherol and retinol was similar in both groups. Feeding CLA decreased milk fat concentration by 23% on average, and during the first 3 wk of the study milk tocopherol concentration tended to be increased by feeding CLA (+17%), but retinol concentrations were not influenced. When related to milk fat, CLA feeding significantly increased both milk tocopherol (+40%) and retinol (+32%) and these effects were evident during the whole experimental period corresponding to the first half of lactation.

Key words: conjugated linoleic acid, milk fat-soluble vitamin, lactating ewe

Short Communication

Conjugated linoleic acids are positional and geometric isomers of linoleic acid that are naturally occurring in ruminant-derived foods. Dietary CLA supplements, usually a mixture of the *trans*-10,*cis*-12 and the *cis*-9,*trans*-11 isomer, have been shown to influence lipid metabolism in a range of species including lactating dairy cows (Baumgard et al., 2002; Schlegel et al., 2012a) and ewes (Hussein et al., 2013). In both ruminants and monogastric animals, supplementing CLA in the diet inhibits lipid synthesis by downregulating expression of several genes involved in lipid synthesis in muscle, adipose tissue, liver (Ringseis et al., 2004; Tous et al., 2012), and mammary gland (Baumgard et al., 2002; Gutgesell et al., 2009). Additionally, CLA inhibits lipoprotein lipase (**LPL**), which is crucial for lipid uptake from chylomicrons and very low density lipoproteins into tissues including the mammary gland (Ringseis et al., 2004; Hussein et al., 2013). Consequently, milk fat concentration is reduced and the FA composition is altered in lactating animals (Baumgard et al., 2002; Ringseis et al., 2004; Lock et al., 2006). In addition to the known effects on lipid metabolism, the metabolism of fat-soluble vitamins might be modified as well due to the fact that the metabolism of lipids and lipid-soluble vitamins are partly interlinked (Lemaire-Ewing et al., 2010; D'Ambrosio et al., 2011). Both vitamin A and E need to be emulsified by bile acids in the intestine and are transported in chylomicrons and lipoproteins together with other lipids (Lemaire-Ewing et al., 2010; D'Ambrosio et al., 2011). Distribution and metabolism of α-tocopherol is closely related to that of other lipids transported in plasma lipoproteins, and LPL is also important for vitamin E uptake into extra-hepatic tissues (Lemaire-Ewing et al., 2010). Likewise, LPL plays a role in tissue uptake of retinol from chylomicrons, and enzymes and proteins that are involved in triglyceride and cholesterol metabolism are also involved in retinoid metabolism (D'Ambrosio et al., 2011).

Due to the described links between metabolism of lipids and vitamin E and A, we hypothesized that supplementing CLA to the diet influences tocopherol and

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retinol concentrations in milk of lactating ewes both absolutely and in relation to milk fat. To investigate this hypothesis, a 42-d experiment was conducted at the Research Station of the Institute of Animal Breeding and Genetics at the University of Giessen, Germany. A total of 23 Merino ewes nursing twin lambs kept in a barn with loose straw bedding were randomly allocated to a treatment group (CLA; n = 11) and a control group (control, n = 12) based on day postpartum (5 ± 2.4; mean ± SD; $P = 0.34$ between groups), average BW (101 ± 13.7 kg; $P = 0.92$), and number of parity (2.7 ± 1.29; $P = 0.91$). Ewes were group-housed together with their lambs and had free access to grass hay from a natural grassland (false oat-grass community type) and water ad libitum. Once daily, 1.5 kg per ewe per day of a commercial concentrate (RWZ Schaf 18 Uni Press, RWZ, Köln, Germany) was fed, which consisted of (g/kg): wheat bran (250), barley (200), rapeseed extraction meal (160), wheat gluten (100), beet pulp (100), dried distillers grains with solubles (44), corn (36), sunflower extraction meal (30), vinasse (30), calcium carbonate (26.7), sugar beet molasses (16), sodium chloride (1.3), magnesium oxide (3), and supplied vitamins and trace elements (per kg): vitamin A, 8,000 IU; vitamin D_3, 1,000 IU; vitamin E, 65 mg; zinc, 40 mg as zinc sulfate monohydrate; manganese, 20 mg as manganese (II) sulfate monohydrate; selenium, 0.2 mg as sodium selenite; cobalt, 0.2 mg as cobalt (II) sulfate monohydrate; and iodine, 0.1 mg as calcium iodate. According to the supplier, the concentrate contained 10.6 MJ of ME/kg and 180 g/kg of CP. The concentrate was supplemented either with 30 g/kg of a hydrogenated vegetable fat (control group; Sila R.r.l., Noale, Italy) or with the rumen-protected CLA supplement Lutrell (CLA group; BASF, Ludwigshafen, Germany). A saturated fat was chosen as the control fat to exclude biohydrogenation and the generation of unknown *trans*-FA and CLA isomers in the rumen. From the start of the experiment (i.e., approximately their 5th day of life), lambs had additional ad libitum access to a commercial concentrate (RWZ Schaf 18 Uni Press). Feed intake of hay per group was estimated based on average bale weight (6.8 ± 1.42 kg; mean ± SD) and number of hay bales consumed weekly. Feed samples were collected weekly and were stored at −20°C until analysis of a pooled sample according to the official German VDLUFA methodology (Bassler and Buchholz, 1993) for DM (no. 4.2.1), crude ash (8.1), crude fat (5.1.1), crude fiber (6.1.1), and CP (N × 6.25; CN-Analysator vario MAX N/CN, elementar Analysesysteme GmbH, Hanau, Germany; 4.1.1), and of FA composition and fat-soluble vitamin concentration. The BW of ewes and their lambs were recorded at the beginning and the end of the experiment. Milk spot samples were collected weekly after lambs had been separated from their mothers for 2 h, but milk yield was not determined. The spot samples were collected from both mammary glands; the first few milliliters of milk were discarded, and afterward, 10 to 15 mL of milk were sampled. The milk was stored at −20°C in aliquots for later analysis of concentrations of milk fat, milk FA, and fat-soluble vitamins.

For milk fat determination, the fat was extracted with HiP (hexane:isopropanol, 3:2, vol/vol; Hara and Radin, 1978) overnight, and after centrifugation at 1,000 × g for 5 min at 15°C the upper fat-containing phase was quantitatively transferred to a dried and weighed test glass. The test glass was dried to constant weight at 105°C and reweighed. The weighed fat was related to the initial milk weight. The coefficient of variation of 1 sample analyzed 6 times was 1.54%. Feed and milk FA were determined by gas chromatography according to Schlegel et al. (2012b). Concentrations of retinol and α-, γ-, and δ-tocopherol were determined in feed and milk by HPLC (L-7100, LaChrom, Merck-Hitachi, Darmstadt, Germany) with fluorescence detection as described for tocopherols in Gessner et al. (2013) and detected by fluorescence (Fluorescence Detector L-7480, LaChrom, Merck-Hitachi, retinol: excitation wavelength, 325 nm, emission wavelength, 475 nm; tocopherols: excitation wavelength, 295 nm, emission wavelength, 325 nm). The δ- und β-tocopherol could not be separated and are summarized as δ-tocopherol. Tocopherol equivalents were calculated using the naturally occurring stereoisomer of α-tocopherol, RRR α-tocopherol, as a reference (RRR α-tocopherol = 1; from now on called tocopherol) and γ-tocopherol was multiplied by 0.25 and δ-tocopherol by 0.01 to consider the lower vitamin activity of those 2 tocopherol isomers (www.dge.de). β-Carotene was extracted in feed samples (0.25 g) using the same procedure, but separated on a LiChroCART DIOL 125–4 column (Merck, Schwalbach, Germany) with hexane as the mobile phase at a flow rate of 1 mL/min and a pressure of 23 bar at 30°C and detected at 455 nm using a UV-VIS detector L4250 (Merck).

Data were statistically analyzed using the software R version 3.1.1 (R Development Core Team, 2011). For analysis of BW data, treatment was considered as fixed and animal as random effect and the function lme, package nlme, was used. Milk fat data were monitored over time, analyzed using treatment and the interaction between treatment and time as fixed effect, animal as random effect, and separate intercepts to account for the initial differences in milk fat content using lme. Milk vitamin and milk FA data were monitored over time as well, and the model included treatment and time and their interaction as fixed effects and animal