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The effect of conjugated linoleic acid supplements on oxidative and antioxidative status of dairy cows

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ABSTRACT

Dairy cows develop frequently negative energy balance around parturition and in early lactation, resulting in excessive mobilization of body fat and subsequently in increased risk of ketosis and other diseases. Dietary conjugated linoleic acid (CLA) supplements are used in dairy cows mainly for their depressing effect on milk fat content, but are also proposed to have antioxidative properties. As negative energy balance is associated with oxidative stress, which is also assumed to contribute to disease development, the present study was conducted to examine effects of CLA on oxidative and antioxidative status of lactating dairy cows. German Holstein cows (primiparous n = 13, multiparous n = 32) were divided into 3 dietary treatment groups receiving 100 g/d of control fat supplement, containing 87% stearic acid (CON; n = 14), 50 g/d of control fat supplement and 50 g/d of CLA supplement (CLA 50; n = 15), or 100 g/d of CLA supplement (CLA 100; n = 16). The CLA supplement was lipid-encapsulated and contained 12% of *trans*-10,*cis*-12 CLA and *cis*-9,*trans*-11 CLA each. Supplementation took place between d 1 and 182 postpartum; d 182 until 252 postpartum served as a depletion period. Blood was sampled at d −21, 1, 21, 70, 105, 140, 182, 224, and 252 relative to calving. The antioxidative status was determined using the ferric-reducing ability of plasma, α-tocopherol, α-tocopherol-to-cholesterol mass ratio, and retinol. For determination of oxidative status concentrations of hydroperoxides, thiobarbituric acid-reactive substances (TBARS), *N*′-formylkynurenine, and bityrosine were measured. Mixed models of fixed and random effects with repeated measures were used to evaluate period 1 (d −21 to 140) and 2 (d 182–252) separately. Cows showed increased oxidative stress and lipid peroxidation during the periparturient period in terms of increased serum concentrations of hydroperoxides and TBARS, which decreased throughout lactation. During period 1, the supplemented cows had lower TBARS concentrations, which was not detectable in period 2. The other determined parameters were not affected by CLA supplementation. The obtained results show that dietary CLA supplementation in the chosen dosage, formulation, and application period had a marginal antioxidative effect in terms of lipid peroxidation in lactating dairy cows.
Key words: conjugated linoleic acid, dairy cow, oxidative stress, antioxidant

INTRODUCTION

Around parturition and in early lactation, negative energy balance is frequently found in dairy cows because of an imbalance between energy intake and energy demands for fetal growth and milk production (Goff and Horst, 1997). Negative energy balance is compensated by mobilization of body energy reserves, in particular fat from adipose tissues. Excessive lipomobilization may lead to health disorders, such as ketosis and fatty liver (Drackley, 1999; Bobe et al., 2004).

The enhanced metabolism of the periparturient cow leads to a rise in production oxidizing agents, including oxygen radicals and certain nonradicals, referred to as reactive oxygen species (**ROS**; Halliwell, 1996; Bionaz et al., 2007). During the periparturient period, free radicals and ROS, such as the strongly oxidizing hydroxyl radical, are formed under physiological and pathological conditions in enzymatic pathways of mitochondria or the respiratory burst of immune defense (Osorio et al., 2014). Reactive oxygen species readily attack biological macromolecules such as lipids and proteins, including enzymes or carrier proteins, causing structural changes and loss of function to the point of cellular dysfunction and apoptosis (Scherer and Deamer, 1986; Stewart, 1994).

To inhibit impaired biological function due to damage to macromolecules by ROS, living organ-

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isms have developed a complex antioxidant defense system, divided into low-molecular mass antioxidants and antioxidative enzymes (Locher et al., 2011). Low-molecular mass antioxidants include the lipid-soluble α-tocopherol, protecting cell membranes from lipid peroxidation by scavenging free radicals (Yu, 2001). Further low-molecular mass antioxidants are ascorbic acid and β-carotene, which are able to quench singlet oxygen and peroxyl radicals and enhance the antioxidative effect of α-tocopherol (Palozza and Krinsky, 1991, 1992). Retinol only shows antioxidant activity in vitro, but not in vivo (Azzi et al., 2004); however, high retinol concentrations have been shown to reduce the risk of disease in dairy cows (LeBlanc et al., 2004).

Increased lipid peroxidation (Bernabucci et al., 2005; Castillo et al., 2005), decreased plasma antioxidant concentrations (Bouwstra et al., 2010b), and low antioxidative enzyme activity (Gaál et al., 2006) have been established as typical findings in blood samples of periparturient dairy cows. This imbalance between ROS and antioxidants favoring the first and disfavoring the latter is commonly referred to as oxidative stress (Sies, 1993). Excessive amounts of ROS during oxidative stress damage cellular membranes or other components, leading to changes in physiological pathways and even cause pathology (Miller et al., 1993). Correlations between decreased antioxidants, oxidative stress, and peripartal production diseases, such as mastitis, retained fetal membranes, and reduced fertility, have been discussed (LeBlanc et al., 2004; Kankofer et al., 2010; Heidarpour et al., 2012).

Mixtures of *trans*-10,*cis*-12 and *cis*-9,*trans*-11 isomers of CLA were repeatedly investigated regarding their effect on improving negative energy balance in periparturient and early-lactation cows. The hypothesis was that CLA may reduce energy expenditure for milk production due to reduced de novo synthesis of milk fat caused by *trans*-10,*cis*-12 CLA (Baumgard et al., 2000). However, recent research has revealed inconsistent results of CLA supplements on energy balance (Castañeda-Gutiérrez et al., 2005; Odens et al., 2007; Hötger et al., 2013). Several studies revealed an increase in overall milk yield next to the milk fat depression (Giesy et al., 2002; Bernal-Santos et al., 2003). These results suggest increased milk yield may countermand the CLA-induced milk fat depression concerning energy balance.

Antioxidative effects have been shown for different CLA isomers in vitro (Leung and Liu, 2000; Basiricò et al., 2015) and in vivo in rats (Ip et al., 1991), mice (Ha et al., 1990), and hens (Qi et al., 2011). Conversely, further in vitro studies delivered contradicting results and showed a possible pro-oxidative effect of CLA (van den Berg et al., 1995; Chen et al., 1997); pro-oxidative properties of CLA have been described in human trials (Basu et al., 2000; Risérus, 2002). The aim of the current study was to investigate effects of long-term supplementation of a commercial CLA isomer mixture, containing *trans*-10,*cis*-12 CLA and *cis*-9,*trans*-11 CLA in equal amounts, on the antioxidative and oxidative profiles of peripartal and lactating dairy cows.

MATERIALS AND METHODS

Animals and Feeding

The trial was conducted in 32 multiparous (second lactation: n = 22; third lactation n = 5; fourth lactation n = 5) and 13 primiparous German Holstein cows at the Institute of Animal Nutrition of the Friedrich-Loeffler-Institut. Details on housing, feeding, CLA supplements, and production parameters of studied cows in this trial are already presented by Pappritz et al. (2011b). Briefly, animals, housed in a freestall, were separated into 3 groups taking into consideration the BW (multiparous from 483–655 kg, primiparous from 391–522 kg), the number of lactations, and the milk yield during the last lactation.

Before parturition, all animals were pasture fed: primiparous animals for 3 mo before calving and multiparous animals up to 5 wk between dry off and study start. Three weeks antepartum, all animals were housed and received the same TMR diet, with 63% silage (60% maize and 40% grass silage) and 37% concentrate on a DM basis until parturition. After parturition, animals were assigned either to a control group (**CON**) or 1 of 2 treatment groups (**CLA 50** and **CLA 100**) and fed a partial mixed ration (**PMR**) ad libitum provided in troughs suitable for measurement of individual feed intake (TYPE RIC, Insentec B.V., Marknesse, the Netherlands). The PMR contained the same amounts of silage and concentrate as TMR. Additionally, all animals received 4 kg of concentrate, which included the fat supplements between d 1 and 182 postpartum, via transponder-controlled feeding stations. Included in the concentrate control cows received 100 g of a commercial fat supplement (Silafat, BASF SE, Ludwigshafen, Germany) and cows of the CLA 100 group received 100 g of a commercial CLA product (Lutrell Pure, BASF SE); cows of the CLA 50 group were fed 50 g of the control fat supplement and 50 g of the CLA product.

The CLA fat supplement contained 11.9% of *trans*-10,*cis*-12 CLA and 12.0% *cis*-9,*trans*-11 CLA (Pappritz et al., 2011b), whereas the control supplement contained 87% of stearic acid (C18:0). In both supplements, 11%