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An unprotected conjugated linoleic acid supplement decreases milk production and secretion of milk components in grazing dairy ewes

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ABSTRACT

Feeding conjugated linoleic acid (CLA) in a rumen-inert form to dairy ewes has been shown to increase milk production, alter milk composition, and increase the milk fat CLA content. However, few studies have tested ruminally unprotected CLA sources. The objective of this study was to evaluate the effects of an unprotected CLA supplement (29.8% of *cis*-9,*trans*-11 and 29.9% of *trans*-10,*cis*-12 isomers as methyl esters) on milk yield and composition of dairy ewes. Twenty-four lactating Lacaune ewes were used in a crossover design and received 2 dietary treatments: (1) control: basal diet containing no supplemental lipid and (2) basal diet plus CLA (30 g/d). The CLA supplement was mixed into the concentrate and fed in 2 equal meals after morning and afternoon milkings. Each experimental period consisted of 21 d: 7 d for adaptation and 14 d for data collection. The CLA supplement decreased milk fat content and yield by 31.3 and 38.0%, respectively. Milk yield and secretion of milk lactose and protein were decreased by 8.0, 9.8, and 5.6%, respectively. On the other hand, milk protein content and linear SCC score were 1.8 and 17.7% higher in ewes fed the CLA supplement. The concentration of milk fatty acids originating from de novo synthesis (<C16) was decreased by 25%, whereas the concentration of milk fatty acids taken up preformed from the plasma (>C16) was increased by 22.6% in ewes fed the CLA supplement. The CLA supplement decreased C14:1/C14:0, C16:1/C16:0, and C18:1/C18:0 desaturase indexes by 25, 18.7, and 0.1%, respectively, but increased the *cis*-9,*trans*-11 CLA/*trans*-11 C18:1 ratio by 8.6%. The concentrations of *trans*-10,*cis*-12 CLA and *cis*-9,*trans*-11 CLA in milk fat was 309 and 33.4% higher in ewes fed CLA. Pronounced milk fat depression coupled with the deleterious effects on milk yield, milk SCC, and secretion of all milk solids observed in ewes fed an unprotected CLA supplement is likely to be associated with high doses of *trans*-10,*cis*-12 CLA reaching the mammary gland, corroborating previous results obtained with dairy cows.
Key words: Lacaune ewe, mammary gland, milk fat depression, milk yield

INTRODUCTION

Conjugated linoleic acid (**CLA**) is a generic term used to describe a mixture of isomers of linoleic acid (C18:2 n-6) containing conjugated double bonds in their molecular structure. These compounds are mainly found in ruminant products as a consequence of partial ruminal hydrogenation of polyunsaturated FA present in feeds. More than 20 CLA isomers have been identified in ruminant milk fat, but *cis*-9,*trans*-11 CLA is by far the most abundant, usually representing 75 to 90% of total CLA in milk fat (Lock and Bauman, 2004). This isomer has been shown to possess health-enhancing properties such as anticarcinogenic and antidiabetogenic activities so that efforts have been made to increase milk CLA content (Parodi, 1997; Bauman et al., 2006). Initial attempts to achieve this goal were made by supplementing dairy cows with synthetically produced CLA, but the increase in milk fat CLA content was accompanied by an unexpected decrease in milk fat yield. This effect was subsequently shown to be caused by another CLA isomer present in the CLA supplement, *trans*-10,*cis*-12 CLA, which is a potent inhibitor of milk fat synthesis (Baumgard et al., 2000).

The methyl ester form of CLA (**ME-CLA**) has several advantages in commercial production as compared with the free FA form (Sæbø, 2003) and studies with dairy cows have indicated comparable results in terms of milk fat depression (**MFD**; de Veth et al., 2004; Perfield et al., 2004). Just as occurs with esterified FA in dietary lipids, the methyl esters of FA are hydrolyzed by bacterial lipases and biohydrogenated by rumen bacteria (Maia et al., 2010). Therefore, lipid encapsulation of ME-CLA has been used as a rumen protection method that offers partial protection against biohydrogenation that is comparable to other rumen

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protection methods for CLA (Perfield et al., 2004). Thus, investigations of CLA effects in dairy cows have often used lipid encapsulated ME-CLA (e.g., de Veth et al., 2006; Castañeda-Gutiérrez et al., 2007; von Soosten et al., 2011).

The antilipogenic effect of *trans*-10,*cis*-12 CLA has been observed in lactating small ruminants in which goats (Lock et al., 2008; Shingfield et al., 2009) were apparently less responsive than cows (de Veth et al., 2004) and ewes (Lock et al., 2006; Sinclair et al., 2007, 2010). Of note, supplements containing *trans*-10,*cis*-12 CLA can be used strategically to improve energy balance of lactating animals by decreasing their energy requirements for milk synthesis. Depending on the physiological status of the animal, the energy spared for milk fat synthesis could be directed to milk production or secretion of other milk components such as protein (Bernal-Santos et al., 2003; Mackle et al., 2003; Odens et al., 2007; Medeiros et al., 2010). Feeding a lipid-encapsulated supplement that contained ME of *trans*-10,*cis*-12 CLA was shown to increase both milk production and milk CLA content in dairy ewes (Lock et al., 2006). However, as reviewed by Chilliard et al. (2003) and Shingfield et al. (2010), the rumen metabolism of lipid supplements differs in small ruminants as compared with dairy cows, and we know of no studies that have evaluated the effects of unprotected CLA supplements on milk yield and composition of dairy ewes. The objective of this study was to evaluate the effects of an unprotected ME-CLA supplement on milk yield, milk composition, and the milk FA profile of Lacaune dairy ewes.

MATERIALS AND METHODS

Animals, Treatments, and Experimental Procedures

Animal Care and Handling procedures were followed according to the Santa Catarina State University Ethical Committee. Twenty-four Lacaune ewes (40 to 70 DIM) were paired by BW, parity, and previous milk production, and randomly assigned to the following dietary treatments in a crossover design: (1) basal diet containing no supplemental lipid (control, **CON**) and (2) basal diet plus 30 g/d of an unprotected ME-CLA supplement. Each experimental period lasted 21 d (7 d for adaptation and 14 d for data collection), and experimental periods were separated by a washout interval in which animals were fed the CON diet for 7 d to minimize any carryover effect (Baumgard et al., 2000). Data from 2 ewes were not included in the analyses: 1 ewe on the CLA diet stopped producing milk over the first week during the CLA treatment and 1 ewe fed the CON diet became lame due to hoof problems.

Ewes were fed 1.2 kg/d (DM basis) of a concentrate mixture according to the NRC (2007) to complement the estimated nutrient intake coming from forage and formulated using the Small Ruminant Nutrition System (SRNS; Tedeschi et al., 2010). The concentrate contained ground corn (56%), soybean meal (40%), and a commercial vitamin-mineral mix (4%), and it was individually fed twice daily (0.6 kg/meal) after the morning and afternoon milkings (Table 1). The unprotected CLA supplement (Luta-60; BASF AG, São Paulo, Brazil) consisted of FA methyl esters (**FAME**) with the following composition: 4.1% palmitic acid, 3.6% stearic acid, 27.4% oleic acid, 1.2% linoleic acid, 29.8% *cis*-9,*trans*-11 CLA, 29.9% *trans*-10,*cis*-12 CLA, and 3.0% other FA. This CLA supplement was mixed into the concentrate twice daily (15 g/meal) to minimize refusals; therefore, each ewe received about 9 g/d of *trans*-10,*cis*-12 CLA via the concentrate. The extent to which the unprotected CLA supplement would be biohydrogenated was unknown, so the dose was chosen based on the assumption that biohydrogenation would be extensive (more than 90%, Jenkins et al., 2008) with only a small percent escaping and being available for absorption in the small intestine. All ewes rotationally grazed 3 paddocks of annual ryegrass and white clover (50:50) with free access to fresh water. A mineral salt and vitamin supplement was also available free choice in all paddocks. After the first milking at 0500 h, the ewes were individually fed 0.6 kg of the daily concentrate and returned to pasture. At 1130 h, animals were brought from pasture to a barn and were individually fed 2.5 kg of corn silage (as-fed basis) until the afternoon milking at 1700 h. Orts were weighed daily to calculate silage intake. After the afternoon milking, the ewes were fed the remaining 0.6 kg of concentrate and subsequently returned to pasture. The BW was recorded at the beginning and at the end of each experimental period.

Milk Production and Composition

The individual milk yield was recorded daily in line meters (Ordemilk Ltda., Treze Tílias, Santa Catarina, Brazil) throughout the study in a milking parlor designed for ewes. Milk samples were taken at each milking during the collection period, pooled by day and stored at 4°C with a preservative (bromopol tablet; D & F Control Systems Inc., San Ramon, CA) before being analyzed for components (fat, protein, lactose, and TS) and SCC using infrared analysis (AOAC, 2000; method #972.160). Additional milk samples were also collected the same way throughout each collecting period and frozen at −20°C without preservative and stored for subsequent FA profile analysis.