A Conjugated Linoleic Acid Supplement Containing *trans*-10, *cis*-12 Reduces Milk Fat Synthesis in Lactating Sheep¹

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ABSTRACT

The efficacy of conjugated linoleic acid (CLA) supplements containing *trans*-10, *cis*-12 for reducing milk fat synthesis has been well documented in dairy cows, but studies with other ruminant species are less convincing, and there have been no investigations of this in sheep. Therefore, the current study was designed to determine whether *trans*-10, *cis*-12 CLA would inhibit milk fat synthesis in sheep. Twenty multiparous ewes in early lactation were paired and randomly allocated to 2 treatments: grass hay plus concentrate either unsupplemented (control) or supplemented with lipid-encapsulated CLA to provide 2.4 g/d of *trans*-10, *cis*-12 CLA. The CLA dose was based on published responses of dairy cows extrapolated to ewes on a metabolic body weight basis. The experimental design was a 2-period crossover with 10-d treatment periods separated by a 10-d interval. Compared with the control, CLA supplementation reduced milk fat content from 6.4 to 4.9% and reduced fat yield from 95 to 80 g/d. The CLA treatment also increased milk yield from 1,471 to 1,611 g/d and increased protein yield from 68 to 73 g/d. Milk protein content and DMI were unaffected by treatment. The reduction in milk fat yield was due to decreases in both *de novo* fatty acid synthesis and uptake of preformed fatty acids. Milk fat content of *trans*-10, *cis*-12 CLA was <0.01 and 0.12 g/100 g of fatty acids for the control and CLA treatments, respectively. The transfer efficiency of *trans*-10, *cis*-12 CLA from the dietary supplement into milk fat was 3.8%. Results of the present study demonstrate that a CLA supplement containing *trans*-10, *cis*-12 CLA reduces milk fat synthesis in lac-

tating sheep in a manner similar to dairy cows when fed at an equivalent dose (metabolic body weight basis). Furthermore, the nutrients spared by the reduction in milk fat coincided with an increase in milk and milk protein yield.

Key words: conjugated linoleic acid, milk fat depression, sheep, lactation

INTRODUCTION

The *trans*-10, *cis*-12 isomer of conjugated linoleic acid (CLA) has been shown to be a potent inhibitor of milk fat synthesis in dairy cows (Baumgard et al., 2000). Subsequent investigations have mainly focused on the mechanism of action of this fatty acid in the regulation of milk fat synthesis, including diet-induced milk fat depression and the potential use of this technology as a management tool in the controlled regulation of milk fat output (Bauman and Griinari, 2003; Bauman et al., 2003; Griinari and Bauman, 2006). Although *trans*-10, *cis*-12 CLA has also been shown to effect body fat accretion in growing animals, response differences among species are well documented, including differences among rodent species and even among strains of mice (Wang and Jones, 2004).

Chilliard et al. (2003) reviewed studies involving effects of lipid supplements on milk fat synthesis in ruminants and concluded that there were many similarities, but often goats and sheep responded differently than cows. Most studies relative to the effects of CLA supplements and *trans*-10, *cis*-12 CLA on milk fat synthesis in ruminants have involved dairy cows. However, 2 recent reports involving goats indicated that CLA supplements had little or no effect on milk fat yield, and researchers emphasized that goats may be not be suitable models for cows in studies of milk fat synthesis (Erasmus et al., 2004; Schmidely and Morand-Fehr, 2004). To date, there have been no investigations in lactating sheep, whose milk is characterized by higher fat and protein concentrations than cow and goat milk (Pulina and Nudda, 2005). Therefore, the objective of the current study was to determine whether a CLA supplement containing *trans*-10, *cis*-12 would reduce milk fat synthesis in lactating sheep. To achieve this

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Table 1. Ingredient composition of concentrate and chemical concentration of concentrate and hay in experimental diets

Composition ¹	Concentrate	Hay
Ingredient, g/kg		
Dried molassed sugarbeet feed ²	294	
Barley	261	
Rapeseed meal	168	
Soybean meal	92	
Corn gluten feed	59	
Megalac ³	46	
Molasses	35	
Urea	18.5	
Minerals and vitamins ⁴	26.5	
Chemical analysis		
DM, g/kg	896	860
ME, Mcal/kg of DM	3.3	2.0
CP, g/kg of DM	211	81
NDF, g/kg of DM	247	712
ADF, g/kg of DM	102	419

¹Ingredient and chemical composition values do not include the lipid-encapsulated conjugated linoleic acid (CLA) supplement that had a fat content of 65%. The fatty acid composition of the supplement was 9% 16:0; 42% 18:0; 12% *cis*-9 18:1; 1% *cis*-9, *cis*-12 18:2; 15% *cis*-9, *trans*-11 CLA; and 15% *trans*-10, *cis*-12 CLA.

²By-product of the manufacture of sugar, consisting of extracted sugar beet slices and sugar beet molasses, which had been dried (Trident Feeds, Peterborough, UK).

³Calcium soap of palm oil fatty acids (Volac Ltd., Royston, Hertfordshire, UK). Fatty acid composition, as reported by the company, is 1.2% 12:0 + 14:0, 48% 16:0, 5% 18:0, 36% *cis*-9 18:1, and 9% 18:2.

⁴Ewe mineral and vitamin premix (SC Feeds, Staffordshire, UK). Major minerals (g/kg) were Ca, 245.7; Na, 78.6; Mg, 50; and P, 20. Trace minerals (mg/kg) were Zn, 4,000; Fe, 4,000; Mn, 2,000; I, 300; Co, 80; and Se, 24. Vitamins (IU/kg) were vitamin A, 250,000; vitamin D₃, 50,000; and vitamin E, 4,000.

goal, a lipid-encapsulated CLA supplement was used, and the dose was extrapolated on metabolic BW (MBW) basis from extensive work with dairy cows.

MATERIALS AND METHODS

The experiment utilized 20 multiparous ewes and was conducted in accordance with the UK Home Office Animals (Scientific Procedures) Act 1986. Immediately postlambing, ewes were group-housed on straw and fed a standard ewe concentrate (Table 1) at the rate of 1.5 kg/d in 3 equal meals at 0800, 1300, and 1600 h. In addition, grass hay (*Lolium perenne*) was fed (Table 1), and water was available at all times. At approximately 2 wk postpartum, lambs were weaned, and the ewes were shorn, individually penned, and bedded on sawdust. The level of concentrates was increased to 1.8 kg/d. Ewes were milked twice daily at 0800 and 1600 h through a standard milking parlor designed for ewes. At approximately 5 wk postpartum, ewes were paired based on their milk and constituent yield, BW, and BCS (Russell et al., 1969), which were measured on 3 occasions during the week prior to allocation. Ewes were then randomly allocated to 1 of 2 treatments.

Treatments were 1) control (unsupplemented diet) and 2) diet supplemented with CLA. The supplement (25 g/d) was mixed into the concentrate allocation on a daily basis and provided 2.4 g of *trans*-10, *cis*-12 CLA/d. The dose of *trans*-10, *cis*-12 CLA was extrapolated from data on cow response as summarized by de Veth et al. (2004a), extrapolation across species was based on MBW (Kleiber, 1961; Blaxter, 1989; Terpstra, 2001), and the supplement was a lipid-encapsulated CLA product. The synthesis of CLA from linoleic acid involved a method that produced methyl esters of 2 CLA isomers in equal proportion (*cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA; BASF AG, Ludwigshafen, Germany). The manufacturing of the lipid-encapsulated supplement involves addition of CLA methyl esters to a silica matrix and then coating this complex using a modified fluid bed system with hydrogenated soybean oil (Balchem Encapsulates, New Hampton, NY). The final supplement product was analyzed as described by Perfield et al. (2004) and had a lipid content of 65% and a fatty acid composition of 15% *trans*-10, *cis*-12 CLA; 15% *cis*-9, *trans*-11 CLA; 9% 16:0; 42% 18:0; 12% *cis*-9 18:1; and 1% *cis*-9, *cis*-12 18:2. The experimental design was a 2-period crossover with 10-d treatment periods separated by a 10-d change-over period. Grass hay was available ad libitum. Fresh amounts were provided daily, and refusals were collected every second day. Concentrate and hay samples were collected weekly, stored at -20°C, and composited across all periods prior to analysis. Milk yield was recorded daily at each milking, and samples were collected for analysis of fat, protein, and lactose. On the final day of each treatment period, an additional milk sample was collected at both milkings for fatty acid analysis. Each ewe was weighed, and BCS was determined every 10 d throughout the experiment.

Chemical Analysis

Concentrate and forage samples were analyzed by methods of AOAC (2000) for DM (934.01) and CP (988.05), and NDF and ADF were determined according to Van Soest et al. (1991). Determination of NDF was conducted without sodium sulfite, but with alpha-amylase, and was corrected for ash. The ME content (Mcal/kg of DM) of the concentrate was estimated by neutral detergent cellulase and gammanase according to the Ministry of Agriculture, Fisheries and Food (1993), and that of the hay was determined by neutral detergent cellulase (Dowman and Collins, 1982) using the prediction equation of Moss and Givens (1990).

Milk samples were analyzed for fat, protein, and lactose using a Lactoscope FTIR Spectrophotometer (Delta Instruments B.V., Drachten, Holland) that was cali-

brated using raw milk samples from sheep. Analytical procedures (AOAC, 2000) were conducted for fat by the Röse-Gottlieb method (method 991.20), for protein by total nitrogen (method 991.20), and for lactose by the polarimetric method (method 896.01).

Milk fat was extracted using the method of Hara and Radin (1978), and fatty acid methyl esters were prepared by base-catalyzed transmethylation according to Christie (1982) with modifications by Chouinard et al. (1999). Fatty acid methyl esters were quantified using a gas chromatograph (GC system 6890+ with flame ionization detector, Agilent Inc., Wilmington, DE) equipped with a CP-SIL 88 fused silica capillary column [100 m × 0.25 mm (i.d.) with 0.2-μm film thickness; Varian, Inc., Walnut Creek, CA]. Hydrogen was the carrier gas, and a programmed temperature sequence was used. Further details and conditions have been described previously (Lock et al., 2004a). Fatty acid identification and recoveries were determined using pure methyl ester standards (Nu-Chek Prep, Elysian, MN; Natural ASA, Hovdebygd, Norway). A butter oil reference standard (CRM 164; Commission of the European Community Bureau of References, Brussels, Belgium) was used as a routine check for recoveries and correction factors for individual fatty acids. Yields of milk fatty acids were calculated as described by Schauff et al. (1992).

Statistical Analysis

Milk production and DMI data for the final 2 d of each treatment period were averaged. Intake, milk production and composition, BW, BCS, and milk fatty acid composition were analyzed as a crossover design. The ANOVA used a sheep (row) and period (column) structure; the error term consisted of the interactions among treatment, sheep, and period. Analysis was conducted using Genstat 7.2 (VSN Int. Ltd., Oxford, UK), and results were presented as treatments means with a SEM. Treatment effects were considered significant at $P < 0.05$.

RESULTS

Ewes consumed all of their daily allocation of concentrates, and the ad libitum intake of hay was similar between treatments. Consequently, there was no effect of treatment on DMI, which averaged 2.45 kg/d for d 9 and 10 of the treatment period (Table 2). Intake was also relatively constant across the 2 experimental periods, averaging 2.45 ± 0.2 kg/d (mean ± SD). There was no effect of treatment on average BW or BCS (Table 2).

The temporal pattern for milk fat content and yield demonstrated a progressive decline when ewes received

Table 2. Performance of ewes either unsupplemented (control) or supplemented with conjugated linoleic acid (CLA)¹

Variable	Treatment		SEM	P
	Control	CLA		
DMI, kg/d				
Hay	0.85	0.82	0.021	0.24
Concentrate	1.61	1.61	—	—
Total	2.47	2.43	0.021	0.24
Milk yield, g/d	1,471	1,611	31.9	<0.01
Milk fat				
%	6.4	4.9	0.18	<0.001
g/d	94.6	79.7	2.53	<0.001
Milk protein				
%	4.6	4.5	0.08	0.24
g/d	67.6	72.5	1.52	<0.05
Milk lactose				
%	4.8	4.6	0.09	0.17
g/d	70.3	74.3	1.49	0.07
BW, kg	59.0	58.8	0.23	0.48
BCS ²	1.9	1.9	0.04	0.94

¹Values represent an average of d 9 and 10 of supplementation.

²Scale 0 = thin to 5 = fat (Russell et al., 1969).

the CLA supplement with a significant deviation from control values beginning by d 4 to 5 of treatment (Figure 1). By d 9 and 10, supplemented ewes had a decrease in milk fat content and yield that averaged 23 and 16%, respectively (Table 2). The CLA-induced reduction in milk fat synthesis was associated with a progressive increase in milk yield (Figure 1), which averaged 10% greater on d 9 and 10 of treatment as compared with unsupplemented animals (Table 2). Although milk protein content was not affected by treatment, there was also a progressive increase in milk protein yield (Figure 1), which averaged 7% higher in ewes fed the CLA supplement on d 9 and 10 of treatment (Table 2).

Yields of all milk fatty acids were reduced in CLA-supplemented ewes. On a molar basis, de novo-derived fatty acids (<C16) accounted for 52% of the decrease, fatty acids that were 16 carbons accounted for 28% of the decrease, and fatty acids derived exclusively by uptake from the circulatory system (>C16) accounted for 20% of the decrease (Figure 2). As a consequence of these differences in the magnitude of the decline among fatty acid groups, the profile of milk fat was shifted toward an increased proportion of long-chain fatty acids (Table 3). Several of the fatty acid ratios constituting the desaturase index were also reduced slightly by CLA treatment (Table 3).

Trans-10, *cis*-12 CLA was undetectable in milk fat of unsupplemented animals (<0.01 g/100 g of fatty acids) and increased to 0.12 g/100 g of fatty acids during CLA supplementation (Table 3). The transfer of *trans*-10, *cis*-12 CLA to milk fat can be estimated by comparing dietary intake and milk fat secretion of *trans*-10, *cis*-

Figure 1. Temporal pattern of milk yield (A), milk fat percentage (B), milk fat yield (C), and milk protein yield (D) of ewes unsupplemented (control; ■) or supplemented with conjugated linoleic acid (CLA; △). Values represent means from 20 ewes; SEM = 34.2 g/d, 0.10%, 2.4 g/d, and 1.7 g/d for milk yield, milk fat percentage, milk fat yield, and milk protein yield, respectively.

12 CLA on an individual ewe basis. Using this approach, the estimated transfer efficiency of *trans*-10, *cis*-12 CLA from the dietary supplement into milk fat was $3.8 \pm 0.8\%$.

DISCUSSION

Effects of CLA supplements on milk fat synthesis have been investigated in dairy cows. de Veth et al.

Figure 2. Daily milk fatty acid yield (mmol) in ewes that were unsupplemented (control; open bars) or supplemented with conjugated linoleic acid (CLA; solid bars). Values represent means ($n = 20$) of d 10 of treatment. Fatty acids are categorized according to origin: <C16 represent de novo-synthesized fatty acids, >C16 represent preformed fatty acids taken up from circulation, and C16 fatty acids are derived from both sources. Standard error is indicated by error bars over each column. $P < 0.01$ for all treatment effects.

(2004a) summarized abomasal infusion studies and demonstrated a curvilinear relationship between the reduction in milk fat yield and the abomasal infusion dose of *trans*-10, *cis*-12 CLA. As reviewed by Chilliard et al. (2003), there are many similarities in milk fat synthesis among ruminant species, but also substantial differences in milk fat response to lipid supplements. The present study represents the first to examine effects of CLA on milk fat synthesis in sheep, and the supplement contained 2 CLA isomers, *trans*-10, *cis*-12 and *cis*-9, *trans*-11, in equal concentrations. Whereas *trans*-10, *cis*-12 CLA has been shown consistently to reduce milk fat yield in dairy cows, abomasal infusions of relatively pure *cis*-9, *trans*-11 CLA had no effect on milk fat production (Baumgard et al., 2000, 2002; Looor and Herbein, 2003). Furthermore, milk fat content of *cis*-9, *trans*-11 CLA varies widely among individual cows, but the concentration is not correlated with either milk fat yield or milk fat content (Kelsey et al., 2003; Lock et al., 2005). Therefore, consideration of effects of the CLA supplement on milk fat synthesis in the present study will focus on the *trans*-10, *cis*-12 isomer.

Choosing a suitable rumen-protected formulation and dose for lactating sheep was fundamental to meeting our objective. We utilized a lipid-encapsulated formulation as the rumen-protection method; this formulation of CLA has proven effective at reducing milk fat synthesis in dairy cows (de Veth et al., 2004b; Lock et al., 2004b; Perfield et al., 2004). Our goal was to select a dose of *trans*-10, *cis*-12 CLA that would achieve a 20 to 25% reduction in milk fat yield. Using the dose-response relationship generated from published studies with dairy cows (de Veth et al., 2004a), we calculated that this reduction would require a postruminal dose

Table 3. Fatty acid composition of milk fat from ewes unsupplemented (control) or supplemented with conjugated linoleic acid (CLA)¹

Fatty acid	Treatment		SEM	P
	Control	CLA		
	(g/100 g)			
4:0	4.22	3.99	0.113	0.17
6:0	1.92	1.14	0.030	<0.001
8:0	1.36	0.70	0.025	<0.001
10:0	3.54	1.89	0.065	<0.001
12:0	2.06	1.60	0.027	<0.001
14:0	6.78	6.38	0.112	<0.01
14:1 <i>cis</i> -9	0.46	0.42	0.016	0.100
15:0	0.72	0.68	0.009	<0.01
16:0	31.10	31.50	0.128	<0.05
16:1 <i>cis</i> -9	0.92	0.72	0.021	<0.001
17:0	0.32	0.33	0.004	<0.05
18:0	9.37	11.36	0.155	<0.001
18:1 <i>trans</i> -6 to 8	0.48	0.64	0.008	<0.001
18:1 <i>trans</i> -9	0.38	0.44	0.007	<0.001
18:1 <i>trans</i> -10	0.65	0.90	0.016	<0.001
18:1 <i>trans</i> -11	1.73	2.50	0.070	<0.001
18:1 <i>trans</i> -12	0.31	0.40	0.018	<0.01
18:1 <i>cis</i> -9	23.60	23.70	0.190	0.71
18:2 <i>cis</i> -9, <i>cis</i> -12	6.83	7.11	0.091	<0.05
18:2 <i>cis</i> -9, <i>trans</i> -11 ²	0.82	1.05	0.030	<0.001
18:2 <i>trans</i> -10, <i>cis</i> -12 ²	<0.01	0.12	0.004	<0.001
18:3 <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	0.22	0.22	0.005	0.38
20:0	0.16	0.18	0.001	<0.001
Other	2.05	2.06	0.033	0.79
Summation				
<C16	20.3	16.1	0.27	<0.001
C16 and C16:1	32.1	32.3	0.13	0.30
>C16	47.6	51.6	0.34	<0.001
Desaturase index ³				
14:1/(14:0 + 14:1)	0.063	0.062	0.0024	0.61
16:1/(16:0 + 16:1)	0.029	0.022	0.0006	<0.001
18:1/(18:0 + 18:1)	0.717	0.677	0.0030	<0.001
<i>cis</i> -9, <i>trans</i> -11 CLA/(18:1 <i>trans</i> -11 + <i>cis</i> -9, <i>trans</i> -11 CLA)	0.322	0.296	0.0038	<0.001

¹Values represent milk fatty acid profile on d 10 of supplementation.

²Milk fat contains a number of additional isomers of CLA (*cis/trans*, *cis/cis*, and *trans/trans*), and these coelute under the analytical conditions used in the present study. See reviews by Kramer et al. (2004) and Lock and Bauman (2004) for details on analytical procedures to determine these minor CLA isomers and the range for their prevalence in milk fat.

³Unless otherwise indicated, double bonds are *cis*-9.

of 2.0 to 2.7 g/d of *trans*-10, *cis*-12 CLA in dairy cows. Based on transfer efficiencies observed in previous studies with lipid-encapsulated CLA supplements in lactating cows (de Veth et al., 2004b; Lock et al., 2004b; Perfield et al., 2004), a dietary supplement supplying 10 to 14 g/d of *trans*-10, *cis*-12 CLA would be needed to deliver this postruminal dose. Extrapolating to lactating sheep on the basis of MBW, this would equal ~2.4 g/d of *trans*-10, *cis*-12 CLA supplied as a lipid-encapsulated supplement that would result in ~0.4 g/d of *trans*-10, *cis*-12 CLA reaching the abomasum of CLA-supplemented ewes. As reviewed by Kleiber (1961) and Blaxter (1989), the concept of MBW was initially developed for comparisons of metabolic rate across species; subsequently, it was used for the inter-species calculation of drug dosages and comparisons

of animal performance variables, including daily milk energy secretion. More recently, Terpstra (2001) summarized published studies with humans and mice and concluded that CLA effects on reducing body fat were more similar between species when compared on the basis of metabolic rate (i.e., MBW).

Feeding of the CLA supplement to lactating ewes resulted in a 23% reduction in milk fat content and a 16% decrease in fat yield (Table 2). These values are comparable with the 20 to 25% reduction that was the basis for our calculations of dosage and similar to the results of de Veth et al. (2004b), who observed a 23 and 21% reduction in milk fat content and yield, respectively, when the same lipid-encapsulated CLA supplement was fed at an equivalent dose (MBW basis) to dairy cows. However, had the dose of CLA been extrapo-

lated on a BW basis, we would have anticipated different values. On a BW basis, the dose of CLA for lactating ewes would have been only about one-half of what we actually used; if the level of CLA supplement we used in ewes had yielded a response comparable with cows on a BW basis (de Veth et al., 2004b), the reduction in milk fat yield should have been about 35%. Thus, the present study provides evidence that *trans*-10, *cis*-12 CLA is effective at reducing milk fat synthesis in sheep, and the magnitude of the reduction appears similar to dairy cows when the CLA dose is expressed on a MBW basis. Recent studies with lactating goats have used doses of CLA extrapolated from cow data on a BW basis, and the marginal or lack of effects on milk fat (Erasmus et al., 2004; Schmidely and Morand-Fehr, 2004) are in striking contrast to our results with lactating sheep. However, data with goats represented brief reports; therefore, additional studies at multiple doses will be required for both goats and sheep to arrive at firm conclusions on comparative responses among ruminant species.

In general, the unsupplemented ewes had a milk fat content and fatty acid profile similar to other studies with lactating sheep (Rotunno et al., 1998; Sevi et al., 2002). Milk content of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA in ewes fed the control diet was, however, lower than that reported in a survey of ewes grazing grass (Nudda et al., 2005), but was comparable with values when a dried, complete diet was fed (Luna et al., 2005). When ewes received the CLA supplement, the transfer efficiency of *trans*-10, *cis*-12 CLA into milk fat averaged 3.8%; previous studies reported a 2.6 to 7.9% transfer efficiency for *trans*-10, *cis*-12 CLA when a lipid-encapsulated CLA supplement was fed to dairy cows (de Veth et al., 2004b; Lock et al., 2004b; Perfield et al., 2004). Transfer of fatty acids from the rumen-protected supplement into milk fat is influenced by both the protection provided from metabolism by rumen bacteria and postruminal availability (Wu and Papas, 1997). There is substantial opportunity to improve the "rumen-protected" formulations of CLA based on the 22% transfer efficiency observed in a summary of published studies involving abomasal infusion of *trans*-10, *cis*-12 CLA in dairy cows (de Veth et al., 2004a).

When ewes received the CLA supplement, the reduction in milk fat yield was gradual over the first few days of treatment (Figure 1) and involved milk fatty acids of all chain lengths, although a relatively greater reduction occurred in de novo-synthesized fatty acids (≤ 16 carbons; Figure 2). Both the temporal pattern and the effects on specific milk fatty acids are similar to what has been observed in lactating dairy cows when *trans*-10, *cis*-12 CLA has been abomasally infused or fed as a rumen-protected supplement (e.g., Baumgard

et al., 2000; 2001; Loor and Herbein, 2003; de Veth et al., 2004a; Perfield et al., 2004). A slight reduction was observed in fatty acid pairs constituting the desaturase index in the current study. This index is a proxy for Δ^9 -desaturase and has been shown to be correlated with Δ^9 -desaturase mRNA and enzyme activity in lactating mice (Singh et al., 2004) and goats (Bernard et al., 2005), and markedly decreased in dairy cows when Δ^9 -desaturase is inhibited by infusions of cyclopropenoic fatty acids (Bauman et al., 2003). Little or no effect has been observed on the desaturase index in dairy cows when low doses of *trans*-10, *cis*-12 CLA have been fed (Perfield et al., 2004), whereas higher doses of *trans*-10, *cis*-12 CLA have been shown to inhibit Δ^9 -desaturase, causing a decrease in the desaturase index (Baumgard et al., 2001; Mackle et al., 2003).

A striking result in the current study was the apparent repartitioning in nutrient use that occurred in the CLA-supplemented ewes. The CLA-induced reduction in milk fat yield coincided with an increase in milk yield (10%) and milk protein yield (7%). Milk fat is the major cost of milk synthesis, as it is energetically equivalent to over one-half of the costs associated with milk synthesis. Consequently, a decrease in milk fat output requires a repartitioning in the use of nutrients. However, studies with lactating cows have typically not observed an effect on the yield of milk or milk protein in response to a CLA-induced reduction in milk fat secretion either in short-term studies, as reviewed by Bauman et al. (2003), or in long-term investigations (Perfield et al., 2002; Selberg et al., 2004; Castañeda-Gutiérrez et al., 2005; Grevais et al., 2005). The only exception has been CLA-supplemented cows in early lactation (Bernal-Santos et al., 2003) and pasture-fed cows (Mackle et al., 2003). Thus, in situations where nutrient supply may be marginal, CLA-induced milk fat depression may allow a repartitioning of nutrients to support an increase in the synthesis of milk and milk protein. However, nutrient status was adequate in our investigation with lactating ewes. The present study is too limited to allow inferences as to mechanism, but clearly further investigations of these effects are warranted.

In conclusion, the results of the present study demonstrate that a CLA supplement containing *trans*-10, *cis*-12 reduced milk fat synthesis in lactating sheep in a manner similar to that observed in lactating dairy cows when fed at an equivalent dose (MBW basis). Furthermore, the reduction in milk fat coincided with an increase in milk and milk protein yield. Although additional studies are necessary to verify and extend these original results, our observations indicate a potential for CLA supplements as a management tool in lactating ewes. In addition, this study has demonstrated that lactating sheep may represent an effective alternative

model to elucidate the mechanism of action by which CLA inhibits milk fat synthesis.

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