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Enhancing α-Tocopherol and Linoleic Acid in Ewe's Milk by Feeding Emulsified Sunflower Oil and DL-α-Tocopheryl Acetate in a Chemically Treated Protein Matrix

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Chemically treated dietary supplements (CTDS) were prepared with defatted soy flour, sunflower oil, DL-α-tocopheryl acetate (TA), and 2,3-butanedione (a generally recognized as safe chemical), and the effects on α-tocopherol (VE) concentration and fatty acid profile in ewe's milk were estimated. Ewes fed control diet (CD) had the lowest levels of milk VE (0.66 μg/mL) and serum VE (1.59 μg/mL). Feeding ewes the CD plus 500 IU of TA increased milk and serum VE by 30 and 70%, respectively, compared to ewes fed the CD. The CTDS-fed ewes showed further increased milk and serum VE levels by 40 and 32%, respectively, over those in ewes fed the CD plus 500 IU of TA. Feeding ewes CTDS did not affect milk fat content (3.13%) but altered the fatty acid composition by decreasing the levels of hypercholesteremic fatty acids, while increasing the content of linoleic acid (8.5%). Inclusion of CTDS in ruminant diets might produce nutritionally enhanced milk products.

KEYWORDS: α-Tocopherol; α-tocopheryl acetate; ewe's milk; linoleic acid

INTRODUCTION

Milk is an important source of nutrients essential to humans, but bovine milk generally contains 70% saturated fat. Consumption of high levels of saturated fat raises low-density lipoprotein (LDL) cholesterol levels in blood and increases the risk of cardiovascular disease (*1*, *2*). Polyunsaturated fats tend to lower blood serum cholesterol and provide essential fatty acids for growth and development (*3*). Dairy scientists have reduced the high levels of saturated fatty acids (SFA) and have increased the levels of unsaturated fatty acids in milk by feeding various dietary fat supplements (*4*, *5*). However, milk fat of ruminants may not be substantially changed through the dietary intake of high levels of polyunsaturated fatty acids (PUFA) because PUFA are biohydrogenated by ruminal microorganisms (*4*−*6*). The most effective way to assuage lipolysis and biohydrogenation in the rumen is through formaldehyde treatment of dietary fat supplements (*4*). This practice has not been adopted in the United States because of the toxicity and carcinogenicity of formaldehyde. Generally recognized as safe, chemicals such as acetaldehyde and diacetyl have been used as alternative agents to form an oil and protein matrix with protective properties similar to those produced with formaldehyde (*7*, *8*). Additional research is needed to substantiate the claim that by feeding ruminants with vegetable oil containing high levels of PUFA the PUFA content in milk is increased.

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In addition, modification of milk fat by feeding various dietary fat supplements containing unsaturated fatty acids increases the risk of developing an oxidized flavor in the milk because of increased susceptibility to oxidation (*9*, *10*). Vitamin E has been fed to ruminants for enhancing immune systems (*11*, *12*) and minimizing off-flavors in milk due to lipid oxidation (*9*, *10*, *13*). However, the absorption of tocopherols is incomplete in ruminants (*14*, *15*). Absorption of tocopheryl acetate is enhanced by optimizing the esterase activity in the intestine (*14*, *16*); however, this approach may not be an effective method to produce the desired changes. Uptake of tocopherols in ruminants can be increased by feeding an emulsifiable form of vitamin E (*17*).

The objectives of this experiment were (1) to produce a chemically treated dietary fat supplement containing emulsified α-tocopheryl acetate in sunflower oil; (2) to estimate the efficacy of feeding dietary fat supplements to incorporate α-tocopherol in blood and milk; and (3) to determine the deposition of linoleic acid in milk fat.

MATERIALS AND METHODS

Peparation of Chemically Treated Dietary Fat (CTDS) Supplement. CTDS containing emulsified DL-α-tocopheryl acetate (BASF Corp., Mt. Olive, NJ) in sunflower oil (AC Humko Inc., Memphis, TN) were embedded in matrices of diacetyl-treated soy proteins (*7*, *8*). To accomplish this, sodium caseinate (5.49%, w/w) and soy lecithin (0.07%, w/w) were dispersed in a preheated (80 °C) 0.1% (w/v) sodium hydroxide (NaOH) solution (55.0%, w/w) in a steam-jacketed stainless kettle (DI Foodservice Co., Jackson, MI). The mixture in the kettle was agitated and further heated to 85 °C. In another container 0.5% (w/v) DL-α-tocopheryl acetate (TA) dispersed in sunflower oil (13.7%,

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Table 1. Chemical Composition of Basal Diet and Chemically Treated Dietary (CTDS) Gel Supplement[a]

	basal	CTDS gel
macrocomponent[b] (%)		
moisture	11.8	50.6
protein	16.1	15.1
fat	3.58	14.9
α-tocopheryl acetate (IU/kg)		466
fatty acid (wt %)		
myristic (C14:0)	0.1	
palmitic (C16:0)	10.5	7.0
stearic (C18:0)	3.2	2.6
oleic (C18:1)	22.3	17.2
linoleic (C18:2)	54.5	71.4
linolenic (C18:3)	8.3	0.6
eicosanic (C20:0)		0.1
9-eicosenoic (C20:1)		0.2
arachidonic (C20:4*n*6)	0.2	0.6
eicosapentenoic (C22:5*n*3)	0.9	0.3

[a] Ground chemically treated dietary supplement gel containing sunflower oil and DL-α-tocopheryl acetate. [b] Weight percent wet basis.

Table 2. Ingredient Composition (Percent) of the Diets Fed to Lactating Ewes on an As-Fed Basis

	diet		
ingredient	control	vitamin E	CTDS gel
coarse ground corn	63.8	63.8	42.5
alfalfa pellets	19.9	19.9	13.3
soybean meal[a]	10.0	10.0	6.7
rolled oats	5.0	5.0	3.3
trace mineral mix[b]	0.5	0.5	0.3
limestone	0.3	0.3	0.2
antibiotic[c]	0.2	0.2	0.1
ammonium chloride	0.3	0.3	0.2
CTDS gel[c]			33.3
α-tocopheryl acetate (IU)		500	500

[a] 48% crude protein. [b] Compositon: NaCl, 45–50%; Ca, 9.0–10.8%; P, >4.5%; Mg, >1.5%; K, >0.9%; S, >0.3%; Zn, >1.55%; I, >180 ppm; Fe, >2000 ppm; Mn, >4000 ppm; Se, >60 ppm; vitamin A, >2,200,000 IU; vitamin D_3, >165,000; vitamin E, >6600 IU/kg. [c] Auromycin 50.

w/w) was prepared. The heated mixture in the kettle was transferred into a Stephan Universal Schnell Cutter (Couch Supply Inc., Kansas City, MO). In addition, defatted soy flour (21.5%, w/w) and sunflower oil containing TA (13.8%, w/w) were poured into the cutter. Shearing forces continuously mixed slurries in the cutter until the internal temperature of the contents reached 85 °C. While the contents were under the continuous shearing motion, 2,3-butanedione (4.18%, w/w) was delivered into the slurries to form a gel. The gels were stored at 5 °C overnight (8 h). The CTDS gel contained ~500 IU of vitamin E/kg and linoleic acid made up to 71.4% of total fatty acids (**Table 1**). The CTDS gel was ground through a 0.625 cm screen in a Hobart grinder (Hobart Co., Troy, OH), packaged in closed plastic bags, and stored at 4 °C until fed.

Feeding Animals and Collecting Samples. Experimental procedures were conducted according to guidelines and approval of The University of Tennessee Animal Care and Use Committee. Six crossbred (Dorset and Suffolk) lactating ewes were selected from The University of Tennessee research flock. A 7-day adjustment period was used to adapt the ewes to new feeding facilities to allow recovery from stress. Two ewes were randomly assigned to each of three pens. Pens were in an enclosed barn, and each ewe was allowed 0.495 m^2 of floor space and provided ad libitum access to water. Ewes were randomly assigned in a 3 × 3 Latin square design to a basal diet (control) or basal diet supplemented with either DL-α-tocopheryl acetate (vitamin E) or emulsified DL-α-tocopheryl acetate (CTDS gel) in sunflower oil (**Table 2**). The experiment consisted of three separate feeding periods; each feeding period consisted of 7 days for adjustment to assigned diets followed by 3 days of sample collection. Twice daily, ewes were fed one of three experimental diets (1.5 kg) plus 2.5 kg of grass hay as a forage source and 20 g of molasses as an α-tocopheryl acetate carrier.

During the collection period, two samples of milk and blood were collected from each ewe on the 8th and 10th days. Blood samples were collected by jugular venipuncture into 20-mL Vacutainer tubes and placed in ice to clot. Serum was separated from the clotted blood sample by centrifugation at 2000*g* for 20 min at 4 °C (Sorvall Super-Speed RC2-B automatic refrigerated centrifuge, Newton, CT). Serum samples were placed in screw-cap vials and were stored at −18 °C until analyzed. Milk was collected in 1.0-L glass jars from each lactating ewe. The raw milk was stored at 4 °C for 8 h, then delivered into a screw-cap brown bottle (50 mL), flushed with nitrogen gas, and stored at −18 °C until further analysis.

α-Tocopherol Analysis. Concentrations of α-tocopherol in serum and milk samples were determined according to procedures adapted from that of Liu et al. (*18*). An aliquot (1.0 mL) of each sample was delivered into a 25-mL screw-cap test tube. Two hundred and fifty milligrams of ascorbic acid (Sigma Chemical Co., St. Louis, MO) and 7.3 mL of a saponification solution that consisted of (8.08%, w/v) NaOH (Fisher Scientific, Pittsburgh, PA) in a mixed solvent of absolute ethanol (55%, v/v) and deionized distilled water (45%, v/v) were placed in each test tube. Test tubes were shaken until ascorbic acid completely dissolved and then placed in a water bath shaker (60 strokes/min) at 80 °C for 15 min. The tubes were removed and cooled in an ice bath. Four milliliters of isooctane was added, and the tubes were vigorously mixed for 2 min by a vortex (Fisher Scientific). The mixture was allowed to stand until the separation of phases occurred. An aliquot from the upper phase was filtered through a 0.45-μm membrane. Fifty microliters of sample was injected into an HPLC (water), separated with a 2.5 × 250 mm BondClone 10 silica column (Phenomenex, Inc., Torrance, CA), and eluted by an isocratic mobile phase of hexane and 2-propanol (99:1 v/v) at a flow rate of 1.5 mL/min. The eluate was detected with a Shimadzu RF-530 fluorescence detector (Shimadzu Corp., Columbia, MD) set at an excitation wavelength of 295 nm and an emission wavelength of 330 nm. The α-tocopherol (Sigma Chemical Co.) was used to calibrate a standard curve. Triplicate standards were prepared between 0.1 and 20 ppm of α-tocopherol in hexane. All samples were analyzed in triplicate.

Milk Lipid Extraction and Fatty Acid Analysis. Total lipids were extracted from each milk sample by means of the chloroform/methanol (2:1 v/v) method of Melton et al. (*19*) as modified by Lee et al. (*7*) and measured gravimetrically. The modification was that the amount of sample was reduced from 50 to 2.0 mL, with solvents and other chemicals reduced accordingly. The extracted lipid was transferred to a screw-cap test tube, flushed with nitrogen gas, and stored at −18 °C until fatty acid analysis.

To determine the fatty acid composition, 100 mg of extracted lipid was saponified with 4 mL of NaOH (0.5 N) in methanol. The mixture was esterified with 5.0 mL of (14%, v/v) boron trifluoride/methanol according to the AOCS method (*20*) of preparation of fatty acid methyl esters (FAME). Gas chromatography (GC) was conducted on the same FAME preparation. A Shimadzu GC-9A gas chromatograph (Shimadzu Corp.) equipped with a flame ionization detector and an automatic injection system (AOC-9, Shimadzu) was used. The column was a fused silica SP-2380 capillary column (60 m × 0.25 mm i.d., Supelco, Inc., Bellefonte, PA), and the FAME preparation was injected in the split mode with a split ratio of 1:30. Helium was selected as the carrier gas and set at a flow rate of 1.5 mL/min. Both injector and detector temperatures were set at 270 °C. The initial oven temperature was 50 °C, held for 2 min and subsequently raised by 4 °C/min to 250 °C, and held for 15 min, resulting in a total analysis time of 67 min. Chromatopac Data Achieve Utility version 3.1 software (Shimadzu Scientific Instruments Inc., Columbia, MD) was used for data analysis. Identification was achieved by comparing the retention time of unknown FAME with those of known FAME standard mixtures (Alltech Associates, Inc., Deerfield, IL; Sigma-Aldrich Corp., Bellefonte, PA). Quantitative analysis of FAME was based on the following procedures: Standards containing known weight percentages of individual FAME were analyzed by the GC, and the correction factors relative to C16:0 were calculated according to the AOCS method for fatty acid

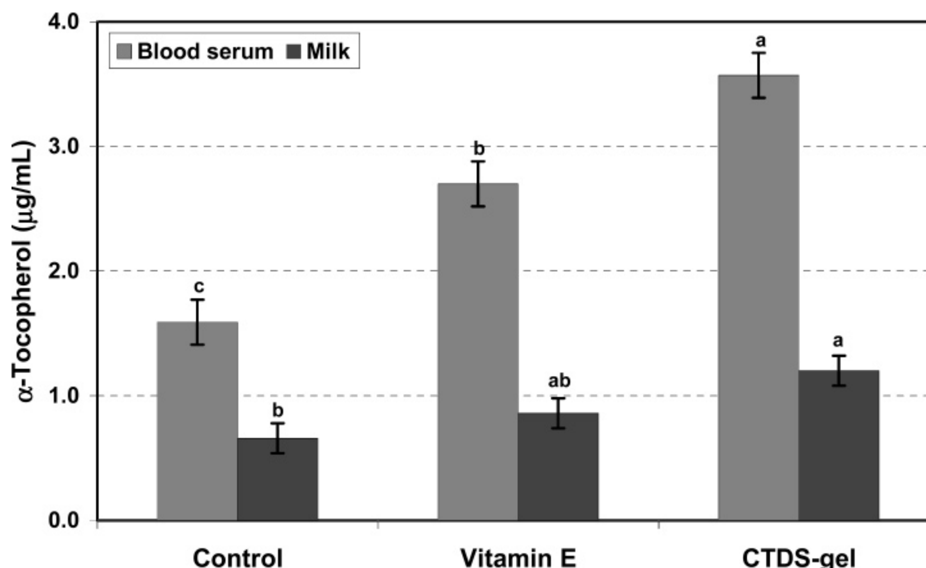

Figure 1. Accumulation of α-tocopherol in blood serum (SE = 0.18) and milk (SE = 0.12) of ewes fed the experimental diets (control, vitamin E, and CTDS gel). Bars bearing unlike letters are different ($P < 0.05$).

analysis (*20*). The area of individual FAME was corrected using the correction factor, and the relative weight percentages of each FAME (C4:0−C22:6) in each sample were calculated using their corrected area (*20*).

Statistical Analysis. All data were analyzed as a 3 × 3 Latin square design using the PROC MIXED procedure of the SAS (release 8.2, SAS Institute Inc., Cary, NC) with ewe considered to be a random effect and experimental period and diet treatment considered to be fixed effects. Least-squares means were calculated for α-tocopherol, total lipid, and fatty acid and statistically separated by pairwise *t* test (PDIFF option) protected by the ANOVA *F* test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Feed Intakes. Average daily dry matter intakes of the ewes fed each dietary treatment during the feeding trial were 1061, 1072, and 879 g for control, vitamin E, and CTDS gel diets, respectively. On the basis of daily intakes, ewes consumed an average of 250 IU (vitamin E diet) and 244 IU (CTDS gel diet) of α-tocopheryl acetate per day. Average daily total fat intakes of the ewes were 136, 144, and 259 g for control, vitamin E, and CTDS gel diets, respectively.

Accumulation of α-Tocopherol in Serum and Milk. Plasma or serum levels of α-tocopherol are reliable indicators of nutritional vitamin E status and are correlated with the intake of vitamin E (*12*, *15*). The α-tocopherol content of serum and milk samples of ewes fed the control, vitamin E, or CTDS gel diet are presented in **Figure 1**. Feeding ewes CTDS gel containing DL-α-tocopheryl acetate (TA) increased the α-tocopherol levels ($P < 0.05$) in serum (3.57 μg/mL) compared with the levels of α-tocopherol in serum (1.59 and 2.07 μg/mL, respectively) of ewes fed the control or vitamin E diet. The increased levels of α-tocopherol were expected in the serum from ewes fed the vitamin E diet, but remained lower than the levels for ewes fed the CTDS gel diet. In previous studies with lactating cows, serum levels of vitamin E were not affected by feeding added fat in the diet (*21*, *22*). In this study, serum levels of α-tocopherol in lactating ewes were further increased by feeding emulsified vitamin E in a chemically treated oil and protein matrix (CTDS gel) over those found when ewes were fed vitamin E alone. These findings are consistent with most recent studies involving feeding vitamin E to dairy cows (*9*, *10*). Feeding ewes with additional vitamin E (8.06 μg/mL) and CTDS gel (1.20 μg/mL) also increased ($P < 0.05$) the α-tocopherol in milk compared to those fed the control diet (0.66 μg/mL). However, no difference ($P > 0.05$) was found between the α-tocopherol content of milk from ewes fed the vitamin E and CTDS gel diets. The ewes fed the CTDS gel diet had higher levels of α-tocopherol in milk than did ewes fed the vitamin E diet ($P = 0.1$). The intake of dietary supplements of α-tocopheryl acetate elevated both serum and milk α-tocopherol. In recent studies, the concentrations of milk α-tocopherol were not substantially changed ($P > 0.1$) in dairy cows by feeding vitamin E incorporated in dietary fat supplements compared with those fed vitamin E without additional fat sources (*10*, *22*, *23*). In general, only ∼2% of ingested α-tocopherol is actually secreted in milk (*22*, *23*). In our study, milk from ewes fed fat protected from rumen degradation (CTDS gel) tended to increase the levels of α-tocopherol compared with those fed vitamin E alone. The changes in serum or milk α-tocopherol levels in ewes fed the CTDS gel diet may be explained by either the CTDS gel matrix enhancing the absorption of α-tocopherol in the small intestinal of ewes (*14*) or the higher concentration of linoleic acid resulting from protected fat from ruminal biohydrogenation (*23*).

α-Tocopherol has been reported to act as a free radical scavenger but has also been shown to quench $^1O_2$ via a charge-transfer quenching mechanism (*24*); it plays a critical role in retarding the lipid oxidation of milk (*25*, *26*). In general, bovine raw milk fat contains α-tocopherol at ∼13−30 μg/g of milk fat (*25*, *26*). In studies of antioxidant incorporation in milk to reduce lipid oxidation, oxidized flavor decreased significantly with increased α-tocopherol levels from 17.6 to 37.8 μg/g of milk fat from dairy cows fed a diet supplemented with 3000 IU of DL-α-tocopheryl acetate for 14 days (*15*), whereas α-tocopherol content was increased by 40.1 μg/g of milk fat in ewes fed CTDS gel (500 IU) for 10 days in our study. Therefore, ovine milk from ewes fed CTDS gel might delay or reduce lipid oxidation as a result of the incorporation of linoleic acid through the intake of sunflower oil protected from ruminal biohydrogenation.

Milk Fat Composition. The concentration of total fat in milk was not different ($P > 0.05$) from that of ewes fed experimental diets (control, vitamin E, and CTDS gel diets). The total fat contents were 3.26, 3.14, and 2.99% for control, vitamin E, and CTDS gel diets, respectively. When dietary fat is fed to

Table 3. Least-Squares Means of Levels[a,b] (Weight Percent) of Fatty Acids in Milk Fat from Ewes Fed the Experimental Diets

fatty acid	diet[c] control	vitamin E	CTDS gel	SEM
C6:0	3.87	3.09	2.76	0.59
C7:0	0.00	0.04	0.00	0.03
C8:0	2.09a	2.05a	0.64b	0.36
C9:0	0.22ab	0.32a	0.16b	0.05
C10:0	8.09a	7.25a	3.18b	0.80
C11:0	0.26a	0.23a	0.11b	0.03
C12:0	4.63a	4.14a	2.13b	0.45
C13:0	0.14	0.14	0.29	0.13
C13:1	0.21a	0.18ab	0.10b	0.03
C14:1	0.52a	0.47a	0.30b	0.05
C15:0	1.13a	0.97a	0.79b	0.06
C16:0	24.39a	22.30a	17.32b	0.82
C16:1*t*9	0.66	0.60	0.57	0.06
C16:1*c*9	1.02	0.94	0.83	0.07
C17:0	0.88	0.96	0.83	0.13
C17:1	0.11	0.10	0.09	0.03
C18:0	11.54	15.09	16.77	1.78
C18:1*t*9	2.30b	3.23b	5.46a	0.49
C18:1*c*9	29.97b	29.61b	33.64a	1.93
C18:2-isomers[d]	1.09b	1.16b	1.40a	0.08
C18:2*t*9*t*12	0.21b	0.23ab	0.27a	0.01
C18:2*c*9*c*12	2.45b	3.79b	6.86a	0.95
C18:3*n*6	0.40	0.41	0.29	0.04
C18:3*n*3	0.80	0.70	1.03	0.17
C20:0	0.73	0.72	0.69	0.09
C20:1	0.20	0.22	0.32	0.16
C20:2*n*6	0.14	0.23	0.05	0.09
C20:3*n*6	0.42	0.28	0.32	0.26
C20:4*n*6	0.15	0.47	0.21	0.14
C21:0	0.11b	0.30a	0.22ab	0.05
C22:1	0.13	0.21	0.05	0.06
C20:5*n*3	0.26	0.24	0.29	0.07
C22:5*n*3	0.15ab	0.30a	0.09b	0.06
C22:6*n*3	0.14	0.17	0.14	0.03

[a] $n = 6$. [b] Least-square means in a row followed by unlike letters are different ($P < 0.05$). [c] Control, basal diet, hay, and molasses; vitamin E, control diet plus 500 IU of α-tocopheryl acetate; CTDS gel, chemically treated gel (sunflower oil + 500 IU of α-tocopheryl acetate) plus control containing less basal diet. [d] Tentatively identified as isomers of linoleic acid (C18:2*n*6); peaks with retention time between those of C18:2*t*9*t*12 and C18:2*c*9*c*12.

ruminants at levels >3−4% of the diet dry matter, rumen microbial activity is reduced and fiber digestibility is decreased. Under these dietary conditions, milk fat content is reduced (*6*, *27*). When dietary fat (>4% in the diet) is protected from ruminal biohydrogenation, the milk fat content in ruminants is not reduced (*28*). The CTDS gel used in our study provided an average of 15% of fat in the diet without adversely affecting the milk fat content. This validates our hypothesis that the fat contained in the CTDS gel escaped ruminal biohydrogenation. Additionally, intake of high levels of vitamin E might eliminate the reduction of milk fat associated with feeding unsaturated fat (*29*).

Fatty acids identified in milk samples (**Table 3**) were divided into three major groups: saturated, monounsaturated, and polyunsaturated fatty acids. Saturated fatty acids were segregated into short-chain (C4−C7), medium-chain (C8−C14), and long-chain (C15−C21) fatty acids. In short-chain saturated fatty acids (C4:0−C7:0), no difference ($P > 0.05$) was found in the mean concentrations of caproic (C6:0) or heptanoic (C7:0) acids in milk samples from ewes fed the three experimental diets. Butyric (C4:0) acid was not detected in milk in our study. In medium-chain saturated fatty acids (C8:0−C14:0), ewes fed the control or vitamin E diet had higher ($P < 0.05$) concentrations of caprylic (C8:0), nonanoic (C9:0), capric (C10:0), undecanoic (C11:0), and lauric (C12:0) acids in milk fat than ewes fed the CTDS gel diet. The level of nonanoic (C9:0) was not significantly different ($P > 0.05$) in milk fat from ewes fed the control and vitamin E diets. In long-chain saturated fatty acids (C15:0−C21:0), there were differences ($P < 0.05$) in the concentrations of pentadecanoic (C15:0), palmitic (C16:0), and uncosanoic (C21:0) acids in milk fat among the experimental diets. The most abundant long-chain saturated fatty acids present were palmitic (C16:0) and stearic (C18:0) acids. Ewes fed the CTDS gel supplement had lower ($P < 0.05$) levels of palmitic (C16:0) acid than ewes fed the control or vitamin E diets. There was no difference ($P > 0.05$) found in the stearic (C18:0) acid in milk from ewes fed the experimental diets. Feeding protected dietary lipid supplements such as calcium salts and encapsulated vegetable oils resulted in lower short- and medium-chain fatty acids in the milk of ruminants when compared to those levels in animals receiving no dietary fat supplementation (*30*−*32*). A similar shift in fatty acids was expected in the present study because protected vegetable oils fed to dairy ruminants usually increases milk fat yield and the proportion of long-chain fatty acids in milk. All of the short- and medium-chain fatty acids (C4−C14) and half of the palmitic acid (C16:0) in milk fat are synthesized de novo in the mammary gland (*28*, *33*). The remaining half of the palmitic acid (C16:0) and the C18 and longer fatty acids in milk fat are obtained directly from blood supply to the mammary gland through dietary or adipose origin (*28*, *33*).

Monounsaturated fatty acids, 9-tridecenoic (C13:1), myristoleic (C14:1), *trans*-7-hexadecenoic (C16:1t9), palmitoleic (C16:1c9), margaroleic (C17:1), elaidic (C18:1*t*9), oleic (C18:1*c*9), 11-eicosaenoic (C20:1), and 13-docosenoic (C22:1) acids, were detected in milk samples. Significant differences ($P < 0.05$) were found in the concentrations of four different monounsaturated fatty acids (C13:1, C14:1, C18:1*t*9, and C18:1*c*9) among milk samples from ewes fed the experimental diets. The levels of 9-tridecenoic (C13:1) and myristoleic (C14:1) acids in milk fat of ewes fed CTDS gel were lower ($P < 0.05$) when compared with the levels of those two fatty acids in milk fat of ewes fed the control or vitamin E diet. The concentrations of elaidic (C18:1*t*9) and oleic (C18:1*c*9) acids were increased ($P < 0.05$) significantly in milk from ewes fed CTDS gel supplements compared with the ewes fed any other diet. The high content of oleic acid (C18:1*c*9) resulted from the actions of desaturase in converting stearic acid (C18:0) to *cis*-oleic acid (C18:1*c*9). Additionally, higher oleic acid, originating from the rumen-protected fat supplement, may be supplied directly from the blood to the mammary gland (*28*, *33*). The higher ($P < 0.05$) concentration of elaidic acid (C18:1*t*9) in milk fat from ewes fed CTDS gel supplements was the result of biohydrogenation of either linoleic (*28*, *33*) or oleic acids (*34*) to *trans*-oleic acid (C18:1*t*9) in the rumen.

In polyunsaturated fatty acids, namely, linoelaidic (C18:2*t*9*t*12), linoleic (C18:2*c*9*c*12), γ-linolenic (C18:3*n*6), linolenic (C18:3*n*3), eicosadienoic (C20:2*n*6), eicosatrienoic (C20:3*n*6), arachadonic (C20:4*n*6), eicosapentaenoic (C20:5*n*3), docosatrienoic (C22:5*n*3), and docosahexaenoic (C22:6*n*3), including unidentified isomers of linoleic acid (isomers-C18:2), significant differences ($P < 0.05$) were found in all three C18:2 (linoelaidic, linoleic, and unidentified linoleic isomers) and docosatrienoic (C22:5*n*3) acids in milk from ewes fed the three different diets. The CTDS gel-fed ewes had higher ($P < 0.05$) levels of linoleic (C18:2*c*9*c*12) acid in their milk than ewes fed the other diets. Levels of linoleic (C18:2*c*9*c*12) acid in milk from ewes fed

either the control or vitamin E diet were not significantly different ($P > 0.05$).

Feeding lactating ewes with CTDS gel supplements containing linoleic acid (71% of total fatty acid) increased the linoleic acid concentrations in their milk fat from 3.8 to 8.5%. This increase in linoleic acid levels resulted in a concomitant decrease in the concentrations of caprylic (C8:0), nonanoic (C9:0), capric (C10:0), undecanoic (C11:0), lauric (C12:0), pentadecylic (C15:0), and palmitic (C16:0) acids from 40.8% in milk from control diet fed ewes to 24.3% in milk from CTDS gel-fed ewes. Others have demonstrated that feeding protected canola seeds or soybean oil to lactating ruminants increased milk C18:1, C18:2, and C18:3 while sharply decreasing the C16:0 percentage (*28*, *30*−*32*). However, feeding unprotected vegetable oils containing the high levels of C18:2 increased mainly C18:0 and C18:1; the increase of the latter of these is probably due to unidentified trans isomers of C18:1. This illustrates that both total and partial hydrogenations of unsaturated fatty acids take place in the rumen (*28*, *30*−*32*). When ewes were fed CTDS gel supplements, the linoleic acid content in milk was almost 3t times higher than ewes fed the control diet; however, the percentage of saturated fatty acids containing C6−C16 carbons was decreased from 45.5 (control diet) to 27.8% (CTDS gel diet). The ratio of long-chain (C14−C18) saturated fatty acids to monounsaturated and polyunsaturated fatty acids in milk fat decreased from 7.5:6.8:1 in ewes fed the control diet to 3.5:4.1:1 in ewes fed the CTDS gel diet. However, the proportion of trans fatty acids (C18:1*t*9) increased ($P < 0.05$) in milk fat form ewes fed the CTDS gel supplement.

The chemically treated dietary supplement containing α-tocopheryl acetate and sunflower oil (71% linoleic acid) increased the concentrations of α-tocopherol in serum and milk. In addition, linoleic acid concentrations in milk fat were enhanced by the gel supplement along with decreasing hypercholesterolemic fatty acids (lauric, myristic, palmitic, and stearic acids). The chemically treated dietary supplement gel can protect unsaturated dietary fats from the biohydrogenation that occurs in the rumen and may provide a means of increasing the absorption of α-tocopherols in the gastrointestinal tract. Feeding lactating dairy ewes with the chemically treated dietary supplement improves the nutritional value of milk and extends the shelf life due to retardation of lipid oxidation of the milk fat.

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