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Incorporation of α-Tocopherol and Linoleic Acid in Fresh Lambs by Feeding Chemically Treated Dietary Supplements Containing DL-α-Tocopheryl Acetate and Sunflower Oil

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The effects of feeding chemically treated dietary supplements (CTDS) containing sunflower oil and DL-α-tocopheryl acetate (TA) on α-tocopherol content and fatty acid profile in edible tissues of lambs were estimated. Compared with lambs fed control diet (CD), lambs fed CD plus 250 IU of either TA or CTDS increased serum α-tocopherol. The CTDS-fed lambs further increased serum α-tocopherol by 29% over those fed CD plus 250 IU of TA. Lambs supplemented with TA or CTDS increased α-tocopherol in muscle and adipose tissues as compared with lambs fed CD. The CTDS-fed lambs had higher levels of α-tocopherol in gluteus medius (7.55 vs 6.05 μg/g), psoas major (7.43 vs 6.02 μg/g), and subcutaneous fat (12.6 vs 9.98 μg/g) compared with the TA-fed lambs. Feeding lambs CTDS also substantially increased levels of linoleic acid in the adipose tissues while decreasing the content of palmitic and oleic acids.

KEYWORDS: α-Tocopherol; α-tocopheryl acetate; lambs; linoleic acid

INTRODUCTION

Vitamin E has been incorporated into fresh meat by either dietary or post-mortem supplementation to improve lipid and color stability of muscle foods (*1*−*3*). The color of fresh meat is an important factor for judging freshness and quality by consumers (*4*, *5*). Changes in meat color are closely associated with lipid and pigment oxidation (*3*) as well as with microbial load (*6*). Meat tissue contains primarily myoglobin (Mb); binding of oxygen to the sixth ligand yields oxymyoglobin (OxyMb) and changes the color of the tissue to a bright cherry red, associated with freshness and high quality by consumers for fresh meat in a market retail case (*3*, *7*). However, in both Mb and OxyMb the ferrous ion (Fe^{2+}) can be changed to ferric (Fe^{3+}) state by autoxidation, forming metmyoglobin (MMb), and the meat acquires the undesirable brownish red color (*3*, *8*). Incorporating vitamin E in red meat inhibits the oxidation of OxyMb and hinders development of lipid-oxidized off-flavor (*3*, *7*, *9*). The principal antioxidant role of vitamin E is to neutralize free radicals that could initiate a chain reaction, particularly among unsaturated fatty acids in membranes (*3*, *7*, *8*).

The absorption of tocopherols is incomplete in ruminants (*10*). Early studies reported that vitamin E was degraded in the rumen (*11*), whereas recent in vitro studies suggested that no destruction of vitamin E was occurring in the rumen (*12*). In general, the rate of absorption of tocopherols is relatively low and quite variable, depending on diet composition and other factors (*10*, *13*). However, the efficiency of absorption might be enhanced by the administration of an emulsified vitamin E form (*14*). Emulsified vitamin E in dietary lipid supplements has the potential to enhance deposition of vitamin E in ruminants.

Reliable scientific evidence supports the assumption that inclusion of polyunsaturated fatty acids (PUFA) in ruminant tissues is favorable to human health and nutrition (*15*−*17*). However, numerous attempts to increase the PUFA content of ruminant-based food products (meat and milk) through dietary lipid supplementation have been significantly limited because of rumen biohydrogenation of dietary PUFA (*15*, *18*). Formaldehyde-treated dietary lipid is by far the most resistant to degradation by rumen microorganisms (*19*), but it is not adopted in the United States because of its toxicity. Lee et al. (*20*, *21*) investigated the possibility of using alternative safer chemical agents nearly as effective as formaldehyde. However, additional research is needed to substantiate the claim that the PUFA content in meat can be increased by feeding ruminants vegetable oil containing high levels of PUFA.

Successful incorporation of vitamin E and PUFA in fresh meat by feeding chemically treated dietary supplements containing α-tocopheryl acetate and sunflower oil may provide healthier meat for humans and extend the shelf life of fresh meat. Therefore, the objectives of this study were to produce emulsified α-tocopherol acetate in dietary supplements consisting of sunflower oil combined with defatted soy flour treated with 2,3-

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Table 1. Chemical Composition of Diacetyl-Treated Dietary Supplement[a]

item	composition
macrocomponent[b] (%)	
moisture	50.6
protein	15.1
fat	14.9
α-tocopheryl acetate (IU/kg)	466
fatty acid (wt %)	
palmitic (C16:0)	7.0
stearic (C18:0)	2.6
oleic (C18:1)	17.2
linoleic (C18:2)	71.4
linolenic (C18:3)	0.6
eicosanic (C20:0)	0.1
9-eicosenic (C20:1)	0.2
arachidonic (C20:4n6)	0.6
eicosapentaenoic (C20:5n3)	0.3

[a] Ground 2,3-butanedione-treated dietary supplement containing sunflower oil and α-tocopheryl acetate. [b] Weight percent wet basis.

Table 2. Ingredient and Nutrient Composition of the Diets Fed to Lambs

item	diet[a]		
	control	TA	CTDS
ingredient component (%)			
coarse ground corn	63.8	63.8	42.5
alfalfa pellets	19.9	19.9	13.3
soybean meal[b]	10.0	10.0	6.7
rolled oats	5.0	5.0	3.3
trace mineral mix	0.5	0.5	0.3
limestone	0.3	0.3	0.2
antibiotic	0.2	0.2	0.1
ammonium chloride	0.3	0.3	0.2
CTDS gel[c]			33.3
α-tocopheryl acetate (IU)		250	250
nutrient composition (%)			
crude protein	16.05	16.05	15.73
calcium	0.46	0.46	0.47
phosphorus	0.33	0.33	0.28
fat	3.58	3.58	7.35

[a] Control, no supplemental vitamin E; TA, supplemented vitamin E; CTDS, 2,3-butanedione-treated vitamin E supplement entrapped in sunflower oil. [b] Forty-eight percent crude protein. [c] Ground 2,3-butanedione treated dietary supplement containing sunflower oil and α-tocopheryl acetate.

butanedione and to estimate the efficacy of the chemically treated dietary lipid supplement to incorporate α-tocopherol and linoleic acid in the blood serum and edible tissues of lambs.

MATERIALS AND METHODS

Preparation of Chemically Treated Dietary Lipid Supplement. Chemically treated dietary lipid supplements (CTDS) were produced by entrapping DL-α-tocopheryl acetate (BASF Corp., Mt. Olive, NJ) in a 2,3-butanedione-treated lipid−protein matrix as described by Lee et al. (*21*). A 0.1% (w/w) sodium hydroxide (NaOH) solution (55.0%, w/w) was heated to 80 °C and poured into a Stephan Universal Schnell Cutter (Couch Supply Inc., Kansas City, MO). Sodium caseinate (5.49%, w/w; Erie National Food, Erie, IL), soy lecithin (0.07%, w/w), defatted soy flour (21.5%, w/w; Archer Daniels Midland, Co., Decatur, IL), and DL-α-tocopheryl acetate (TA; 0.5%, w/w) dispersed in sunflower oil (13.7%, w/w; AC Humko Inc., Memphis, TN) were poured into the cutter. Shearing forces continuously mixed the contents in the cutter. When the internal temperature of the content reached 85 °C, 2,3-butanedione (4.18%, w/w) was delivered into the content to form a gel. The gel was stored at refrigerated temperature (4 °C) overnight (8 h). The CTDS gel consisted of ∼500 IU of vitamin E/kg and linoleic acid (C18:2) 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 for no longer than 3 weeks before being fed to experimental animals.

Feeding Trial and Collecting Samples. Experimental procedures involving animals were conducted according to the guidelines of and with approval from the University of Tennessee Institutional Animal Care and Use Committee. Experimental animals were obtained from The University of Tennessee Research Flock. Twenty-four crossbred (Dorset × Suffolk) wether feeder lambs weighing an average of 32.5 kg were assigned in a completely randomized experimental design to a feeding trial consisting of three dietary treatments. Each treatment was replicated in two pens, with four lambs per pen. All experimental animals were dewormed and given appropriate vaccinations prior to the start of a feeding trial. A 14-day adjustment period was used to adapt lambs to the new feeding facilities and allowed lambs to recover from stresses associated with weaning. Lambs were placed in feeding pens located at the Cherokee Farm of the Knoxville Experimental Station. Pens were in a closed barn, and each lamb was provided 1.56 m^2 of floor space with ad libitum access to water. Each pen of four lambs was individually fed one of three dietary treatments for the 10-week feeding period. The dietary treatments consisted of (1) control diet containing 1.28 kg dry matter (DM) of corn basal diet (BD), 375 g DM of grass hay, and 10 g of molasses; (2) TA diet consisting of α-tocopherol acetate plus control diet; or (3) CTDS diet containing 0.5 kg of CTDS and control diet (**Table 2**). The control diet consisted of a ground corn basal diet composed predominantly of cracked corn and alfalfa pellets. Vitamin E supplementation (TA and CTDS) diets offered equal amounts of α-tocopherol acetate (250 IU/day) to individual lambs.

Blood samples were taken from each lamb biweekly during the feeding trial prior to slaughter of experimental animals. The blood samples were collected via jugular vein puncture into 20-mL Vacutainer tubes and immediately placed on an ice bath. Serum was separated from the blood sample by centrifugation at 2000*g* for 20 min in a Sorvall Superspeed model 5RC2-B automatic refrigerated centrifuge (Ivan Sorvall Inc., Newton, CT) and stored in a 10-mL vial at −28 °C for further analysis.

At the conclusion of the feeding trial, gluteus medius, longissimus dorsi, and psoas major muscles, as well as intermuscular and subcutaneous fat, were obtained from each lamb carcass. The longissimus dorsi muscle from the fifth through seventh ribs was excised from the right side of each carcass, subcutaneous fat was removed, and the intermuscular fat between the longissimus dorsi muscle and the subcutaneous fat layer was also sampled. Gluteus medius and psoas major muscles were procured from sirloin chops, taken from the right side of each carcass. All muscle and fat samples from each carcass were ground under liquid nitrogen, packaged in polyethylene bags (NASCO Inc., Fort Atkinson, WI), sealed, and stored at −28 °C for α-tocopherol and fatty acid analyses.

Chemical Analysis of Blood Serum and Tissue Samples. Extraction of α-tocopherol in blood serum samples was conducted according to the method of McMurray and Blanchflower (*22*), with each sample extracted in triplicate. Blood serum (1.0 mL) was delivered into a 25-mL screw-cap test tube. One milliliter of pure ethanol was added with mixing to precipitate proteins followed by 5 mL of hexane in the tube. The tube was vigorously mixed for 5 min by a Fisher vortex mixer (Fisher Scientific) and then centrifuged for 15 min at 3000*g* at 0 °C (model TJ-6 centrifuge, Beckman Instruments Inc., Palo Alto, CA). The contents were allowed to stand until the separation of phases occurred. A portion (4 mL) of the hexane layer was collected and filtered through a 0.45-μm membrane filter. A 20-μL aliquot was injected into a Waters HPLC system as described subsequently. α-Tocopherol (Sigma Chemical Co., St. Louis, MO) was used to create a standard curve. Triplicate standards were prepared between 0.1 and 200 ppm of α-tocopherol in hexane. All samples were analyzed in triplicate.

The α-tocopherol in muscle and adipose tissue samples was also extracted according to the method of Liu et al. (*23*) as modified by Lee et al. (*21*). One gram of muscle tissue or 0.1 g of adipose tissue sample was weighed into a 25-mL screw-cap test tube to which was

added 0.25 g of ascorbic acid (Sigma Chemical Co.) and 7.3 mL of a saponification solution, prepared with NaOH (8.08%, w/v) in a mixture of deionized−distilled water (45%, v/v) and ethanol (55%, v/v). The test tube was placed in a water bath shaker (60 strokes/min) at 80 °C for 15 min and then cooled in an ice bath. Four milliliters of isooctane was added to the tube and vigorously vortexed for 2 min. After the separation of phases occurred in the contents, an aliquot of the upper layer was transferred to a HPLC filtering syringe and filtered through 0.45-μm membrane. Twenty microliters of the filtrate was injected into a Waters HPLC system, 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.

Total lipids of adipose tissue samples were extracted by means of the chloroform/methanol (2:1, v/v) method of Lee et al. (*20*). Heneicosanoic acid used as an internal standard was added to the sample. One hundred milligrams of extracted lipid from each sample was saponified with 4 mL of NaOH (0.5 N) in methanol and esterified with 5.0 mL of (14%, v/v) boron trifluoride/methanol according to the AOCS method (*24*) for the preparation of fatty acid methyl esters (FAME). The fatty acid composition of tissue lipids was determined by a Shimadzu gas chromatograph (Shimadzu Corp.) equipped with a flame ionization detector and an automatic injection system (AOC-9, Shimadzu) using a fused silica SP2330 column (30 m × 0.25 mm i.d., Supelco, Inc., Bellefonte, PA). The FAME preparation was injected in the split mode with a split ratio of 1:30. Helium was used as the carrier gas with a 2.0 mL/min flow rate. Both injector and detector temperatures were set at 270 °C. The initial column temperature was 130 °C and continuously raised at 2 °C/min to 220 °C and then held for 20 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 heneicosanoic acid as an internal standard and on relative peak areas of the fatty acids.

Statistical Analysis. Blood serum data were analyzed as a completely randomized repeated measures design using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC), with an individual lamb serving as the experimental unit. The effects of dietary treatment, length of supplementation, and their two-way interaction were considered to be fixed. "Animal within dietary treatment" was used as a random error term for dietary treatment. Muscle and adipose tissue data were analyzed with a completely randomized design using the GLM procedure of SAS. Least-squares means were generated and separated using the PDIFF option of SAS for main or interaction effects. Significance was determined at $P < 0.05$, but differences of $0.05 \leq P < 0.1$ were considered as trends.

RESULTS AND DISCUSSION

On the basis of daily intakes, lambs consumed an average of 250 IU (TA diet) or 240 IU (CTDS diet) of α-tocopheryl acetate per day. Average daily fat intakes were 38, 38, and 114 g in control, TA, and CTDS diets, respectively, and the average daily weight gain by the lambs was 167, 145, or 188 g for control, TA, and CTDS diets, respectively. Average weight during the feeding trial was 39.2, 40.8, and 40.3 kg, respectively.

Concentrations of α-Tocopherol in Blood Serum and Edible Tissues. The effect of feeding emulsified vitamin E (α-tocopheryl acetate) in dietary lipid supplements to lambs on blood serum α-tocopherol concentrations across the feeding period is presented in **Figure 1**. Dietary vitamin E supplementation (TA and CTDS diets) increased ($P < 0.05$) serum α-tocopherol concentrations compared with the control (unsupplemented vitamin E) diet. Increased levels of α-tocopherol were expected in the blood serum from lambs fed TA diet, but the increase was less than that from lambs fed the CTDS

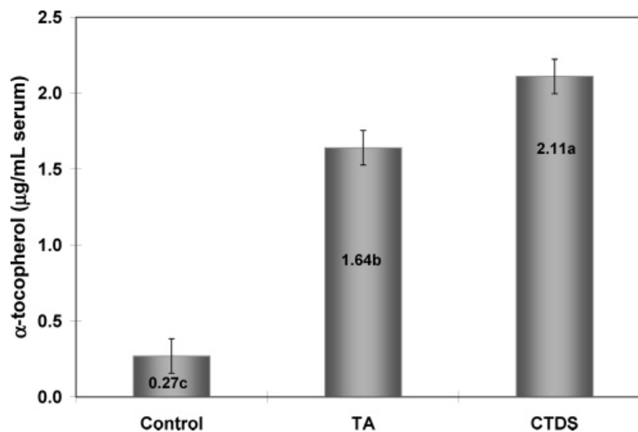

Figure 1. Accumulation of α-tocopherol in blood serum (SE = 0.12) of lambs fed the experimental diet across feeding times: control, no supplemental vitamin E; TA, supplemented vitamin E (250 IU/day) with molasses; CTDS, 2,3-butanedione-treated vitamin E supplement (250 IU/day). Bars bearing unlike letters are different ($P < 0.05$).

diet. Although TA and CTDS diets provided equal amounts of dietary vitamin E (250 IU/day) to each lamb, levels of serum α-tocopherol in lambs fed the CTDS were 28.7% higher than those fed the TA diet. This suggests that CTDS may enhance the absorption of α-tocopheryl acetate in the small intestine of ruminants. According to ruminant nutritionists (*25*, *26*), mechanical actions (chewing and gastric churning) facilitate emulsification of dietary lipid and fat-soluble vitamins in rumen. Thus, vitamin E becomes dissolved in the emulsion droplets upon entering the duodenum. Large droplets are broken down in the small intestine, and this process depends on the release of lipase enzyme and bile from the gall bladder. Both bile and pancreatic fluid are necessary for maximum absorption of vitamin E. The acetate form is hydrolyzed by a pancreatic esterase before it can be absorbed. The products of the hydrolysis of these large droplets form small particles called mixed micelles. The hydrophobic part of these mixed micelles attracts nonpolar lipids such as cholesterol and fat-soluble vitamins such as vitamin E. Mixed micelles serve as a carrier for vitamin E to enhance the absorption of vitamin E in the small intestine. Consequently, the α-tocopherol is absorbed as a lipid-bile micelle by penetrating the epithelial cells in the intestine. The transfer of α-tocopherol across the epithelial cell may involve diffusion processes, but the actual mechanism is not clearly understood. In the present study vitamin E (α-tocopheryl acetate) was emulsified in a sunflower oil and soy protein complex in the CTDS dietary supplement, which might be re-emulsified in the rumen and produce well-defined micelles compared with the TA dietary supplement prepared by mixing α-tocopheryl acetate with molasses as a delivery agent.

Extending time on feeding across experimental diets significantly affected ($P < 0.05$) serum α-tocopherol levels in lambs (**Figure 2**). Both applications of α-tocopheryl acetate (TA and CTDS diets) increased ($P < 0.05$) serum α-tocopherol content continuously in the blood serum of lambs with some deviation during the 10-week feeding trial. However, serum α-tocopherol levels were almost constant in lambs fed the control diet. Feeding of α-tocopheryl acetate supplements and duration of feeding significantly influenced ($P < 0.05$) the serum levels of α-tocopherol in lambs fed the vitamin E supplemented (TA and CTDS) diets. After the first 2 weeks, the vitamin E supplemented diets had serum α-tocopherol concentrations approximately 2-fold (TA diet) or 5-fold (CTDS diet) higher than did the control diet (0.31 μg/mL serum). Lambs fed the CTDS diet

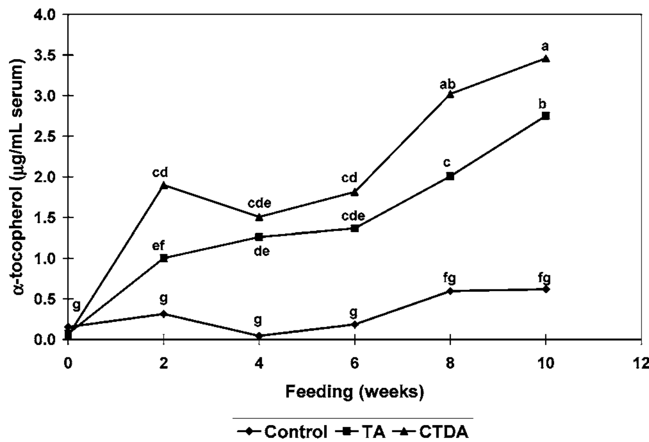

Figure 2. Effect of time and vitamin E supplementation on lamb blood serum α-tocopherol contents over a 10-week feeding trial (SE = 0.26): control, no supplemental vitamin E; TA, supplemented vitamin E; CTDS, 2,3-butanedione-treated vitamin E supplement entrapped in sunflower oil. Points with no common letter are different ($P < 0.05$).

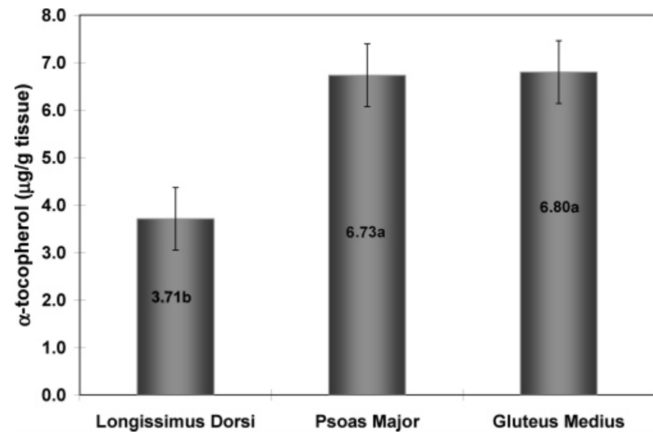

Figure 3. α-Tocopherol concentrations of different muscles across dietary treatments from experimental lambs ($n = 24$). Bars bearing unlike letters are different ($P < 0.05$).

Table 3. Least-Squares Means ($n = 8$) of α-Tocopherol Concentrations in Muscle and Adipose Tissues from Lambs Fed Different Dietary Supplements[a]

	dietary supplement			
component	control	TA	CTDS	SE
muscle, μg/g				
longissimus dorsi	1.46c	3.20b	4.21b	0.36
psoas major	2.16d	6.02c	7.43b	0.31
gluteus medius	3.19d	6.05c	7.55b	0.35
adipose, μg/g				
intermuscular	3.77c	15.64b	14.49b	0.57
subcutaneous	3.19d	9.98c	12.55b	0.56

[a] Control, no supplemental vitamin E; TA, supplemented vitamin E (250 IU/day) with molasses; CTDS, 2,3-butanedione-treated vitamin E supplement (250 IU/dau). Within a row, least-squares means that do not have a common letter differ ($P < 0.05$).

had the highest concentration of α-tocopherol in blood serum in the first 2 weeks. This result may be due to the emulsified α-tocopheryl acetate in the CTDS, which might enhance the absorption of vitamin E in the small intestine; moreover, the length of feeding time also affected the increment of serum α-tocopherol. However, there was no further increase of serum α-tocopherol levels in lambs fed the vitamin E supplemented diets (TA and CTDS diets) until week 6. This pattern might indicate that a saturation level of serum α-tocopherol was reached after 2 weeks of vitamin E supplementation. It also suggests that after blood was saturated with α-tocopherol by the second week, the surplus α-tocopherol started to deposit in the tissues. This trend of elevation of plasma α-tocopherol and saturation in the levels of dietary vitamin E supplementation has been reported previously (*14*, *27*−*29*). However, further increment of serum α-tocopherol in lambs fed the vitamin E supplemented diets was shown in the present study. The time required for serum α-tocopherol to reach its peak level depends on sources of vitamin E and method of administration (*14*, *29*). It also might be related to the concentration of vitamin E intake. In the previous studies, when lambs were fed supranutritional levels (300−1000 IU/day) of vitamin E (α-tocopheryl acetate), the saturation levels of serum α-tocopherol were reached in <2 weeks (*14*, *29*); moreover, the time required for serum α-tocopherol to reach its saturation level was inversely related to the concentration of supplemented vitamin E. The further increment of serum α-tocopherol in the present study might be explained by difference in levels of vitamin E supplemented. In the present study lambs were fed 250 IU of vitamin E per day, which might require additional feeding time to reach the peak level of serum α-tocopherol compared with those fed the supranutritional levels of vitamin E, although differences were small (50 IU/day). However, more research is needed to estimate the maximum bioavailability of α-toopherol in blood serum based on types and dosage levels of vitamin E supplements. The vitamin E supplemented diets significantly increased ($P < 0.05$) the serum content of α-tocopherol in lambs after the 8th (CTDS diet) and 10th (TA diet) weeks in the feeding trial (**Figure 2**). Lambs fed the CTDS had 25.8% higher levels of serum α-tocopherol than those fed the TA diet at week 10. Clearly, the α-tocopheryl acetate emulsified in the sunflower oil and protein matrix was more effective in raising the circulating blood serum levels of α-tocopherol than mixing α-tocopheryl acetate with molasses. In general, serum levels of α-tocopherol are a reliable indicator of nutritional vitamin E status, which are correlated with intakes of vitamin E (*13*, *29*).

The α-tocopherol concentrations in muscles and adipose tissues of lambs fed the control, TA, or CTDS diet are presented in **Table 3**. Lambs supplemented with vitamin E (TA and CTDS diets) had higher ($P < 0.05$) levels of α-tocopherol in all types of tissue samples than unsupplemented lambs (control diet). Feeding lambs with the CTDS further increased ($P < 0.05$) α-tocopherol concentration in gluteus medius and psoas major muscles, as well as subcutaneous fat, compared with lambs fed the TA diet, but no significant differences ($P > 0.05$) were found in α-tocopherol concentrations of longissimus dorsi muscle and intermuscular fat from lambs fed two supplemented vitamin E diets. However, the lambs fed the CTDS tended to have higher ($P < 0.07$) levels of α-tocopherol in longissimus dorsi muscles than did lambs fed the TA. Recent research indicates that vitamin E not only delays lipid oxidation in fresh meat but also prevents OxyMb oxidation (*1*, *3*, *9*). OxyMb oxidation in the muscle tissue is dependent on lipid oxidation, which is substantially reduced by the addition of dietary vitamin E (*3*). The strategy for supplementing ruminants with α-tocopheryl acetate is to achieve the ideal concentration to maximize the antioxidant ability (*7*, *30*, *31*). Meat containing α-tocopherol concentrations of <3.0 μg/g has shorter case life in terms of reduced MMb) accumulation or lipid oxidation; however, scientists have shown that the case life of beef retail products can be improved if cattle achieve muscle concentration of vitamin E in excess of 3−4 μg/g of muscle tissue (*7*, *30*, *31*).

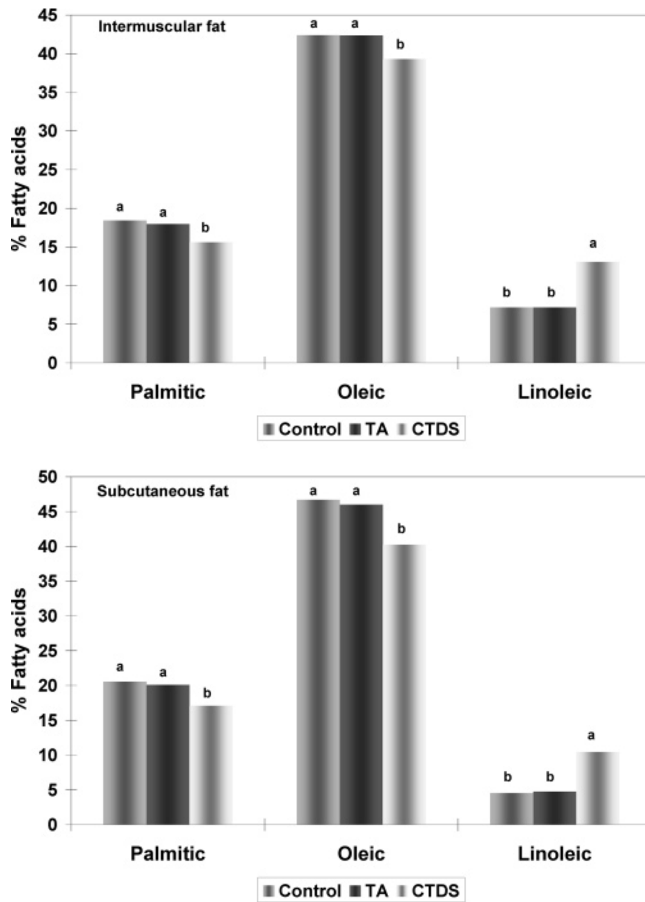

Figure 4. Percentages of three major fatty acids (palmitic, oleic, and linoleic acids) in fat depots (intermuscular and subcutaneous fats) of lambs fed different dietary supplements: control, no supplemental vitamin E; TA, supplemented vitamin E; CTDS, 2,3-butanedione-treated vitamin E supplement entrapped in sunflower oil. For any one acid, bars bearing unlike letters are different ($P < 0.05$).

In the present study supplementation of vitamin E (TA and CTDS diet) in the feed achieved the critical concentration of α-tocopherol in the longissimus dorsi, gluteus medius, and psoas major muscle tissues of lambs.

The α-tocopherol accumulation differs among major muscles within a bovine carcass (*27*, *28*, *32*). Ochoa et al. (*14*) reported that tissue concentrations of vitamin E in lambs increased in proportion to the logarithm of the dose consumed. Higher ($P < 0.05$) α-tocopherol accumulation in adipose tissues (9.94 μg/g of tissue) than in skeletal muscles (4.59 μg/g of tissue) across dietary treatments was observed in the present study and has been also reported from experiments with meat-producing animals (*32*, *33*). The degree of α-tocopherol deposition was different ($P < 0.05$) among the major muscles within the carcass across dietary treatments in the present study (**Figure 3**). The α-tocopherol concentrations in gluteus medius and psoas major muscles were higher ($P < 0.05$) than in longissimus dorsi muscle, yet no significant difference ($P > 0.05$) was found in the levels of α-tocopherol between gluteus medius and psoas major muscles. Guidera et al. (*34*) reported that there was no significant difference in the levels of α-tocopherol in gluteus medius and psoas major muscles from lambs fed a high-dose supplementation of vitamin E (1000 mg of α-tocopherol acetate/day) for 9 weeks. Tissue accumulation of α-tocopherol in vitamin E supplemented animals generally depends on dose and feeding time (*27*, *31*). Animals fed higher levels of α-tocopherol daily accumulate higher tissue levels of α-tocopherol more quickly than those fed lower levels. After vitamin E supplementation to beef cattle, the levels of α-tocopherol accumulation in the major muscles followed the order: gluteus medius > semimembranosus > longissimus dorsi (*27*, *35*). The deposition of α-tocopherol in skeletal muscles may depend on the proportion of red muscle fibers because red muscle fibers contain numerous small mitochondria between myofibrils that may provide more membrane volume for potential retention of α-tocopherol than white fibers containing fewer but larger mitochondria. Red muscle fibers also have a much greater capillary blood supply than do white fibers, so that the residual blood and its associated tocopherol would also provide increased concentrations of α-tocopherol in red muscle. According to Njeru et al. (*29*), the α-tocopherol concentration in muscle tissue varies depending on the need for antioxidant protection. However, there are no clear explanations for muscle differences in α-tocopherol concentration.

Major Fatty Acid Contents in Adipose Tissues. The protection of dietary lipid of CTDS gel from biohydrogenation in rumen significantly changed ($P < 0.05$) the relative weight concentrations of palmitic (C16:0), oleic (C18:1n9), and linoleic (C18:2n6) acids in adipose tissues of lambs. **Figure 4** shows the proportion of major fatty acids in the fat depots from lambs fed three different diets after the 10-week feeding period. The sunflower oil-containing supplement (CTDS diet) caused substantial increases ($P < 0.05$) in the proportion of linoleic (C18:2n6) acid with decreases ($P < 0.05$) in the proportion of oleic (C18:1n9) and palmitic (C16:0) acids in intermuscular and subcutaneous fat. The proportion of stearic acid (C18:0) in fat depots was not altered by feeding the sunflower oil supplement. Cook et al. (*36*) reported that the depot fat of sheep was altered by feeding supplements of formaldehyde-treated particles containing safflower oil and casein for a period of 6 weeks immediately prior to slaughter. In this study, the proportion of linoleic (C18:2n6) acid increased from 2 to 29% in subcutaneous fat, and there were corresponding decreases in the proportions of palmitic (C16:0), stearic (C18:0), and oleic (C18:1n9) acids. In the present study, the levels of linoleic (C18:2n6) acid increased from 4.5 to 10.4% in subcutaneous fats and from 7.2 to 13.0% in intermuscular fat, respectively. Consequently, the 2,3-butanedione-treated supplements containing defatted soy flour and sunflower oil may protect linoleic (C18:2n6) acid from ruminal biohydrogenation and readily incorporate it into adipose tissues.

Dietary protein−lipid supplements made with a safe chemical agent and containing polyunsaturated fat and vitamin E supplement could enhance the deposition of α-tocopherol and increase levels of polyunsaturated fat in fresh meat when fed to lambs. Thus, vitamin E and linoleic acid enriched fresh meat will provide health benefits to consumers because increasing the intake of vitamin E and polyunsaturated fat reduces the risk of cardiovascular disease. Furthermore, the case life of lamb retail products might be improved because feeder lambs could achieve muscle concentrations of vitamin E in excess of 3 μg/g.

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