Biodiscrimination of α -tocopherol stereoisomers in plasma and tissues of lambs fed different proportions of *all-rac*- α -tocopheryl acetate and RRR- α -tocopheryl acetate^{1,2}

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ABSTRACT: A ratio of 1.36:1 in relative bioactivity of RRR- α -tocopheryl acetate as a natural (Nat- α -T) source to *all-rac*- α -tocopheryl acetate, as a synthetic (Syn- α -T) source, is generally accepted. This factor also largely reflects the difference in bioavailability. However, studies indicate that neither bioavailability of α -tocopherol stereoisomers nor relative bioavailability between them are constant, but are dose-dependent and differ between organs. However, no information is available about how different ratios between synthetic and natural α -tocopherol affect bioavailability of α -tocopherol stereoisomers. Thirty lambs were randomly assigned to diets supplied with additives containing 5 different Syn- α -T to Nat- α -T ratios, including 100:0, 75:25, 50:50, 25:75, and 0:100. The experiment lasted for 70 d after which

the lambs were slaughtered. The amount of RRR- α -tocopherol generally increased in plasma and organs with increasing the proportion of Nat- α -T in the diet ($P < 0.05$). However, the relative bioavailability of RRR- and RRS- α -tocopherol in plasma, organs, and abdominal fat generally decreased with increasing the proportion of Nat- α -T in the diet ($P < 0.05$), whereas the other stereoisomers only showed minor changes with the exception of liver. However, a linear response was maintained between the ratio of stereoisomers in the feed and the ratio in plasma and organs. In conclusion, regardless of Syn- α -T to Nat- α -T ratio in the diets, amounts of α -tocopherol stereoisomers in plasma, brain, heart, lungs, and abdominal fat were in the following order: RRR > RRS, RSR, RSS > Σ 2S.

Key words: α -tocopherol stereoisomers, biodiscrimination, lambs

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INTRODUCTION

The term vitamin E was first introduced by Evans and Bishop (1922) to describe an important nutrient for animal reproduction. There is important evidence that supplemental vitamin

E may have multiple health and nutritional benefits for domestic livestock. Generally, positive responses seem to be related either to improved immune competence or to the antioxidant system (Baldi, 2005).

α -tocopherol consists of 8 different isomeric configurations, including 2R configurations (RSS, RRS, RSR, and RRR) and 2S configurations (SRR, SSR, SRS, and SSS). The RRR isomer is the only isomer of α -tocopherol occurring in nature (Vagni *et al.*, 2011), whereas α -tocopherol used for feed additives consists of a racemic mixture (*all-rac*) of all 8 stereoisomers. To stabilize the functional phenol group during storage, the

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commercial vitamin mixtures, *all-rac*- α -tocopherol (Syn- α -T), are typically acetylated and added to the ration as *all-rac*- α -tocopheryl acetate. This acetylated form of α -tocopherol must be hydrolyzed by carboxyl ester hydrolase (Jensen and Lauridsen, 2007) before absorption from the gastro-intestinal tract. Absorption of α -tocopherol is also stimulated by high fat levels in the diet (Weiss and Wyatt, 2003).

The official bioactivity factors of 1.00 for *all-rac*- α -tocopheryl acetate and 1.36 for RRR- α -tocopheryl (Nat- α -T) acetate are mainly based on results of the rat resorption–gestation test (Harris and Ludwig, 1948). Weiser and Vecchi (1982) showed the relative bioavailability of all 8 stereoisomers of α -tocopherol by the rat resorption–gestation test and reported that α -tocopherol with 2R configuration had a higher biopotency factor than α -tocopherol with 2S configuration. It has been well established that the bioavailability of the RRR-stereoisomer of α -tocopherol is higher than the bioavailability of the synthetic stereoisomers of α -tocopherol, and the ratio of 1.36:1 in biopotency of RRR- α -tocopheryl acetate to *all-rac*- α -tocopheryl acetate is generally reported when working with *all-rac*- α -tocopherol supplementation (Blatt *et al.*, 2004). Weiser *et al.* (1996) stated that differences in α -tocopherol stereoisomers biopotency are related to corresponding differences in α -tocopherol concentrations. The bioavailability of RRR- α -tocopherol relative to *all-rac*- α -tocopherol has been questioned for decades because α -tocopherol stereoisomers are treated and metabolized within the tissues in different ways, and for this reason the difference in vitamin E activity between Nat- α -T and Syn- α -T cannot be expressed by one single ratio (Blatt *et al.*, 2004; Jensen *et al.*, 2006). Studies in humans (Burton *et al.*, 1998), dairy cows (Meglia *et al.*, 2006), and sows (Lauridsen *et al.*, 2002) show a higher bioavailability of the natural isomer compared with the synthetic isomers. This has been ascribed to the presence of α -tocopherol transfer protein (α -TTP), which discriminates between forms of α -tocopherol and maintains α -tocopherol concentrations in plasma (Hosomi *et al.*, 1997; Jishage *et al.*, 2001).

Hidiroglou *et al.* (1992) reported higher plasma concentrations of RRR- α -tocopherol in sheep fed RRR- α -tocopheryl acetate compared with *all-rac*- α -tocopheryl acetate. However, the response in different organs was not evaluated in their study. Dersjant-Li *et al.* (2009) studied the α -tocopherol stereoisomers distribution in veal calves and reported that the amount of RRR- α -tocopherol

was higher than the other stereoisomers. The purpose of the present experiment was to follow the distribution and change in amounts of α -tocopherol stereoisomers in lambs fed constant amounts but different ratios of synthetic α -tocopherol (*all-rac*- α -tocopheryl acetate) to natural α -tocopherol (RRR- α -tocopherol).

MATERIAL AND METHODS

All animals were registered and cared for according to guidelines approved by the Swedish University of Agricultural Science Animal Care and Use Committee and the National Animal Research Authority, and the study was carried out in accordance with the laws and regulations controlling experiments performed with live animals in Sweden. The studies were carried out in the Department of Agricultural Research of Northern Sweden (63°45'N, 20°17'E).

Treatments and Study Design

The study was carried out with supplementation with different proportions of *all-rac*- α -tocopheryl acetate as synthetic source of α -tocopherol and RRR- α -tocopheryl acetate as natural source of α -tocopherol. Thirty ewe lambs, on average 120 d old, were randomly assigned to 5 treatment groups and put in straw-bedded pens. They were fed the same diet consisting of hay, barley, and crushed peas. The diet was formulated to supply a low amount of α -tocopherol. The feed rations were weighed daily for each group, and refusals were weighed 3 times per week. The barley and peas were mixed before feeding with a mineral compound and an additive with different ratios of Syn- α -T to Nat- α -T, including 100:0, 75:25, 50:50, 25:75, and 0:100%. The 5 vitamin E mixtures were manufactured by mixing *all-rac*- α -tocopheryl acetate (Rovimix E50-Ads from DSM Nutritional Products, Brøndby, Denmark) and RRR- α -tocopheryl acetate (Natur-E granulate 40%, Pharmalett A/S, Kolding, Denmark). All the experimental additives contained the same α -tocopherol content and were formulated to supply 200 mg·d⁻¹·lamb⁻¹. The analyzed content of α -tocopherol in the vitamin E mixtures averaged 99% (95%–105%) of the expected content, and the analyzed distributions of stereoisomers in the different mixtures of Syn- α -T and Nat- α -T and experimental diets are shown in Table 1.

The experiment was a long lasting experiment for 10 wk. The lambs were weighed at the experiment start, after 5 wk, and at the end of the experiment.

Blood samples were collected from the jugular vein into 10-mL evacuated test tubes containing heparin (BD Vacutainer Systems, Preanalytical Solutions, Plymouth, United Kingdom) at the beginning, in the middle, and at the end of experiment. The tubes were centrifuged at 5000 U/min for 15 min; plasma was collected and stored at -20°C until analysis. The lambs were slaughtered on 2 consecutive days. Samples of liver, spleen, lungs, heart, muscle, and abdominal fat were collected at the abattoir and stored in -20°C until analysis.

Vitamin E Analysis

One analysis of α -tocopherol was made of each feed, based on samples collected twice a week during the whole experiment. Quantitative determination of α -tocopherol concentration in plasma, abdominal fat, and organs, including liver, spleen, lungs, heart, muscle, and abdominal fat, was carried out by HPLC after saponification and extraction into heptane as described by [Jensen et al. \(2006\)](#). In brief, plasma (1000 μL) was diluted with 2.0-mL ethanol (96% vol/vol), 0.5-mL methanol (100%), 1.0-mL ascorbic acid (20% wt/vol), 0.3-mL KOH-water (1:1, wt/vol), and 0.7-mL water. Samples were saponified at 80°C for 20 min and cooled in the dark. Tocopherol was extracted into 2 volumes of 5-mL heptane, and 100 μL of the combined heptane phase was injected into the HPLC. All solvents used were of HPLC quality. Organs were homogenized in twice the amount of ethanol by an Ultra-Turrax homogenizer while kept on ice. Aliquots of the homogenates corresponding to 167-mg liver were saponified in a mixture of ethanol, methanol, ascorbic acid (20% wt/vol), and KOH-water (1:1 wt/vol) at 80°C for 30 min, subsequently cooled and extracted into 2 portions of 5-mL heptane, and 100 μL of the combined extract was injected

into the HPLC. The HPLC column for determination of tocopherol consisted of a 4.0×125 mm Perkin-Elmer HS-5-Silica column (Perkin-Elmer GmbH, D-7770 Überlingen, Germany). The mobile phase consisted of heptane containing 2-propanol (3.0 mL/liter) and was degassed with helium. The flow rate was 3.0 mL/min. A comparison of retention time and peak areas with Merck (D-6100 Darmstadt, Germany) external standards was used to obtain the identification and quantification of the tocopherol. Fluorescence detection was performed with an excitation wavelength of 290 nm and an emission wavelength of 327 nm.

Stereoisomers of α -tocopherol were analyzed by HPLC. The remaining heptane extract was evaporated to exact dryness under a stream of nitrogen. Then the α -tocopherol was derivatized to its methyl ether according to the method described by [Jensen et al. \(2006\)](#). The methyl ether derivative was extracted with 1.00-mL heptane of which 100 μL was injected into the HPLC. Chromatographic separation was achieved on a Chiralcel OD-H column (25×0.46 cm, 5 μm particle size), cellulose tris (3,5-dimethylphenylcarbamate) from Daicel Chemical industries, Ltd. (Tokyo, 100-6077, Japan) with heptane as eluent. This method allows the separation of the 8 stereoisomers of α -tocopherol into 5 peaks. The first peak contains the 2SR, SR, S forms ($\Sigma 2\text{S}$), the second peak contains the RSS- α -tocopherol (RSS), the third peak contains RRS- α -tocopherol (RRS), the fourth peak contains RRR- α -tocopherol (RRR), and the fifth peak contains RSR- α -tocopherol (RSR).

Statistical Analyses

The weight change of individual lambs was analyzed in the program NCSS with start weight as a covariate. The relative bioavailability of α -tocopherol stereoisomers in plasma and organs to the

Table 1. Distribution of α -tocopherol stereoisomers in the additives and experimental diets including different proportions of synthetic to natural α -tocopherol¹

Syn- α -T to Nat- α -T ratio in diets ²	α -Tocopherol stereoisomers in the additives										α -Tocopherol stereoisomers in the diets				
	Calculated proportion					Analyzed proportion					Analyzed proportion				
	RRR	RRS	RSR	RSS	$\Sigma 2\text{S}$	RRR	RRS	RSR	RSS	$\Sigma 2\text{S}$	RRR	RRS	RSR	RSS	$\Sigma 2\text{S}$
100:0	12.5	12.5	12.5	12.5	50.0	17.0	12.7	11.7	11.2	47.3	22.5	11.9	10.9	10.5	44.2
75:25	34.4	9.4	9.4	9.4	37.5	32.7	10.4	10.4	8.6	37.9	37.1	9.7	9.7	8.0	35.4
50:50	56.3	6.3	6.3	6.3	25.0	54.8	8.2	6.4	5.6	25.0	57.8	7.7	6.0	5.2	23.4
25:75	78.1	3.1	3.1	3.1	12.5	68.3	7.5	4.7	3.3	16.1	70.4	7.0	4.4	3.1	15.1
0:100	100	0	0	0	0	91.1	3.6	0.3	0.7	4.3	91.7	3.4	0.3	0.7	4.0

¹All the diets were formulated to supply 200-mg α -tocopherol·d⁻¹·lamb⁻¹.

²Synthetic α -tocopherol to natural α -tocopherol ratio in diets.

diet was calculated as percentage of a given stereoisomer in plasma, organs, or abdominal fat divided by percentage of the stereoisomer in the diet. Differences between treatments within stereoisomer were analyzed by using the model: $Y_{ij} = \mu + \alpha_i + e_{ij}$; where Y_{ij} is the dependent variable (α -tocopherol; stereoisomer amount; relative bioavailability), α_i the effect of treatment i , and e_{ij} the random residual error. Differences in total α -tocopherol content between organs were analyzed using the model, $Y_{ij} = \mu + \beta_i + e_{ij}$; where Y_{ij} is the dependent variable (total α -tocopherol), β_i the effect of organs i , and e_{ij} the random residual error. Random effects were assumed normally distributed with mean value 0 and constant variance $e \sim N(0, \sigma^2)$. The results are presented as least squares means and differences considered statistically significant if $P < 0.05$. Pearson correlation coefficients between proportion of RRR- α -tocopherol in diets and plasma, organs, and abdominal fat were calculated by using PROC REG of SAS.

RESULTS

The average daily gain of lambs was 156, 143, 158, 145, and 160 g/d in diets containing 100:0, 75:25, 50:50, 25:75, and 0:100 Syn- α -T to Nat- α -T ratio during the experiment, respectively, with no significant difference between the different treatment groups. Dry matter intake was 1.22 kg DM per lamb per day on average (13.8 MJ ME, 150 g CP). The α -tocopherol content was 3.8 mg/kg DM in hay, 17.4 mg/kg DM in barley, and 3.5 mg/kg DM in peas, which resulted in an average

intake of α -tocopherol from the feedstuffs of $7 \text{ mg} \cdot \text{lamb}^{-1} \cdot \text{d}^{-1}$.

At the beginning of the experiment, all lambs had only RRR- α -tocopherol circulating in the plasma; the concentration averaged $0.60 \pm 0.26 \text{ } \mu\text{g/mL}$. Figure 1 shows that regardless of proportion of Syn- α -T to Nat- α -T, the plasma α -tocopherol amount increased 5 and 10 wk after receiving α -tocopherol supplement. In addition, with the exception of diets containing 100% of Syn- α -T, the amount of RRR- α -tocopherol in plasma followed the same trend as α -tocopherol content and increased 5 and 10 weeks after receiving the α -tocopherol. As expected with replacing the Syn- α -T by Nat- α -T, the amount of RRR- α -tocopherol and non-RRR- α -tocopherol in plasma, organs, and abdominal fat increased and decreased, respectively (Table 2).

The total α -tocopherol content in spleen increased with increasing the proportion of Nat- α -T content in the diet, whereas the α -tocopherol content in plasma, other organs, and abdominal fat was not affected by different proportions of Syn- α -T to Nat- α -T (Table 2). The greater RRR- α -tocopherol amount in plasma, and organs is related to the increased intake of RRR- α -tocopherol stereoisomers with increasing the proportion of Nat- α -T in the diet (Table 2).

Comparison of the α -tocopherol content between plasma, organs, and abdominal fat in the diets containing different proportions of Syn- α -T and Nat- α -T is shown in Figure 2. Irrespective of the Syn- α -T to Nat- α -T ratio in the diets, the lowest amount of total α -tocopherol was observed in plasma, lungs, and muscle, and the greatest amount

Figure 1. Total- α -tocopherol content and $\Sigma 2S$, RRS, RSR, RSS and RRR- α -tocopherol stereoisomer distribution of plasma in lambs fed different ratios of synthetic to natural α -tocopherol during the experiment (week 0, at the beginning; week 5, in the middle and week 10, at the end of experiment).

of α -tocopherol was observed in liver and abdominal fat ($P < 0.05$).

Comparison of amounts of α -tocopherol stereoisomers in plasma, organs, and abdominal fat

in diets containing different ratios of Syn- α -T and Nat- α -T is shown in Table 2. As expected, the RRR- α -tocopherol amount increased in plasma ($P < 0.002$), liver ($P < 0.001$), and muscle ($P < 0.004$)

Table 2. Amount of total α -tocopherol and α -tocopherol stereoisomers in plasma, different organs, and abdominal fat of lambs fed different proportions of synthetic to natural α -tocopherol

Syn- α -T to Nat- α -T ratio in diets ¹	Total α -tocopherol	α -Tocopherol stereoisomers					<i>P</i> value	SEM
		RRR	RRS	RSS	RSR	Σ 2S		
Plasma, $\mu\text{g/mL}$								
100:0	1.62	0.67 ^{W,c}	0.35 ^{X,a}	0.26 ^{XY,a}	0.23 ^{XY,a}	0.11 ^Y	0.002	0.04
75:25	1.62	1.04 ^{W,b}	0.21 ^{X,ab}	0.14 ^{XY,b}	0.15 ^{XY,b}	0.07 ^Y	<0.001	0.06
50:50	1.94	1.46 ^{W,a}	0.17 ^{X,b}	0.12 ^{X,b}	0.12 ^{X,b}	0.07 ^X	<0.001	0.10
25:75	2.04	1.79 ^{W,a}	0.08 ^{X,b}	0.04 ^{X,b}	0.08 ^{X,b}	0.05 ^X	<0.001	0.13
0:100	1.66	1.66 ^a	ND	ND	ND	ND	—	0.12
<i>P</i> -value	0.39	0.002	0.006	0.001	<0.001	0.09	—	—
SEM	0.09	0.09	0.03	0.02	0.01	0.008	—	—
Liver, $\mu\text{g/g}$								
100:0	8.18	2.79 ^{W,b}	1.20 ^{X,a}	1.11 ^{X,a}	1.31 ^{X,a}	1.76 ^{X,a}	0.001	0.04
75:25	8.80	4.10 ^{W,b}	0.97 ^{X,ab}	0.86 ^{X,a}	1.11 ^{X,a}	1.77 ^{X,a}	<0.001	0.06
50:50	9.07	6.25 ^{W,a}	0.64 ^{X,bc}	0.55 ^{X,b}	0.66 ^{X,b}	0.97 ^{X,b}	<0.001	0.10
25:75	10.37	8.20 ^{W,a}	0.47 ^{X,c}	0.40 ^{X,b}	0.48 ^{X,b}	0.82 ^{X,b}	<0.001	0.13
0:100	7.72	7.45 ^{W,a}	0.07 ^{X,d}	0.02 ^{X,c}	0.06 ^{X,c}	0.12 ^{X,c}	0.002	0.12
<i>P</i> -value	0.48	<0.001	<0.001	<0.001	<0.001	0.92	—	—
SEM	0.47	2.23	0.08	0.13	0.47	3.98	—	—
Heart, $\mu\text{g/g}$								
100:0	6.45	2.80 ^{W,d}	1.36 ^{X,a}	0.90 ^{Y,a}	1.03 ^{Y,a}	0.36 ^{Z,a}	<0.001	0.15
75:25	6.63	4.16 ^{W,c}	0.88 ^{X,b}	0.62 ^{XY,b}	0.70 ^{XY,b}	0.27 ^b	<0.001	0.27
50:50	6.91	5.33 ^{W,bc}	0.57 ^{X,c}	0.39 ^{X,c}	0.45 ^{X,c}	0.17 ^c	<0.001	0.37
25:75	6.49	5.73 ^{W,ab}	0.28 ^{X,d}	0.18 ^{X,d}	0.22 ^{X,d}	0.09 ^d	<0.001	0.41
0:100	7.06	6.88 ^{W,a}	0.06 ^{X,d}	0.03 ^{X,e}	0.05 ^{X,e}	0.03 ^e	<0.001	0.52
<i>P</i> -value	0.91	<0.001	<0.001	<0.001	<0.001	<0.001	—	—
SEM	0.23	0.31	0.09	0.06	0.06	0.02	—	—
Lungs, $\mu\text{g/g}$								
100:0	2.48	1.25 ^{W,c}	0.48 ^{X,a}	0.26 ^{X,a}	0.39 ^{XY,a}	0.10 ^{Y,a}	<0.001	0.08
75:25	2.57	1.75 ^{W,c}	0.28 ^{X,ab}	0.16 ^{X,ab}	0.28 ^{X,ab}	0.10 ^{X,a}	<0.001	0.17
50:50	3.10	2.48 ^{W,b}	0.22 ^{X,bc}	0.15 ^{X,ab}	0.17 ^{X,bc}	0.07 ^{X,a}	<0.001	0.24
25:75	2.74	2.34 ^{W,b}	0.13 ^{Y,bc}	0.10 ^{Y,bc}	0.09 ^{Y,c}	0.09 ^{Y,ab}	<0.001	0.19
0:100	3.18	3.10 ^{W,a}	0.02 ^{X,c}	0.01 ^{X,c}	0.02 ^{X,c}	0.03 ^{X,b}	<0.001	0.24
<i>P</i> -value	0.89	0.09	0.003	0.008	<0.001	0.057	—	—
SEM	0.26	0.22	0.04	0.02	0.03	0.009	—	—
Spleen, $\mu\text{g/g}$								
100:0	3.83 ^b	1.69 ^{W,d}	0.76 ^{X,a}	0.54 ^{XY,a}	0.62 ^{XY,a}	0.22 ^{Y,b}	<0.001	0.11
75:25	5.50 ^{ab}	3.26 ^{W,c}	0.77 ^{X,a}	0.54 ^{XY,a}	0.58 ^{XY,a}	0.35 ^{Y,a}	<0.001	0.20
50:50	5.55 ^{ab}	4.31 ^{W,bc}	0.44 ^{X,b}	0.31 ^{X,b}	0.35 ^{X,b}	0.14 ^{X,b}	<0.001	0.30
25:75	6.88 ^a	5.94 ^{W,ab}	0.34 ^{X,b}	0.20 ^{X,b}	0.26 ^{X,b}	0.15 ^{X,b}	<0.001	0.42
0:100	5.37 ^{ab}	5.34 ^{W,a}	0.01 ^{X,c}	ND	0.01 ^{X,c}	0.005 ^{X,c}	<0.001	0.42
<i>P</i> -value	0.01	<0.001	<0.001	<0.001	<0.001	<0.001	—	—
SEM	0.29	0.33	0.06	0.04	0.04	0.02	—	—
Muscle, $\mu\text{g/g}$								
100:0	2.32	1.12 ^{W,b}	0.39 ^{X,a}	0.39 ^{X,a}	0.35 ^{X,a}	0.11 ^{X,a}	<0.001	0.08
75:25	1.64	1.06 ^{W,b}	0.18 ^{X,b}	0.20 ^{X,b}	0.16 ^{X,b}	0.05 ^{X,b}	<0.001	0.07
50:50	2.33	1.81 ^{W,a}	0.15 ^{X,b}	0.20 ^{X,b}	0.13 ^{X,b}	0.04 ^{X,bc}	<0.001	0.13
25:75	2.23	1.93 ^{W,a}	0.10 ^{X,b}	0.10 ^{X,bc}	0.08 ^{X,bc}	0.02 ^{X,bc}	<0.001	0.13
0:100	2.11	2.04 ^{W,a}	0.02 ^{X,b}	0.02 ^{X,c}	0.02 ^{X,c}	0.01 ^{X,c}	<0.001	0.15
<i>P</i> -value	0.38	0.004	0.003	<0.001	0.001	0.004	—	—
SEM	0.12	0.11	0.033	0.032	0.024	0.007	—	—

Table 2. Continued

Syn- α -T to Nat- α -T ratio in diets ¹	Total α -tocopherol	α -Tocopherol stereoisomers					<i>P</i> value	SEM
		RRR	RRS	RSS	RSR	Σ 2S		
Abdominal fat, μ g/g								
100:0	8.23	3.68 ^W	1.38 ^{X,a}	1.28 ^{X,a}	0.90 ^X	0.98 ^{X,a}	0.002	0.28
75:25	7.52	4.91 ^W	0.59 ^{X,b}	0.54 ^{X,b}	0.94 ^X	0.54 ^{X,b}	<0.001	0.38
50:50	7.24	5.52 ^W	0.64 ^{X,b}	0.44 ^{X,bc}	0.37 ^X	0.27 ^{X,bc}	<0.001	0.40
25:75	8.35	7.25 ^W	0.36 ^{X,b}	0.15 ^{X,bc}	0.46 ^X	0.13 ^{X,bc}	<0.001	0.53
0:100	7.29	6.75 ^W	0.20 ^{X,b}	0.10 ^{X,c}	0.23 ^X	0.02 ^{X,c}	<0.001	0.52
<i>P</i> -value	0.97	0.15	<0.001	<0.001	0.12	<0.001	—	—
SEM	0.63	0.49	0.09	0.09	0.10	0.08	—	—

^{a-d}Means within a column with different letters indicate significant differences ($P < 0.05$).

^{x-z}Means within a row with different letters indicate significant differences ($P < 0.05$).

¹Synthetic α -tocopherol to natural α -tocopherol ratio in diets.

ND = not detected; SEM = standard error of means.

Figure 2. Total- α -tocopherol content and Σ 2S, RRS, RSR, RSS and RRR- α -tocopherol stereoisomer distribution of plasma (μ g/mL), organs (μ g/g) and abdominal fat (μ g/g) in lambs fed diets with different synthetic to natural α -tocopherol ratios. ^{a-c}Means within different letters indicate significant differences in total- α -tocopherol content between the different organs in the same diets ($P < 0.05$).

with increasing the ratio of RRR- α -tocopherol in the diet (Table 2). The amount of RRR- α -tocopherol was greatest in spleen and heart in the diets containing 75% and 100% of Nat- α -T ($P < 0.001$). In lungs, the greatest RRR- α -tocopherol amount was found with the diet containing 100% of Nat- α -T ($P < 0.004$). As expected, the amount of the synthetic 2R stereoisomers and the Σ 2S- α -tocopherol stereoisomers decreased in plasma, organs, and abdominal fat when the proportion of Syn- α -T in diets decreased. The liver was the organ that contained the greatest amount of non-RRR- α -tocopherol stereoisomers.

Comparison of α -tocopherol stereoisomers within plasma, organs, and abdominal fat within the same diets is summarized in Table 2. The amounts

of α -tocopherol stereoisomers in plasma, brain, heart, lungs, and abdominal fat were observed in the following order: RRR > RRS, RSR, RSS > Σ 2S, regardless of Syn- α -T to Nat- α -T ratio in the diets. The amount of α -tocopherol in liver was not affected by different Syn- α -T to Nat- α -T ratios, and RRR- α -tocopherol was the most abundant stereoisomers followed by Σ 2S-, RRS-, RSS-, and RSR- α -tocopherol stereoisomers.

There was linear correlation between the proportion of each stereoisomer in the diet and the relative amount in plasma, organs, and abdominal fat as shown for RRR- α -tocopherol in Figure 3. Despite this linear correlation, the calculated relative bioavailability of RRR- α -tocopherol generally increased in organs, abdominal fat,

Figure 3. Linear correlation between the relative proportion of RRR-α-tocopherol in diet and relative proportion of RRR-α-tocopherol in plasma, organs, and abdominal fat of lambs fed different ratios of synthetic to natural α-tocopherol.

and plasma with increasing the proportion of Syn-α-T in the diet ($P < 0.05$). Similar to RRR-α-tocopherol, the relative bioavailability of RRS-α-tocopherol in organs with the exception of muscle increased with increasing the Syn-α-T proportion in the diet. In addition, the relative bioavailability of RSR-α-tocopherol in plasma, spleen, and muscle decreased with increasing the proportion of Nat-α-T in the diets. However, the relative bioavailability of RSR-α-tocopherol in liver and heart increased with increasing the proportion of Nat-α-T in the diets. In the liver, the relative bioavailability of Σ2S-α-tocopherol stereoisomers was greatest ($P < 0.001$; Table 3).

The relative bioavailability of Σ2S-α-tocopherol stereoisomers was high in the liver, but low in other organs and plasma and was generally very modestly affected by different Syn-α-T to Nat-α-T ratios in the other tissues and plasma. The relative bioavailability of Σ2R-α-tocopherol stereoisomers had the same trend in plasma, organs, and abdominal fat and increased with increasing the proportion of Syn-α-T.

DISCUSSION

All the experimental diets contained the same total amount of α-tocopherol. The dietary

α -tocopherol supplementation source had no effect on average daily gain as reported by [Leal *et al.* \(2018\)](#). This is in agreement with previous findings in lambs ([López-Bote *et al.*, 2001](#);

[Turner *et al.*, 2002](#); [de la Fuente *et al.*, 2007](#)) and beef ([Arnold *et al.*, 1992](#); [Lee *et al.*, 2008](#); [Nassu *et al.*, 2011](#)) where vitamin E supplementation had no effect on growth performance.

Table 3. Relative bioavailability of α -tocopherol stereoisomers in plasma, different organs, and abdominal fat of lambs fed different proportions of synthetic to natural α -tocopherol¹

Syn- α -T to Nat- α -T ratio in diets ²	α -tocopherol stereoisomers					
	RRR	RRS	RSS	RSR	Σ 2S	Σ 2R
Plasma						
100:0	2.4 ^a	1.6 ^a	1.4 ^a	1.2 ^b	0.14	1.8 ^a
75:25	1.9 ^b	1.2 ^b	1.0 ^b	1.4 ^a	0.11	1.5 ^b
50:50	1.3 ^c	1.0 ^b	1.0 ^b	0.9 ^c	0.15	1.3 ^c
25:75	1.2 ^c	0.5 ^c	0.6 ^c	0.8 ^c	0.15	1.2 ^d
0:100	1.0 ^d	ND	ND	ND	ND	1.0 ^e
<i>P</i> -value	<0.001	<0.001	<0.001	<0.001	0.76	<.0001
SEM	0.09	0.09	0.06	0.05	0.01	0.04
Liver						
100:0	2.0 ^a	1.1 ^a	1.9 ^b	1.3 ^b	0.47 ^b	1.5 ^a
75:25	1.4 ^b	1.0 ^a	2.4 ^b	1.1 ^b	0.18 ^b	1.3 ^b
50:50	1.2 ^c	0.8 ^b	1.9 ^b	1.1 ^b	0.82 ^b	1.2 ^c
25:75	1.1 ^d	0.6 ^c	2.3 ^b	0.9 ^b	0.59 ^b	1.1 ^d
0:100	1.0 ^e	0.2 ^d	2.2 ^{ab}	2.8 ^a	4.14 ^a	1.0 ^e
<i>P</i> -value	<0.001	<0.001	0.05	<0.001	<0.001	<.0001
SEM	0.06	0.06	0.07	0.16	0.32	0.03
Heart						
100:0	2.5 ^a	1.6 ^a	1.2	1.3 ^b	0.11	1.8 ^a
75:25	1.9 ^b	1.2 ^b	1.0	1.0 ^{bc}	0.10	1.5 ^b
50:50	1.4 ^c	1.0 ^c	1.0	1.0 ^{bc}	0.09	1.3 ^c
25:75	1.2 ^d	0.5 ^d	0.8	0.7 ^c	0.08	1.2 ^d
0:100	1.0 ^e	0.2 ^e	0.9	2.6 ^a	0.10	1.0 ^e
<i>P</i> -value	<0.001	<0.001	0.61	<0.001	0.06	<.0001
SEM	0.10	0.09	0.08	0.14	0.004	0.04
Lungs						
100:0	3.0 ^a	1.4 ^a	0.9	1.3	0.09	1.8 ^a
75:25	2.1 ^b	0.9 ^b	0.6	1.0	0.11	1.5 ^b
50:50	1.4 ^c	0.7 ^{bc}	0.9	0.8	0.11	1.3 ^c
25:75	1.2 ^c	0.6 ^{bc}	0.1	0.6	0.26	1.1 ^d
0:100	1.0 ^c	0.2 ^c	0.6	1.9	0.25	1.0 ^e
<i>P</i> -value	<0.001	<0.001	0.58	0.33	0.13	<.0001
SEM	0.14	0.10	0.10	0.21	0.03	0.05
Spleen						
100:0	2.6 ^a	1.5 ^a	1.2 ^a	1.3 ^a	0.12 ^{bc}	1.8 ^a
75:25	1.8 ^b	1.3 ^b	1.1 ^b	1.0 ^{ab}	0.16 ^a	1.5 ^b
50:50	1.4 ^c	0.9 ^c	0.9 ^c	0.9 ^{ab}	0.10 ^c	1.3 ^c
25:75	1.2 ^d	0.6 ^d	0.8 ^d	0.7 ^{bc}	0.13 ^b	1.2 ^d
0:100	1.0 ^e	0.06 ^e	ND	0.3 ^c	0.01 ^d	1.0 ^e
<i>P</i> -value	<0.001	<0.001	0.01	0.01	<0.001	<.0001
SEM	0.10	0.09	0.03	0.09	0.01	0.04
Muscle						
100:0	2.8 ^a	1.1	1.6	1.2 ^b	0.09	1.8 ^a
75:25	1.9 ^b	1.0	1.3	0.9 ^b	0.08	1.6 ^b
50:50	1.4 ^c	0.8	1.4	0.9 ^b	0.07	1.3 ^c
25:75	1.2 ^d	0.5	1.3	0.7 ^b	0.06	1.2 ^d
0:100	1.0 ^e	0.2	1.2	3.1 ^c	0.12	1.0 ^e
<i>P</i> -value	<0.001	0.11	0.9	0.01	0.50	<.0001
SEM	0.11	0.11	0.26	0.20	0.01	0.05

Table 3. Continued

Syn- α -T to Nat- α -T ratio in diets ²	α -tocopherol stereoisomers					
	RRR	RRS	RSS	RSR	Σ 2S	Σ 2R
Abdominal fat						
100:0	2.5 ^a	1.3	1.4	0.9 ^b	0.25 ^a	1.7 ^a
75:25	1.9 ^b	0.9	0.8	1.0 ^b	0.16 ^b	1.5 ^b
50:50	1.3 ^c	1.1	1.0	1.0 ^b	0.15 ^b	1.3 ^c
25:75	1.2 ^{cd}	0.5	0.5	1.1 ^b	0.09 ^b	1.2 ^d
0:100	1.0 ^d	0.8	2.0	8.1 ^a	0.03 ^c	1.0 ^e
<i>P</i> -value	<0.001	0.11	0.24	<0.001	<0.001	<.0001
SEM	0.11	0.09	0.21	0.78	0.01	0.04

^{a-e}Means within a column with different letters indicate significant differences ($P < 0.05$).

¹The relative bioavailability was calculated as percentage of a given stereoisomer in plasma, organs, and abdominal fat divided by percentage of the stereoisomer in the diet.

²Synthetic α -tocopherol to natural α -tocopherol ratio in diets.

ND = not detected; SEM: standard error of means.

The total α -tocopherol content in spleen was affected by different proportions of Syn- α -T to Nat- α -T; however, no significant difference was observed in plasma, other organs, and abdominal fat. Although a synergistic action between Syn- α -T and Nat- α -T forms of α -tocopherol has been reported by Weber *et al.* (1964) when these 2 forms were given orally, no such synergistic action was observed in the present study. Different concentrations of total α -tocopherol in different organs revealed that uptake by various organs is selective depending on the specific requirement of organs as reported by Njeru *et al.* (1994) and Hidioglou and Charmley (1990). Comparison of the α -tocopherol content between the plasma, organs, and abdominal fat showed that the organs with the greatest α -tocopherol content were the liver followed by heart and spleen, regardless of Syn- α -T to Nat- α -T ratio (Figure 2). The key role of the liver as a regulatory organ in terms of vitamin E storage and transport could be the reason for the highest total α -tocopherol content in the liver. Furthermore, the higher total α -tocopherol content in the heart may be due to the greater phospholipid content and therefore a greater oxidative capacity in cardiac muscle (Njeru *et al.*, 1994). In agreement with our findings, Hidioglou (1987) reported the greatest α -tocopherol concentration in the liver of sheep fed a single oral dose of RRR- α -tocopheryl acetate. However, they reported a greater α -tocopherol content in spleen when compared with heart. Moreover, Ochoa *et al.* (1992) reported consistently greater α -tocopherol concentrations in liver when compared with heart and muscle tissues in sheep fed 1,000 IU per day of different α -tocopherol sources.

Although the bioactivity of various vitamin E compounds has been tested in a few experiments with sheep, only one experiment dealing with the distribution of stereoisomers of α -tocopherol has been reported (Hidioglou *et al.*, 1988). In agreement with our findings, this study showed that sheep fed diets containing either *all-rac*- α -tocopheryl acetate or RRR- α -tocopheryl acetate had greater concentration of RRR- α -tocopherol in heart, kidney, and lungs. Furthermore, Dersjant-Li *et al.* (2009) showed a similar preferential uptake of RRR- α -tocopherol and a similar discrimination against non-RRR- α -tocopherol in calf. In the current study, the biodiscrimination against the α -tocopherol stereoisomers in diets containing the *all-rac*- α -tocopheryl acetate occurred in the following order: Σ 2S > RRS, RSS, RSR > RRR. The greatest amount of RRR- α -tocopherol in plasma, organs, and abdominal fat showed a discrimination against systemic circulation and uptake of synthetic stereoisomers of α -tocopherol into organs. Between 2R- α -tocopherol stereoisomers, the strongest discrimination was observed against the RSS- α -tocopherol stereoisomer in liver, lungs, and spleen. However, in plasma, muscle, and abdominal fat, the strongest discrimination was observed against RSR- α -tocopherol. These results showed that different organs respond differently from the 2R- α -tocopherol stereoisomers. In contrast, Weiser *et al.* (1996) and Jensen *et al.* (2006) reported that rats showed very little discrimination between the four 2R stereoisomers in plasma, liver, brain, and adipose tissues.

Different organs showed different amounts of non-RRR- α -tocopherol stereoisomers; thus, biodiscrimination against 2R-stereoisomers is

dependent on target organ. This study confirms that the different α -tocopherol stereoisomers are not equally transferred to the plasma and tissues. The preference for 2R- α -tocopherol has been reported to be dependent on α -TTP activity which recognizes the 2S stereoisomers poorly. It is well established that after the uptake α -tocopherol into the intestinal cells, α -tocopherol is secreted into the chylomicrons. Therefore, every time that plasma lipoproteins containing the tocopherols are taken up by the liver due to biodiscrimination against the Σ 2S, the 2R- α -tocopherols are preferentially secreted into the plasma (Traber *et al.*, 1990; Leonard *et al.*, 2002). It is likely to expect that the Σ 2S- α -tocopherol stereoisomers fit very poorly into the cavity of the α -TTP and therefore are transported very poorly within the blood. The greatest amount of Σ 2S- α -tocopherol stereoisomers in the liver and the lower Σ 2S- α -tocopherol stereoisomers content in plasma and other organs could be due to the key role of the liver in elimination of Σ 2S- α -tocopherol stereoisomers.

The results showed that with increasing the proportion of Syn- α -T, the amount of non-RRR- α -tocopherol increased in plasma, organs, and abdominal fat. These findings indicate that at high level of Syn- α -T, the stereospecificity of α -TTP allows other 2R- α -tocopherol stereoisomers than RRR- α -tocopherol to be transported with α -TTP. It has been reported that Σ 2S- α -tocopherol stereoisomers are recognized as xenobiotic molecules and are therefore predominantly catabolized (Lauridsen *et al.*, 2002; Traber *et al.*, 2017). In addition, the 2R- α -tocopherol stereoisomers are recognized by α -TTP and resecreted from the liver back into the plasma (Brigelius-Flohe *et al.*, 2002). However, there is a high content of 2R and Σ 2S- α -tocopherol stereoisomers in the organs and abdominal fat. Most likely, organs and abdominal fat take up 2R and Σ 2S- α -tocopherol stereoisomers because this uptake occurs nonspecifically via the mechanisms of lipid uptake (Hymøller *et al.*, 2018). In agreement with our findings, Hosomi *et al.* (1997) demonstrated that the tissues take up vitamin E by a variety of nonspecific mechanisms that depend on the metabolism of lipoproteins. However, there is a variation in content of 2R and Σ 2S- α -tocopherol stereoisomers in the plasma and organs. It seems that the receptors on each tissue could be a reason why different lipoproteins may get delivered in different amounts.

The amount of RRR- α -tocopherol increased with increasing the proportion of Nat- α -T, and the same trend was observed in the proportion of

RRR- α -tocopherol. Figure 3 shows that the proportion of RRR- α -tocopherol increased linearly with increasing the proportion of Nat- α -T in the diet. The linearity in RRR- α -tocopherol proportion was quite surprising because the enzymes with the key role in elimination and clearance of α -tocopherol stereoisomers are saturable, and a curve linear response was expected (Blatt *et al.*, 2004). However, the level of α -tocopherol fed to the lambs was within normal physiological range and may have been below the saturation level. The key role of α -TTP as a determinant of relative bioavailability (Leonard *et al.*, 2002; Blatt *et al.*, 2004; Jensen and Lauridsen, 2007) at the physiological level of α -tocopherol may be an explanation for contradictory results between different relative bioavailability and linearity between the relative proportions of each stereoisomer in diet and plasma or tissues. In addition, competition between α -tocopherol stereoisomers for binding to enzymes involved in transferring and elimination may be an explanation for a different relative bioavailability (Blatt *et al.*, 2004). The different slopes and intercepts may show that plasma, liver, heart, lungs, and spleen had different trends for circulation and accumulation of RRR- α -tocopherol.

In the present study, the relative bioavailability reflects proportion of α -tocopherol stereoisomers that are absorbed, transferred, reached, and accumulated in the organs. The relative bioavailability of the RRR-stereoisomer of α -tocopherol increased when the proportion of Syn- α -T increased from 0 to 100% in the diets. The greater elimination of Σ 2S and 2R- α -tocopherols may increase the relative amount and therefore the relative bioavailability of RRR- α -tocopherols in diets containing Syn- α -T. In contrast, the relative bioavailability of plasma Σ 2S- α -tocopherol stereoisomers remained relatively stable regardless of percent of Syn- α -T. These results showed that the relative bioavailability of α -tocopherol stereoisomers is affected by the absolute and/or relative amount of α -tocopherol stereoisomers. Based on Table 3, relative bioavailability of α -tocopherol stereoisomers cannot be constant because the elimination and distribution of α -tocopherol stereoisomers involve processes that are stereospecific and saturable, and therefore, the relative bioavailability will change with the saturation of those processes (Blatt *et al.*, 2004). Generally, the ratio 1.36:1 between RRR- α -tocopheryl acetate and *all-rac*- α -tocopheryl acetate has been accepted by researchers (Blatt *et al.*, 2004). The present results demonstrated that relative bioavailability of α -tocopherol

stereoisomers changes with different proportions of Syn- α -T to Nat- α -T, despite the observed linearity between ratio of stereoisomers in the diets and the ratio in plasma and tissues. Our findings also confirm results of other researchers who found that bioavailability of α -tocopherol stereoisomers is dose or dose-time dependent (Blatt *et al.*, 2004; Jensen *et al.*, 2006; Meglia *et al.*, 2006). This implies that the relative bioavailability of Nat- α -T and Syn- α -T stereoisomers of α -tocopherol is not constant but heavily dependent on the ratio between Nat- α -T and Syn- α -T, dose of α -tocopherol stereoisomers, and the target organ (Jensen *et al.*, 2006). With the exception of RSR α -tocopherol in the liver, the bioavailability of 2R- α -tocopherol in all diets followed the same trend as the RRR- α -tocopherol. However, the relative bioavailability of 2R- α -tocopherol was lower than RRR- α -tocopherol. The RRS- α -tocopherol, the stereoisomers most similar to RRR- α -tocopherol, is the synthetic stereoisomer which also shows an increase in bioavailability with increasing the proportion of Syn- α -T to Nat- α -T in diets, revealing the importance of the stereochemical configuration as a determinant for relative bioavailability.

The results of the present study showed that lamb organs express discrimination against synthetic stereoisomers of α -tocopherol, and plasma, organs, and abdominal fat responded differently in favor of α -tocopherol stereoisomers. The results indicated that the relative bioavailability of the RRR-stereoisomer of α -tocopherol is variable when lambs are fed different proportions of *all-rac*- α -tocopheryl acetate to RRR- α -tocopheryl acetate. The results indicated that distribution and the relative bioavailability of α -tocopherol stereoisomers are dependent on proportions of *all-rac*- α -tocopheryl acetate to RRR- α -tocopheryl acetate.

Conflict of interest statement. None declared.

LITERATURE CITED

- Arnold, R. N., K. K. Scheller, S. C. Arp, S. N. Williams, D. R. Buege, and D. M. Schaefer. 1992. Effect of long- or short-term feeding of alpha-tocopheryl acetate to Holstein and crossbred beef steers on performance, carcass characteristics, and beef color stability. *J. Anim. Sci.* 70:3055–3065.
- Baldi, A. 2005. Vitamin E in dairy cows. *Livest. Prod. Sci.* 98:117–122. doi:10.1016/j.livprodsci.2005.10.004
- Blatt, D. H., W. A. Pryor, J. E. Mata, and R. Rodriguez-Proteau. 2004. Re-evaluation of the relative potency of synthetic and natural alpha-tocopherol: experimental and clinical observations. *J. Nutr. Biochem.* 15:380–395. doi:10.1016/j.jnutbio.2003.12.011
- Brigelius-Flohe, R., F. J. Kelly, J. T. Salonen, J. Neuzil, J. M. Zingg, and A. Azzi. 2002. The European perspective on vitamin E: current knowledge and future research. *Am. J. Clin. Nutr.* 76:703–716. doi:10.1093/ajcn/76.4.703
- Burton, G. W., M. G. Traber, R. V. Acuff, D. N. Walters, H. Kayden, L. Hughes, and K. U. Ingold. 1998. Human plasma and tissue alpha-tocopherol concentrations in response to supplementation with deuterated natural and synthetic vitamin E. *Am. J. Clin. Nutr.* 67:669–684. doi:10.1093/ajcn/67.4.669
- Dersjant-Li, Y.S. K. Jensen, L. W. Bos, and M. R. Peisker. 2009. Bio-discrimination of α -tocopherol Stereoisomers in rearing and veal calves fed milk replacer supplemented with all-rac- α -tocopheryl acetate. *Int. J. Vitam. Nutr. Res.* 79:199–211. doi:10.1024/0300-9831.79.4.199.
- Evans, H., and K. S. Bishop. 1922. On an invariable and characteristic disturbance of reproductive function in animals reared on a diet poor in fat soluble vitamins A. *Anat. Rec.* 23:17.
- de la Fuente, J., M. T. Díaz, I. Alvarez, S. Lauzurica, V. Cañeque, and C. Pérez. 2007. Effect of dietary supplementation with vitamin E on characteristics of vacuum-packed lamb. *J. Sci. Food Agric.* 87:651–659. doi:10.1002/jsfa.2759.
- Harris, P., and M. Ludwig. 1948. Relative vitamin E potency of natural vitamin E and of synthetic α -tocopherol. *J. Biol. Chem.* 179:1111–1115
- Hidiroglou, M. 1987. Vitamin E levels in sheep tissues at various times after a single oral administration of d-alpha-tocopherol acetate. *Int. J. Vitam. Nutr. Res.* 57:381–384.
- Hidiroglou, M., and E. Charmley. 1990. Vitamin E concentrations in blood plasma of sheep and in sheep tissues after a single intraruminal or intraperitoneal administration of DL-alpha-tocopheryl acetate. *Res. Vet. Sci.* 48:158–161. doi:10.1016/S0034-5288(18)30982-2
- Hidiroglou, N., L. F. Laflamme, and L. R. McDowell. 1988. Blood plasma and tissue concentrations of vitamin E in beef cattle as influenced by supplementation of various tocopherol compounds. *J. Anim. Sci.* 66:3227–3234. doi:10.2527/jas1988.66123227x.
- Hidiroglou, N., L. R. McDowell, A. M. Papas, M. Antapli, and N. S. Wilkinson. 1992. Bioavailability of vitamin E compounds in lambs. *J. Anim. Sci.* 70:2556–2561. doi:10.2527/1992.7082556x
- Hosomi, A., M. Arita, Y. Sato, C. Kiyose, T. Ueda, O. Igarashi, H. Arai, and K. Inoue. 1997. Affinity for alpha-tocopherol transfer protein as a determinant of the biological activities of vitamin E analogs. *FEBS Lett.* 409:105–108. doi:10.1016/S0014-5793(97)00499-7.
- Hymøller, L., S. Lashkari, T. N. Clausen, and S. K. Jensen. 2018. Distribution of α -tocopherol stereoisomers in mink (*Mustela vison*) organs varies with the amount of all-rac- α -tocopheryl acetate in the diet. *Br. J. Nutr.* pp.1–6. doi:10.1017/S0007114518002878
- Jensen, S. K., and C. Lauridsen. 2007. Alpha-tocopherol stereoisomers. *Vitam. Horm.* 76:281–308. doi:10.1016/S0083-6729(07)76010-7
- Jensen, S. K., J. V. Nørgaard, and C. Lauridsen. 2006. Bioavailability of alpha-tocopherol stereoisomers in rats depends on dietary doses of all-rac- or RRR-alpha-tocopheryl acetate. *Br. J. Nutr.* 95:477–487. doi:10.1079/BJN20051667.
- Jishage, K., M. Arita, K. Igarashi, T. Iwata, M. Watanabe, M. Ogawa, O. Ueda, N. Kamada, K. Inoue, H. Arai, et al. 2001. Alpha-tocopherol transfer protein is important for the normal development of placental labyrinthine

- trophoblasts in mice. *J. Biol. Chem.* 276:1669–1672. doi:10.1074/jbc.C000676200
- Lauridsen, C., H. Engel, A. M. Craig, and M. G. Traber. 2002. Relative bioactivity of dietary RRR- and all-rac-alpha-tocopheryl acetates in swine assessed with deuterium-labeled vitamin E. *J. Anim. Sci.* 80:702–707. doi:10.2527/2002.803702x
- Leal, L. N., J. A. Beltrán, V. Alonso, J. M. Bello, L. A. den Hartog, W. H. Hendriks, and J. Martín-Tereso. 2018. Dietary vitamin E dosage and source affects meat quality parameters in light weight lambs. *J. Sci. Food Agric.* 98:1606–1614. doi:10.1002/jsfa.8635
- Lee, S. K., Panjono, S. M. Kang, T. S. Kim, and Y. S. Park. 2008. The effects of dietary sulfur and vitamin E supplementation on the quality of beef from the longissimus muscle of Hanwoo bulls. *Asian-Australas. J. Anim. Sci.* 21:1059–1066. doi:10.5713/ajas.2008.70372
- Leonard, S. W., Y. Terasawa, R. V. Farese, Jr., and M. G. Traber. 2002. Incorporation of deuterated RRR- or all-rac-alpha-tocopherol in plasma and tissues of alpha-tocopherol transfer protein-null mice. *Am. J. Clin. Nutr.* 75:555–560. doi:10.1093/ajcn/75.3.555
- López-Bote, C., A. Daza, M. Soares, and E. Berges. 2001. Dose-response effect of dietary vitamin E concentration on meat quality characteristics in light-weight lambs. *Anim. Sci.* 73:451–457. doi:10.1017/S1357729800058422
- Meglia, G. E., S. K. Jensen, C. Lauridsen, and K. Persson Waller. 2006. Alpha-tocopherol concentration and stereoisomer composition in plasma and milk from dairy cows fed natural or synthetic vitamin E around calving. *J. Dairy Res.* 73:227–234. doi:10.1017/S0022029906001701
- Nassu, R. T., M. E. Dugan, M. Juárez, J. A. Basarab, V. S. Baron, and J. L. Aalhus. 2011. Effect of α -tocopherol tissue levels on beef quality. *Animal* 5:2010–2018. doi:10.1017/S1751731111001182
- Njeru, C. A., L. R. McDowell, N. S. Wilkinson, S. B. Linda, L. X. Rojas, and S. N. Williams. 1994. Serum and tissue tocopherol in sheep after intramuscular injection and/or dietary vitamin E supplementation. *J. Anim. Sci.* 72:739–745. doi:10.2527/1994.723739x
- Ochoa, L., L. R. McDowell, S. N. Williams, N. Wilkinson, J. Boucher, and E. L. Lentz. 1992. Alpha-tocopherol concentrations in serum and tissues of sheep fed different sources of vitamin E. *J. Anim. Sci.* 70:2568–2573.
- Traber, M. G., G. W. Burton, K. U. Ingold, and H. J. Kayden. 1990. RRR- and SRR-alpha-tocopherols are secreted without discrimination in human chylomicrons, but RRR-alpha-tocopherol is preferentially secreted in very low density lipoproteins. *J. Lipid Res.* 31:675–685.
- Traber, M. G., E. Mah, S. W. Leonard, G. Bobe, and R. S. Bruno. 2017. Metabolic syndrome increases dietary α -tocopherol requirements as assessed using urinary and plasma vitamin E catabolites: a double-blind, crossover clinical trial. *Am. J. Clin. Nutr.* 105:571–579. doi:10.3945/ajcn.116.138495
- Turner, K. E., K. E. McClure, W. P. Weiss, R. J. Borton, and J. G. Foster. 2002. Alpha-tocopherol concentrations and case life of lamb muscle as influenced by concentrate or pasture finishing. *J. Anim. Sci.* 80:2513–2521. doi:10.1093/ansci/80.10.2513
- Vagni, S., F. Saccone, L. Pinotti, and A. Baldi. 2011. Vitamin E bioavailability: past and present insights. *Food Nutr. Sci.* 2:1088–1096. doi:10.4236/2Fns.2011.210146
- Weber, F., U. Gloor, J. Wüsch, and O. Wiss. 1964. Synergism of d- and l-alpha-tocopherol during absorption. *Biochem. Biophys. Res. Commun.* 14:186–188. doi:10.1016/0006-291X(64)90252-9
- Weiser, H., G. Riss, and A. W. Kormann. 1996. Biodiscrimination of the eight alpha-tocopherol stereoisomers results in preferential accumulation of the four 2R forms in tissues and plasma of rats. *J. Nutr.* 126:2539–2549. doi:10.1093/jn/126.10.2539
- Weiser, H., and M. Vecchi. 1982. Stereoisomers of alpha-tocopheryl acetate. II. Biopotencies of all eight stereoisomers, individually or in mixtures, as determined by rat resorption-gestation tests. *Int. J. Vitam. Nutr. Res.* 52:351–370.
- Weiss, W. P., and D. J. Wyatt. 2003. Effect of dietary fat and vitamin E on alpha-tocopherol in milk from dairy cows. *J. Dairy Sci.* 86:3582–3591. doi:10.3168/jds.S0022-0302(03)73964-2