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Original Research Article

Formation of *RRR*-α-tocopherol in rumen and intestinal digestibility of tocopherols in dairy cows

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ABSTRACT

Tocopherol sources in diets are often a combination of *all-rac*-α-tocopheryl acetate (synthetic α-tocopherol) from vitamin supplements and natural tocopherols and 2R-(4′R, 8′R)-5,7,8-trimethyltocotrienol (α-tocotrienols) from the feed sources. Synthetic α-tocopherol consists of 8 different stereoisomers including 2R-(4′R, 8′R)-5,7,8-trimethyltocol (*RRR*-α-tocopherol), 2R-(4′S, 8′R)-5,7,8-trimethyltocol (*RSR*-α-tocopherol), 2R-(4′R, 8′S)-5,7,8-trimethyltocol (*RRS*-α-tocopherol), 2R-(4′S, 8′S)-5,7,8-trimethyltocol (*RSS*-α-tocopherol), 2S-(4′S, 8′S)-5,7,8-trimethyltocol (*SSS*-α-tocopherol), 2S-(4′R, 8′S)-5,7,8-trimethyltocol (*SRS*-α-tocopherol), 2S-(4′S, 8′R)-5,7,8-trimethyltocol (*SSR*-α-tocopherol), and 2S-(4′R, 8′R)-5,7,8-trimethyltocol (*SRR*-α-tocopherol). The pre-absorption metabolism of tocopherols and tocotrienols in ruminants differs from monogastric animals due to the extensive microbial fermentation in the anaerobic rumen. The current study investigated the impact of toasting and decortication of oats on metabolism in the digestive tract (synthesis, digestion), and intestinal digestibility of tocopherols in dairy cows by using 4 ruminal and intestinal cannulated Danish Holstein cows in a 4 × 4 Latin square design for 4 periods. Cows were fed a total mixed ration ad libitum containing different forms of oats: whole oat, decorticated oat, toasted oat, and decorticated toasted oat, all rolled before mixed ration. Overall means across 4 treatments were statistically analyzed, testing whether overall means were different from zero. Decortication or toasting did not affect the balance or digestibility of α-tocopherols in rumen. Average across treatments showed the ruminal degradation of synthetic α-tocopherol (279 mg/d, $P = 0.02$; *P*-value shows that average across treatments is different from zero), synthetic 2R-α-tocopherol (133 mg/d, $P < 0.01$; summation of *RRS*-, *RSR*- and *RSS*-α-tocopherol), and 2S-α-tocopherol (190 mg/d; $P < 0.01$, summation of *SSS*-, *SRS*-, *SSR*, and *SRR*-α-tocopherol), while *RRR*-α-tocopherol was formed in the rumen (221 mg/d, $P = 0.10$). The average across treatments showed that small intestinal digestibility of tocopherols ranked in the following order: α-tocotrienol > natural α-tocopherol > synthetic α-tocopherols > 2R-(4′R, 8′R)-,7,8-dimethyltocol (γ-tocopherol). The average across treatments for small intestinal and feed-ileum digestibility ranked in the following order: *RRR*-α-tocopherol > synthetic 2R-α-tocopherol > 2S-α-tocopherol. Results showed the first evidence for *RRR*-α-tocopherol formation under anaerobic conditions in the rumen. In addition, synthetic α-tocopherol stereoisomers, γ-tocopherol and α-tocotrienol were degraded in the rumen. There was a discrimination against absorption of synthetic 2R- and 2S-α-tocopherol in the small intestine.

1. Introduction

Vitamin E is a fat soluble cellular antioxidant of importance for immune function (Lashkari et al., 2021b), reproduction and oxidative stability of milk and meat (Baldi, 2005). Vitamin E consists of 4 tocopherols and 4 tocotrienols, of which α-tocopherol is biologically the most important compound. α-Tocopherol has 3 asymmetric carbons and can occur in 8 different isomeric configurations, including four 2R configurations (2R-(4′R, 8′R)-5,7,8-trimethyltocol

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[*RRR*-α-tocopherol], 2R-(4′S, 8′R)-5,7,8-trimethyltocol [*RSR*-α-tocopherol], 2R-(4′R, 8′S)-5,7,8-trimethyltocol [*RRS*-α-tocopherol], 2R-(4′S, 8′S)-5,7,8-trimethyltocol [*RSS*-α-tocopherol]), and four 2S configurations (2S-(4′S, 8′S)-5,7,8-trimethyltocol [*SSS*-α-tocopherol], 2S-(4′R, 8′S)-5,7,8-trimethyltocol [*SRS*-α-tocopherol], 2S-(4′S, 8′R)-5,7,8-trimethyltocol [*SSR*-α-tocopherol], and 2S-(4′R, 8′R)-5,7,8-trimethyltocol [*SRR*-α-tocopherol]) (Jensen and Lauridsen, 2007). *RRR*-α-tocopherols are synthesized only by plants and other oxygenic, photosynthetic organisms (DellaPenna, 2005), while synthetic α-tocopherol, used in most feed and food applications, consists of a racemic mixture of all 8 stereoisomers. In addition, synthetic *all-rac*-α-tocopherol is mostly acetylated and thus fed as *all-rac*-α-tocopheryl acetate (Jensen and Lauridsen, 2007). The acetylated form of α-tocopherol must be hydrolyzed by pancreatic carboxyl ester hydrolase prior to absorption (Hymøller et al., 2018; Jensen and Lauridsen, 2007; Lashkari et al., 2021a), as only free tocopherols are absorbed from the gastro-intestinal tract (Hidiroglou and Ivan, 1992).

Tocopherol sources in ruminant diets are often a combination of synthetic *all-rac*-α-tocopheryl acetate from vitamin supplements and natural tocopherols and tocotrienols from the feed sources. The pre-absorption metabolism of tocopherols and 2R-(4′R, 8′R)-5,7,8-trimethyltocotrienol (α-tocotrienols) in ruminants differs from monogastric animals due to the extensive microbial fermentation in the anaerobic rumen. No reports are available on whether tocotrienols are exposed to ruminal biohydrogenation in the same way as unsaturated fatty acids (Lashkari et al., 2017, 2020). Thus, absorption of tocopherols in ruminants is only sparsely described, and literature regarding the susceptibility of α-tocopherol to ruminal degradation is inconsistent. Some studies showed that pre-intestinal vitamin E disappearance is around 42% in sheep (Alderson et al., 1971) and up to 52% in cattle (Shin and Owens, 1990). In contrast, other studies showed that tocopherols are stable in the rumen (Astrup et al., 1974; Hymøller and Jensen, 2010; Leedle et al., 1993; Weiss et al., 1995). Therefore, due to high price of vitamin E supplements, it is critical to obtain knowledge on the metabolism of tocopherol in the gastro-intestinal tract of cows to avoid under or over-supply.

The development of advanced chromatographic separation methods that allow separation of the individual α-tocopherol stereoisomers (Jensen and Lauridsen, 2007) makes it possible to distinguish between natural and synthetic source of α-tocopherol in a feed mixture and the fate of the α-tocopherols in the digestive tract. To the best of our knowledge, there are no published data on digestibility of α-tocopherols in the different sections of the digestive tract in cows; thus, the aim of the present experiment was to investigate differences in metabolism and absorption through the digestive tract in dairy cows of tocopherols originating from natural or synthetic sources and to investigate possible biohydrogenation of α-tocotrienol to their corresponding α-tocopherol.

2. Materials and methods

2.1. Animal ethics statement

This study was carried out according to the guidelines of the Danish Ministry (Justice Law No. 474; 15 May 2014) concerning experiments with animals and care of animals used for experimental purposes under the approval of the Danish Veterinary and Food Administration. The present study was carried out in compliance with the ARRIVE guidelines.

2.2. Animals and experimental design

Two primiparous and two multiparous lactating Danish Holstein dairy cows were fitted with rumen cannulas (#4C; Bar Diamond, Parma, ID) and duodenal and ileal simple polyvinyl chloride T-cannulas (i.d. = 2.5 cm) placed 60 cm caudal to the pylorus (duodenal) and 20 cm cranial to the cecum (ileum). Cows were assigned to receive 1 of 4 experimental diets over 4 periods in a 4 × 4 Latin square design. Cows were randomly assigned to 1 of 4 experimental diets for the first period. In each period, which lasted for 22 d, d 1 to 13 were allocated for adaptation, and d 13 to 17 for collection of digesta (i.e., rumen, duodenum, ileum, feces). The experimental diets contained whole grain oat (Oat), decorticated oat (D), toasted whole oat (T), or decorticated toasted oat (DT), and all the types were rolled and offered in a TMR. In this study, the main effect of decortication and toasting is reported in full terms, i.e., "decortication" for the main effect of decortication in D and DT diets; and "toasting" represented the main effect of toasting in T and DT diets. Animals were housed in tie-stalls, and cubicles were bedded with rubber mats and sawdust. The average body weight (mean ± standard deviation) of cows at the onset of the experiment was 618 ± 48 kg (Panah et al., 2020a), and body condition score, days in milk, daily milk yield, and energy corrected milk were 3.00 ± 0.20, 61.3 ± 49.9 d and 32.6 ± 7.0 kg/d, and 33.1 ± 7.4 kg/d, respectively (mean ± standard deviation).

2.3. Diets and feeding

Oat (*Avena sativa*) cultivar Dominik grown in Denmark in 2017 was used in this study. A mobile decorticator with 3 MHSA dehuller (Buhler AG, Uzwil, Switzerland) mounted on a truck was used for decortication on-farm (Gl. Buurholt ApS, Bronderslev, Denmark), and the efficiency of decortication was 83% (83% fully decorticated), and the remainder was partially decorticated. Toasting at 121 °C was done for 35 s on-farm using a Bulldog Toaster (Mecmar S.p.a., Minerbe, Italy) with the flow of 2,500 kg/h. All 4 forms of oat were rolled by an MS 1325-4 roller (Skjold, Saby, Denmark) with a 3-mm roller distance.

Formulation of the diet was based on NorFor (Volden, 2011), and the feed ration and composition of the nutrients are reported in Table 1 (Panah et al., 2020a). In order to calculate the ration composition, all feedstuffs were analyzed for dry matter (DM), ash, crude protein, crude fat, amino acids, and neutral detergent fiber, which have been reported in Panah et al. (2020a, 2020b). Cows

Table 1
Total mixed ration and nutrient composition of experimental diets (g/kg DM, unless mentioned).

Item	Diets[1]			
	Oat	D	T	DT
Ingredients				
Whole oat grain	217.1	–	–	–
Decorticated oat grain	–	217.1	–	–
Toasted oat grain	–	–	217.1	–
Decorticated toasted oat grain	–	–	–	217.1
Toasted fava beans	165.2	165.2	165.2	165.2
Grass clover silage	609	609	609	609
Mineral and vitamin supplements[2]	8.70	8.70	8.70	8.70
Nutrients				
Dry matter, g/kg	540	540	563	561
Ash	77.6	77.4	77.8	77.4
Crude fat	43.9	48.1	40.5	45.1
Crude protein	194	197	193	198
Starch	156	175	151	174
Neutral detergent fiber	331	310	331	301

[1] D = decorticated oat; T = toasted oat; DT = decorticated and toasted oat.

[2] Composition per kilogram DM: calcium carbonate 420 g, NaCl 259 g, magnesium sulfate 150 g, magnesium oxide 74 g, sugar beet molasses 31 g, calcium-magnesium carbonate 5 g, vitamin A 900,000 IU, vitamin D_3 190,000 IU, synthetic α-tocopheryl acetate 6,000 IU, Mn 4,000 mg, Cu 1,500 mg, Zn 4,500 mg, I 225 mg, Co 25 mg, Se 50 mg.

were fed twice a day at 06:00 and 16:30. Dry matter concentration of feed and residues were recorded on daily basis on d 12 through 17 in each period to estimate the dry matter intake (DMI). The ingredient samples were taken in each period on a weekly basis and analyses were performed on pooled samples of period 1 and 2, and pooled samples of periods 3 and 4.

2.4. Rumen, digesta, and feces sampling

Details for rumen, digesta, and feces sampling have been reported (Panah et al., 2020a, 2020b). Briefly, duodenal, ileal, and fecal flow were estimated using two markers; chromium oxide (Cr_2O_3; 10 g) and titanium dioxide (TiO_2; 13 g), which were weighed in degradable coffee filter bags and placed into the rumen simultaneously with milking times (05:20 and 15:45). Over 5 d, intestinal digesta and feces were sampled 12 times (i.e., d 13 to 17; at 10:00 and 18:00 on d 13; 02:00, 12:00, and 20:00 on d 14; 04:00, 14:00, and 22:00 on d 15; 06:00, 16:00, and 24:00 on d 16; 08:00 on d 17). In order to collect duodenal (0.5 L) and ileal (0.2 L) contents from the T-cannula, plastic bags, mounted on L-shaped polyvinylchloride pipe connectors, were used. Fecal samples (approx. 50 g) were collected either during defecation or grabbed from the rectum. At the end of each experiment, the 12 samples for duodenal and ileal contents and feces were pooled by sample, cow, and period for the downstream analysis.

2.5. Chemical analysis

All feed and digesta samples were freeze-dried before analysis. Dry matter was determined by oven drying at 60 °C for 48 h (Åkerlind et al., 2011). The ash content was determined by combustion at 525 °C for 6 h. Nitrogen content of feed was determined (Hansen, 1989) by the Dumas method using a Vario MAX CN (Elementar Analysesysteme GmbH, Hanau, Germany), and converted to crude protein by multiplying with 6.25. Crude fat was measured by gravimetric method according to a modified Bligh and Dyer method (Bligh and Dyer, 1959; Jensen, 2008). Samples were hydrolyzed with 3 M HCl (Merck, Germany) for 1 h at 80 °C, and then a mixture of 3 mL of methanol (VWR Chemical BDH, Norway), 1.5 mL of distilled water, and 3 mL of chloroform (VWR Chemical BDH, Norway) was used for fat extraction. After phase separation, approximately 2 mL of the chloroform phase was transferred to tared tube, weighed, oven-dried at 100 °C, and weighed for determination of total fat. An enzymatic colorimetric method was used to determine the starch concentration of feedstuffs (Knudsen et al., 1987). Neutral detergent fiber concentration was analyzed by Fibertec M6 System using sodium sulfite and heat-stable amylase (Mertens, 2002), and reported as ash free. Amino acids were determined using the EEC (98/64/EC) method (European Commission, 1998).

The concentration of α-tocopherol in feedstuffs and digesta was quantified by high performance liquid chromatography (HPLC) after saponification and extraction into heptane (Jensen et al., 2006). Accordingly, digesta and feed samples were diluted with 2.0 mL ethanol (96% vol/vol; VWR Chemical BDH, Norway), 0.5 mL methanol (100%), 1.0 mL ascorbic acid (20% wt/vol; VWR Chemical BDH, China), 0.3 mL KOH-water (1:1, wt/vol; Merck, Germany), and 0.7 mL water. Saponification of the samples was carried out at 80 °C for 20 min and cooled in the dark. Tocopherol was extracted into 2 volumes of 5 mL heptane (VWR Chemical BDH, Poland), and 100 μL of the combined heptane phase was injected into the HPLC. The HPLC column for determination of tocopherol consisted of a 4.0 × 250 mm Perkin-Elmer HS-5-Silica column (Perkin-Elmer GmbH, D-7770 Uberlingen, Germany). The mobile phase consisted of heptane and 2-propanol (3.0 mL/L; VWR Chemical BDH, Norway) and was degassed by helium with the flow rate of 3.0 mL/min. The identification and quantification of the tocopherols were obtained by a comparison of retention time and peak areas with Merck (D-6100 Darmstadt, Germany) external standards. An excitation wavelength of 290 nm and an emission wavelength of 327 nm were performed for fluorescence detection. The following extinction coefficients for the standards in ethanol (96%, vol/vol) were used: α-tocopherol, $A^{1\%}{}_{1\ \mathrm{cm}} = 71.0$ at 294 nm; γ-tocopherol, $A^{1\%}{}_{1\ \mathrm{cm}} = 92.8$ at 298 nm and δ-tocopherol, $A^{1\%}{}_{1\ \mathrm{cm}} = 91.2$ at 298 nm (Merck; D-6100 Darmstadt, Germany). The tocotrienols were quantified by means of the extinction coefficient for the corresponding tocopherol.

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 1.0 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 of which the first peak represents the four 2S forms (*SSR*, *SRR*, *SRS* and *SSS*), the second peak contains the synthetic *RSS*-, third peak contains *RRS*-, the fourth peak contains *RRR*-, while the fifth peak contains *RSR*-α-tocopherol. The ratio between natural and synthetic α-tocopherol in the samples was calculated on basis of the relative abundance of each stereoisomer.

2.6. Calculations and statistical analyses

The data on DMI (d 12-17) were averaged per cow per period, and the daily DMI was calculated as DM offered minus DM in refusals. From day 13 to day 17 in each period, DM flow for digesta DM was calculated as an average of flows for each marker. Digestibility in the rumen, small intestine, and total tract was calculated from the respective intake and flow at the duodenum, ileum, and fecal output (Supplementary S1).

The effect of different treatments on intake, balance, and digestibility was analyzed using SAS (version 9.4, SAS Institute Inc., Cary, NC). The least square means of response variables were measured using the general linear model procedure in SAS through the following model:

$$Y_{ijkl} = \mu + D_i + T_j + DT_{ij} + P_k + C_l + \text{Й}_{ijkl},$$

where Y is the dependent variable, μ is the overall mean, and the model includes the fixed effects of decortication (D_i), toasting (T_j), the interaction between decortication and toasting (DT_{ij}), the kth period (P_k) and the random effect of lth cow (C_l), and the random error Й_{ijkl}. The main effects of decortication (Dec) are reported as the effect of decortication in D and DT diets, and the main effect of toasting (Toa) is based on the effects of toasting in T and DT diets. Experimental unit was cow × period. For feed-ileum balance of α-tocotrienol and 2R-(4′R, 8′R)-,7,8-dimethyltocol (γ-tocopherol), hindgut balance of γ-tocopherol, and digestibility of some of the tocopherols, the covariance for the random effect of cow could not be estimated in MIXED. Thus, the *F*-test for the effect of Dec, Toa, and interaction between them was made using the mean squares of cows as the error term and the test option from a similar GLM model (Jensen et al., 2020).

Overall means across treatments were analyzed by using proc mixed procedure in SAS with Maximum Likelihood method, and the model was:

$$Y_{ijkl} = \mu + P_k + C_l + \breve{И}_{ikl},$$

where Y is the dependent variable, μ is the overall mean, and the model includes random effect of kth period (P_k), random effect of lth cow (C_l), and the random error $\breve{И}_{ikl}$. The approximation of degree of freedoms was specified by DDFM = KENWARDROGER option in the stated model. Except for Tables 1 and 2, values presented in Tables are least square means with corresponding SEM.

All the collected data were included in the analysis, and values in Fig. presented the least square means across treatments with corresponding SEM as error bars. Significance for main effects was declared at $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.10$. When significant interactions were found, least squares means were compared using Tukey–Kramer test in $\alpha = 0.05$. Data for small intestinal digestibility and feed-ileum digestibility of α-tocotrienol were not normally distributed; therefore, the data were transformed by root square, and back transformed data were reported.

3. Results

3.1. Tocopherol composition of experimental feedstuffs

α-Tocotrienol accounts for about half of the tocopherol content in oat (Table 2). Decortication increased, while toasting reduced tocopherol concentration. γ-Tocopherol accounts for 80% of the tocopherols in fava beans, while α-tocopherol is the dominant tocopherol in grass clover silage. The vitamin and mineral supplement contributed only with synthetic α-tocopherol.

3.2. Nutrient intake and flow of tocopherols

Intake of nutrients is reported in Table 3 (Panah et al., 2020a, 2020b). Dry matter intake (kg/d) was 21.7, 20.7, 22.0 and 21.9 in oat, D, T, and DT, respectively, and tended to be higher in toasted ($P = 0.08$). Average across treatments showed that tocopherols and α-tocopherol intake were higher than zero ($P < 0.01$ for all cases; Fig. 1).

Composition of tocopherols in duodenal and ileal digesta and feces flow is presented in Table S1. Average across treatments showed that duodenal, ileal, and fecal flow of tocopherols and α-tocopherol stereoisomers were higher than zero ($P < 0.01$ for all cases; results not shown in tables or figures).

3.3. Balance and digestibility

Balance and digestibility of tocopherols in rumen, intestine, hindgut, and total tract are shown in Tables 4 and 5, respectively. Decortication and toasting did not influence the balance of tocopherols in the rumen, with the exception of an interaction between decortication and toasting for α-tocotrienol (interaction $P < 0.01$). Balance of α-tocotrienol in the rumen for oat and decorticated oat was higher than for toasted decorticated oat and toasted oat. Across treatments, average values are presented in Fig. 2 and tested if they are different from zero (Tables S2 and S3). Thus, Fig. 2 shows that 279 mg/d of synthetic α-tocopherol, 133 mg/d of synthetic 2R-α-tocopherol and 190 mg/d of 2S-α-tocopherol were degraded in the rumen. Similarly, α-tocotrienol (23 mg/d) and γ-tocopherol (60 mg/

Table 2
α-Tocopherol composition of feedstuffs (mg/kg of DM ± standard deviation).

Item	Total α-tocopherol	Natural α-tocopherol[1]	Synthetic α-tocopherol[2]	*RRR*-α-tocopherol[3]	Synthetic 2R-α-tocopherol[4]	2S-α-tocopherol[5]	α-Tocotrienol[6]	γ-Tocopherol[7]
Oat	6.9 ± 0.16	6.9 ± 0.16	ND	6.9 ± 0.16	ND	ND	13.2 ± 0.90	1.6 ± 1.24
Decorticated oat	7.7 ± 1.14	7.7 ± 1.14	ND	7.7 ± 1.14	ND	ND	17.7 ± 2.19	1.6 ± 0.52
Toasted oat	5.1 ± 0.98	5.1 ± 0.98	ND	5.1 ± 0.98	ND	ND	2.4 ± 1.26	0.2 ± 0.07
Toasted decorticated oat	3.6 ± 1.39	3.6 ± 1.39	ND	3.6 ± 1.39	ND	ND	1.5 ± 1.24	ND
Toasted fava beans	2.0 ± 2.79	2.0 ± 2.79	ND	2.0 ± 2.79	ND	ND	0.3 ± 0.29	40.3 ± 4.67
Grass clover silage	57.2 ± 16.11	57.2 ± 16.11	ND	57.2 ± 16.11	ND	ND	ND	6.4 ± 3.11
Mineral and vitamin supplement	5,534 ± 0.0	ND	5,534 ± 0.0	728 ± 0.0	2,075 ± 0.0	2,767 ± 0.0	ND	ND

ND = not detected.

[1] Natural α-tocopherol originates from feedstuffs.

[2] Synthetic α-tocopherol originates from vitamin supplement.

[3] *RRR*-α-tocopherol (2R-(4′R, 8′R)-5,7,8-trimethyltocol) originates from feedstuffs and vitamin supplement.

[4] Synthetic 2R-α-tocopherol is summation of *RRS* (2R-(4′R, 8′S)-5,7,8-trimethyltocol), *RSR* (2R-(4′S, 8′R)-5,7,8-trimethyltocol) and *RSS*-α-tocopherol (2R-(4′S, 8′S)-5,7,8-trimethyltocol).

[5] 2S-α-tocopherol is summation of *SRR* (2S-(4′R, 8′R)-5,7,8-trimethyltocol), *SSR* (2S-(4′S, 8′R)-5,7,8-trimethyltocol), *SRS* (2S-(4′R, 8′S)-5,7,8-trimethyltocol), and *SSS*-α-tocopherol (2S-(4′S, 8′S)-5,7,8-trimethyltocol).

[6] α-Tocotrienol (2R-(4′R, 8′R)-5,7,8-trimethyltocotrienol).

[7] γ-Tocopherol (2R-(4′R, 8′R)-,7,8-dimethyltocol).

Table 3
Dry matter and nutrient intake (kg/d).[1]

Item	Diets[2]				SEM	*P*-values[3]		
	Oat	D	T	DT		Dec	Toa	Dec × Toa
Dry matter	21.7	20.7	22.0	21.9	2.07	0.25	0.08	0.25
Organic matter	20.4	19.5	20.7	20.7	2.05	0.25	0.08	0.25
Starch	3.4	3.6	3.3	3.8	0.34	0.01	0.53	0.19
Neutral detergent fiber	7.0	5.8	7.0	6.4	0.62	0.01	0.22	0.35
Crude protein	4.1	4.0	4.1	4.3	0.40	0.92	0.12	0.13
Amino acids	3.0	3.0	3.0	3.1	0.30	0.79	0.10	0.24
Crude fat	0.90	0.91	0.89	0.94	0.089	0.19	0.59	0.49

[1] Reported in Panah et al. (2020a, 2020b).

[2] Oat = whole grain oat; D = decorticated oat; T = toasted oat; DT = decorticated and toasted oat.

[3] Dec = effect of decortication; Toa = effect of toasting; Dec × Toa = interaction between toasting and decortication.

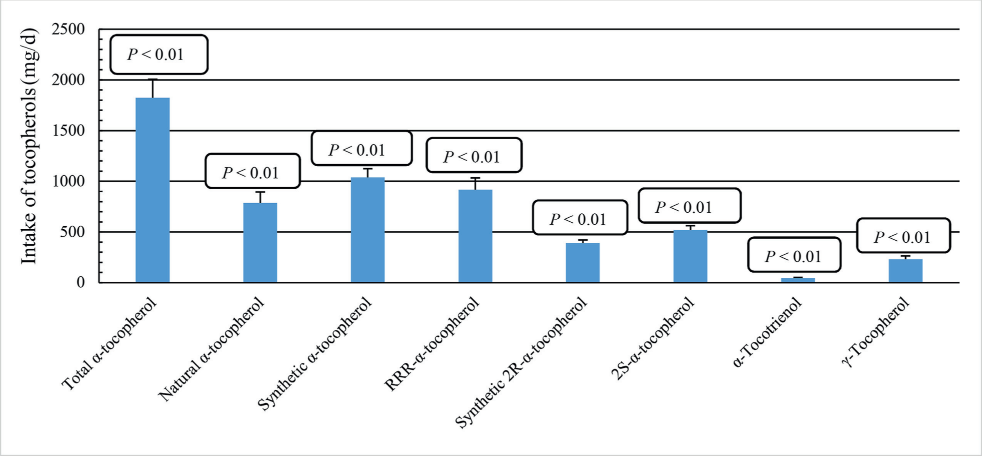


Fig. 1. Intake of tocopherols from diets. Total α-tocopherol originates from feedstuffs and vitamin supplement. Natural α-tocopherol originates from feedstuffs. Synthetic α-tocopherol originates from vitamin supplements. *RRR*-α-tocopherol (2R-(4′R, 8′R)-5,7,8-trimethyltocol) originates from feedstuffs and vitamin supplement. Synthetic 2R-α-tocopherol is summation of *RRS* (2R-(4′R, 8′S)-5,7,8-trimethyltocol), *RSR* (2R-(4′S, 8′R)-5,7,8-trimethyltocol), and *RSS*-α-tocopherol (2R-(4′S, 8′S)-5,7,8-trimethyltocol). 2S-α-tocopherol is summation of *SRR* (2S-(4′R, 8′R)-5,7,8-trimethyltocol), *SSR* (2S-(4′S, 8′R)-5,7,8-trimethyltocol), *SRS* (2S-(4′R, 8′S)-5,7,8-trimethyltocol), and *SSS*-α-tocopherol (2S-(4′S, 8′S)-5,7,8-trimethyltocol). α-Tocotrienol and γ-tocopherol are 2R-(4′R, 8′R)-5,7,8-trimethyltocotrienol and 2R-(4′R, 8′R)-,7,8-dimethyltocol, respectively. *P*-values show that average across treatments is different from zero. Error bars represent the standard error of means.

d) were degraded in the rumen. On the other hand, there was a formation of *RRR*-α-tocopherol in each of the 4 oat diets, and lsmeans of oat ($P = 0.02$), D ($P < 0.01$), T ($P < 0.01$), and DT ($P < 0.01$) were different from zero (*P*-values not shown in Tables or Figures). Likewise, the average across treatments for the balance of *RRR*-α-tocopherol (221 mg/d) in the rumen tended to be higher than zero ($P = 0.10$). Similar to the balance, average ruminal digestibility across treatments was higher than zero for synthetic α-tocopherol ($P < 0.01$), 2S-α-tocopherol ($P < 0.01$), α-tocotrienol ($P < 0.01$; Fig. 3).

3.4. Intestinal balance and digestibility

Decortication and toasting did not influence the balance of tocopherols in the small intestine with the exception of α-tocotrienol, which decreased upon toasting (Table 4; $P < 0.01$). However, the average of tocopherols across treatments (Fig. 2) showed that the balance in the small intestine of tocopherols was higher than zero ($P < 0.01$), with the exception of α-tocotrienol. Average of balance in the small intestine of tocopherols across treatments was highest for total α-tocopherol (690 mg/d; $P = 0.02$) and it was higher for natural α-tocopherol (421 mg/d; $P = 0.01$) and *RRR*-α-tocopherol (485 mg/d; $P = 0.01$) than for synthetic α-tocopherol (269 mg/d; $P = 0.03$) and synthetic 2R-α-tocopherol (92 mg/d; $P = 0.02$). In the small intestine, the balance of *RRR*-tocopherols across treatments was more than five times higher than the synthetic 2R-α-tocopherol. In addition, the balance of natural α-tocopherol across treatments in the small intestine was higher than for α-tocotrienol and γ-tocopherol. For the small intestine digestibility, the average across treatments was higher than zero for all tocopherols and was highest for α-tocotrienol (511 mg/g).

Decortication or toasting did not affect the feed-ileum balance of the various α-tocopherol stereoisomers without interaction between toasting and decortication, except for α-tocotrienol, which decreased upon toasting. Feed-ileum balance was higher than zero for total α-tocopherol ($P = 0.02$), synthetic α-tocopherol, synthetic 2R-α-tocopherol, 2S-α-tocopherol, α-tocotrienol and γ-tocopherol (Fig. 2; $P < 0.01$ for all tocopherols). Furthermore, average of digestibility across treatments showed (Fig. 3) that feed-ileum digestibility of tocopherols was higher than zero ($P < 0.01$ for all tocopherols) and was highest for natural α-tocopherol (555 mg/g) and lowest for 2S-α-tocopherol (211 mg/g).

For total tract balance of *RRR*-α-tocopherol, an interaction was found between toasting and decortication ($P = 0.02$), and it was higher in oat compared to toasted oat and toasted decorticated oat. Average of total tract balance across treatments was higher than zero for all tocopherols ($P < 0.01$ for all tocopherols) except for natural α-tocopherol and *RRR*-α-tocopherol. Average across treatments showed that total tract digestibility of total α-tocopherol, synthetic α-tocopherol, synthetic 2R-α-tocopherol, 2S-α-tocopherol ($P < 0.01$ for all), and γ-tocopherol ($P = 0.02$) was higher than zero. Average of balance in the hindgut across treatments was not different from zero with the exception of α-tocotrienol ($P = 0.04$) and γ-tocopherol ($P = 0.01$). In addition, except for total α-tocopherol ($P = 0.03$) and γ-tocopherol ($P < 0.01$), averages of hindgut digestibility across treatments for other tocopherols were not different from zero.

4. Discussion

Our previous data from the same experiment showed adverse effects of toasting on unsaturated fatty acid content of oat (Panah et al., 2020b). Therefore, we aimed to investigate the effect of decortication and toasting on tocopherol content as a susceptible compound to toasting and their consequence on ruminal and intestinal digestibility of tocopherols. Although there was an effect on α-tocopherol and α-tocotrienol content in oat due to decortication and toasting; the effect on overall intake of α-tocopherol was negligible, thus, in the discussion the overall fate of tocopherols across treatments throughout the gastrointestinal tract is discussed. Results for ruminal and post ruminal digestibility of protein (Panah et al., 2020c), amino acids (Panah et al., 2020a) and fatty acids (Panah et al., 2020b) have previously been presented and discussed.

4.1. Tocopherol composition of experimental feedstuffs

Minor changes in concentration of total α-tocopherol and α-tocotrienol after decortication showed that α-tocopherol and α-tocotrienol are mainly located in the germ or endosperm, hence contribution of hull is negligible. Total α-tocopherol decreased 26%

Table 4
Intake of tocopherols and balance of tocopherols in the rumen, intestine, and total-tract (mg/d).[1]

Item	Diets[2]				SEM	*P*-values[3]		
	Oat	D	T	DT		Dec	Toa	Dec × Toa
Total α-tocopherol								
Intake	1,862	1,753	1,837	1,850	176.0	0.24	0.36	0.15
Rumen	267	39	64	37	94.9	0.17	0.26	0.26
Small intestine	534	648	789	790	224.7	0.77	0.34	0.78
Feed-ileum	802	686	853	827	244.2	0.67	0.57	0.79
Hindgut	67	−44	−266	−343	168.4	0.56	0.08	0.92
Total tract	869	643	587	483	120.1	0.04	0.01	0.36
Natural α-tocopherol[4]								
Intake	819	755	780	793	77.9	0.28	0.99	0.12
Rumen	−88	−189	−213	−216	46.3	0.21	0.09	0.23
Small intestine	349	377	480	476	131.6	0.92	0.34	0.89
Feed-ileum	261	188	267	260	127.3	0.70	0.70	0.75
Hindgut	55	−17	−141	−190	93.5	0.51	0.08	0.90
Total tract	316	171	125	71	52.8	0.03	0.01	0.25
Synthetic α-tocopherol[5]								
Intake	1,043	998	1,057	1,056	99.6	0.25	0.08	0.25
Rumen	356	228	278	253	58.8	0.14	0.58	0.30
Small intestine	185	271	308	314	96.0	0.59	0.34	0.64
Feed-ileum	541	499	586	567	120.1	0.65	0.41	0.86
Hindgut	11	−27	−125	−154	78.1	0.65	0.11	0.95
Total tract	553	472	461	413	69.2	0.08	0.05	0.61
RRR-α-tocopherol[6]								
Intake	949	880	912	925	89.3	0.26	0.86	0.12
Rumen	−120	−248	−254	−264	51.5	0.16	0.13	0.21
Small intestine	393	446	549	552	150.0	0.83	0.34	0.85
Feed-ileum	273	198	295	288	144.4	0.72	0.62	0.76
Hindgut	61	−18	−181	−235	108.4	0.53	0.06	0.90
Total tract	334^{a}	180^{ab}	114^{b}	54^{b}	59.8	0.05	0.11	0.02
Synthetic 2R-α-tocopherol[7]								
Intake	391	374	396	396	37.6	0.25	0.08	0.25
Rumen	153	113	139	127	22.2	0.11	0.98	0.35
Small intestine	68	92	100	107	32.2	0.59	0.41	0.75
Feed-ileum	222	205	239	233	44.8	0.64	0.35	0.81
Hindgut	14	6	−50	−53	29.0	0.85	0.06	0.92
Total tract	236	211	189	181	25.5	0.23	0.02	0.52
2S-α-tocopherol[8]								
Intake	521	499	528	528	49.8	0.25	0.08	0.25
Rumen	234	174	180	174	34.3	0.27	0.36	0.35
Small intestine	74	110	139	131	47.2	0.75	0.34	0.61
Feed-ileum	307	284	318	305	58.8	0.57	0.62	0.88
Hindgut	−8	−32	−34	−56	36.2	0.53	0.49	0.98
Total tract	299	252	284	249	40.1	0.04	0.61	0.72
α-Tocotrienol[9]								
Intake	63	81	13	20	5.6	0.01	<0.01	0.15
Rumen	37^{a}	42^{a}	5^{b}	8^{b}	5.3	0.25	0.42	<0.01
Small intestine	11.4	21.5	0.4	−2.2	5.30	0.38	<0.01	0.16
Feed-ileum	48	64	5	6	6.1	0.15	<0.01	0.18
Hindgut	15	17	8	14	5.5	0.41	0.37	0.72
Total tract	63	81	13	20	5.6	<0.01	0.06	0.15
γ-Tocopherol[10]								
Intake	244	225	228	233	23.5	0.36	0.60	0.15
Rumen	92	54	52	51	13.5	0.15	0.10	0.18
Small intestine	50	59	61	56	11.4	0.86	0.74	0.53
Feed-ileum	131	113	113	106	19.5	0.42	0.39	0.70
Hindgut	−27	−42	−36	−32	9.1	0.57	0.97	0.35
Total tract	104	80	78	88	15.6	0.10	0.20	0.16

[a,b] Means in the same row with different superscripts differ ($P < 0.05$).

[1] Balance values in rumen, small intestine, hindgut, and total tract were calculated by milligram of digested minus milligram of intake, duodenal flow, ileal flow, and intake, respectively (Supplementary S1). Negative values represent the ruminal or hindgut formation.

[2] Oat = whole grain oat; D = decorticated oat; T = toasted oat; DT = decorticated and toasted oat.

[3] Dec = effect of decortication; Toa = effect of toasting; Dec × Toa = interaction between toasting and decortication.

[4] Natural α-tocopherol originates from feedstuffs.

[5] Synthetic α-tocopherol originates from vitamin supplement.

[6] *RRR*-α-tocopherol (2R-(4′R, 8′R)-5,7,8-trimethyltocol) originates from feedstuffs and supplement.

[7] Synthetic 2R-α-tocopherol is summation of *RRS* (2R-(4′R, 8′S)-5,7,8-trimethyltocol), *RSR* (2R-(4′S, 8′R)-5,7,8-trimethyltocol), and *RSS*-α-tocopherol (2R-(4′S, 8′S)-5,7,8-trimethyltocol).

[8] 2S-α-tocopherol is summation of *SRR* (2S-(4′R, 8′R)-5,7,8-trimethyltocol), *SSR* (2S-(4′S, 8′R)-5,7,8-trimethyltocol), *SRS* (2S-(4′R, 8′S)-5,7,8-trimethyltocol), and *SSS*-α-tocopherol (2S-(4′S, 8′S)-5,7,8-trimethyltocol).

[9] α-Tocotrienol (2R-(4′R, 8′R)-5,7,8-trimethyltocotrienol).

[10] γ-Tocopherol (2R-(4′R, 8′R)-,7,8-dimethyltocol).

Table 5
Digestibility of tocopherol in rumen, intestine, and total-tract (mg/g).[1]

Item	Diets[2]				SEM	*P*-values[3]		
	Oat	D	T	DT		Dec	Toa	Dec × Toa
Total α-tocopherol								
Rumen	91	25	37	−9	47.2	0.22	0.33	0.82
Small intestine	340	365	404	437	108.6	0.78	0.53	0.97
Feed-ileum	310	363	415	409	102.9	0.82	0.49	0.79
Hindgut	20	−60	−377	−1,033	506.5	0.47	0.20	0.57
Total tract	426	369	317	234	42.3	0.10	0.02	0.72
Natural α-tocopherol[4]								
Rumen	−227	−276	−296	−357	63.9	0.31	0.18	0.91
Small intestine	394	388	443	466	110.2	0.94	0.56	0.90
Feed-ileum	481	514	636	591	144.7	0.97	0.45	0.80
Hindgut	24	−64	−403	−1,010	493.1	0.49	0.19	0.60
Total tract	310	213	153	21	57.8	0.06	0.01	0.73
Synthetic α-tocopherol[5]								
Rumen	314	235	270	227	39.7	0.17	0.53	0.66
Small intestine	268	339	353	402	109.5	0.57	0.48	0.92
Feed-ileum	189	263	268	284	108.1	0.58	0.53	0.72
Hindgut	3	−53	−353	−1,066	528.9	0.47	0.22	0.53
Total tract	506	482	435	379	35.0	0.26	0.04	0.64
RRR-α-tocopherol[6]								
Rumen	−231	−301	−292	−355	61.5	0.23	0.28	0.94
Small intestine	375	385	433	459	107.0	0.86	0.54	0.94
Feed-ileum	458	517	611	580	138.4	0.92	0.47	0.76
Hindgut	29	−56	−418	−1,040	499.7	0.48	0.18	0.59
Total tract	280	196	119	−2	58.0	0.10	0.02	0.73
Synthetic 2R-α-tocopherol[7]								
Rumen	372	307	354	309	34.7	0.13	0.82	0.76
Small intestine	289	331	354	404	111.6	0.66	0.51	0.97
Feed-ileum	182	234	235	258	98.5	0.61	0.60	0.84
Hindgut	654	17	−419	−1,052	529.2	0.51	0.16	0.57
Total tract	584	578	475	447	35.1	0.65	0.01	0.76
2S-α-tocopherol[8]								
Rumen	418	359	349	318	43.2	0.34	0.25	0.75
Small intestine	231	322	341	390	128.0	0.59	0.49	0.87
Feed-ileum	156	211	239	237	79.0	0.75	0.51	0.73
Hindgut	−61	−160	−222	−1,006	518.3	0.40	0.35	0.51
Total tract	550	506	538	458	35.6	0.05	0.27	0.49
α-Tocotrienol[9]								
Rumen	563	514	256	385	142.9	0.63	0.04	0.32
Small intestine	412	551	60	−93	682.8	0.99	0.25	0.72
Feed-ileum	203	270	272	−102	431.2	0.55	0.42	0.43
Hindgut	1,000	1,000	1,000	1,000	–	–	–	–
Total tract	1,000	1,000	1,000	1,000	–	–	–	–
γ-Tocopherol[10]								
Rumen	261	226	216	174	54.9	0.76	0.94	0.66
Small intestine	309	334	334	311	53.5	0.98	0.11	0.35
Feed-ileum	228	269	271	258	54.5	0.80	0.78	0.64
Hindgut	−271	−316	−396	−278	100.8	0.72	0.77	0.51
Total tract	345	295	329	268	58.4	0.15	0.54	0.89

[1] Digestibility values in rumen, small intestine, hindgut, and total tract are reported in milligram of digested per g of intake, duodenal flow, ileal flow, and intake, respectively (Supplementary S1). Negative values represent the ruminal or hindgut formation.
[2] Oat = whole grain oat; D = decorticated oat; T = toasted oat; DT = decorticated and toasted oat.
[3] Dec = effect of decortication; Toa = effect of toasting; Dec × Toa = interaction between toasting and decortication.
[4] Natural α-tocopherol originates from feedstuffs.
[5] Synthetic α-tocopherol originates from vitamin supplement.
[6] *RRR*-α-tocopherol (2R-(4′R, 8′R)-5,7,8-trimethyltocol) originates from feedstuffs and supplement.
[7] Synthetic 2R-α-tocopherol is summation of *RRS* (2R-(4′R, 8′S)-5,7,8-trimethyltocol), *RSR* (2R-(4′S, 8′R)-5,7,8-trimethyltocol), and *RSS*-α-tocopherol (2R-(4′S, 8′S)-5,7,8-trimethyltocol).
[8] 2S-α-tocopherol is summation of *SRR* (2S-(4′R, 8′R)-5,7,8-trimethyltocol), *SSR* (2S-(4′S, 8′R)-5,7,8-trimethyltocol), *SRS* (2S-(4′R, 8′S)-5,7,8-trimethyltocol), and *SSS*-α-tocopherol (2S-(4′S, 8′S)-5,7,8-trimethyltocol).
[9] α-Tocotrienol (2R-(4′R, 8′R)-5,7,8-trimethyltocotrienol).
[10] γ-Tocopherol (2R-(4′R, 8′R)-,7,8-dimethyltocol).

in toasted oat compared to oat and decreased 53% in toasted decorticated compared to decorticated oat, which showed the pronounced degrading effect of toasting on tocopherols. In addition, these results showed more severe adverse effect of toasting on α-tocopherol after decortication, which can be justified by the removal of physical barrier made up by the hull. In agreement with our findings, Ko et al. (2003) reported a decreased α-tocopherol and α-tocotrienol concentration in rice bran roasted at 170 °C. Even though the oxidation end products of fatty acids are different from tocopherols; the degradation of tocopherols by toasting is in line with more than 23% reduction of total fatty acids in toasted oat compared to oat, and 34% reduction in total fatty acids in toasted decorticated oat compared to decorticated oat (Panah et al., 2020b). This reduction in both tocopherols and poly-unsaturated fatty acids is most likely caused by non-enzymatic free radicals, generating oxidation processes, which may use some of the tocopherols as the first defensive antioxidative mechanism to protect against the fatty acid oxidation during and after toasting. Our previous data from the same study showed a reduction of linoleic acid from 20 to 11 g/kg of DM in oat compared to decorticated toasted oat. The significant reduction in α-tocotrienol concentration for toasted and decorticated toasted oat is in line with the observed oxidation of polyunsaturated fatty acids such as linoleic acid (Panah et al., 2020b) because tocotrienols might be more susceptible to oxidation due to the unsaturated side chain. Higher destruction of α-tocotrienol and γ-tocopherol in toasted oat compared to non-toasted may reflect the higher antioxidant properties of α-tocotrienol and γ-tocopherol compared to α-tocopherols. In support of our results, higher antioxidant activity was reported in γ-tocopherol compared to α-tocopherol in coconut fat heated at 60 and 160 °C (Wagner et al., 2001).

4.2. Intake and ruminal balance and digestibility

The slightly higher DMI due to toasting can explain the slightly higher intake of synthetic α-tocopherol, synthetic 2R-α-tocopherol, and 2S-α-tocopherol. The greater flow of *RRR*-α-tocopherol from the rumen compared to the dietary intake indicates formation of *RRR*-α-tocopherol in the rumen.

Overall means for ruminal balance and digestibility of tocopherols showed ruminal formation of *RRR*-α-tocopherol and degradation of the synthetic stereoisomers of α-tocopherol, α-tocotrienol, and γ-tocopherol (Fig. 2). Part of the formation of *RRR*-α-tocopherol can be explained by rumen microbial biohydrogenation of the unsaturated phytyl side chain of α-tocotrienol; however, even if this biohydrogenation was complete, it would account for less than 44 mg per day, which equal approximately 20% of total ruminal *RRR*-α-tocopherol formation. In biological systems no reports are available of racemization of α-tocopherol; therefore, formation of *RRR*-α-tocopherol from synthetic α-tocopherol is less probable. Lindqvist et al. (2014) reported an increased α-tocopherol content of legume–grass mixtures silage treated with an inoculant-enzyme preparation and a formic acid-based additive than in untreated silage, and they speculated that α-tocopherol-producing microorganisms present on plants may enhance the α-tocopherol production when cultured in an optimal medium. Similarly, Liu et al. (2019) reported a higher α-tocopherol concentration in whole-crop oat silage treated with *Lactobacillus plantarum* and propionic acid additive. In support of our data, synthesis of fat-soluble substances, such as menaquinones by *Flavobacterium* spp. has been reported (Tani and Sakurai, 1987). In addition, it has recently been reported that *S. cerevisiae* through a systematic metabolic engineering are capable of synthesis β-farnesene, which can be converted into isophytol (Ye et al., 2022). It could be speculated that part of the *RRR*-α-tocopherol present in the feed is not liberated and extracted by the sample preparation, but then we should have expected to see the same for γ-tocopherol. Since the synthetic isomers of α-tocopherol in the rumen did not increase, only *RRR*-α-tocopherol, it reflects precision of analytical method used.

More than 10% loss of α-tocopherol has been reported during ensiling of whole-crop oat silage due to wilting (Liu et al., 2019). In addition, our results showed toasting decreased α-tocopherol and α-tocotrienol by 3.4 and 6.8 mg/kg of DM, respectively, caused by lipid oxidation and/or peroxidation. The reason for these losses could be that α-tocopherol acts as an electron donor to lipid peroxyl radicals during ensiling and heat treatment, resulting in formation of α-tocopherol radicals. Therefore, a part of the *RRR*-α-tocopherol formation could be a result of regeneration of oxidized *RRR*-α-tocopherol by electrons donated by nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) in the reduced environment in the rumen. In support of our results, Ishida et al. (2020) reported an increase in regeneration of α-tocopherol due to H^+ derived from the fermentation of non-digestible saccharides in colon of rats. However, rumen formation of *RRR*-α-tocopherol due to biohydrogenation of α-tocotrienol is still unknown. Degradation of synthetic 2R- and 2S-α-tocopherol in the rumen is in disagreement with other reports, who showed no degradation of α-tocopherol in the rumen of cows (Hymøller and Jensen, 2010), sheep (Astrup et al., 1974) and steers (Leedle et al., 1993). However, there are still conflicting results in reported literature regarding the stability of tocopherols against degradation by rumen microbes. Shin and Owens (1990) reported up to 52% pre-intestinal tocopherol degradation in cattle, and Alderson et al. (1971) reported more than 40% pre-intestinal tocopherol degradation in sheep. The discrepancy between the current results and the abovementioned research could be that in the previous studies, total α-tocopherol was reported, while in the present study the fractioned α-tocopherol stereoisomers are reported.

4.3. Intestinal balance and digestibility

The lack of effect of decortication on small intestine and feed-ileum balance (Table 3) and digestibility (Table 4) of tocopherols showed that removal of hulls had no effect on intestinal digestibility. Small intestinal balance of tocopherols took place in the following order: natural α-tocopherol > synthetic α-tocopherols > γ-tocopherol > α-tocotrienol (Fig. 2), which reflects an obvious discrimination against the digestion and absorption of synthetic α-tocopherol in the small intestine. The higher small intestinal balance and digestibility of natural α-tocopherol compared to synthetic α-tocopherol may be caused by limited hydrolysis of the ester bonds of synthetic α-tocopherol (*all-rac*-α-tocopheryl acetate) due to limited activity of the necessary carboxyl ester hydrolase (Jensen et al., 1999; Lashkari et al., 2021a). In line with the observed intestinal digestibility in our study, Bjørneboe et al. (1986) indicated approximately 40% absorption for α-tocopherol in rats; while, Jensen et al. (2006) reported an average absorption of 82% for *all-rac*-α-tocopheryl acetate and 88% for *RRR*-α-tocopheryl acetate in growing rats, and 71% in growing broilers for *all-rac*-α-tocopheryl acetate (Jensen et al., 1999).

Considerably higher small intestinal balance and digestibility in total α-tocopherol compared to γ-tocopherol could be due to preferential absorption of α-tocopherol as a result of its capability for better dispersion and micellization, higher intake of total α-tocopherol (1,825 g/d) compared to γ-tocopherol (76 g/d), and preferential secretion of γ-tocopherol into bile (Traber and Kayden, 1989). In agreement with our findings, high intake levels of α-tocopherol resulted in a considerably lowered plasma γ-tocopherol in humans (Baker et al., 1986). Intestinal absorption mechanism of fat-soluble vitamins is similar to lipids, which is dependent on pancreatic function, biliary secretion, micellar formation and absorption/diffusion across intestinal membranes (Muller et al., 1974). Differences between small intestinal digestibility of tocopherols and fatty acids [787 g/kg of total fatty acids (Panah et al., 2020b), average across treatments from the same study] could be due to duodenal fatty acid composition. Fatty acid composition of duodenal flow showed approximately 700 g saturated fatty acid (C14:0 + C16:0 + C18:0 + C20:0) per kilogram of fatty acid, which may reduce the dispersion and micellarization of tocopherols during digestion and intestinal transportation. In support of our findings, Failla et al. (2014) reported that mono- and polyunsaturated fatty acids seem to increase vitamin E absorption compared to saturated fatty acids. In addition, Lashkari et al. (2021b) demonstrated that plasma vitamin E concentration was higher for 245 mg/kg of *RRR*-α-tocopherol in calf concentrate mixed

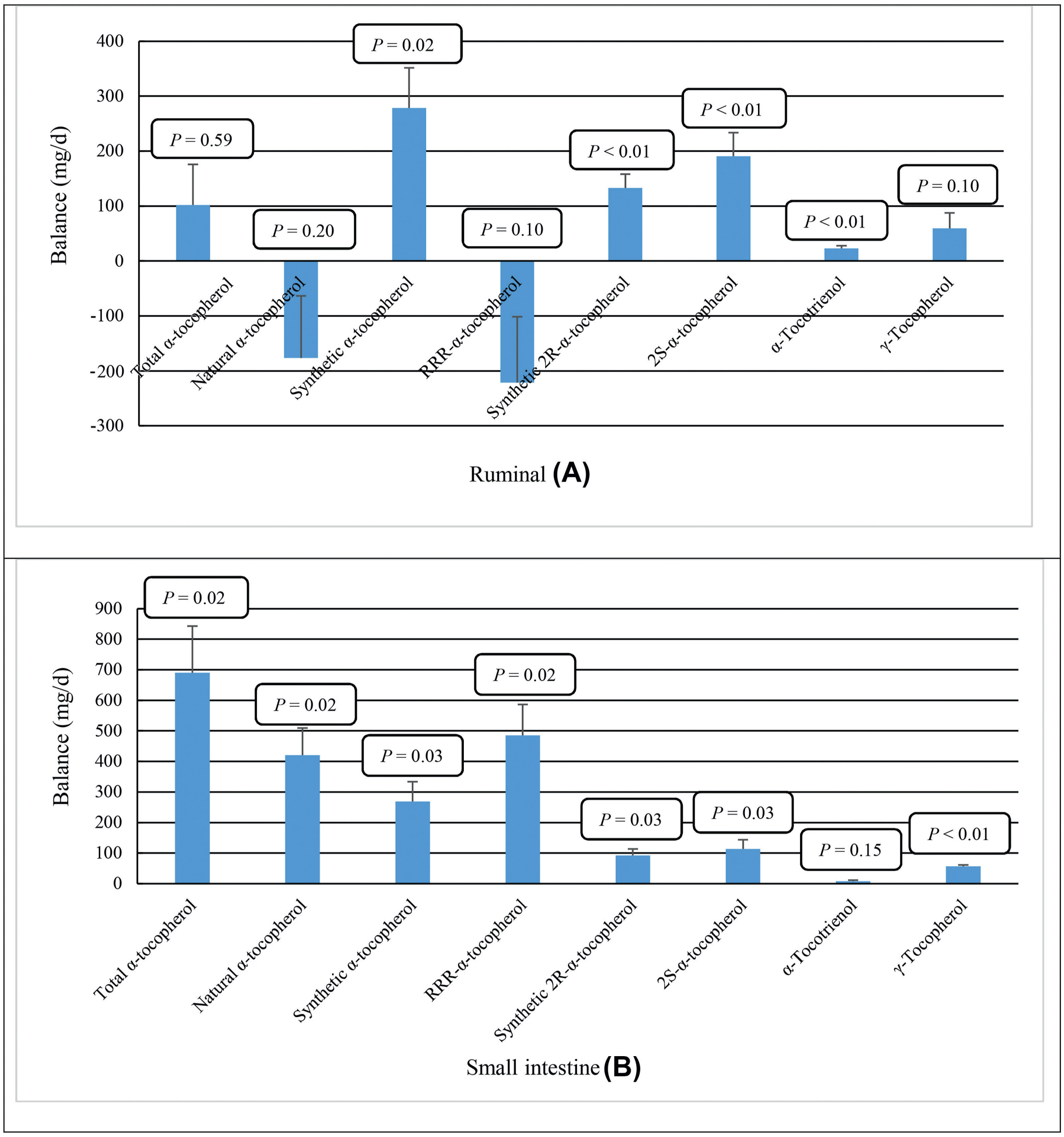
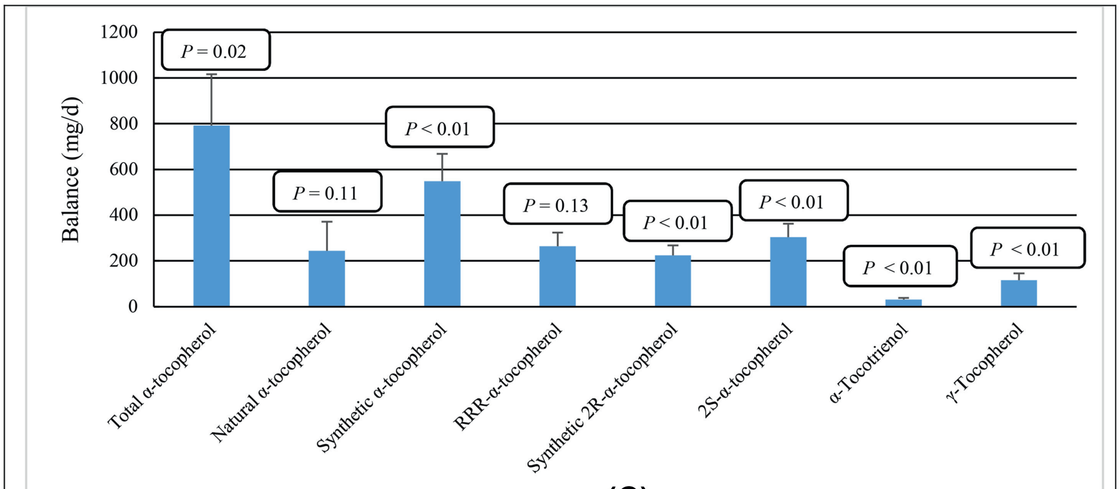


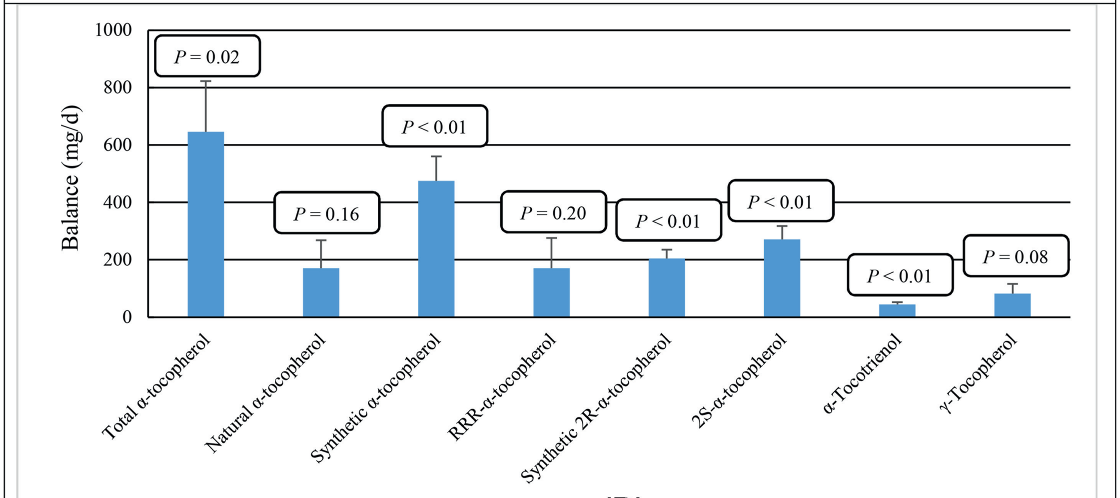
Fig. 2. Tocopherol balance in rumen (A), small intestine (B), feed-ileum (C), total tract (D), and hindgut (E). Total α-tocopherol originates from feedstuffs and vitamin supplement. Natural α-tocopherol originates from feedstuffs. Synthetic α-tocopherol originates from vitamin supplements. *RRR*-α-tocopherol (2R-(4′R, 8′R)-5,7,8-trimethyltocol) originates from feedstuffs and vitamin supplement. Synthetic 2R-α-tocopherol is summation of *RRS* (2R-(4′R, 8′S)-5,7,8-trimethyltocol), *RSR* (2R-(4′S, 8′R)-5,7,8-trimethyltocol), and *RSS*-α-tocopherol (2R-(4′S, 8′S)-5,7,8-trimethyltocol). 2S-α-tocopherol is summation of *SRR* (2S-(4′R, 8′R)-5,7,8-trimethyltocol), *SSR* (2S-(4′S, 8′R)-5,7,8-trimethyltocol), *SRS* (2S-(4′R, 8′S)-5,7,8-trimethyltocol), and *SSS*-α-tocopherol (2S-(4′S, 8′S)-5,7,8-trimethyltocol). α-Tocotrienol and γ-tocopherol are 2R-(4′R, 8′R)-5,7,8-trimethyltocotrienol and 2R-(4′R, 8′R)-,7,8-dimethyltocol, respectively. *P*-values show that average across treatments is different from zero. Error bars represent the standard error of means. Negative values represent the ruminal or hindgut formation.

with 3% of lecithin mixture (contained 40% of lecithin, 30% of rapeseed oil, and 30% of free fatty acids from rapeseed oil distillates) compared to 490 mg/kg of *RRR*-α-tocopherol without lecithin as fat supplements in the calf concentrate, highlighting the importance of fat for tocopherol absorption. The highest small intestinal digestibility observed in α-tocotrienol could be due to effect of unsaturated side chain, which makes α-tocotrienol better dispersed into micelles as seen for higher absorption of unsaturated fatty acids over saturated fatty acids in the small intestine (Panah et al., 2020b).

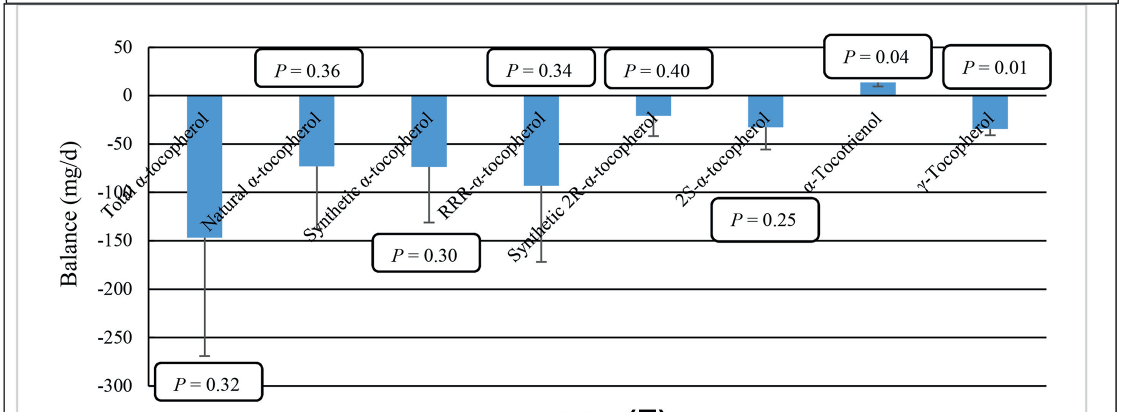
Primary findings, obtained in rat intestinal everted sacs (Hollander et al., 1975), showed passive diffusion through enterocyte apical membrane for vitamin E absorption. However, it has been shown that α- and γ-tocopherol absorption is mediated by scavenger receptor class B type I (Reboul et al., 2006), intracellular cholesterol transporter 1 (Reboul et al., 2012), and CD36 molecule (Abuasal et al., 2010). These transporters were reported to selectively mediate the transport of some molecules present in mixed micelles, which may result in favor of a direct interaction with their ligands. Interestingly, it was recently demonstrated that α-



Feed-ileum **(C)**



Total tract **(D)**



Hindgut **(E)**

Fig. 2. (*continued*).

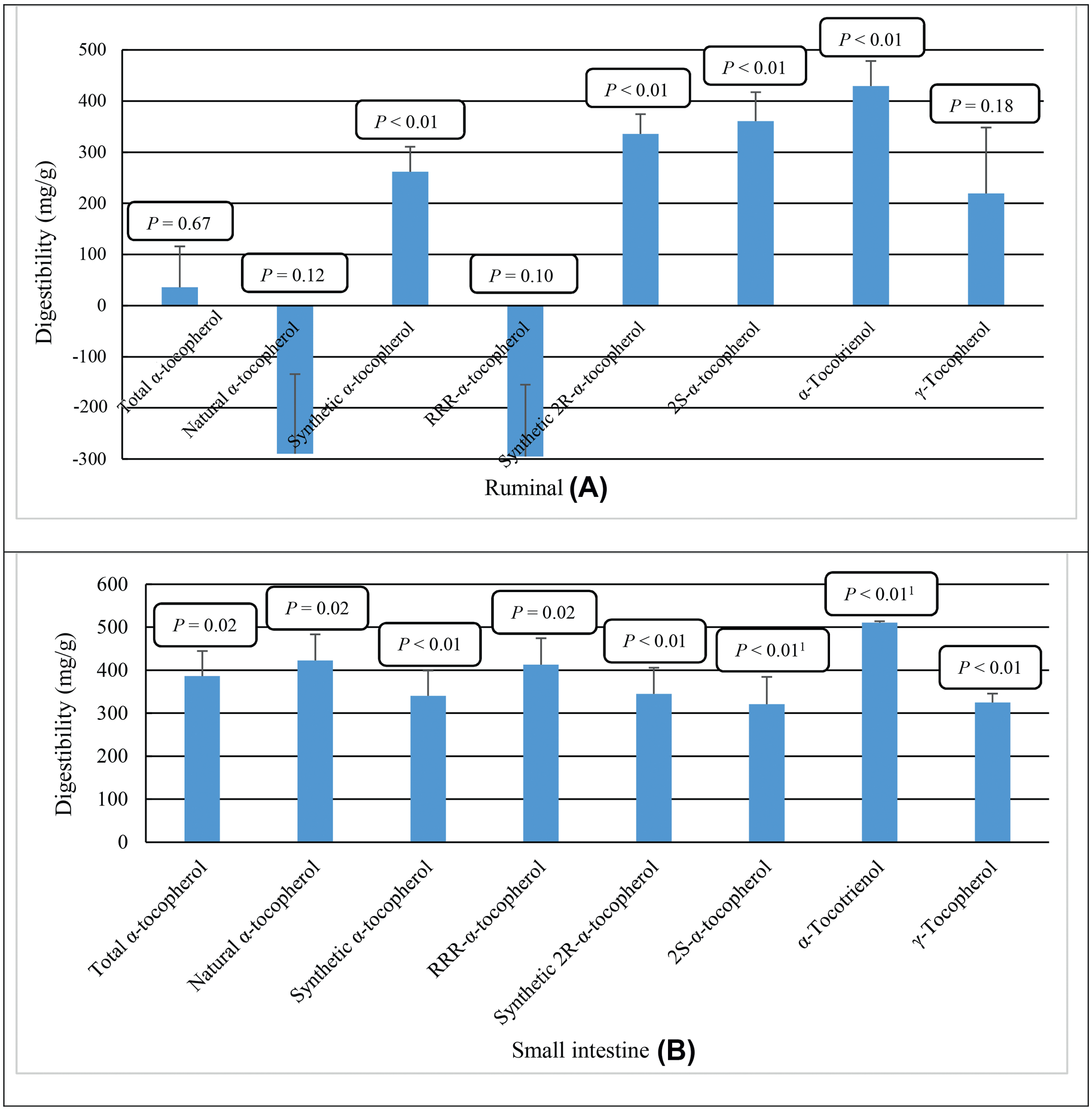


Fig. 3. Tocopherol digestibility in rumen (A), small intestine (B), feed-ileum (C), total tract (D), and hindgut (E). Total α-tocopherol originates from feedstuffs and vitamin supplement. Natural α-tocopherol originates from feedstuffs. Synthetic α-tocopherol originates from vitamin supplements. *RRR*-α-tocopherol (2R-(4′R, 8′R)-5,7,8-trimethyltocol) originates from feedstuffs and vitamin supplement. Synthetic 2R-α-tocopherol is summation of *RRS* (2R-(4′R, 8′S)-5,7,8-trimethyltocol), *RSR* (2R-(4′S, 8′R)-5,7,8-trimethyltocol), and *RSS*-α-tocopherol (2R-(4′S, 8′S)-5,7,8-trimethyltocol). 2S-α-tocopherol is summation of *SRR* (2S-(4′R, 8′R)-5,7,8-trimethyltocol), *SSR* (2S-(4′S, 8′R)-5,7,8-trimethyltocol), *SRS* (2S-(4′R, 8′S)-5,7,8-trimethyltocol), and *SSS*-α-tocopherol (2S-(4′S, 8′S)-5,7,8-trimethyltocol). α-Tocotrienol and γ-tocopherol are 2R-(4′R, 8′R)-5,7,8-trimethyltocotrienol and 2R-(4′R, 8′R)-,7,8-dimethyltocol, respectively. P-values show that average across treatments is different from zero. Error bars represent the standard error of means. ^{1}Data for small intestinal digestibility and feed-ileum digestibility of α-tocotrienol were not normally distributed; therefore, the data were transformed by root square, and back transformed was reported.

tocopherol competed with cholesterol to bind to the intracellular cholesterol transporter 1 L1-N terminal domain (Kamishikiryo et al., 2017). Therefore, we speculate interactions between α-tocopherol and other lipid compounds may explain the low intestinal digestibility of tocopherols in cows. Additionally, the endogenous α-tocopherol losses originating from mucus, epithelial cells, and digestive enzymes secreted in the abomasum and duodenum may also explain the lower intestinal digestibility of α-tocopherol.

In contrast to the small intestine, feed-ileum balance (synthetic α-tocopherols > natural α-tocopherol > γ-tocopherol > α-tocotrienol) and feed-ileum digestibility (natural α-tocopherol > α-tocotrienol > γ-tocopherol > synthetic α-tocopherols) showed a different pattern compared to small intestine balance and digestibility. These discrepancies could be due to ruminal degradation of synthetic α-tocopherol and synthesis of natural form, which obviously were reflected in duodenal and ileal flow of different tocopherols. Digestibility of different stereoisomers of α-tocopherol in the small intestine and feed-ileum occurred in the following order: *RRR*-α-tocopherol > synthetic 2R-α-tocopherol > 2S-α-tocopherol. These findings showed that *RRR*-α-tocopherols is obviously more absorbable than synthetic stereoisomers of α-tocopherol in the small intestine, which is in line with a previous study in calves (Lashkari et al., 2022). Highest *RRR*-α-tocopherol

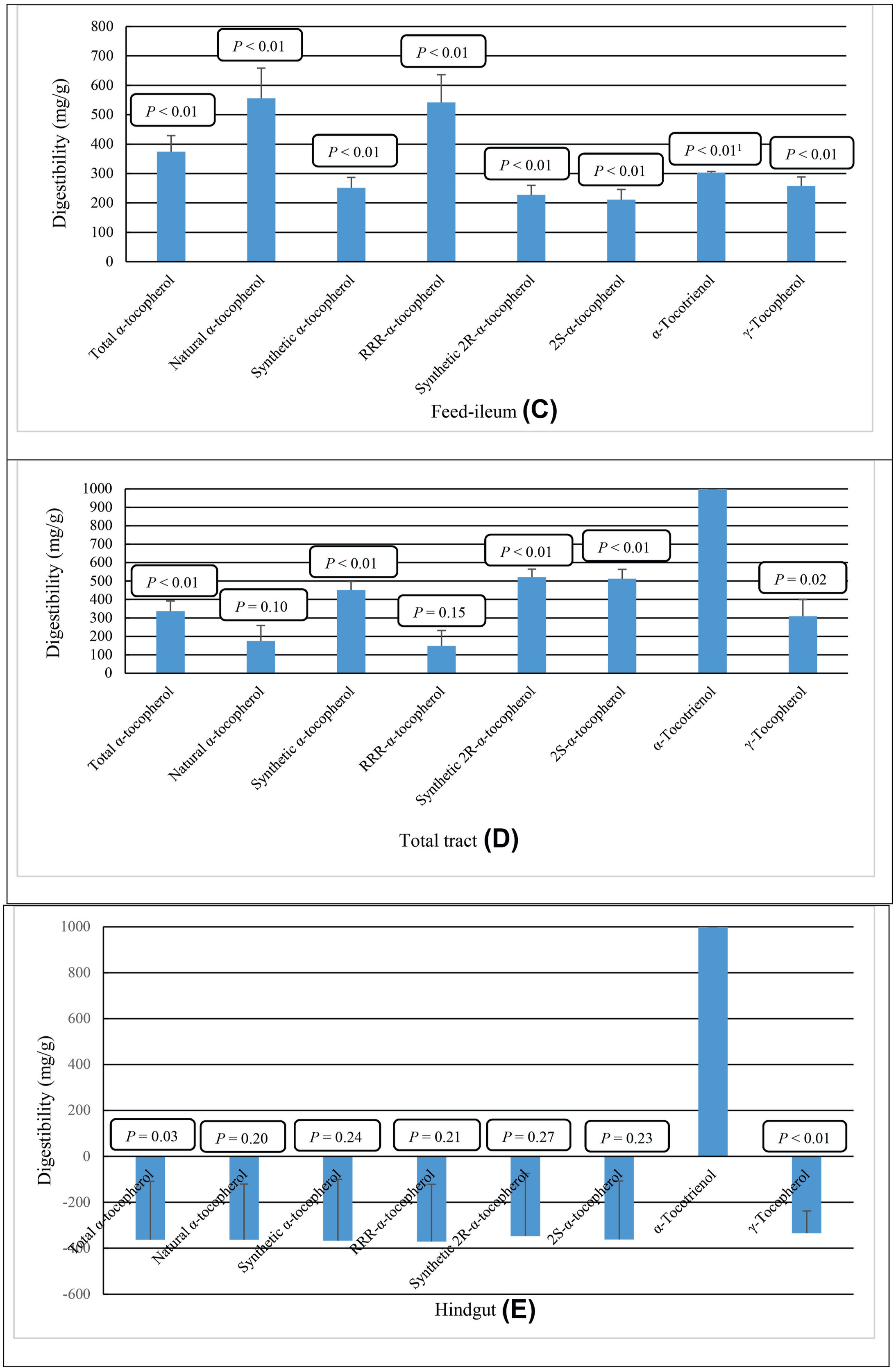


Fig. 3. (*continued*).

balance and digestibility could be due to the stereoselectivity of carboxyl ester hydrolase of bile salts (Moore et al., 1995; Zahalka et al., 1991), which reflects the higher hydrolysis rate of the acetate esters for *RRR*-α-tocopherol than the other synthetic α-tocopherol stereoisomers (Moore et al., 1995; Zahalka et al., 1991). Likewise, Zahalka et al. (1991) showed more extensive and rapid hydrolysis of *RRR*-α-tocopherol than the *SRR*-α-tocopherol in rats, fed deuterium-labeled α-tocopherol.

Hindgut balance of α-tocotrienol and γ-tocopherol could be due to low small intestinal digestibility, which provides the substrate for hindgut microbes. In addition, the balance of α-tocotrienol in the hindgut could be due to biohydrogenation of α-tocotrienol by hindgut microbes, as shown for unsaturated fatty acids. Hindgut balance and digestibility indicated neither digestion nor absorption of tocopherols in the hindgut with exception of α-tocotrienol and γ-tocopherol. Results of hindgut digestibility showed that study of tocopherols digestibility based on total tract is an acceptable approach due to negligible changes in the hindgut. However, the results of hindgut digestibility need to be interpreted with caution due to a difficulty to take representative samples from ileal cannulas (Olijhoek et al., 2016; Panah et al., 2020b) and very low tocopherol concentration in collected samples.

5. Conclusion

This study provided the first evidence for *RRR*-α-tocopherol formation in the rumen. In addition, synthetic α-tocopherol, γ-tocopherol and α-tocotrienol were degraded in the rumen. The results showed a low intestinal digestibility of natural α-tocopherol. The small intestine discriminated against absorption of synthetic 2R- and 2S-α-tocopherol, and small intestine digestibility of synthetic 2R- and 2S-α-tocopherol were much lower compared to the *RRR*-α-tocopherol. In addition, results demonstrated that the hindgut had no impact on the tocopherol digestion and absorption. Further, the absorption of α-tocopherol in the small intestine was higher than the absorption of γ-tocopherol and α-tocotrienol.

Author contributions

Saman Lashkari: Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Writing – original draft, Writing – review & editing. **Farhad M. Panah:** Formal analysis, Validation, Visualization, Writing – original draft, Writing – review & editing. **Martin R. Weisbjerg:** Data curation, Formal analysis, Investigation, Methodology, Software, Methodology, Validation, Project administration, Writing – review & editing, Writing – original draft. **Søren K. Jensen:** Investigation, Data curation, Methodology, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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Appendix supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2023.07.007.

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