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A comparison between feeding systems (pasture and TMR) and the effect of vitamin E supplementation on plasma and milk fatty acid profiles in dairy cows

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Unidentified constituents in fresh pasture increase milk fat *cis*-9, *trans*-11 conjugated linoleic acid (CLA) concentration, and prevent milk fat depression, even though ruminal conditions conducive to reducing milk fat synthesis exist. One possible explanation is vitamin E (α-tocopherol), a constituent high in fresh pasture, but naturally low in conserved/dried forages and cereal grains. Twenty late-lactating dairy cows previously consuming a total mixed ration (TMR) were randomly allocated to one of two dietary treatments for 21 d: TMR (control; $n=10$); and TMR plus an additional 10 000 i.u. α-tocopherol/d (VIT E; $n=10$). These cows were simultaneously compared with 13 late-lactation dairy cows previously grazing fresh pasture (PAS) balanced for age, parity and genetic merit. Average daily α-tocopherol intakes were approximately 468, 10 520 and 1590 i.u./cow for the control, VIT E and PAS treatments, respectively. Dietary α-tocopherol supplementation (VIT E *v.* control) slightly increased milk fat content by 0·23 percentage units, but did not significantly alter milk fatty acid composition. Plasma *trans*-11 18:1 (VA) content tended to increase and *trans*-10 18:1 levels numerically declined following α-tocopherol supplementation suggesting possible changes in rumen biohydrogenation products. In addition, increased α-tocopherol intake in TMR-fed cows decreased serum urea levels and tended to alter milk fat 15:0 suggesting changes in rumen microbial populations. However, when compared with cows grazing pasture, TMR-fed cows supplemented with α-tocopherol, still produced milk with lower *cis*-9, *trans*-11 CLA and VA, and higher *trans*-10 18:1 concentrations suggesting α-tocopherol is not a primary reason for milk fatty acid profile differences between pasture and TMR-fed cows. Therefore, additional unknown pasture constituents favour production of fatty acids originating from the *cis*-9, *trans*-11 instead of the *trans*-10, *cis*-12 CLA biohydrogenation pathways.

Keywords: Vitamin E, milk fat depression, conjugated linoleic acid.

Reductions in ruminal pH and diets high in polyunsaturated fatty acids (PUFA) are strongly linked with decreased milk fat content, a phenomenon commonly referred to as milk fat depression (MFD; Kalscheur et al. 1997; Griinari et al. 1998; Bauman & Griinari, 2001; NRC, 2001). The lipid of rotationally grazed ryegrass pastures contains more than 60% PUFA (Hawke, 1963; Dewhurst et al. 2002; Kay et al. 2004); however, reductions in rumen pH in pasture-fed dairy cows do not reduce milk fat content to the same extent as in cows consuming a total mixed ration (TMR) (Kolver & de Veth, 2002) suggesting that factors additional to PUFA content and ruminal pH may influence milk fat synthesis in pasture-fed cows. Cows grazing pasture produced milk with a higher fat content than cows fed a TMR (Kolver et al. 2000) and higher (approximately 3-fold) *cis*-9, *trans*-11 conjugated linoleic acid (CLA) concentrations (Kelly et al. 1998; Dhiman et al. 1999; Auldist et al. 2002; Kay et al. 2004). *Cis*-9, *trans*-11 CLA, the predominant CLA isomer in milk fat has numerous putative health benefits (Belury, 2002) and is produced primarily (>65%) from endogenous conversion of *trans*-11 vaccenic acid (VA), a PUFA biohydrogenation intermediate, via Δ^9-desaturase (Griinari et al. 2000; Corl et al. 2001a; Kay et al. 2004). In addition, a small portion of milk fat *cis*-9, *trans*-11 CLA arises

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directly from ruminal escape of *cis*-9, *trans*-11 CLA during incomplete biohydrogenation of PUFA. Consequently, milk fat *cis*-9, *trans*-11 CLA levels can be enhanced by increasing VA supply to the mammary gland, by increasing ruminal *cis*-9, *trans*-11 CLA production or by upregulating the Δ^9-desaturase system (Griinari et al. 2000; Bauman et al. 2001; Corl et al. 2001a). Depending upon the initial isomerization step, PUFA biohydrogenation can also produce intermediates containing a *trans*-10 double bond. *Trans*-10, *cis*-12 CLA and *trans*-10 18 : 1 are two fatty acids associated with MFD (Griinari et al. 1998; Baumgard et al. 2000; Piperova et al. 2000) that are almost undetectable in milk fat from pasture-fed dairy cows (Mackle et al. 2003; Kay et al. 2004). TMR diets containing high-concentrate levels, finely chopped forages, or large amounts of PUFA, typically result in an increased milk fat *trans*-10 18 : 1/VA ratio (Griinari et al. 1998) suggesting a change in PUFA biohydrogenation pathways towards production of *trans*-10, *cis*-12 CLA and subsequent intermediates.

In attempting to reduce milk fatty acid oxidation and thus increase dairy product shelf life, Focant et al. (1998) serendipitously discovered that high doses of dietary α-tocopherol (9619 i.u./cow per d) partly alleviated diet-induced MFD in TMR-fed dairy cows. The α-tocopherol concentration in fresh pasture (approximately 106 i.u./kg DM) is 4–5 times greater than found in a typical TMR based on NRC values (15 i.u./kg; NRC, 2001). We hypothesized that α-tocopherol might be a pasture constituent that alters ruminal biohydrogenation to favour fatty acids produced from the *cis*-9, *trans*-11 instead of the *trans*-10, *cis*-12 CLA pathway. The aforementioned scenario would therefore increase milk fat *cis*-9, *trans*-11 CLA and VA concentrations and might be why pasture-fed cows typically produce milk with a higher fat content.

This experiment examined the effects of supplementing TMR-fed cows with a large dose of α-tocopherol (10 000 i.u./cow per d) on production parameters, blood metabolites and milk and plasma fatty acid composition. These results were also compared with those from cows consuming fresh pasture, a diet naturally high in α-tocopherol.

Materials and Methods

Animals and treatments

All procedures were approved by the Ruakura Animal Ethics Committee (Hamilton, NZ). Thirty-three multiparous Holstein-Friesian dairy cows in late lactation (267 ± 14 days in milk) that were part of a multiyear comparison trial were used. The multiyear comparison trial (Kolver et al. 2000) contained lactating dairy cows assigned to a TMR or pasture diet prior to their first parturition. Cows in the pasture treatment were offered a fresh break of pasture twice daily (>45 kg fresh pasture DM/cow per d) with the intention to feed cows generously and not mimic a constrained pasture system. Pastures used in the comparison trial for the 2 months prior to the start of the present study had 3–4 weeks regrowth and were grazed at a pre-grazing mass of 3587 ± 230·6 kg DM/ha with post grazing residuals 2751 ± 242·8 kg DM/ha. TMR-fed cows were confined to a loafing paddock that was sheltered from the wind. TMR was mixed in a Jaylor vertical mixing wagon and fed twice daily at 8.00 and 16.00 in 5-m long mobile fibreglass troughs. At the time of treatment allocation to the multiyear comparison trial, TMR and pasture treatments were balanced for age, parity and genetic merit for milk production.

For the present study, 20 cows (675 ± 78 kg in weight) previously consuming a TMR, were allocated to one of two TMR treatments balanced for age, parity, genetic merit for milk production, milk yield and milk fat concentration. The two treatments were (1) TMR (control; approx. 468 i.u. α-tocopherol/cow per d; n=10) and (2) TMR plus 10 000 i.u. α-tocopherol/cow per d (VIT E; approx. 10 468 i.u. α-tocopherol/cow per d; n=10). These two TMR treatment groups were then compared with pasture-fed cows from the same multiyear comparison trial (balanced for age, parity and genetic merit for milk production) that had previously been grazing pasture (PAS; approx. 1590 i.u. α-tocopherol/cow per d; n=13; 557 ± 23 kg in weight). The vitamin E dose (10 000 i.u. α-tocopherol/cow per d) and study length (21 d) were chosen, based on data from Focant et al. (1998), who reported that supplementation of 10 000 i.u. α-tocopherol/cow per d for 21 d increased milk fat content by 17% and alleviated diet-induced MFD.

Animal and feed management

All cows were milked twice daily at 7.00 and 16.00. Prior to each milking, cows on the VIT E treatment were orally dosed, using a bolus gun, with 5000 i.u. of α-tocopherol (dl-α-tocoperhyl acetate, a non-rumen protected water-soluble vitamin E dietary supplement; BASF, Germany) in gelatin capsules (Torpac Inc, Fairfield NJ, USA). The daily dose of 10 000 i.u. α-tocopherol is similar to the amount administered by Focant et al. (1998) for 21 d, which resulted in significant increases in milk fat content. Control and VIT E cows were confined to a loafing paddock (0·25 ha) and given access *ad libitum* to TMR 7.00 and 16.00 from four 5-m mobile fibreglass troughs. TMR offered and refused was measured daily and the average DMI calculated. TMR was formulated using the Spartan ration formulation program (van de Haar et al. 1992) and the Cornell Net Carbohydrate and Protein System model (Fox et al. 1992) and ingredient and nutrient composition is presented in Table 1.

Animals on the PAS treatment were rotationally grazed as one herd and offered a fresh pasture allocation twice daily. To maintain similar consumption to the multiyear comparison trial, cows were offered >45 kg DM/d (69 ± 4·4 kg DM/d) and paddocks measured 0·25 ha (defined daily grazing area) and contained 3–4 weeks pasture

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Table 1. Ingredient and nutritive composition of TMR and pasture offered

Composition	TMR	Pasture
Ingredient, % of DM		
Corn silage	35·0	
Pasture silage†	30·0	
Grass hay	7·5	
Whole cotton seed	10·0	
Barley	2·9	
Soybean meal	7·3	
Chilean aquaquality fish meal	1·8	
Corn gluten meal	1·6	
Molasses	2·5	
Vitamin and Mineral mix‡	0·4	
Chemical analysis, % of DM		
CP	18·2	30·9
ME (MJ/kg)	13·4	13·0
NDF	30·8	39·3
ADF	22·1	21·1
Ash	9·0	11·2
Non-fibre carbohydrates	26·9	7·8
Total Fatty Acids	4·6	5·0
Fatty acids (g/100 g total fatty acids)		
16 : 0	22·0	10·3
cis-9 16 : 1	0·3	2·5
18 : 0	12·5	2·0
cis-9 18 : 1	13·7	3·9
cis-9, *cis*-12 18 : 2	26·1	9·2
cis-9, *cis*-12, *cis*-15 18 : 3	11·2	57·3
Other	12·6	14·8

† Pasture consisted of 75% perennial ryegrass dominant (*Lolium perenne L*), approximately 15% other grasses (*Dactylus, Poa*, spp), 7·5%white clover (*trifolium repens*) and 2·5% weeds

‡ Contained 14·0% Ca, 7·0% P, 4·0% Na, 4·5% Mg, 1·0% Cu, 2·3% Mn, 3·5% Zn, 0·6% Fe, 5000 i.u. Vitamin A/g, 500 i.u. of vitamin D/g and 6 i.u. of vitamin E/g

re-growth. Pasture allocations and residuals were visually assessed by the same trained assessor throughout the experiment and calibrated twice weekly through cutting a range of pasture yields (n=15) representative of pre- and post grazing yields (Campbell & Arnold, 1973; O'Donovan et al. 2002). Group intake was estimated by calculating herbage disappearance rate (pasture offered less pasture remaining) in the area grazed as previously described (Roche et al. 2002). On a DM basis, the pasture sward consisted of approximately 68·0% perennial ryegrass (*Lolium perenne* L.), 27·5% other grasses (*Dactylus glomerata, Holcus lanatus* and some *Poa* species), 1·5% white clover (*Trifolium repens* L.), and 3·0% weeds. Pasture composition is presented in Table 1.

Feed sampling and analysis

Representative samples of pasture and TMR were collected every second day by hand plucking pasture to grazing height from paddocks due to be grazed, and selecting handfuls of feed from TMR troughs prior to consumption. Triplicate subsamples were either frozen immediately, dried at 100 °C for DM analysis or dried at 60 °C for nutrient composition analysis.

Nutrient composition samples were bulked weekly, ground to pass through a 1·0-mm sieve (Christy Lab Mill, Suffolk, UK) and analysed for CP, NDF, ADF, ash, non-fibre carbohydrates and organic matter digestibility (OMD) by near infra-red analysis (NIRS system 650; Corson et al. 1999; Kolver et al. 2002). Metabolizable energy (ME) was derived directly from predicted OMD, on the basis of an cellulase digestibility assay *in vitro* (Dowman & Collins, 1982; Roughan & Hollan, 1977), which had been calibrated against standards derived from measurements *in vivo*.

Frozen feed samples were bulked weekly, freeze-dried (Cuddon instrument model 0610, Blenheim, NZ), and ground to pass through a 0·5-mm sieve (Christy Lab Mill) One subsample was analysed for α-tocopherol by the method of Brubacher et al. (1985) and another subsample was stored at −20 °C for subsequent fatty acid analysis.

Pasture and TMR lipids were extracted and methylated by the one-step method of Garces & Mancha (1993) as outlined in Kay et al. (2004). Fatty acid analysis was performed on a Hewlett Packard 5890 Series II gas chromatograph equipped with a 30-m RTX-2330 column (30 m × 0·32 mm i.d. and 0·2 µm film thickness; Restek Corp, Bellefonte CA, USA) and gas chromatogram conditions were as previously described (Kay et al. 2004). Fatty acid content of pasture and TMR are presented in Table 1.

Milk sampling and analysis

Milk yields were recorded and milk collected from four consecutive milkings prior to treatment initiation and at the end of the 21-d treatment period using in-line milk meters (TruTest, Palmerston North, NZ). Individual composites were formed based on weight proportionality according to milk yields and split into three aliquots. One aliquot was analysed immediately for fat, lactose, casein, protein and total solids using an infrared milk analyser (FT120; Foss Electric, Hillerød, Denmark) while a second aliquot was analysed for α-tocopherol by the method of Brubacher et al. (1985). Milk fat was extracted from the third aliquot using the Röese-Gottlieb fat extraction procedure (IDF, 1987) and stored at −20 °C until analysed for fatty acid composition.

Milk fatty acid methyl esters were quantified by gas chromatography (GC) after methylation using sodium methoxide as described by MacGibbon (1988). GC analyses of fatty acid methyl esters were performed on a Hewlett Packard series 6890 equipped with a flame ionization detector, and a Hewlett Packard series 6890 auto-injector (Hewlett Packard, Pan Alto CA, USA). A 105-m RTX-2330 column (105 m × 0·25 mm i.d. and 0·20 µm film thickness; Restek Corp.) was used and 1 µl solvent solution was injected using split injection with a split ratio of 65 : 1. Helium was the carrier gas and column flow was

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1·4 ml/min or 22 cm/s in constant flow mode. The initial oven temperature was 100 °C for 8 min, ramped at a rate of 50 deg C/min to 150 °C, then ramped at a rate of 1·5 deg C/min to 180 °C and held for 12 min, then ramped at 5 deg C/min to 260 °C and held for 5 min. Injector temperature was 260 °C and detector temperature was 300 °C. Standards for CLA and other fatty acids were obtained from Matreya Inc. (Pleasant Gap PA, USA) and CLA isomer mixes from Sigma (St Louis MO, USA) and NuCheck Prep (Elysian MN, USA). In addition, a butter reference standard (CRM 164; Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was used as a qualitative reference for individual fatty acids and GLC 87 and 74X (NuCheck Prep) were used as a quantitative methyl ester reference.

Blood sampling and analysis

Two evacuated blood tubes, one containing a sodium heparin pellet (100 U of heparin/ml of blood) and one with no additive were collected by venipuncture of the coccygeal vessel immediately prior to treatment initiation and at the end of the 21-d treatment period. Plasma was harvested from heparinized tubes following centrifugation at 2800 ***g*** at 4 °C for 12 min, split into two aliquots and stored at −20 °C until further analysis for insulin and fatty acids. Insulin was analysed by a double-antibody radioimmunoassay as previously described (McMahon et al. 1998; Coat-A-Count; Diagnostic Products Corporation, Los Angeles CA, USA). All samples were completed within one assay and the intra-assay CV was <10%.

Plasma lipids were extracted by the method of Hara & Radin (1978) as modified by Corl et al. (2001a) and methylated according to Christie (1982) with modifications (Corl et al. 2001a). GC analyses of plasma fatty acid methyl esters and equipment used were as described for milk fatty acid analysis with the following GC condition differences. Initial oven temperature was 135 °C for 2 min, then ramped to 250 °C at a rate of 1·5 deg C/min and held for 2 min. Injector temperature was 260 °C and detector temperature was 300 °C.

Serum from plain vacutainers was analysed for urea nitrogen (UN; urease method), glucose (hexokinase method), BHBA and NEFA (colourimetric method) using commercially available enzymic colourimetric kits (Boehringer Mannheim, Germany) and a spectrophotometric auto-analyser (Hitachi 717; Hitachi Ltd., Tokyo, Japan). All samples were completed within one assay with intra-assay CV <5%.

Calculations

The Δ^9-desaturase index serves as a proxy for Δ^9-desaturase activity and/or expression and was calculated using four pairs of fatty acids that represent products and substrates for Δ^9-desaturase. The Δ^9-desaturase index was defined as: [sum of Δ^9-desaturase products]/[sum of Δ^9-desaturase products+substrates] and was calculated as follows:

[14 : 1+16 : 1+*cis*-9 18 : 1+*cis*-9, *trans*-11 CLA]/[14 : 0+14 : 1 +16 : 0+16 : 1+18 : 0+*cis*-9 18 : 1+VA+*cis*-9, *trans*-11 CLA]

Statistical Analysis

Data were analysed using the Proc Mixed procedure of SAS (1999) and the model included treatment as the dependent variable. Statistical comparisons included control *v.* VIT E, control *v.* PAS and VIT E *v.* PAS. Comparisons between control and VIT E were analysed using day 0 as a covariate; however, as cows were consuming either TMR or pasture diets prior to initiation of the present trial, comparisons between control *v.* PAS and VIT E *v.* PAS could not be analysed using a covariate. Results are presented as least square means ± SEM and considered significant when $P<0{\cdot}05$.

Results

Based on average group daily DMI estimates (20 ± 4 kg/cow), α-tocopherol daily intakes were approx. 468 and 10 468 i.u./cow for the control and VIT E treatments, respectively. Increasing α-tocopherol intake for 21 d enhanced ($P<0{\cdot}01$; Table 2) milk α-tocopherol concentrations by 125% (0·12 *v.* 0·27 i.u./100 ml for the control and VIT E groups, respectively.

There was no effect of α-tocopherol supplementation on milk yield or the yield of milk constituents (Table 2); however, α-tocopherol supplementation increased ($P<0{\cdot}05$) milk fat content by 0·23 percentage units (3·81% *v.* 4·04% for control and VIT E groups, respectively) and total solids content by 0·34 percentage units (13·34% *v.* 13·68% for control and VIT E groups, respectively; Table 2.) Concentrations of milk protein, lactose and casein were not affected by α-tocopherol supplementation (Table 2), nor was the Δ^9-desaturase index or milk fatty acid profile, with the exception of 15 : 0 which tended to increase with α-tocopherol supplementation (Table 3).

Increased intake of α-tocopherol tended to increase ($P<0{\cdot}08$) plasma VA concentration and numerically decreased ($P<0{\cdot}14$) plasma *trans*-10 18 : 1 (Table 4). There was no effect of dietary α-tocopherol supplementation on plasma insulin or serum BHBA, glucose or NEFA concentrations; however, serum urea concentrations were reduced ($P<0{\cdot}01$; 6·57 *v.* 5·82 mmol/l for control and VIT E groups, respectively; Table 5).

Cows grazing fresh pasture consumed less estimated DMI (16 ± 3 kg/d) than cows offered TMR and average α-tocopherol intake for grazing cows was approximately 1590 i.u./d. Milk α-tocopherol content from grazing cows reflected the high pasture α-tocopherol content and was approximately 58% higher and 30% lower than control and VIT E cows, respectively (Table 2). Cows consuming TMR, (control and VIT E treatments), produced more

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Table 2. The effect of α-tocopherol supplementation and diet on milk production†

	Treatment‡			SEM§		Comparison¶		
Variable	control	VIT E	PAS	1	2	1	2	3
Milk Yield, kg	18·9	19·8	12·5	1·06	2·03	0·55	0·03	0·02
Milk Fat								
%	3·81	4·04	4·50	0·08	0·28	0·05	0·06	0·30
g/d	707	768	562	38	78	0·28	0·20	0·06
Milk Protein								
%	4·00	4·00	3·89	0·05	0·09	0·98	0·32	0·43
g/d	750	791	485	33	77	0·40	0·02	0·01
Milk Lactose								
%	4·86	4·95	4·51	0·03	0·10	0·08	0·01	0·02
g/d	922	988	571	55	104	0·41	0·01	0·01
Milk Casein								
%	2·95	2·96	2·88	0·04	0·08	0·76	0·50	0·50
g/d	554	596	359	24	58	0·36	0·02	0·01
Milk Total Solids								
%	13·34	13·68	13·42	0·10	0·36	0·03	0·91	0·63
g/d	2510	2680	1680	131	259	0·37	0·02	0·01
Milk α-tocopherol								
i.u./100 ml	0·12	0·27	0·19	0·02	0·03	<0·01	0·04	0·01

† Values are means of individual milk samples composited from four consecutive milkings at the end of the 21-d treatment period
‡ Treatments were total mixed ration (control), total mixed ration+10 000 i.u./d α-tocopherol (VIT E) and pasture (PAS)
§ SEM 1=standard error of the means for comparison 1 (control *v.* VIT E), SEM 2=standard error of the means for comparison 2 (control *v.* PAS) and comparison 3 (VIT E *v.* PAS)
¶ Comparison 1 (control *v.* VIT E), 2 (control v. PAS), 3 (VIT E *v.* PAS)

($P<0·05$; Table 2) milk and milk protein, lactose, casein and total solids than cows grazing fresh pasture. Pasture-fed cows tended to produce milk with a higher ($P<0·06$) fat content than control cows. However, there was no difference in milk fat levels between pasture-fed cows and cows in the VIT E treatment (Table 2).

There were distinct differences in the milk fatty acid profile from cows grazing pasture or offered TMR (both control and VIT E; Table 3). Milk fat *cis*-9, *trans*-11 CLA and VA concentrations were on average 2·4–2·5-times greater ($P<0·01$) in cows consuming fresh pasture compared with both TMR treatments. Conversely, the concentration of *trans*-10 18:1 was 36-times lower ($P<0·01$) in grazing cows. Average linoleic and palmitic acid contents were 2·2 and 1·2-times higher ($P<0·01$), respectively, in milk fat from control and VIT E cows compared with pasture-fed cows, while linolenic acid content was 3-times higher ($P<0·01$) in PAS cows. There was no effect of diet on the Δ^9-desaturase index (Table 3).

The yield of total *trans* 18:1 milk fatty acids (mmol/d) was similar across diets but the contribution of specific isomers varied greatly (Fig. 1). On a percentage of total trans yield (mmol/d), VA accounted for 69% while *trans*-10 18:1 contributed <1·0% in PAS cows. In contrast, total *trans* 18:1 milk fatty acids from cows consuming a TMR (both control and VIT E) consisted of approximately 33% VA and 22% *trans*-10 18:1.

Consistent with milk fatty acids, cows in the PAS treatment had higher ($P<0·01$) plasma concentrations of linolenic acid and lower ($P<0·01$) levels of linoleic and palmitic acids than cows consuming TMR or TMR supplemented with α-tocopherol (Table 4). In addition, cows grazing fresh pasture had higher ($P<0·01$) plasma concentrations of total *trans* 18:1 fatty acids, VA, oleic acid and *cis*-9, *trans*-11 CLA and lower ($P<0·01$) concentrations of *trans*-10 18:1.

Grazing cows had higher ($P<0·05$) concentrations of urea, and lower ($P<0·01$) levels of glucose and insulin, than cows on both TMR treatments (Table 5). Grazing cows also had higher ($P<0·05$) concentrations of NEFA than control cows and tended ($P<0·06$) to have higher NEFA levels compared with the VIT E treatment. Plasma BHBA content was not affected by treatment.

Discussion

Supplementing TMR-fed cows with 10 000 i.u./d of α-tocopherol slightly increased (6%) milk fat content, supporting earlier findings of Focant et al. (1998). Ruminal biohydrogenation of PUFA, in particular linoleic acid, proceeds via two pathways, depending on the initial isomerization, to produce either *cis*-9, *trans*-11 CLA and subsequent VA (Harfoot & Hazlewood, 1988) or *trans*-10, *cis*-12 CLA and subsequent *trans*-10 18:1 (Bauman et al. 2001). The predominant biohydrogenation pathway and consequent production of specific CLA and octadecenoic fatty acid isomers is relevant for both milk fat synthesis and overall milk healthfulness. *Trans*-10, *cis*-12 CLA is a potent inhibitor of milk fat synthesis in dairy cows (Baumgard

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Table 3. The effect of α-tocopherol supplementation and diet on milk fatty acid composition†

	Treatment‡			SEM§		Comparison¶		
Fatty acids	control	VIT E	PAS	1	2	1	2	3
	(g/100 g total fatty acids)							
4 : 0	2·46	2·47	3·14	0·06	0·10	0·84	<0·01	<0·01
6 : 0	1·30	1·33	1·64	0·03	0·08	0·53	<0·01	<0·01
8 : 0	0·61	0·64	0·74	0·02	0·05	0·19	0·18	0·10
10 : 0	1·41	1·43	1·48	0·06	0·13	0·81	0·82	0·40
12 : 0	1·68	1·69	1·51	0·07	0·14	0·99	0·15	0·78
14 : 0	9·73	9·63	9·95	0·15	0·35	0·64	0·84	0·39
cis-9 14 : 1	1·23	1·21	1·13	0·04	0·09	0·42	0·21	0·61
15 : 0	0·82	0·88	1·09	0·02	0·30	0·06	<0·01	<0·01
16 : 0	31·87	31·52	27·57	0·27	0·73	0·37	<0·01	<0·01
cis-9 16 : 1	1·22	1·06	1·00	0·27	0·25	0·69	0·67	0·99
17 : 0	0·37	0·39	0·50	0·01	0·01	0·23	<0·01	<0·01
17 : 1	0·14	0·14	0·21	0·01	0·01	0·96	<0·01	<0·01
18 : 0	11·3	11·6	12·16	0·22	0·76	0·30	0·40	0·61
Total *trans* 18 : 1	4·91	4·79	5·75	0·18	0·29	0·57	0·11	<0·01
trans-6,7,8 18 : 1	0·42	0·41	0·23	0·01	0·02	0·63	<0·01	<0·01
trans-9 18 : 1	0·54	0·49	0·29	0·03	0·03	0·33	<0·01	<0·01
trans-10 18 : 1	1·14	1·12	0·03	0·13	0·13	0·93	<0·01	<0·01
trans-11 18 : 1	1·61	1·56	3·93	0·08	0·21	0·62	<0·01	<0·01
trans-12 18 : 1	1·20	1·21	1·27	0·08	0·08	0·92	0·53	0·62
cis-9 18 : 1	23·03	23·18	21·64	0·42	0·85	0·79	0·41	0·11
cis-9, *trans*-11 CLA	0·71	0·72	1·84	0·04	0·02	0·92	<0·01	<0·01
cis-9, *cis*-12 18 : 2	1·68	1·60	0·73	0·04	0·06	0·18	<0·01	<0·01
cis-9, *cis*-12, *cis*-15 18 : 3	0·32	0·32	0·95	0·03	0·06	0·93	<0·01	<0·01
20 : 0	0·19	0·19	0·10	0·01	0·01	0·33	<0·01	<0·01
Others††	5·10	5·14	6·91	0·33	0·31	0·94	<0·01	<0·01
Fatty acid origin								
de novo (4 : 0–15 : 1)	19·27	19·29	20·66	0·32	0·08	0·97	0·24	0·24
16 : 0+16 : 1	33·04	32·63	28·57	0·04	0·08	0·50	<0·01	<0·01
Preformed (>17 : 0)	42·74	42·79	43·86	0·43	1·18	0·94	0·37	0·67
Δ^9 desaturase ratios								
cis-9, *trans*-11CLA/VA	0·45	0·46	0·45	0·03	0·03	0·72	0·92	0·92
Δ^9- desaturase index	0·32	0·32	0·32	0·01	0·97	0·94	0·91	0·97

† Values are means of individual milk samples composited from four consecutive milkings at the end of the 21-d treatment period

‡ Treatments were total mixed ration (control), total mixed ration+10 000 i.u./d α-tocopherol (VIT E) and pasture (PAS)

§ SEM1 = standard error of the means for comparison 1 (control *v.* VIT E), SEM 2 = standard error of the means for comparison 2 (control *v.* PAS) and comparison 3 (VIT E *v.* PAS)

¶ Comparison 1 (control *v.* VIT E), 2 (control *v.* PAS), 3 (VIT E *v.* PAS)

†† Represent unidentified fatty acids

et al. 2000, 2001) and reduces the expression of genes responsible for fatty acid uptake, intracellular fatty acid transport, desaturation, fatty acid synthesis *de novo* and triglyceride production (Baumgard et al. 2002). In addition, *trans*-10 18 : 1, the putative product of *trans*-10, *cis*-12 CLA hydrogenation (Bauman et al. 2001), has also been associated with MFD (Griinari et al. 1998, Piperova et al. 2000). In contrast, *cis*-9, *trans*-11 CLA does not affect milk fat synthesis (Baumgard et al. 2000) and both *cis*-9, *trans*-11 CLA and VA are potent anticarcinogens in rodent mammary cancer models (Ip et al. 1999; Corl et al. 2003). Therefore, shifting rumen biohydrogenation towards production of *cis*-9, *trans*-11 CLA and VA instead of *trans*-10, *cis*-12 CLA and *trans*-10 18 : 1 may have two specific benefits: MFD prevention and enhancing the overall healthfulness of milk and milk products.

Although in the present study, α-tocopherol supplementation slightly enhanced milk fat content in TMR-fed cows, there were little or no changes in specific individual milk fatty acids (an indirect proxy of fatty acids exiting the rumen) nor were there any changes when milk fatty acids were classified into origin (synthesized *de novo* *v.* incorporated preformed). Furthermore, α-tocopherol had no effect on the mammary Δ^9-desaturase system, as the individual fatty acid ratios (results not presented) and the overall Δ^9-desaturase index did not differ (Table 3). This lack of effect on individual milk fatty acid synthesis suggests that ruminal biohydrogenation may not have been

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Table 4. The effect of α-tocopherol supplementation and diet on blood fatty acid composition†

	Treatment‡			SEM§		Comparison¶		
Fatty acids	control	VIT E	PAS	1	2	1	2	3
	g/100 g total fatty acids							
14 : 0	1·15	1·15	1·31	0·06	0·07	0·98	0·44	0·43
15 : 0	0·39	0·40	0·72	0·01	0·02	0·48	<0·01	<0·01
16 : 0	10·85	10·76	9·36	0·31	0·31	0·85	<0·01	<0·01
cis-9 16 : 1	0·96	0·91	0·91	0·04	0·08	0·43	0·17	0·40
17 : 0	0·51	0·54	0·70	0·02	0·02	0·22	<0·01	<0·01
18 : 0	15·87	16·47	17·52	0·08	0·73	0·60	0·11	0·32
Total *trans* 18 : 1	1·51	1·59	2·54	0·05	0·17	0·48	<0·01	<0·01
trans-6,7,8 18 : 1	0·10	0·12	0·11	0·01	0·01	0·16	0·31	0·67
trans-9 18 : 1	0·17	0·16	0·13	0·01	0·01	0·66	<0·01	<0·01
trans-10 18 : 1	0·31	0·23	0·01	0·04	0·04	0·14	<0·01	<0·01
trans-11 18 : 1	0·52	0·62	1·95	0·04	0·16	0·08	<0·01	<0·01
trans-12 18 : 1	0·41	0·46	0·34	0·03	0·03	0·23	0·03	<0·01
cis-9 18 : 1	7·87	8·10	10·58	0·25	0·40	0·52	<0·01	<0·01
cis-9, *trans*-11 CLA	0·02	0·02	0·21	0·01	0·02	0·91	<0·01	<0·01
cis-9, *cis*-12 18 : 2	41·30	39·00	18·00	0·98	0·86	0·11	<0·01	<0·01
cis-9, *cis*-12, *cis*-15 18 : 3	5·81	6·13	20·90	0·32	0·59	0·50	<0·01	<0·01
Others††	13·97	14·69	17·36	0·22	0·49	0·04	<0·01	<0·01
Δ^9 desaturase ratios								
cis-9, *trans*-11CLA/VA	0·03	0·03	0·10	0·01	0·01	0·90	<0·01	<0·01
Δ^9-desaturase index	0·25	0·25	0·29	0·01	0·01	0·94	<0·01	<0·01

† Values are means of individual plasma collected at the end of the 21-d treatment period
‡ Treatments were total mixed ration (control), total mixed ration + 10 000 i.u./d α-tocopherol (VIT E) and pasture (PAS)
§ SEM 1 = standard error of the mean for comparison 1 (CONT *v.* VIT E), SEM 2 = standard error of the mean for comparison 2 (control *v.* PAS) and comparison 3 (VIT E *v.* PAS)
¶ Comparison 1 (control *v.* VIT E), 2 (control *v.* PAS), 3 (VIT E *v.* PAS)
†† Represent unidentified fatty acids

altered by dietary α-tocopherol as we postulated. However, as fatty acids exiting the rumen are intestinally absorbed and transported in blood, total plasma fatty acids may also be used as an indirect proxy of rumen biohydrogenation. In contrast to milk, increasing α-tocopherol intake of TMR-fed cows tended to increase ($P<0·08$) total plasma VA and numerically decreased ($P<0·14$) *trans*-10 18 : 1, suggesting a shift in rumen biohydrogenation towards production of fatty acids from the *cis*-9, *trans*-11 CLA pathway. The reason α-tocopherol putatively induced changes in rumen biohydrogenation were not reflected in the milk fatty acid profile is not known, but may be due to the fact that blood triacylglyceride and NEFA lipid classes are thought to be the major contributors to preformed milk fatty acids, but these two lipid fractions make up less than 5% of total plasma lipid (Moore & Christie, 1979).

The mechanism by which α-tocopherol or its derivatives may alter biohydrogenation is unclear, but may include modifying rumen microbial populations/dynamics and consequent fatty acid hydration. Two quinol derivatives of α-tocopherol, α-tocopherolquinol and deoxy-α-tocopherolquinol, which are present in the cell extract of *Butyrivibrio fibrisolvens* act as endogenous electron donors during hydration of *cis*-9, *trans*-11 CLA to VA (Hughes & Tove, 1980a, b; 1982) thus increasing the synthesis of fatty acids by this pathway. Further evidence suggesting α-tocopherol altered the rumen microbial milieu is that plasma urea concentrations decreased by ~11% in cows supplemented with α-tocopherol possibly suggesting greater rumen ammonia utilization or reduced ammonia synthesis. In agreement, milk 15 : 0 content tended ($P<0·06$; Table 3) to increase in the VIT E group compared with control and milk odd chain fatty acids are thought to be useful markers of rumen microbial populations (Dewhurst et al. 2000).

As anticipated, owing to distinct differences in nutrient intake, there were marked differences in milk production between pasture and TMR-fed cows, and with the exception of milk fat content, these differences were not altered by α-tocopherol supplementation (Table 2). TMR-fed cows (control and VIT E) consumed more feed and produced more milk and consequently more milk protein, lactose, casein and total solids than grazing cows. There was a trend ($P<0·06$) for milk fat concentration to be higher in pasture-fed than in control cows and these differences are consistent with previous reports (Kolver et al. 2000). However, following α-tocopherol supplementation, milk fat content increased and was not statistically different between VIT E and PAS cows (Table 2).

Milk fatty acid composition also differed markedly between TMR (both control and VIT E treatments) and pasture-fed cows. Linolenic acid content was 3-times

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Table 5. The effect of α-tocopherol supplementation and diet on blood metabolites†

	Treatment‡			SEM§		Comparison¶		
Blood metabolite	Control	VIT E	PAS	1	2	1	2	3
NEFA, mmol/l	0·10	0·12	0·15	0·01	0·01	0·22	0·02	0·07
BHBA, mmo/l	0·37	0·34	0·39	0·04	0·04	0·62	0·56	0·45
Urea, mmol/l	6·57	5·82	11·85	0·18	0·29	<0·01	<0·01	<0·01
Glucose, mmol/l	4·12	4·02	3·53	0·05	0·06	0·19	<0·01	<0·01
Insulin, pmol/l	8·44	6·36	1·77	1·53	1·14	0·36	<0·01	<0·01

† Values are means of individual plasma collected at the end of the 21-d treatment period
‡ Treatments were total mixed ration (control), total mixed ration+10 000 i.u./d α-tocopherol (VIT E) and pasture (PAS)
§ SEM 1 = standard error of the means for comparison 1 (control *v.* VIT E), SEM 2 = standard error of the means for comparison 2 (control *v.* PAS) and comparison 3 (VIT E *v.* PAS)
¶ Comparison 1 (control *v.* VIT E), 2 (control *v.* PAS), 3 (VIT E *v.* PAS)

greater in milk fat from pasture-fed cows than from cows on both TMR treatments and although this fatty acid is readily biohydrogenated in the rumen, the increase probably reflects the high pasture linolenic acid content (57%; Table 1) and the higher rumen passage rate in grazing compared with TMR-fed cows (Harrison & McAllan, 1980). Predominant TMR fatty acids are linoleic (26%) and palmitic acids (22%), and these fatty acids were more prevalent in milk fat from control and VIT E treatments compared with PAS. The concentration of *cis*-9, *trans*-11 CLA in milk fat from pasture-fed cows was 2·4-times higher than in those fed a TMR (both control and VIT E), which is consistent with previous literature (Kelly et al. 1998; Dhiman et al. 1999; Auldist et al. 2002). Most of the *cis*-9, *trans*-11 CLA in milk is produced by endogenous mammary conversion of VA via Δ^9-desaturase (Griinari et al. 2000; Corl et al. 2001a; Kay et al. 2004) and Lock & Garnsworthy (2003) suggest that increasing dietary pasture enhances *cis*-9, *trans*-11 CLA by elevating Δ^9-desaturase activity. However, in the present study, there was no difference in the Δ^9-desaturase system between TMR and pasture-fed cows, based on the Δ^9-desaturase index and individual ratios. The fact there were no differences in the Δ^9-desaturase system is surprising as insulin up-regulates Δ^9-desaturase gene expression (Miyazaki & Ntambi, 2003), and even though the mammary gland is thought to be insulin insensitive (Hove, 1978), elevated insulin levels (>8 fold) increase the Δ^9-desaturase index and consequently enhance *cis*-9, *trans*-11 CLA milk fat content (Corl et al. 2001b). Pasture-fed cows in our study had markedly lower (>4 fold) plasma insulin levels (Table 5), but higher (~155%) *cis*-9, *trans*-11 CLA milk fat content (Table 3). This suggests that increased milk fat *cis*-9, *trans*-11 CLA content from pasture-fed cows is primarily due to increased ruminal VA output and to a much lesser extent to increased Δ^9-desaturase activity or expression. This postulation is also supported by the elevated VA concentration in plasma from pasture-fed cows (Table 4). Furthermore, Fievez et al. (2003) used principal component analysis to show that *cis*-9, *trans*-11 CLA production depends primarily on VA supply and not on variation in Δ^9-desaturase activity in cows consuming grass and legume silages.

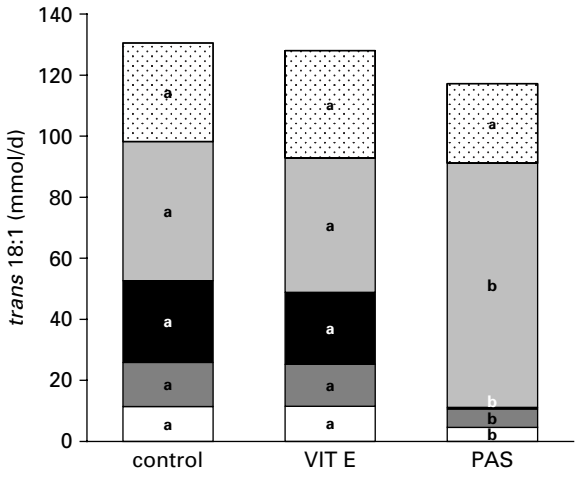

Fig. 1. Effect of total mixed ration (control), total mixed ration plus 10 000 i.u./d α-tocopherol (VIT E) and pasture (PAS) on yield of individual *trans* 18 : 1 isomers. *Trans* 6–8 18 : 1 (white bar), *trans*-9 18 : 1 (dark grey bar), *trans*-10 18 : 1 (black bar), *trans*-11 18 : 1 (VA; light grey bar), *trans*-12 18 : 1 (spotted bar). Bars with different superscripts differ significantly ($P<0·01$). Total *trans* 18 : 1 fatty acid yield was not different among treatments ($P>0·63$).

To our knowledge this is the first study to compare individual *trans* 18 : 1 fatty acids from cows consuming solely TMR or pasture for a prolonged period of time. Although the total *trans* 18 : 1 fatty acid yield (mmol/d) in milk was similar between treatments (Fig. 1) there were distinct differences in the amount of individual *trans* 18 : 1 isomers secreted, with VA being the predominant (69%) *trans* 18 : 1 fatty acid in milk fat from pasture-fed cows while the *trans*-10 18 : 1 isomer was detected only in trace amounts. In contrast, the contribution of VA and *trans*-10 18 : 1 to total *trans* 18 : 1 milk fatty acid yield was 33 and 22%, respectively, for control cows fed TMR. These results indicate that the predominant rumen biohydrogenation

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pathway in grazing dairy cows favours an initial *trans*-11 isomerization, ensuring production of a *cis*-9, *trans*-11 fatty acid metabolite and subsequent VA. These high milk fat contents of VA and *cis*-9, *trans*-11 CLA (two fatty acids shown to inhibit cancer in animal models; Ip et al. 1999; Corl et al. 2003) combined with the subsequent reduction in *trans*-10, *cis*-12 CLA and *trans*-10 18 : 1 may explain the increased fat content and the 'healthier' fatty acid profile frequently observed in milk from pasture-fed cows.

As expected, the plasma fatty acid profile also differed between pasture and TMR-fed (both control and VIT E) cows and largely reflected the pattern of fatty acid intake. Although increased α-tocopherol intake in TMR-fed cows numerically decreased plasma *trans*-10 18 : 1 and tended to increase VA, these fatty acids remained much lower and higher, respectively, in pasture-fed cows, suggesting that α-tocopherol was not the primary factor influencing PUFA biohydrogenation in grazing cows. An interesting finding in the present study was the increased plasma *cis*-9, *trans*-11 CLA/VA ratio in grazing cows compared with TMR-fed cows (Table 4). Ruminal biohydrogenation of linolenic acid, although documented (Dawson & Kemp, 1970; Kemp et al. 1975), has not been as intensively investigated as that of linoleic acid, and while linolenic acid is reported to produce VA but not *cis*-9, *trans*-11 CLA as an intermediate (Harfoot & Hazlewood, 1988), the higher plasma *cis*-9, *trans*-11 CLA/VA ratio in pasture-fed cows (where linolenic acid predominates) suggests that *cis*-9, *trans*-11 CLA could be a linolenic acid biohydrogenation intermediate. In addition, this supports Kolver et al. (2002) who proposed an alternative pathway that yielded CLA intermediates (including *cis*-9, *trans*-11 CLA) from linolenic acid biohydrogenation in continuous culture fermentors. Although the substrate supply for ruminal fatty acid production (Table 1), may partly explain the different milk fatty acid composition from pasture and TMR-fed cows, Lock & Garnsworthy (2003) suggest differences in the amount of linoleic and linolenic acid entering the rumen does not fully explain the significant increases in milk fat *cis*-9, *trans*-11 CLA and VA concentrations in pasture-fed cows. Additional factors such as increased α-tocopherol content, increased rumen passage rate (Harrison & McAllan, 1980), increased soluble carbohydrates (de Veth & Kolver, 2001), altered feeding frequency, meal size, and ruminating time (Kelly et al. 1998) may affect rumen microbial population (as indicated by differences in milk fat 15 : 0 and 17 : 0 content; Fievez et al. 2003) and subsequent PUFA biohydrogenation in grazing cows. Further research involving direct rumen fluid fatty acid analysis is required to determine in more detail the effect of pasture constituents and individual fatty acid intake on rumen PUFA biohydrogenation.

Blood metabolites were also affected by diet as both blood glucose and insulin concentrations were higher in TMR-fed (both control and VIT E) cows, which is consistent with previous findings (Kolver et al. 2001) and is probably due to nutrient intake differences and decreased production of gluconeogenic precursors (i.e., propionic acid) during fermentation of a pasture diet compared with a conventional TMR diet. In addition, the increased DMI and energy intake (and possibly whole animal energy balance) of the control and VIT E cows, may result in increased glucose and subsequently elevated insulin concentrations. In addition to the estimated reduced nutrient intake, decreased insulin levels may help explain enhanced NEFA concentration in pasture-fed compared with control cows, as insulin is a potent antilipolytic agent that prevents NEFA mobilization from adipocytes (Vernon & Pond, 1997). Increased plasma urea concentrations in pasture-fed cows reflects the greater pasture protein content compared with TMR (31 *v.* 18%, respectively) and is consistent with findings of Bargo et al. (2002) who reported a positive association between plasma urea levels and pasture protein content.

In conclusion, increasing α-tocopherol intake of TMR-fed cows slightly increased milk fat content by 6% but did not alter milk fatty acid profiles as hypothesized. Total plasma fatty acid composition may indicate a shift towards increased production of fatty acids from the *cis*-9, *trans*-11 CLA pathway, following α-tocopherol supplementation, and reduced serum urea levels and a tendency for a change in milk fat 15 : 0 content suggest that there may have been an effect on rumen microflora metabolism. However, distinct differences in milk production and milk and plasma fatty acids profiles remained between pasture-fed and TMR-fed cows supplemented with α-tocopherol and this suggests that α-tocopherol is not a primary reason for differences in the fatty acid profiles and milk fat content between pasture and TMR-fed cows. Further research is required to determine the effect of a more constant delivery of α-tocopherol (synchronized with feed intake) and α-tocopherol effects in a TMR containing higher levels of linolenic acid in an attempt to better mimic the fatty acid intake of pasture-fed dairy cows. Additionally, evaluating direct α-tocopherol effects on rumen biohydrogenation and identifying alternative pasture constituents that may cause pasture-fed cows to produce milk with a 'healthier' fatty acid profile is of interest.

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