Journal of Dairy Research (2006) **73** 227–234. © *Proprietors of Journal of Dairy Research 2006*
doi:10.1017/S0022029906001701 *Printed in the United Kingdom*

α-Tocopherol concentration and stereoisomer composition in plasma and milk from dairy cows fed natural or synthetic vitamin E around calving

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Received 21 February 2005 and accepted for publication 19 September 2005

The aim of this study was to compare the effects of supplementing dairy cows with 1000 IU/day of *all-rac*-α-tocopheryl acetate (SynAc), *RRR*-α-tocopheryl acetate (NatAc), or *RRR*-α-tocopherol (NatAlc), from approximately 3 weeks before estimated calving until 2 weeks after calving, on the concentration of α-tocopherol and its stereoisomers (*RRR*-, *RSS*-, *RRS*-, *RSR*- and the four 2*S*-forms of α-tocopherol) in blood and milk. An unsupplemented group was included as control. Blood samples were collected at 3, 2 and 1 weeks before estimated calving, at calving, and 3, 7 and 14 days after calving, while milk samples were taken twice within 24 h after calving and at 7 and 14 days in milk. Overall, time and treatment had significant effects on plasma α-tocopherol with higher concentrations in NatAc than in the other groups. In addition, SynAc had higher concentrations than Control, and NatAlc tended to be higher than Control. The lowest plasma concentrations were observed at calving and 3 days after calving. Independent of treatment, the concentration was higher in colostrum than in milk day 7 and 14 after calving. Analyses of the stereoisomer distribution in plasma and milk showed that, irrespective of dietary treatment, *RRR*-α-tocopherol was the most predominant form, constituting more than 86%, whereas the remaining part of α-tocopherol was made up by the three synthetic 2*R* isomers, while the 2*S* isomers only contributed less than 1% of the total α-tocopherol. In control cows and cows supplemented with natural vitamin E, the proportion of *RRR*-α-tocopherol in plasma and milk constituted more than 98% of the total α-tocopherol. In conclusion, the results indicate that daily oral supplementation of dairy cows with *RRR*-α-tocopheryl acetate gives the highest blood concentrations of α-tocopherol in the periparturient period. Analyses of the distribution of the individual stereoisomers of α-tocopherol further indicate that the bioavailability of *RRR*-α-tocopherol relative to synthetic stereoisomers in cattle is considerably higher than officially accepted until now.

Keywords: Vitamin E, supplementation, natural, synthetic, stereoisomers, dairy cows, periparturient period.

Vitamin E is the generic name of a group of lipid-soluble compounds, which are present in eight different forms, i.e. α-, β-, γ-, δ-tocopherols and -tocotrienols, respectively (Kayden & Traber, 1993). α-Tocopherol is the major form found in feedstuffs, but the contents of tocotrienols and γ-tocopherol in feed are in certain cases also high. However, α-tocopherol is the most biologically active form of vitamin E in cattle, as it is the most predominant form in blood and milk of these animals (Pehrson & Hakkarainen, 1986).

Vitamin E is an important biological antioxidant in mammalian cell membranes, preventing the oxidation of unsaturated fatty acids (Reddy & Frey, 1990; Bendich, 1993). In this context, vitamin E has attracted great interest due to its efficacy in preventing oxidation problems in milk fat (Charmley & Nicholson, 1994). Vitamin E has been found to increase cellular and humoral immunity, and during vitamin E insufficiencies in cattle, important neutrophil functions, such as migration, phagocytosis and killing

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capacity, are impaired with negative consequences for the defence against infectious diseases (e.g. Hogan et al. 1992; Ndiweni & Finch, 1996; Politis et al. 1996).

The vitamin E status of dairy cows can easily be determined by blood analysis, and the lowest concentration of vitamin E in blood is normally obtained around calving (e.g. Goff & Stabel, 1990; Weiss et al. 1990; Politis et al. 1996; Meglia et al. 2001; Goff et al. 2002). Several factors have been implicated in the drop detected around calving, but reduced feed intake and transfer of vitamin E from blood to colostrum are considered most important (Goff & Stabel, 1990; Weiss et al. 1990). The drop in blood concentrations of vitamin E around calving coincides with suppression of the immune system and increased disease incidence. This has lead to studies investigating if vitamin E supplementation around calving can improve the animal's vitamin E status at parturition and its resistance against infections. In several reports, preventive effects of extra vitamin E on the occurrence of mastitis, the most common infectious disease in dairy cows, have been studied. In some studies positive effects have been found (Smith et al. 1984; Weiss et al. 1997), while other studies reported no such effects (Batra et al. 1992; LeBlanc et al. 2002).

Supplementation of vitamin E has mainly been done using synthetic vitamin E (*all-rac*-α-tocopheryl acetate [2, 5, 7, 8-tetramethyl-2*RS*- (4′*RS*, 8′*RS*, 12 trimethyltridecyl)-6-chromanol]). This form consists of an equal proportion of all eight possible stereoisomers of α-tocopherol with four stereoisomers possessing the 2*R* configuration (*RRR*, *RRS*, *RSS*, *RSR*), and four stereoisomers possessing the 2*S* configuration (*SRR*, *SSR*, *SRS* and *SSS*), whereas *RRR*-α-tocopherol [2, 5, 7, 8-tetramethyl-2*R*- (4′*R*, 8′*R*, 12 trimethyltridecyl)-6-chromanol] is the only form found in nature. The relative vitamin E activity of *RRR*-α-tocopherol compared with *all-rac*-α-tocopherol has been questioned for decades (Blatt et al. 2004). α-Tocopherol stereoisomers are treated and metabolised within the body in different ways, therefore a single ratio cannot express the difference in vitamin E activity between natural and synthetic α-tocopherol (Blatt et al. 2004). However, studies in humans (Burton et al. 1998) and sows (Lauridsen et al. 2002a, b) suggest a higher biological activity of the natural isomer compared with the synthetic isomers. This has been ascribed to the presence of α-tocopherol transfer protein (α-TTP), which discriminates between forms of α-tocopherol, and maintains α-tocopherol concentrations in plasma (Hosomi et al. 1998; Jishage et al. 2001).

Hidiroglou et al. (1988), reported higher plasma concentrations of α-tocopherol after feeding beef cows equal amounts, on IU basis, of *RRR*-α-tocopheryl acetate compared with *all-rac*-α-tocopheryl acetate. However, few studies (Hidiroglou et al. 1997) have compared the effects of supplementing natural and synthetic α-tocopherol in periparturient dairy cows, and, to our knowledge, there are no studies on the distribution of α-tocopherol stereoisomers in blood and milk. Therefore, the aim of the present study was to compare the concentrations of α-tocopherol and its stereoisomers (*RRR*-, *RSS*-, *RRS*-, *RSR*- and the four 2*S*-forms of α-tocopherol) in blood and milk when feeding synthetic or natural vitamin E to cows around calving.

Materials and Methods

Animals and feeding

The study was performed during the indoor season, from December to April, with thirty-six Holstein-Friesian cows from the experimental herd at Research Centre Foulum, Danish Institute of Agricultural Sciences, Tjele, Denmark. All cows were dried off 2 months before expected calving using the same routine, and were randomly assigned to the experiment from 3 weeks before estimated calving to 14 days after calving. Before the experiment, the animals were supplemented with a vitamin-mineral mix containing synthetic vitamin E (800 IU *all-rac*-α-tocopheryl acetate/day) recommended for dry cows. During this time, the ratio between corn silage and clover grass silage in the diet was 1 : 1, otherwise the feed was similar to that used during the experiment. The animals were housed in individual tie stalls and fed a total mixed ration (TMR) according to Danish recommendations for the dry and lactation periods. The TMR consisted of clover grass silage (28%), corn silage (18%), sugar beet pellets (10%), barley straw (3%) and concentrates (41%). The same TMR was used throughout the experiment but the amounts per day differed. The mean amount of energy fed during the dry period until 10 days before expected calving was 21·6 Mcal of NE_L/day, and during the close up and early lactation it was 30·3 Mcal of NE_L/day. The individual dry matter intake (DMI) was recorded daily, and the disease incidence was recorded during the experimental period.

Experimental design and sample collection

The cow with the earliest expected calving date was assigned to the first experimental group, the cow with the second earliest calving date was assigned to the second experimental group and so on until all 36 cows was equally distributed in the four experimental groups with nine cows in each group. Each cow was followed during approximately 5 weeks, and the total experiment lasted 4 months. Vitamin E supplementation was given daily, from three weeks before estimated calving to 14 days after calving, as top dressing on the feed. Three groups were supplemented with 1000 IU per day of *all-rac*-α-tocopheryl acetate (SynAc, Rovimix E50-Ads, Roche A/S, Denmark), *RRR*-α-tocopheryl acetate (NatAc, Natur-E granulate 40%, Pharmalett A/S, Kolding, Denmark) or *RRR*-α-tocopherol (NatAlc, Natur-E micelle, Pharmalett A/S, Kolding, Denmark), respectively, while one group was not supplemented (Control). The daily doses of 1000 IU were equal to 917 mg *all-rac*-α-tocopherol in the

https://doi.org/10.1017/S0022029906001701 Published online by Cambridge University Press

SynAc group, and 671 mg *RRR*-α-tocopherol in both NatAc and NatAlc groups, using the USP conversion factors (United States Pharmacopeia, 1985).

Blood samples were taken from the jugular vein at seven time points from each animal, i.e. at 3, 2 and 1 weeks before estimated calving, within 12 h after calving, and at 3, 7 and 14 d after calving, into heparinized tubes (Becton Dickinson Vacutainer Systems Europe, Meylan Cedex, France). Blood plasma was collected after centrifugation at 1000 ***g*** for 10 min, and stored at −20 °C until analysed. The experiment complied with guidelines of the Danish Ministry of Justice with respect to animal experimentation and care of animals under study.

From each cow, one composite colostrum sample was taken within 12 h after calving and one sample between 12 and 24 h after calving. These samples were analysed separately, but prior to statistical analysis the data were computed into one figure in order to decrease the individual variation between cows. Composite milk samples were also taken from the morning and evening milkings at d 7 and 14. The morning and evening samples for each day were mixed and aliquots were stored in plastic tubes at −20 °C until analysed. The total daily milk production was also measured at days of samplings.

Representative feed samples were taken two weeks, two months and four months after the start of the experiment. The samples were frozen in plastic bags at −20 °C until analysis of α-tocopherol.

Tocopherol analyses

Feed samples were freeze dried and milled prior to analysis, and 1 g of a finely milled feed sample was suspended in a mixture of 24 ml ethanol (960 ml/l), 9 ml methanol, 10 ml ascorbic acid in water (200 g/l) and 7 ml KOH-water (1 : 1 w/v). This mixture was saponified for 30 min at 80 °C (boiling) in the dark, and cooled in cold water. Exactly 2 ml of the saponified mixture were diluted with 0·5 ml water after which tocopherols were quantitatively extracted with two portions of 5·0 ml heptane. Plasma and milk samples were thawed, and milk samples were heated at 40 °C for 20 min and mixed thoroughly prior to analysis. The extraction procedure for tocopherols was as follows: 1000 mg milk, or 500 µl blood plasma, was diluted with 0·5 ml ascorbic acid solution (200 g/l), 0·5 ml methanol, 2·0 ml ethanol, and 0·5 ml KOH-water (1 : 1 w/v). The final volume of the saponification mixture was adjusted to 5·5 ml with water. Saponification was carried out at 80 °C for 20 min. After cooling, tocopherol was extracted twice using 5 ml heptane per occasion. The two extracts were mixed, and 100 µl were injected onto the HPLC for normal tocopherol analysis, while 9 ml was used for analysis of stereoisomers. All solvents used were of HPLC quality. The HPLC column consisted of a 4·0 × 125 mm Perkin Elmer HS-5-Silica column. Heptane containing 2-propanol (3·0 ml/l) and degassed with helium constituted the mobile phase. The flow rate was 3·0 ml/min and the column was held at room temperature. Fluorescence detection was performed with excitation and emission wavelengths of 290 and 327 nm, respectively. Identification and quantification of α-tocopherol was obtained by comparison of retention time, as well as peak areas, with external standards (Merck, D-64293 Darmstadt, Germany), and an extinction coefficient of $A^{1\%}_{1\ cm}$=71·0 at 294 nm was used for α-tocopherol, as previously described by Jensen & Nielsen (1996).

Stereoisomers of α-tocopherol were analysed by HPLC as follows: The remaining 9 ml heptane extract was evaporated to exact dryness under a stream of nitrogen. Then the extracted α-tocopherol was derivatized to its methyl ether following the method described by Drotleff & Ternes (2001). The methyl ether derivative was extracted with 1000 µl 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). The mobile phase consisted of heptane modified with 2-propanol (0·065 ml/l) with a flow rate of 1 ml/min and fluorescence detection was monitored with excitation and emission wavelengths of 290 nm and 327 nm. This method allows the separation of the eight stereoisomers of α-tocopherol into five peaks, the first peak contains the four 2*S* forms (*SSS/SSR/SRS/SRR*), peak two contains the *RSS*-α-tocopherol, peak three contains *RRS*-α-tocopherol, peak four contains *RRR*-α-tocopherol and peak five contains RSR-α-tocopherol.

Statistical analyses

The repeated measures of α-tocopherol and the *RRR, RSS, RRS, RSR* and 2*S* stereoisomers in blood and milk were analysed with the MIXED procedure (SAS, 1999). Interaction between treatment and time was excluded from the final model when it was insignificant ($P>0·20$) in the preliminary analysis. One-way analysis of variance (ANOVA) was used to compare groups within day, and time points within group.

Results

Animal health, dry matter and α-tocopherol intake

In total, 11 (30%) cows suffered from mastitis or puerperal paresis during the study period, the number of diseased cows was 4, 2, 3, and 2 for SynAc, NatAc, NatAlc and Control groups, respectively.

From drying off until the start of the experiment, the total daily dietary intake of α-tocopherol was approximately 800 IU from the vitamin and mineral feed plus 200–300 IU from the roughage. The TMR used during the experimental period contained on average 34·3 mg α-tocopherol/kg DM (SD=8·2) corresponding to 51 IU vitamin E/kg DM. The average DMI and daily vitamin E

https://doi.org/10.1017/S0022029906001701 Published online by Cambridge University Press

Table 1. Daily dry matter intake (DMI) and calculated vitamin E intake from the TMR diet used during the experiment

Values are means ± SD for $n=36$

Days relative to calving	DMI, kg/d	Vitamin E intake, IU/d
−24†	10·6 ± 2·7	454 ± 114
−16	14·5 ± 3·6	621 ± 155
−9	14·2 ± 3·8	608 ± 164
0	11·4 ± 3·9	475 ± 165
3	14·8 ± 3·7	636 ± 159
7	15·8 ± 3·3	679 ± 142
14	17·8 ± 4·0	785 ± 171

Time had a significant ($P<0·001$) effect on the DMI and vitamin E intake
† This time point was just before the start of feed supplementation

intake from the TMR are listed in Table 1. The DMI and basal vitamin E intake did not differ between treatments ($P=0·35$), but time had a significant effect on this variable ($P<0·001$) and a decreased DMI was observed at calving.

Blood and milk α-tocopherol

Treatment had a significant effect on the content of α-tocopherol in plasma ($P<0·001$). Overall, the NatAc group had higher ($P<0·01$) concentrations than the other groups. In addition, the α-tocopherol concentration was higher ($P=0·037$) in SynAc cows than in Control cows, and the concentration tended to be higher ($P=0·06$) in NatAlc compared with Control. No other differences were observed between treatments.

Overall, a significant effect of time on blood α-tocopherol was also observed ($P<0·001$). The concentration was numerically lowest at calving and 3 d after calving (Table 2).

The α-tocopherol concentration in milk varied significantly over time ($P<0·001$; Table 3) with higher α-tocopherol concentrations in colostrum (day 1) than in milk samples taken 7 and 14 d after calving. A large between-cow variation was observed in this response, and overall, treatment did not have a significant effect on the α-tocopherol concentration in milk. However, according to the one-way ANOVA colostrum from cows fed NatAlc had a higher α-tocopherol concentration than colostrum from SynAc cows ($P=0·05$). The total daily amount of α-tocopherol in milk is given in Table 4. Overall, the daily secretion into milk did not differ significantly between groups, due to a high individual variation day 1. However, at day 7 cows fed NatAc had a higher daily secretion compared with the control cows ($P=0·03$) according to the one-way ANOVA.

Blood and milk α-tocopherol stereoisomers

At day −24 all cows were fed the same diet containing *all-rac*-α-tocopheryl acetate. Thus, no differences between groups were found in the relative distribution of individual stereoisomers of α-tocopherol in plasma at this time point (Table 5). The natural isomer (*RRR*-α-tocopherol) accounted for 91·5%, on average, of the total α-tocopherol. The distribution of stereoisomers of α-tocopherol in plasma from cows in the SynAc group did not differ significantly throughout the experiment, whereas cows from the NatAc, NatAlc and Control groups had a significant increase in the proportion of *RRR*-α-tocopherol from −24 d to −16 d ($P<0·001$) and this difference was persistent throughout the remaining experimental period ($P<0·001$). At all sampling occasions after day −24 there was a significant difference between cows fed SynAc and the other three groups ($P<0·001$).

In plasma from cows on Control, NatAc or NatAlc diets, *RRR*-α-tocopherol accounted for 98–100% of the analysed α-tocopherol. *RRR*-α-tocopherol was also the dominating isomer (88–93%) in plasma from cows fed SynAc, followed by the three synthetic 2*R*-isomers (*RSS, RRS* and *RSR*). The four 2*S*-isomers accounted for less than 1%, although these isomers were dominating in the feed in this group.

Irrespective of dietary group, *RRR*-α-tocopherol was the dominating isomer in all milk samples, but milk from cows fed SynAc had a significantly lower proportion of *RRR*-α-tocopherol compared with cows on the other three diets (Table 6). Small quantities of the synthetic isomers were detected in most milk samples, independent of the dietary treatment. However, the proportion of the synthetic isomers was highest in cows fed SynAc (Table 6).

Discussion

This study demonstrates that α-tocopherol with natural stereochemistry (*RRR*) is by far the most predominating form in blood and milk of cows, irrespective of dietary treatment. In experiments with rats (Weiser et al. 1996) and broilers (Cortinas et al. 2004) it has been shown that the 2*R* forms of α-tocopherol are favoured in the circulating plasma compared with the 2*S* forms. Using deuterated *RRR*-α-tocopheryl acetate and *all-rac*-α-tocopheryl acetate, Lauridsen et al. (2002a, b) showed a preferential incorporation of *RRR*- compared with *all-rac*-α-tocopherol into blood and milk of sows, resulting in a 2 : 1 ratio between the two forms. Analyses of the individual stereoisomers in samples of monogastric animal species have shown that the *RRR*-form contributes approximately 33% of the α-tocopherol content when fed *all-rac*-α-tocopheryl acetate (Cortinas et al. 2004; Lauridsen & Jensen, 2005). This is considerably lower than in the present experiment in which *RRR*-α-tocopherol contributed at least 86% of the total α-tocopherol in plasma and milk from cows on the SynAc diet. When added to the other dietary treatments, the *RRR*-form accounted for almost the whole α-tocopherol concentration of the cows. The present study therefore indicates

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Table 2. Plasma concentrations (mg/l) of α-tocopherol in 36 dairy cows fed daily supplements of no vitamin E (Control), *all-rac*-α-tocopheryl acetate (SynAc), *RRR*-α-tocopheryl acetate (NatAc), and *RRR*-α-tocopherol (NatAc) from approximately three weeks before calving to 14 days after calving

Values are means ± SEM for $n=9$ per group

Days relative to calving	Control	SynAc	NatAc	NatAc	*P*-values
−24†	2·24 ± 0·26	2·48 ± 0·31	2·56 ± 0·21	2·16 ± 0·26	0·67
−16	2·54 ± 0·24	3·33 ± 0·26	3·76 ± 0·41	3·06 ± 0·47	0·13
−9	2·40 ± 0·28^{b}	2·86 ± 0·29^{ab}	3·92 ± 0·35^{a}	3·24 ± 0·37^{ab}	0·01
0	1·92 ± 0·23	2·52 ± 0·34	2·89 ± 0·24	2·39 ± 0·29	0·13
3	1·95 ± 0·18$^{(b)}$	2·11 ± 0·28$^{(ab)}$	2·83 ± 0·19$^{(a)}$	2·60 ± 0·37$^{(ab)}$	0·08
7	2·15 ± 0·21^{b}	2·54 ± 0·28^{ab}	3·39 ± 0·33^{a}	2·62 ± 0·37^{ab}	0·05
14	2·62 ± 0·25	3·36 ± 0·41	4·00 ± 0·52	2·90 ± 0·48	0·14

Treatment ($P<0·001$) and time ($P<0·001$) had significant effects on the concentrations
† At the sampling before the start of the experiment (d −24) all cows were fed a diet containing synthetic vitamin E
^{a,b} Values within a row without a common superscript letter are significantly different ($P<0·05$)
$^{(a,b)}$ Values within a row without a common superscript letter differ at $0·05>P>0·01$

Table 3. Composite milk concentration (mg/kg) of α-tocopherol on days 1, 7 and 14 postpartum in 36 dairy cows fed daily supplements of no vitamin E (Control), *all-rac*-α-tocopheryl acetate (SynAc), *RRR*-α-tocopheryl acetate (NatAc), and *RRR*-α-tocopherol (NatAc) from approximately three weeks before calving to 14 days after calving

Values are means ± SEM for $n=9$ per group

Day	Control	SynAc	NatAc	NatAc	*P*-values
1	5·94 ± 0·71^{ab}	4·69 ± 0·60^{b}	7·15 ± 0·92^{ab}	7·35 ± 0·65^{a}	0·05
7	0·73 ± 0·12	0·85 ± 0·08	1·00 ± 0·13	1·13 ± 0·20	0·21
14	0·67 ± 0·08	0·78 ± 0·09	0·90 ± 0·09	0·77 ± 0·05	0·26

Time ($P<0·001$), but not treatment, had a significant effect on the concentration
^{a,b} Values within a row without a common superscript letter are significantly different ($P<0·05$)

Table 4. Daily milk yield (kg/day) and secretion of α-tocopherol in milk (mg/day) from day 1, 7 and 14 postpartum in 36 dairy cows fed daily supplements of no vitamin E (Control), *all-rac*-α-tocopheryl acetate (SynAc), *RRR*-α-tocopheryl acetate (NatAc), and *RRR*-α-tocopherol (NatAc) from approximately three weeks before calving to 14 days after calving

Values are means ± SEM for $n=9$ per group

Day	Milk yield†	Control	SynAc	NatAc	NatAc	*P*-values
1	10·3 ± 0·7	55·1 ± 10·8	48·5 ± 11·1	76·1 ± 17·5	78·6 ± 14·7	0·33
7	32·0 ± 1·3	19·9 ± 2·6^{b}	28·1 ± 3·2^{ab}	34·5 ± 3·9^{a}	30·6 ± 3·2^{ab}	0·03
14	33·3 ± 1·2	18·7 ± 2·4	28·2 ± 4·4	30·6 ± 3·4	25·9 ± 2·9	0·09

Overall, time ($P<0·001$), but not treatment, had a significant effect on the concentration
† Mean ± SEM of all four groups ($n=36$ cows)
^{a,b} α-tocopherol values within a row without a common superscript letter are significantly different ($P<0·05$)

that periparturient cows to a greater extent than pigs (Lauridsen et al. 2002a, b) and humans (e.g. Burton et al. 1998), have a preferential uptake of *RRR*-α-tocopherol compared with the other stereoisomers of α-tocopherol.

In the present study, a higher blood concentration of α-tocopherol was detected in periparturient cows supplemented with NatAc compared with in cows supplemented with NatAlc or SynAc, or no supplementation (Control). A superior effect of the *RRR*-form compared with the *all-rac*-form has been demonstrated before in cattle and lambs (Hidiroglou et al. 1988, 1992, 1997), even when compared on IU basis, and it was therefore expected that higher blood concentrations of α-tocopherol in cows fed NatAc when compared with SynAc would be found. However, it was surprising that the highest concentration of α-tocopherol in plasma was observed in cows fed NatAc rather than NatAlc. This result was in contrast to previous experiments with beef cattle (Hidiroglou et al. 1988) and lambs (Hidiroglou et al. 1992) in which the alcohol form of α-tocopherol was superior to the acetate form. The reasons for the discrepancy between studies are not clear. Conflicting results regarding ruminal

https://doi.org/10.1017/S0022029906001701 Published online by Cambridge University Press

Table 5. Relative proportions (%) of α-tocopherol stereoisomers in blood from 36 dairy cows fed daily supplements of no vitamin E (Control), *all-rac*-α-tocopheryl acetate (SynAc), *RRR*-α-tocopheryl acetate (NatAc), and *RRR*-α-tocopherol (NatAlc) from approximately three weeks before to 14 d after calving

Values are means ± SEM for $n=9$ per group

	Days relative to calving							
	−24†	−16	−9	0	3	7	14	*P*-values
				RRR				
Control	$92{\cdot}4^{B}$	$98{\cdot}5^{Aa}$	$99{\cdot}2^{Aa}$	$98{\cdot}5^{Aa}$	$98{\cdot}2^{Aa}$	$98{\cdot}4^{Aa}$	$97{\cdot}6^{Aa}$	<0·001
SynAc	$89{\cdot}8$	$91{\cdot}3^{b}$	$92{\cdot}7^{b}$	$88{\cdot}7^{b}$	$90{\cdot}2^{b}$	$87{\cdot}5^{b}$	$88{\cdot}1^{b}$	0·24
NatAc	$92{\cdot}4^{B}$	$99{\cdot}5^{Aa}$	$99{\cdot}5^{Aa}$	$100{\cdot}0^{Aa}$	$98{\cdot}7^{Aa}$	$98{\cdot}5^{Aa}$	$99{\cdot}2^{Aa}$	<0·001
NatAlc	$91{\cdot}1^{B}$	$99{\cdot}1^{Aa}$	$98{\cdot}7^{Aa}$	$97{\cdot}5^{Aa}$	$97{\cdot}8^{Aa}$	$97{\cdot}9^{Aa}$	$98{\cdot}3^{Aa}$	0·006
SEM	1·3	1·0	0·8	0·9	0·9	1·1	1·0	
P-values	0·88	<0·001	<0·001	<0·001	<0·001	<0·001	<0·001	
				RSS				
Control	$2{\cdot}6^{A}$	$0{\cdot}4^{Bb}$	$0{\cdot}2^{Bb}$	$0{\cdot}5^{Bb}$	$0{\cdot}6^{Bb}$	$0{\cdot}4^{Bb}$	$0{\cdot}6^{Bb}$	<0·001
SynAc	2·3	$2{\cdot}7^{a}$	$2{\cdot}1^{a}$	$3{\cdot}6^{a}$	$3{\cdot}3^{a}$	$3{\cdot}6^{a}$	$4{\cdot}3^{a}$	0·30
NatAc	$2{\cdot}7^{A}$	$0{\cdot}2^{Bb}$	$0{\cdot}1^{Bb}$	$0{\cdot}0^{Bb}$	$0{\cdot}4^{Bb}$	$0{\cdot}5^{Bb}$	$0{\cdot}2^{Bb}$	<0·001
NatAlc	$2{\cdot}8^{A}$	$0{\cdot}4^{Bb}$	$0{\cdot}4^{Bb}$	$0{\cdot}8^{Bb}$	$0{\cdot}5^{Bb}$	$0{\cdot}6^{Bb}$	$0{\cdot}4^{Bb}$	0·01
SEM	0·6	0·3	0·2	0·3	0·3	0·3	0·4	
P-values	0·10	<0·001	<0·001	<0·001	<0·001	<0·001	<0·001	
				RRS				
Control	$2{\cdot}1^{A}$	$0{\cdot}5^{Bb}$	$0{\cdot}2^{Bb}$	$0{\cdot}6^{Bb}$	$0{\cdot}6^{Bb}$	$0{\cdot}6^{Bb}$	$0{\cdot}7^{Bb}$	0·002
SynAc	2·3	$2{\cdot}8^{a}$	$2{\cdot}3^{a}$	$3{\cdot}8^{a}$	$3{\cdot}5^{a}$	$4{\cdot}2^{a}$	$4{\cdot}7^{a}$	0·32
NatAc	$2{\cdot}6^{A}$	$0{\cdot}2^{Bb}$	$0{\cdot}2^{Bb}$	$0{\cdot}0^{Bb}$	$0{\cdot}5^{Bb}$	$0{\cdot}6^{Bb}$	$0{\cdot}3^{Bb}$	<0·001
NatAlc	$3{\cdot}0^{A}$	$0{\cdot}4^{Bb}$	$0{\cdot}5^{Bb}$	$0{\cdot}8^{Bb}$	$0{\cdot}8^{Bb}$	$0{\cdot}7^{Bb}$	$0{\cdot}4^{Bb}$	<0·001
SEM	0·4	0·3	0·2	0·3	0·3	0·4	0·4	
P-values	0·95	<0·001	<0·001	<0·001	<0·001	<0·001	<0·001	
				RSR				
Control	$1{\cdot}6^{A}$	$0{\cdot}6^{Bb}$	$0{\cdot}3^{Bb}$	$0{\cdot}4^{Bb}$	$0{\cdot}6^{Bb}$	$0{\cdot}5^{Bb}$	$1{\cdot}0^{ABb}$	0·04
SynAc	2·4	$2{\cdot}6^{a}$	$2{\cdot}5^{a}$	$3{\cdot}4^{a}$	$3{\cdot}0^{a}$	$3{\cdot}4^{a}$	$2{\cdot}3^{a}$	0·51
NatAc	$2{\cdot}0^{A}$	$0{\cdot}0^{Bb}$	$0{\cdot}1^{Bb}$	$0{\cdot}0^{Bb}$	$0{\cdot}4^{Bb}$	$0{\cdot}4^{Bb}$	$0{\cdot}3^{Bb}$	<0·001
NatAlc	$2{\cdot}3^{A}$	$0{\cdot}3^{Bb}$	$0{\cdot}3^{Bb}$	$0{\cdot}8^{Bb}$	$1{\cdot}0^{Bb}$	$0{\cdot}7^{Bb}$	$0{\cdot}6^{Bb}$	0·06
SEM	0·4	0·3	0·3	0·3	0·3	0·3	0·2	
P-values	0·98	<0·001	<0·001	<0·001	<0·001	<0·001	<0·001	
				2SR/SR/S				
Control	0·3	$0{\cdot}0^{b}$	0·0	$0{\cdot}0^{b}$	0·1	$0{\cdot}2^{ab}$	0·2	0·38
SynAc	0·5	$0{\cdot}7^{a}$	0·3	$0{\cdot}4^{a}$	0·0	$1{\cdot}3^{a}$	0·6	0·09
NatAc	$0{\cdot}2^{A}$	$0{\cdot}0^{Bb}$	$0{\cdot}1^{B}$	$0{\cdot}0^{Bb}$	$0{\cdot}0^{B}$	$0{\cdot}0^{Bb}$	$0{\cdot}0^{B}$	0·006
NatAlc	0·8	$0{\cdot}1^{ab}$	0·1	$0{\cdot}1^{ab}$	0·0	$0{\cdot}0^{b}$	0·4	0·06
SEM	0·1	0·1	0·0	0·1	0·0	0·2	0·1	
P-values	0·39	0·004	0·08	0·03	0·11	0·02	0·09	

Treatment ($P<0{\cdot}001$) and time ($P<0{\cdot}05$) had significant effects on the distribution of all stereoisomers
† At the sampling before the start of the experiment (d −24) all cows were fed a diet containing synthetic vitamin E
[a,b] Values within a column without a common superscript letter are significantly different ($P<0{\cdot}05$)
[A,B] Values within a row without a common superscript letter are significantly different ($P<0{\cdot}05$)

degradation of α-tocopherol have been found in the literature. According to Alderson et al. (1971) up to 50% of the α-tocopheryl acetate added to the diet could be degraded in the rumen when a high proportion of concentrate was fed, but in newer experiments by Weiss et al. (1995) this high intraruminal degradation could not be confirmed. In contrast to the acetate form, the alcohol form acts as an antioxidant in the feed and the intestine (Halliwell et al. 2000), which may result in a loss prior to its absorption by the cow. However, the product was stored in sealed containers and distributed as top-dressing just after feeding of fresh TMR every morning, and the concentration of α-tocopherol in the product was checked by HPLC each time a new solution was made (i.e. four times during the experiment). The concentration and total amount of α-tocopherol in mammary secretions varied substantially between cows. However, the total amount of α-tocopherol secreted daily into milk was lower in control than in supplemented groups days 7 and 14 after calving, reflecting the lower blood concentration in this group.

https://doi.org/10.1017/S0022029906001701 Published online by Cambridge University Press

Table 6. Relative proportions (%) of α-tocopherol stereoisomers in milk from 36 dairy cows sampled during the first two weeks after calving. The cows were fed daily supplements of no vitamin E (Control), *all-rac*-α-tocopheryl acetate (SynAc), *RRR*-α-tocopheryl acetate (NatAc), and *RRR*-α-tocopherol (NatAlc) from approximately three weeks before calving to 14 days after calving

Values are means ± SEM for $n = 9$ per group

	Time after calving			
	0–12 h	12–24 h	7 d	14 d
			RRR	
Control	96·0^{ab}	98·0^{ab}	96·6^{ab}	99·2^{a}
SynAc	91·2^{b}	89·6^{b}	90·5^{b}	87·4^{b}
NatAc	99·8^{a}	99·2^{a}	100·0^{a}	100·0^{a}
NatAlc	97·5^{a}	98·3^{a}	99·2^{ab}	100·0^{a}
SEM	0·9	1·0	1·0	0·4
P-values	0·008	0·005	0·03	<0·001
			RSS	
Control	1·3^{ab}	0·5^{b}	0·9^{ab}	0·3^{b}
SynAc	2·4^{a}	2·7^{a}	2·9^{a}	2·9^{a}
NatAc	0·1^{b}	0·3^{b}	0·0^{b}	0·0^{b}
NatAlc	0·5^{b}	0·5^{b}	0·2^{b}	0·0^{b}
SEM	0·3	0·3	0·3	0·1
P-values	0·02	0·04	0·04	<0·001
			RRS	
Control	1·2^{ab}	0·5^{ab}	0·7^{ab}	0·3^{b}
SynAc	2·2^{a}	3·2^{a}	2·6^{a}	2·8^{a}
NatAc	0·0^{b}	0·3^{b}	0·0^{b}	0·0^{b}
NatAlc	0·7^{ab}	0·5^{ab}	0·2^{b}	0·0^{b}
SEM	0·2	0·4	0·3	0·1
*P*values	0·03	0·03	0·03	<0·001
			RSR	
Control	1·4^{ab}	0·8^{ab}	1·6^{ab}	0·3^{b}
SynAc	3·7^{a}	3·6^{a}	3·7^{a}	5·1^{a}
NatAc	0·1^{b}	0·4^{b}	0·0^{b}	0·0^{b}
NatAlc	1·1^{b}	0·6^{ab}	0·3^{ab}	0·0^{b}
SEM	0·4	0·3	0·5	0·2
P-values	0·01	0·002	0·006	<0·001
			2SR/SR/S	
Control	0·22	0·11	0·21	0·0^{b}
SynAc	0·60	0·92	0·27	1·83^{a}
NatAc	0·06	0·15	0·00	0·00^{b}
NatAlc	0·26	0·06	0·00	0·00^{b}
SEM	0·13	0·13	0·11	0·18
P-values	0·48	0·05	0·71	0·009

Treatment ($P<0·001$), but not time, had significant effects on the distribution of all stereoisomers

[a,b] Values within a column without a common superscript letter are significantly different ($P<0·05$)

The stereoisomer composition in milk was similar to blood within treatment, which suggests that mammary cells do not have a preferential uptake of *RRR*-stereoisomer, but take up what is available in blood. These findings agree with studies in pigs (Lauridsen et al. 2002b). The placental transfer of vitamin E is very low in ruminants, as well as in pigs, making colostrum the most important source of vitamin E for the calf (Van Saun et al. 1989). It may be speculated that a high *RRR*-stereoisomer proportion in colostrum/milk is better for the calf due to a higher biological activity of this isomer (Burton et al. 1998; Lauridsen et al. 2002a, b).

Despite the positive effect of NatAc supplementation on plasma α-tocopherol concentration, the concentration at calving dropped below 3 mg/l in most cows, both at calving and 3 d after calving. Similar patterns were observed also in the other groups. The decrease at calving was in agreement with earlier findings (e.g. Goff & Stabel, 1990; Weiss et al. 1990; Politis et al. 1996; Meglia et al. 2001; Goff et al. 2002), and was probably due to different factors such as a reduced DMI, an increased transfer of α-tocopherol to colostrum, and an increased demand for antioxidants at this time. In line with earlier studies (Hidiroglou, 1989; Batra et al. 1992), colostrum contained much higher amounts of α-tocopherol than milk.

In conclusion, the results indicate that daily oral supplementation of dairy cows with *RRR*-α-tocopheryl acetate (NatAc) in the periparturient period is superior in terms of increased plasma vitamin E status of cows when compared with supplementation of *all-rac*-α-tocopheryl acetate (SynAc), *RRR*-α-tocopherol (NatAlc), or no supplementation (Control). Moreover, irrespective of dietary treatment, the bioavailability of the *RRR*-stereoisomer was greater compared with the other stereoisomer forms of α-tocopherol, which led to an enrichment of the *RRR*-form of α-tocopherol in milk to more than 86% of the total concentration. Thus, relative to the other stereoisomers, the bioavailability of *RRR*-α-tocopherol in cattle is considerably higher than generally accepted until now.

The authors want to thank Dr Patrik Öhagen for statistical assistance, Dr Nicolai Agger and Dr Rob Stuart for kindly providing the vitamin products, and Ms Elsebeth L. Pedersen, Ms Mette Würtz and Mr Lars B. Gildberg for technical assistance. The financial support from the Swedish Farmer's Foundation for Agricultural Research and the Swedish Institute is gratefully acknowledged.

References

Alderson NE, Mitchel GE, Little CO, Warner RE & Tucker RE 1971 Preintestinal disappearence of vitamin E in ruminants. *Journal of Nutrition* **101** 655–659

Batra AG, Hidiroglou M & Smith WM 1992 Effect of vitamin E on incidence of clinical mastitis. *Canadian Journal of Animal Science* **72** 287–297

Bendich A 1993 Antioxidant, immune response, and animal function. *Journal of Dairy Science* **76** 2789–2794

Blatt DH, Pryor WA, Mata JE & Rodriguez-Proteau R 2004 Re-evaluation of the relative potency of synthetic and natural α-tocopherol: experimental and clinical observations. *Journal of Nutritional Biochemistry* **15** 380–395

Burton GW, Traber MG, Acuff RV, Walters DN, Kayden H, Hughes L & Ingold KU 1998 Human plasma and tissue α-tocopherol concentrations in response to supplementation with deuterated natural

and synthetic vitamin E. *American Journal of Clinical Nutrition* **67** 669–684

Charmley E & Nicholson JWG 1994 Influence of dietary fat source on oxidative stability and fatty acids composition of milk from cows receiving a low or high level of dietary vitamin E. *Canadian Journal of Animal Science* **74** 657–664

Cortinas L, Baroeta AC, Galobart J & Jensen SK 2004 Distribution of α-tocopherol stereoisomers in liver and thigh of chickens. *British Journal of Nutrition* **92** 295–301

Drotleff AM & Ternes W 2001 Determination of RS,E/Z-tocotrienols by HPLC. *Journal of Chromatography A* **909** 215–223

Goff JP & Stabel JR 1990 Decreased plasma retinol, alpha-tocopherol and zinc concentration during the periparturient period: effect of milk fever. *Journal of Dairy Science* **73** 3195–3199

Goff JP, Kimura K & Horst RL 2002 Effect of mastectomy on milk fever, energy, and vitamins A and E, and β-carotene status at parturition. *Journal of Dairy Science* **85** 1427–1436

Halliwell B, Zhao K & Whiteman M 2000 The gastrointestinal tract: A major site of antioxidant action. *Free Radical Research* **33** 819–830

Hidiroglou N, Laflamme LF & McDowell LR 1988 Blood plasma and tissue concentrations of vitamin E in beef cattle as influenced by supplementation of various tocopherol compounds. *Journal of Animal Science* **66** 3227–3234

Hidiroglou M 1989 Mammary transfer of vitamin E in dairy cows. *Journal of Dairy Science* **72** 1067–1071

Hidiroglou N, McDowell LR, Papas AM, Antapli M & Wilkinson NS 1992 Bioavailability of vitamin E compounds in lambs. *Journal of Animal Science* **70** 2556–2561

Hidiroglou M, Batra TR & Zhao X 1997 Bioavailability of vitamin E compounds and the effect of supplementation on release of superoxide and hydrogen peroxide by bovine neutrophils. *Journal of Dairy Science* **80** 187–193

Hogan JS, Weiss WP, Todhunter DA, Smith KL & Schoenberger PS 1992 Bovine neutrophil responses to parenteral vitamin E. *Journal of Dairy Science* **75** 399–405

Hosomi A, Arita M, Sato Y, Kiyose C, Ueda T, Igarashi O, Arai H & Inoue K 1998 Affinity for α-tocopherol transfer protein as a determinant of the biological activities of vitamin E analogs. *FEBS Letter* **409** 105–108

Jensen SK & Nielsen KN 1996 Tocopherols, retinol, β-carotene and fatty acids in fat globule membrane and fat globule core in cows' milk. *Journal of Dairy Research* **63** 565–574

Jishage K, Arita M, Igarashi K, Iwata T, Watanabe M, Ogawa M, Ueda O, Kamada N, Inoue K, Arai H & Suzuki H 2001 α-Tocopherol transfer protein is important for the normal development of placental labyrinthine trophoblasts in mice. *Journal of Biological Chemistry* **273** 1669–1672

Kayden HJ & Traber MG 1993 Absorption, lipoprotein transport, and regulation of plasma concentrations of vitamin E in humans. *Journal of Lipid Research* **34** 343–358

Lauridsen C, Engel H, Craig AM & Traber MG 2002a Relative bioactivity of dietary *RRR*- and *all-rac*-α-tocopheryl acetates in swine assessed with deuterium-labeled vitamin E. *Journal of Animal Science* **80** 702–707

Lauridsen C, Engel H, Jensen SK, Craig AM & Traber MG 2002b Lactating sows and suckling piglets preferentially incorporate *RRR*- over *all-rac*-α-tocopherol into milk, plasma and tissue. *Journal of Nutrition* **132** 1258–1264

Lauridsen C & Jensen SK 2005 Influence of supplementation of *all-rac*-α-tocopheryl acetate preweaning and vitamin C postweaning on α-tocopherol and immune responses of piglets. *Journal of Animal Science* **83** 1274–1286

LeBlanc SJ, Duffield TF, Leslie KE, Bateman KG, TenHag J, Walton JS & Johnson WH 2002 The effect of prepartum injection of vitamin E on health in transition dairy cows. *Journal of Dairy Science* **85** 1416–1425

Meglia GE, Johannisson A, Petersson L & Persson Waller K 2001 Changes in blood micronutrients, leukocytes and neutrophil expression of adhesion molecules in dairy cows. *Acta Veterinary Scandinavica* **42** 109–120

Ndiweni N & Finch JM 1996 Effects of in vitro supplementation with α-tocopherol and selenium on bovine neutrophil functions: implications for resistance to mastitis. *Veterinary. Immunology and Immunopathology* **51** 67–78

Pehrson B & Hakkarainen J 1986 Vitamin E status of healthy Swedish cattle. *Acta Veterinary Scandinavica* **27** 351–360

Politis I, Hidiroglou M, White JH, Gilmore JA, Williams SN, Scherf H & Frig M 1996 Effects of vitamin E on mammary and blood leukocyte function, with emphasis on chemotaxis, in periparturient dairy cows. *American Journal of Veterinary Research* **57** 468–471

Reddy PG & Frey RA 1990 Nutritional modulation of immunity in domestic food animals. *Advances in Veterinary Science and Comparative Medicine* **35** 255–281

SAS 1999 SAS version 8.0, SAS Institute, Cary, NC, USA

Smith LK, Harrison JH, Hancock DD, Todhunter DA & Conrad HR 1984 Effect of vitamin E and selenium supplementation on incidence of clinical mastitis and duration of clinical symptoms. *Journal of Dairy Science* **67** 1293–1300

United States Pharmacopeia 1985 The United States Pharmacopeial Convention, Inc. 21st revision. Rockville, MD, USA

Van Saun RJ, Herdt TH & Stowe HD 1989 Maternal and fetal vitamin E concentrations and selenium-vitamin E interrelationships in dairy cattle. *Journal of Nutrition* **119** 1156–1164

Weiser H, Riss G & Kormann AW 1996 Biodiscrimination of the eight alpha-tocopherol stereoisomers result in preferential accumulation of the four 2*R* forms in tissues and plasma of rats. *Journal of Nutrition* **126** 2539–2549

Weiss WP, Todhunter DA, Hogan JS & Smith KL 1990 Effect of duration of supplementation of selenium and vitamin E on periparturient dairy cows. *Journal of Dairy Science* **73** 3187–3194

Weiss WP, Smith KL, Hogan JS & Steiner TE 1995 Effect of forage to concentrate ratio on disappearance of vitamins A and E during in vitro ruminal fermentation. *Journal of Dairy Science* **78** 1837–1842

Weiss WP, Hogan JS, Todhunter DA & Smith KL 1997 Effect of vitamin E supplementation in diets with a low concentration of selenium on mammary gland health of dairy cows. *Journal of Dairy Science* **80** 1728–1737