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# Supplementing dairy steers and organically managed dairy cows with synthetic vitamin $D_3$ is unnecessary at pasture during exposure to summer sunlight

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Use of synthetic feed additives, including synthetic vitamin $D_3$ ($D_3$) in the feed for cows and other ruminants, is not consistent with the international principles of organic farming. If dairy farmers wish to produce in accordance with the organic principles, production animals would be left with only their endogenous production of $D_3$ from summer sunlight as a source of $D_3$. To examine the impact of supplemental synthetic $D_3$ from the feed on the $D_3$ status of dairy cattle in organic production in Nordic countries, 20 high-yielding dairy cows and 30 dairy steers were divided into two groups: one supplemented with synthetic $D_3$ in the feed and one not supplemented with synthetic $D_3$. Vitamin $D_3$ status of the animals was assessed by measuring the concentration of the liver-derived 25-hydroxyvitamin $D_3$ ($25OHD_3$) in plasma. Results showed that $25OHD_3$ concentration in plasma from dairy cattle as well as from steers decreased during winter for both supplemented and unsupplemented groups. Unsupplemented cows and steers had approximately 2 ng $25OHD_3$ per ml plasma during winter, whereas supplemented animals had between 10 (cows) and 30 (steers) ng/ml. During summer and autumn there was no additive effect of supplementing with synthetic $D_3$ since unsupplemented and supplemented animals had the same $D_3$ status at this time of year. In all cows summer concentrations of $25OHD_3$ were 20–25 ng/ml and in all steers 40–50 ng/ml plasma. The decrease in vitamin $D_3$ status during winter indicates that cows and steers are able to store $D_3$ only to a limited extent. The results also show that cows or steers fed supplemental $D_3$ according to Swedish recommendation throughout the year are not able to maintain their summer value of $25OHD_3$ during winter.

**Keywords:** Vitamin $D_3$, cholecalciferol, supplementation, blood plasma status, dairy cows, steers, organic production.

In northern Europe dairy cattle have two sources of vitamin $D_3$ ($D_3$): dietary $D_3$ from artificial vitamin $D_3$ additives added to feed or endogenous $D_3$ produced in the skin during exposure to summer sunlight. Production of $D_3$ in the skin starts by epithelial cells producing 7-dehydrocholesterol, the immediate precursor of $D_3$, from acetate (Gaylor & Sault, 1964). Irradiation with u.v. light cleaves the bond between C9 and C10 of 7-dehydrocholesterol and renders pre-vitamin $D_3$ which spontaneously isomerizes into $D_3$ (MacLaughlin et al. 1982; Stryer, 1995).

In the liver $D_3$ is hydroxylated at C25 into 25-hydroxyvitamin $D_3$ ($25OHD_3$) which in the blood stream is transported to the kidneys where it is hydroxylated at C1 into 1,25-dihydroxyvitamin $D_3$ ($1,25OH_2D_3$) which is biologically active agent in regulating the calcium (Ca) turnover in the body of animals (Horst et al. 1994). The most important functions of $1,25OH_2D_3$ are to stimulate the production of calcium-binding protein (CABP) in the gastro intestinal tract responsible for absorption of Ca; to

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control the resorption of Ca from the bones and the excretion of Ca from the kidneys (Horst et al. 1994), thereby facilitating and maintaining normal Ca homeostasis in the body against the risk of hypocalcaemia, or milking fever, a problem in high-yielding dairy cattle (Goff et al. 1991).

The main difficulty in predicting $D_3$ supply, compared with the supply of other fat-soluble vitamins, is that there are the afore mentioned two sources of $D_3$: synthetic $D_3$ supplied in feed and natural $D_3$ synthesized in the skin. These $D_3$ sources both supplement and substitute each other. The need for supplemental $D_3$ in the feed therefore varies, i.e. it is low when production of $D_3$ in the skin is high and vice versa. This supplementary action makes it difficult to get an overview of the actual need for providing $D_3$ in the feed for dairy cattle.

The international principles for organic farming do not approve the use of synthetic vitamins in organic dairy production (IFOAM, 2008). Producing according to the organic principles would therefore cause animals in organic dairy production to be left with only their endogenously produced $D_3$ as their source of $D_3$.

According to EU Council Regulation (EC) No. 1804/1999 on organic production etc., herbivores should have access to pasture whenever conditions allow it (EC, 1999). In southern Sweden at 58–59°N dairy cows are usually confined in the stable from mid-autumn until mid-spring owing to the meteorological and geographical conditions in that part of Sweden. However, it is unknown whether organic dairy cattle with seasonal access to outside areas in Nordic countries will have their need for $D_3$ covered by their endogenous production during summer. Furthermore, it is not known for how long the endogenously produced $D_3$ can be stored in the body and maintain an appreciable $D_3$ status in the cows since it was shown by Hymøller et al. (2008) that organic dairy cows that are not supplemented with $D_3$ during winter have a very low $D_3$ status in early spring.

The aim of this study was to examine whether dairy breed steers and high-yielding organic dairy cows in southern Sweden at 58–59°N can maintain a $D_3$ status during autumn, winter and spring similar to summer levels through supplementation with synthetic $D_3$ in their feed according to Swedish recommendations, and whether supplementation with synthetic $D_3$ is necessary during summer, when endogenous production of $D_3$ is high.

## Materials and Methods

### *Animals and housing*

*Organic dairy cows*: This study was carried out from September 2003 to September 2005 at the organic research farm Tingvall in the southern part of Sweden at 59°N. On the research farm, there were a total of 70 milking cows of Swedish Holstein breed with an average annual energy-corrected milk yield of 9873 and 10383 kg/cow during the first and the second year, respectively, of the study.

Cows were assigned to the following two treatments according to expected calving date, lactation number and the previous 12-month milk yield or their breeding index in the case of first-calving heifers: (1) a 100% organic ration with a mineral feed from Lactamin Inc. (SE-10425 Stockholm, Sweden) including vitamins $D_3$, E and A, fed at a level according to the Swedish recommendations (Spörndly, 2003), and (2) the same 100% organic ration with a similar mineral feed but excluding vitamins $D_3$, E and A. Lactating supplemented cows received a daily dose of approx. 20 000 i.u. and dry cows approximately 12 000 i.u. $D_3$ in the diet. The mineral feed and part of the concentrate was fed to the cows individually in transponder-controlled automatic feeders. Cows were housed in the stable from approx. 15 October to 15 May both years and the rest of the time let out to pasture [10·9 MJ metabolizable energy (ME), 380 g neutral detergent fibre (NDF), 182 g crude protein (CP) in 2004 and 10·9 MJ ME, 392 g NDF, 144 g CP in 2005] (in May and October both years only during the daytime). Twenty of the cows, ten from each treatment, which had completed two entire lactations during the 2-year study, were chosen for retrospectively establishing the $D_3$ status of the herd throughout the study period.

All cows were housed in a loose housing barn with cubicles during winter. All feed for the cows was 100% organic and was fed as a mixed ration (nutrient content per kg dry matter is given in parentheses) containing grass-clover silage (9·7 MJ ME, 548 g NDF, 140 g CP in 2003/2004 and 11·4 MJ ME, 488 g NDF, 129 g CP in 2004/2005) and rolled barley (13·2 MJ ME, 200 g NDF, 112 g CP in 2003/2004 and 13·1 MJ, 165 g NDF, 123 g CP in 2004/2005). In 2004/05 triticale was also used (14·1 MJ ME, 125 g NDF, 118 g CP). The mixed ration was supplemented with a barley/pea mixture (peas: 13·8 MJ ME, 209 g NDF, 210 g CP in 2003/2004 and 14·0 MJ ME, 108 g NDF, 236 g CP in 2004/2005) and cold-pressed rapeseed cake (17·0 MJ ME, 250 g NDF, 263 g CP in 2003/2004 and 17·0 MJ ME, 192 g NDF, 281 g CP in 2004/2005) in automatic feeders according to stage of lactation. Cows were fed a minimum of 50% of their dry matter (DM) intake as forage during their first 3 months of lactation and thereafter they were fed a minimum of 60% of their DM intake as forage according to the organic regulations (EC, 1999). Milking was carried out twice a day at 5.30 and 15.30.

*Steers*: This study was carried out in order to rule out effects of lactation stage that might have been encountered in the cow study. The steers were placed at the conventional research station Götala at 58°N in the southern part of Sweden. Samples were taken between January 2006 and November 2006 but the steers were started on the treatments in October 2005. The 30 steers used in

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this study were of the dairy breeds Swedish Red and Swedish Holstein with an average initial live weight of 128±27 kg and an average daily weight gain of 800 g/d. The steers were housed in pens on deep straw bedding during the first winter and in pens on slatted floors during the second winter. During the housing seasons the steers were fed a total mixed ration of grass-clover silage (9·7 MJ ME, 588 g NDF, 133 g CP), rolled barley (13·3 MJ ME, 189 g NDF, 93 g CP), peas and cold-pressed rape seed cake. The steers were equally divided into two treatment groups according to their initial live weights. The treatments were: (1) a mineral feed from Lactamin Inc. (SE-10425 Stockholm, Sweden) including the vitamins $D_3$, E and A, fed at a level according to the Swedish recommendations (Spörndly, 2003) and (2) a similar mineral feed without the vitamins $D_3$, E and A. Steers received a daily dose of approx. 12 000 i.u. $D_3$ per steer. From the end of April 2006 until the end of October 2006 all steers were let out to pasture (10·2 MJ ME, 533 g NDF and 143 g CP) where steers in the vitamin $D_3$ treated group had access to the mineral feed including the vitamins $D_3$, E and A and the unsupplemented group had access to the mineral feed without vitamins $D_3$, E and A. Vitamins were available ad libitum in specifically designed feeders, and it was not possible to measure the mineral feed intake of individual steers at pasture.

### Samples

*Organic dairy cows*: Blood was drawn from the tail vein. One blood sample was taken prior to the beginning of the study (zero sample) to establish the initial $D_3$ status of the cows. Five blood samples were taken during each lactation according to the time of calving: sample 1, 3 weeks before calving; sample 2, within 24 h after calving; sample 3, 3–4 weeks after calving; sample 4, 3–5 months after calving; and sample 5, 7–9 months after calving. All cows calved between November and February. Blood was collected in heparin-coated Vacutainer tubes, centrifuged at 1500 ***g*** for 10 min at and the plasma was transferred to sample tubes and stored at −18 °C until analysis.

*Steers*: Blood samples from all 30 steers were collected once a month from either the jugular vein or the tail vein. Blood was collected in heparin-coated Vacutainer tubes, centrifuged at 1500 ***g*** for 10 min and the plasma was transferred to sample tubes and stored at −18 °C until analysis.

### Analytical specifications

All plasma samples were analysed by HPLC for content of the liver-derived $25OHD_3$. All analyses were performed in the laboratories at Aarhus University, Faculty of Agricultural Sciences, DK-8830 Tjele, Denmark.

*Chemicals*: Water quality was at all times secured by treatment on a Milli-Q 185 filter provided by Millipore S.A.S. (F-67120 Molsheim, France). Methanol and heptane of HPLC grade were purchased from POCH S.A. (PL-44102 Gliwice, Poland) and acetonitrile of HPLC far u.v. grade from Lab-Scan Ltd. (Stillorgan Dublin, Ireland). Ethanol (96%) was purchased from Danisco (DK-1001 Copenhagen, Denmark). L(+)-Ascorbic acid, 40 g, (JT Baker, NL-7400 Deventer, Holland), was dissolved in 200 ml of water to obtain a 20% (w/v) solution. The ascorbic acid solution was prepared fresh every week. KOH (AppliChem (D-64291 Darmstadt, Germany) was prepared in a 50% (w/v) solution with water every month. Technical gases were purchased from Air Liquide (DK-8700 Horsens, Denmark). HPLC grade 1α-hydroxyvitamin $D_3$ ($1\alpha OHD_3$) used as internal standard was purchased from Fluka (CH-9471 Buchs, Switzerland) and dissolved in ethanol. The exact concentration of $1\alpha OHD_3$ in the standard solution was determined before use according to the extinction coefficient in ether: $E_{264\ nm}=18\,000\ mol^{-1}\ cm^{-1}$ (Merck Index) to 1130 ng/ml on a Hitachi U-2000 double beam u.v./vis. spectrophotometer from Hitachi Instruments Inc. (JP-1008280 Tokyo, Japan).

*Sample preparation*: Samples were at all times protected from light during preparation. Plasma (2 ml) and 150 µl of internal standard was placed in a culture tube and the following added: 2·0 ml 96% ethanol, 0·5 ml methanol, 1·0 ml of 20% ascorbic acid solution, and 0·3 ml of 50% KOH. Culture tubes were placed in a water bath at 80 °C where the samples were saponified for 20 min. After saponification samples were rapidly cooled in cold water. All samples were extracted with 2 × 5·0 ml heptane. Heptane fractions were quantitatively transferred to a clean culture tube after centrifugation at 1500 ***g*** for 10 min. The heptane fraction was evaporated to exact dryness over $N_2$ at 40 °C.

Samples from the organic dairy cows were re-dissolved in 100 µl of 80% (v/v) acetonitrile, vortex mixed, centrifuged at 1500 ***g*** for 10 min, and transferred to micro vials. Samples from the steers were re-dissolved in 200 µl of 90% (v/v) methanol, vortex mixed, centrifuged at 1500 ***g*** for 10 min, and transferred to micro vials.

### High Pressure Liquid Chromatography

*Organic dairy cows*: The HPLC equipment from Perkin-Elmer Inc. (MA-02451 Waltham, USA) was a Perkin-Elmer Series 200 auto sampler and pump. Detection was carried out using a Perkin-Elmer 235C diode array u.v. detector at a fixed wavelength of 265 nm, scanning the spectrum between 190 and 340 nm every 5 s for identification of peaks by their u.v. spectra. Data was collected and handled in the Total Chrom Workstation version 6.3 also from Perkin-Elmer Inc. The guard column was a Supelcogel ODP-50 Supelcoguard cartridge (20 × 4·0 mm

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ID) with 5·0 μm particle size. The analytical column was a $C_{18}$, Supelcogel ODP-50 (150 × 4·0 mm ID) with 5·0 μm particle size both from Supelco (MO-63178 St. Louis MO, USA). The columns were kept at 30 °C during elution.

Twenty μl of sample was injected and gradient elution performed at a flow rate of 1·0 ml/min with acetonitrile/methanol/$H_2O$ and a gradient from 58·8% /10%/31·2% to 89%/10%/0·4% during 60 min. Eluents were degassed with helium before use. The following peaks were identified at the given retention times (Rt) and quantified: $25OHD_3$ (Rt=11·4 min) $1\alpha OHD_3$ (Rt=21·2 min).

The detection limit on this HPLC equipment was established as 3-times the random baseline noise to 0·6 ng/ml and the quantification limit as 10-times the random baseline noise to 1·8 ng/ml. Error percentages were calculated based on 10 plasma samples analysed five by five on two consecutive days. The maximum uncertainty of the analysis within day was 3·9% and the reproducibility, or day to day error percentage, was 0·6%.

*Steers*: The HPLC equipment from Dionex Corporation (CA-94088-3603, Sunnyvale, USA) was a Dionex UltiMate 3000 vacuum degasser, auto sampler, pump and column compartment. Detection was carried out using a Dionex UltiMate 3000 variable wavelength u.v. detector at a fixed wavelength of 265 nm. Data were collected and stored in the Chromelion software version 6.80 from Dionex Corporation.

The analytical column was an YMC $C_{30}$ column (250 × 4·6 mm ID) with 5·0 μm particle size from YMC Europe GmbH (D-46539 Dinslagen, Germany) and a 10-mm guard column made from the same material was placed in front of the analytical column. Both columns were kept at 35 °C during elution. Fifty μl of sample was injected and gradient elution performed at a flow rate of 1·0 ml/min with acetonitrile/methanol/$H_2O$ and a gradient from 3%/87·4%/9·6% to 97%/3%/0% during 45 min. It was possible to identify and quantify the following peaks: $25OHD_3$ (Rt=8·7), $25OHD_2$ (Rt=9·4), $1\alpha OHD_3$ (Rt=15·6), $D_2$ (Rt=23·6), and $D_3$ (Rt=24·2). The maximum uncertainty of the analysis within day was 6·2% and the reproducibility, or day to day error percentage, was 1·8%.

### *Statistical analysis*

*Organic dairy cows*: A relatively small number of blood samples were taken from the cows every month and the samples were therefore assigned to a quarter of the year (3 months) rather than a month of the year for statistical analysis. Plasma values of $25OHD_3$ below the detection limit of the HPLC equipment were set to zero ng/ml.

Analysis of variance on plasma concentrations of $25OHD_3$ was performed using the MIXED models procedure of SAS® (SAS Institute Inc., Cary NC, USA). Systematic effects included effects of sampling time divided into nine quarters throughout the 2-year study, treatment, and interaction between sampling time and treatment. Cow was introduced as random effect. The statistical model used was: $Y_{ijk}=\mu+\alpha_i+\beta_j+(\alpha\beta)_{ij}+C_{ijk}+\varepsilon_{ikj}$, where $Y_{ijk}$ is the plasma concentration of $25OHD_3$, μ is the overall mean, $\alpha_i$ is the fixed effect of treatment $i$ (synthetic $D_3$ supplements; no synthetic $D_3$ supplements), $\beta_j$ is the fixed effect of sampling time $j$ (1st quarter; 2nd quarter; 3rd quarter; …; 9th quarter), $(\alpha\beta)_{ij}$ is the effect of the interaction between treatment $i$ and sampling time $j$, $C_k$ is the random effect of cow, and $\varepsilon_{ijk}$ is the random residual error.

Random effects were assumed normally distributed with mean value zero and constant variance $C_{ijk}\sim N(0,\sigma_C^2)$ and $\varepsilon_{ikj}\sim N(0,\sigma^2)$. Differences were considered statistically significant when $P\leqslant 0{\cdot}05$.

*Steers:* Analysis of variance on plasma concentrations of $25(OH)D_3$ was performed using the MIXED models procedure of SAS®. Systematic effects included the effects of sampling month and vitamin supplementation regime together with the interaction between the two. Steer was used as a random effect. To account for the covariance structure of the repeated measures during consecutive months within steers the covariance was modelled using the *repeated* statement for the MIXED procedure in SAS® (Littell et al. 2006). The best model fit was obtained using the auto regressive 1st order covariance structure [AR(1)]. The statistical model used was: $Y_{ijk}=\mu+\alpha_i+\beta_j+(\alpha\beta)_{ij}+C_{ijk}+\varepsilon_{ikj}$, where $Y_{ijk}$ is the plasma concentration of $25OHD_3$, μ is the overall mean, $\alpha_i$ is the fixed effect of vitamin supplementation regime $i$ (synthetic $D_3$ supplements; no synthetic $D_3$ supplements), $\beta_j$ is the fixed effect of month $j$ (January, February, …, November), $(\alpha\beta)_{ij}$ is the effect of the interaction between vitamin supplementation regime $i$ and housing season $j$, $C_k$ is the random effect of steer $k$, and $\varepsilon_{ijk}$ is the random residual error.

Random effects were assumed normally distributed with mean value zero and constant variance $C_{ijk}\sim N(0, \sigma_C^2)$ and $\varepsilon_{ikj}\sim N(0, \sigma^2)$. Differences were considered statistically significant when $P\leqslant 0{\cdot}05$. Results are presented as least squares means ± SE.

## Results

### *Organic dairy cows*

Results of the statistical analysis are shown in Fig. 1. There was a general effect of treatment ($P\leqslant 0{\cdot}01$) and sampling quarter ($P\leqslant 0{\cdot}001$) together with a significant interaction between the two ($P\leqslant 0{\cdot}001$) probably because the plasma concentration of $25OHD_3$ of the unsupplemented cows declined less than the plasma concentrations of supplemented cows between the July–September and the October–December quarters in 2003 as shown in Fig. 1. In the July–September quarter 2003 there were no differences in plasma $25OHD_3$ between $D_3$ supplemented and

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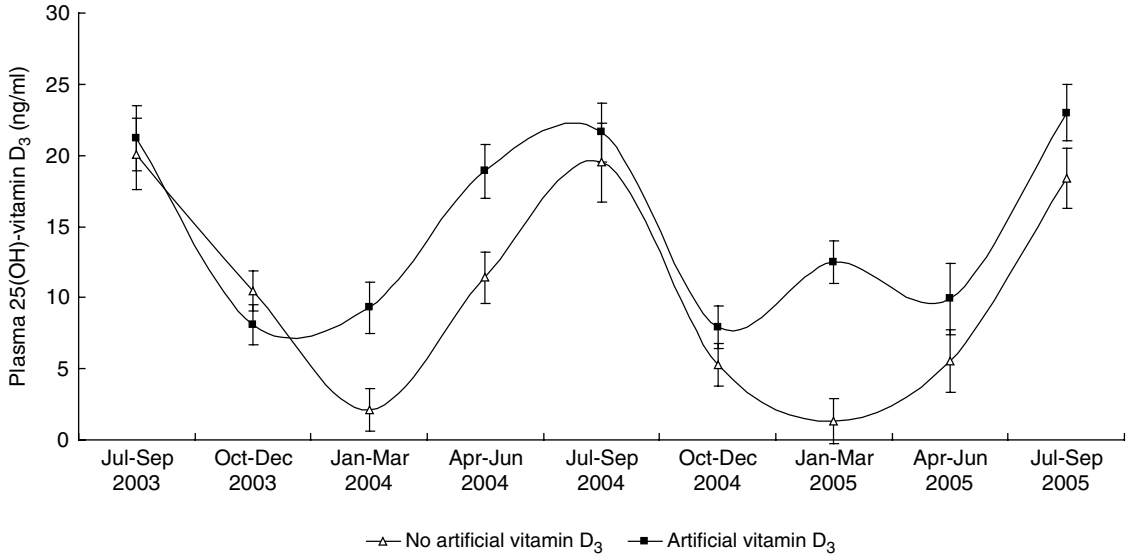


**Fig. 1.** Results from the statistical analysis illustrating quarterly plasma concentration of 25(OH)-vitamin $D_3$ in high-yielding organic dairy cows at Tingvall fed with ($n=10$) or without ($n=10$) synthetic vitamin $D_3$ in the feed (least squares means ± SE)

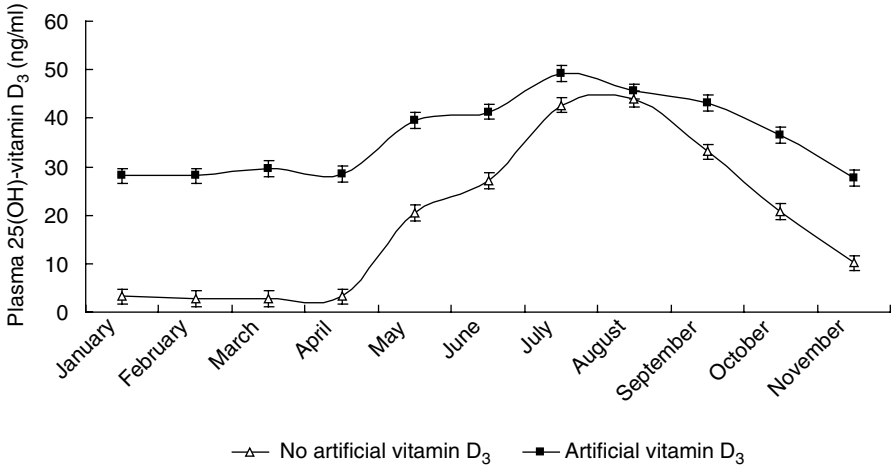


**Fig. 2.** Results from the statistical analysis illustrating plasma concentration of 25(OH)-vitamin $D_3$ throughout the year 2006 in Swedish dairy breed steers at Götala fed with ($n=15$) or without ($n=15$) synthetic vitamin $D_3$ in the feed (least squares means ± SE)

unsupplemented cows and there was no difference between the July–September quarters between the two years (Fig. 1). In the January–March quarters in both years, the concentration of $25OHD_3$ in the plasma of unsupplemented cows had decreased to about one-tenth of the concentrations in the July–September quarters in unsupplemented cows. In $D_3$ supplemented cows the concentration decreased less than in unsupplemented cows and was significantly higher than in unsupplemented cows ($P \leqslant 0{\cdot}01$) in the January–March quarter in both years as shown in Fig. 1. Within supplemented and unsupplemented cows, respectively, there was no difference between the same respective quarters across years. October–December and April–June quarters showed intermediate plasma concentrations of $25OHD_3$ and there was no difference between treatments in those quarters (Fig. 1).

### *Steers*

Results from the statistical analysis are shown in Fig. 2. A general effect of treatment ($P \leqslant 0{\cdot}001$) and sampling month ($P \leqslant 0{\cdot}001$) was found but also a significant interaction between treatment and sampling month ($P \leqslant 0{\cdot}001$) probably due to the much steeper increase in plasma concentration of $25OHD_3$ due to summer sunlight in steers not supplemented with synthetic $D_3$ than in steers supplemented with $D_3$. In the stable between January and April the $D_3$ supplemented steers had an almost 10-times higher concentration of $25OHD_3$ in their plasma than

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unsupplemented steers ($P \leqslant 0{\cdot}001$). Between the April and the May samples the plasma concentration of $25OHD_3$ increased significantly in both treatment groups ($P \leqslant 0{\cdot}001$) and continued to increase significantly month by month in the unsupplemented steers until the July blood samples ($P \leqslant 0{\cdot}001$). In supplemented steers, however, the increase in plasma concentration of $25OHD_3$ slowed down and there was no significant increase between the May and June samples ($P = 0{\cdot}24$) or between the June and July samples ($P = 0{\cdot}52$). By June the difference between the two treatment groups had decreased to $14{\cdot}2 \pm 2{\cdot}2$ ng/ml ($P \leqslant 0{\cdot}001$) and by July to $6{\cdot}4 \pm 2{\cdot}2$ ng/ml ($P \leqslant 0{\cdot}01$) whereas by August there was no statistically significant difference between the groups as the unsupplemented steers had a mean plasma concentration of $25OHD_3$ amounting to $44{\cdot}0 \pm 1{\cdot}6$ ng/ml and supplemented steers $45{\cdot}5 \pm 1{\cdot}6$ ng/ml ($P = 0{\cdot}50$). From August to November, the decrease in plasma $25OHD_3$ was more pronounced in the unsupplemented steers, resulting in lower $25OHD_3$ values in unsupplemented than in supplemented steers. By the time of the November samples plasma concentrations of $25OHD_3$ were approaching the levels found during the previous winter in both treatment groups but had not yet reached the lowest detected level from the previous winter ($P \leqslant 0{\cdot}001$).

## Discussion

Consistent with the finding of the present studies, Miller & Thompson (2007), in a 2-year survey on non-lactating cows grazing extensive native pastures all year round on the Falkland Islands at latitudes between 51°S and 53°S, found significantly decreased concentrations of $25OHD_3$ in plasma during winter and early spring compared with during summer and early autumn. Summer concentrations were 3·5-times higher than the concentrations found in winter samples. Similar differences between summer and winter plasma levels of $25OHD_3$ were found in the unsupplemented cows in the present study. However, in supplemented cows the difference between winter and summer was less pronounced. In a study on the effects on the $D_3$ status in heifers in response to $D_3$ supplementation and sunlight, Hidiroglou et al. (1979) observed that plasma concentrations of $25OHD_3$ in January were 2·5-times higher in $D_3$ supplemented heifers than in unsupplemented heifers, when both groups were confined indoors. By late June, after 3 weeks on pasture, there was no difference between supplemented and unsupplemented heifers (Hidiroglou et al. 1979) probably owing to the endogenous production of $D_3$ induced by the sunlight (Webb et al. 1988). In the present study, supplemented cows had an almost 7-times higher plasma concentration of $25OHD_3$ than unsupplemented cows during the first winter of the study, and an almost 10-times higher concentration during the second winter. The reason for the larger difference between supplemented and unsupplemented cows in the present study, in comparison with the heifers in the study of Hidiroglou et al. (1979), might be a consequence of an additional loss of $D_3$ into milk in high-yielding dairy cows. This loss of $D_3$ into milk in the dairy cows may also explain why supplemented steers in the present study in general reached much higher plasma levels of $25OHD_3$ than the supplemented cows. In unsupplemented steers and cows the lowest winter plasma concentrations of $25OHD_3$ were practically the same, at 2–5 ng/ml (Figs 1 and 2).

Sunlight's ability to induce endogenous $D_3$ production in the skin of animals or humans, was shown by Webb et al. (1988) to depend on the season in a study on the production of pre-vitamin $D_3$, a precursor of $D_3$ that isomerizes into $D_3$ catalysed by body heat (MacLaughlin et al. 1982), in human skin samples. They found that increasing latitude was negatively correlated to the length of time during which production of pre-vitamin $D_3$ in the skin took place. In Canada at 52°N the production of pre-vitamin $D_3$ ceased in October and did not reappear until mid-April even on cloudless days (Webb et al. 1988).

Despite the much lower plasma concentration of $25OHD_3$ found in unsupplemented steers and cows than in supplemented animals during winter, both treatment groups reached similar plasma concentrations of $25OHD_3$ when let out to pasture during summer, as did the heifers in the study by Hidiroglou et al. (1979). However, the fact that there is a significant difference in plasma $25OHD_3$ between winter and summer within both treatment groups in both steers and cows indicates that the endogenous production of $D_3$ by sunlight is a much more potent source for maintaining high plasma concentrations of $25OHD_3$ than synthetic $D_3$ supplemented in feed according to the Swedish recommendations. This is even further supported by the lack of difference in plasma $25OHD_3$ concentrations of supplemented and unsupplemented steers and cows during summer, which shows that there is no additional effect of $D_3$ supplements on the $D_3$ status of steers and cows when they have access to sufficient amounts of summer sunlight, a finding which is consistent with Hidiroglou et al. (1979). The physiology behind this finding could be either that the intestinal absorption of $D_3$ in supplemented cows is down-regulated when plasma concentrations of $25OHD_3$ reach a threshold value, or that the endogenous production of $D_3$ in both groups of cows is down-regulated either when plasma concentrations of $25OHD_3$ reach a threshold value or when sunlight exposure exceeds a certain intensity. Since $D_3$ from feed has been shown to be absorbed through passive diffusion into the mesenteric lymph following the fat fraction of the feed (Maislos et al. 1981) the latter explanation seems the most likely, as shown by MacLaughlin et al. (1982) who found that the pre-vitamin $D_3$ produced in human skin started to be degraded by the sunlight itself during prolonged exposure to sunlight instead of isomerizing into $D_3$. It also becomes evident that the endogenous $D_3$ produced by the summer sunlight is not stored in the body of steers and

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cows to a degree that can maintain sufficient $D_3$ status throughout winter when there is no access to sunlight (Figs 1 and 2), findings consistent with results obtained from sheep (Quarterman et al. 1961).

In conclusion, supplementation with $D_3$ in feed for dairy breed steers and organic dairy cows during periods without access to summer sunlight is a good precaution in order to prevent problems related to $D_3$ deficiency during winter and spring, as the animals experience a very low $D_3$ status during winter when not supplied with synthetic $D_3$ in their feed. During summer and autumn, however, it seems unnecessary to supplement dairy steers and high yielding organic dairy cows with $D_3$ beyond their endogenous $D_3$ production. The effect of summer sunlight on the $D_3$ status does not last throughout winter, hence it appears that cows and steers are able to store endogenously produced $D_3$ only to a limited extent.

Experiments conducted on more breeds of cattle differing in productivity and repeated over several locations and years are needed to deliver recommendations for $D_3$ supplementation for cattle in Nordic countries. However, based on the present study it appears that the current Swedish recommendations are too low to supply organic dairy cattle with enough $D_3$ to maintain the same vitamin $D_3$ status in the animals during winter as during exposure to summer sunlight.

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