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# Environmental and genetic factors influence the vitamin D content of cows’ milk 

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#### Abstract

Vitamin D is obtained by cattle from the diet and from skin production via UVB exposure from sunlight. The vitamin D status of the cow impacts the vitamin D content of the milk produced, much like human breast milk, with seasonal variation in the vitamin D content of milk well documented. Factors such as changes in husbandry practices therefore have the potential to impact the vitamin D content of milk. For example, a shift to year-round housing from traditional practices of cattle being out to graze during the summer months and housed during the winter only, minimises exposure to the sun and has been shown to negatively influence the vitamin D content of the milk produced. Other practices such as changing dietary sources of vitamin D may also influence the vitamin D content of milk, and evidence exists to suggest genetic factors such as breed can cause variation in the concentrations of vitamin D in the milk produced. The present review aims to provide an overview of the current understanding of how genetic and environmental factors influence the vitamin D content of the milk produced by dairy cattle. A number of environmental and genetic factors have previously been identified as having influence on the nutritional content of the milk produced. The present review highlights a need for further research to fully elucidate how farmers could manipulate the factors identified to their advantage with respect to increasing the vitamin D content of milk and standardising it across the year.


Environmental factors: Genetic factors: Vitamin D: Cows' milk

Cattle require vitamin D to aid the excretion of calcium from the kidneys and in the reabsorption of calcium from the bones, maintaining calcium homeostasis ${ }^{(1)}$. Vitamin D is also important in preventing the development of hypocalcaemia ${ }^{(2)}$ and milk fever which is a debilitating disorder typically seen close to calving, characterised by decreased blood calcium concentrations, and in severe cases can result in fatalities ${ }^{(3)}$.

In a similar manner to human subjects, cattle can obtain vitamin D through both endogenous, or dermal synthesis, as well as dietary sources. Only vitamin $D_{3}$ (cholecalciferol) is produced through dermal synthesis following exposure to UVB emitted from the sun ${ }^{(4)}$. Dietary sources, however, can provide both vitamins
$D_{3}$ and $D_{2}$ (ergocalciferol). Vitamin $D_{2}$ is typically obtained naturally through the ingestion of fungi growing among the vegetation cattle consume ${ }^{(5)}$, and dietary vitamin $D_{3}$ is provided through synthetic additives in the feed concentrates ${ }^{(6)}$, usually in regulated quantities (per kg/d). Therefore, differences in husbandry practices can cause an inherent variation in the vitamin D content of the milk produced between different farms and throughout the year (e.g. housed $v$. grazing on pasture and grass $v$. concentrate feed). The amount of vitamin D consumed or synthesised by cattle impacts the vitamin D status of the animal, and much like human breast milk, the vitamin D status of the cow subsequently impacts the vitamin D content of the milk produced ${ }^{(7-9)}$.

Cows' milk provides many nutrients in the human diet (e.g. protein, calcium, riboflavin, vitamin $\mathrm{B}_{12}$, potassium, iodine and phosphorus) and has been associated with a number of health benefits ${ }^{(10)}$. In the face of limited dietary sources of vitamin D , dairy products remain an important contribution to adults' overall vitamin D intake ${ }^{(11)}$, with several countries across the globe implementing a mandatory or voluntary fortification policy for fluid milk to improve the vitamin D content of the milk on sale ${ }^{(12-15)}$.

The aims of the present review were to provide an overview of: (1) the genetic and the environmental factors that influence the vitamin D status of dairy cattle; (2) how these factors influence the vitamin D content of the milk produced.

## Environmental factors

## Seasonal changes in vitamin $D$ content

Seasonal variations in vitamin D content of milk are well documented, with concentrations found to be higher in the summer months than in the winter, most likely due to differences in both husbandry and feeding practices between the seasons. Reports dating back as far as the 1920s demonstrated that a single cow pasture-fed between May and July had a higher 'anti-rachitic' (vitamin D) content than the milk produced when the same cow was fed in-house and kept in the dark ${ }^{(16)}$. The same cow was then involved in another study, which collected milk samples for 18 months. In support of the initial findings, a 23 -fold increase in the vitamin D content of the milk produced was observed when the cow was out to pasture, compared with the milk produced when the cow was housed in a dark stall ${ }^{(17)}$. Evidence suggests that this seasonal variation is the result of insufficient stores of vita$\min \mathrm{D}$ in the liver and fat tissues for mobilisation in times when dietary intake of the vitamin is low ${ }^{(18)}$. Many subsequent studies have confirmed the seasonal variation of the vitamin D content of milk (approximate differences ranging between 0.004 and $0.0014 \mu \mathrm{~g} / \mathrm{g}$ fat) across different countries and breeds of cattle (Table 1) ${ }^{(19-22)}$. Although seasonal variation in vita$\min \mathrm{D}$ content is widely reported in the literature, units of measurement are inconsistent, which makes it difficult to compare between studies. In the previous edition of the UK Food Composition Tables, no seasonal variation in the vitamin D content of milk was noted for whole, semiskimmed and skimmed milk, but was observed in the whole milk samples from the Channel Islands, where mean concentrations for summer and winter were 0.04 $\mu \mathrm{g} / 100 \mathrm{~g}$ and $0.03 \mu \mathrm{~g} / 100 \mathrm{~g}$, respectively ${ }^{(23)}$. In the most recent edition, a lack of seasonal variation is still apparent, with vitamin D only quantified for Channel Island whole milk, listed as $0.01 \mu \mathrm{~g} / 100 \mathrm{~g}$ and trace for whole, semi-skimmed, skimmed and $1 \%$ milks ${ }^{(24)}$.

While the seasonal variation in the vitamin D content of milk is established, not all studies or databases, such as the recent editions of the UK Food Composition Tables, report such variations, and a more comprehensive update
Table 1. Studies investigating the seasonal variation of vitamin D concentrations in cow's milk
*Seasonal means not reported.
of vitamin D in milk across the UK and Ireland is warranted.

## UVB exposure

In a study by Hymøller et al., cows from two organic dairy farms in Denmark were selected to determine the effect of sunlight on the vitamin D status in March and April, and on each farm, cattle were allocated based on milk yield, parity and lactation stage to have daily outdoor access (from February to April) or to be confined indoors for the duration of the study (NovemberApril) ${ }^{(25)}$. Results from Farm 1 found no significant effect of treatment allocation on plasma 25 -hydroxyvitamin $\mathrm{D}_{3}$ $\left(25(\mathrm{OH}) \mathrm{D}_{3}\right)$ concentration in March ( $P=0.350$ ) or April ( $P=0.060$ ), with mean plasma $25(\mathrm{OH}) \mathrm{D}_{3}$ concentrations of 7.84 and $5.85 \mathrm{ng} / \mathrm{ml}$ for the outdoor and indoor groups, respectively ${ }^{(25)}$. On Farm 2, the outdoor group had a significantly higher $25(\mathrm{OH}) \mathrm{D}_{3}$ concentration in March, compared with the indoor group ( 5.71 v. $3.36 \mathrm{ng} / \mathrm{ml}$; $P<0.05$ ), but the same difference was not reported in April $(P=0.100)^{(25)}$. Hymoller et al. ${ }^{(25)}$ concluded that the assumption was that supplemental vitamin $\mathrm{D}_{3}$ may still be required in the spring as a means to maintain a healthy vitamin D status.
In the field of bio-fortification/bio-addition, a recent Danish study ${ }^{(9)}$ investigated the potential impact of supplemental UVB light on vitamin $D_{3}$ synthesis in sixteen housed Holstein cattle, a common dairy breed, which had been severely depleted of their vitamin D stores. The cows were randomised to receive artificial UVB light 30,90 or 120 min daily for 24 d or 60 min for 73 d; the length of UVB exposure was designed to be equivalent to $1,2,3$ and 4 h of sunlight at pasture at $56^{\circ} \mathrm{N}$, respectively ${ }^{(9)}$. After 24 d , the exposure to supplemental UVB light significantly increased the vitamin $\mathrm{D}_{3}$ and $25(\mathrm{OH}) \mathrm{D}_{3}$ concentrations in the milk in a dosedependent manner over 30,90 and $120 \min ^{(9)}$. In the cattle allocated to receive 60 min daily, a significant increase ( $P=0.029$ ) in the vitamin $\mathrm{D}_{3}$ (but not the $25(\mathrm{OH}) \mathrm{D}_{3}$ ) concentration of the milk produced between days 0 and 24 was noted, but this did not increase further up to day $73(P=0.400)^{(9)}$.

This important preliminary evidence, albeit from a limited number of studies, suggests that vitamin D biofortification of cow's milk does, at least in theory seem probable. Future studies therefore should investigate this novel on-farm method as a means of minimising the seasonal variation in cow's vitamin D status and the milk produced.

## Diet

The seasonal changes in the vitamin D content of milk, have long been associated with the change in UV intensity and a reduction in the time spent outdoors, rather than as a result of the change in feed ${ }^{(22,26)}$. That being stated, in the UK cattle are solely reliant on dietary vitamin D during the winter, obtained through grass stores (hay, silage or haylage) or feed concentrates. Prior to 2010, both vitamins $\mathrm{D}_{2}$ and $\mathrm{D}_{3}$ were authorised by the European Commission as sources of vitamin D, which
could be added to feeds intended for cattle; however, in November 2010 no submission was made for the reauthorisation of a vitamin $\mathrm{D}_{2}$ dossier, and as a result cattle can now only obtain vitamin $\mathrm{D}_{2}$ from the consumption of fungi growing among the vegetation (fresh grass, hay, silage or haylage) used as roughage in the diet ${ }^{(5)}$ and not from concentrates. Within the EU , vitamin $\mathrm{D}_{3}$ is now the only authorised source of supplemental vitamin D for cattle ${ }^{(27)}$, with the maximum permitted levels set at $4000 \mathrm{IU}(100 \mu \mathrm{~g}) / \mathrm{kg}$ of feed ${ }^{(28)}$.

Although cattle are reliant on dietary vitamin D during the winter months, it has been suggested that fatsoluble vitamins from such dietary sources are destroyed once they enter the rumen, owing to the fermentative environment ${ }^{(29,30)}$. Research using a fistula model was designed to test this hypothesis in vitamin $D^{(4)}$. A maximum of 15 kg ruminal contents were removed and mixed with a vitamin $\mathrm{D}_{2}$ and $\mathrm{D}_{3}$ (both 250 mg ) and vita$\min \mathrm{E}$ pre-mix ${ }^{(4)}$. The contents were then returned to the rumen; ruminal and blood samples were then collected over the subsequent 30 h period ${ }^{(4)}$. Once collected, ruminal samples were freeze-dried (in vivo samples), additional ruminal samples were collected at the 1 h time-point, and stored in plastic bottles, which were then placed in a water-bath ( $37^{\circ} \mathrm{C}$; in vitro samples). The concentrations of both vitamins $D_{2}$ and $D_{3}$ declined over the study period in the in vivo samples, with concentrations remaining stable in the in vitro samples, suggesting no degradation in the intact ruminal sample ${ }^{(4)}$. Results showed that the plasma concentrations of both vitamins $D_{2}$ and $D_{3}$ increased over the first few hours, from levels below the limit of detection, and reached a maximum concentration after $24 \mathrm{~h}(99(15)$ and $163(16) \mathrm{ng} / \mathrm{ml}$, respectively), with vitamin $\mathrm{D}_{3}$ concentrations significantly higher than those for vitamin $\mathrm{D}_{2}^{(4)}$. It has previously been hypothesised that vitamin D degradation in the rumen may be a natural protective detoxification process when large quantities of the vitamin are consumed ${ }^{(31)}$, and this may also be a possible reason for the rapid conversion to 25 -hydroxyvitamin D observed by Hymoller and Jensen ${ }^{(4)}$.

Previously the potential of intravenous supplements to improve the vitamin D status of the cow and the milk produced have also been considered. Thompson and Hidiroglou ${ }^{(32)}$ orally administered 1000000 IU ( $25000 \mu \mathrm{~g}$ ) vitamin $\mathrm{D}_{2}$ and $1000000 \mathrm{IU}(25000 \mu \mathrm{~g})$ vitamin $\mathrm{D}_{3}$ mixed in maize oil to two dairy cows, collecting milk and blood samples for 10 d after. The results showed that the maximum plasma vitamin D concentrations were observed after 2-3 d, with the maximum concentrations in the milk $1-3 \mathrm{~d}$ after ${ }^{(32)}$. At the same time twelve additional cows were allocated to be orally or intravenously administered with vitamin $D_{3}$ in doses of 5000 $000 \mathrm{IU}(125000 \mu \mathrm{~g})$ or $10000000 \mathrm{IU}(250000 \mu \mathrm{~g})$. Increases in the vitamin D content of the milk produced varied between animals, with the maximum levels reached between 3 and 7 d for the oral doses and up to 10 d for the intravenous doses, with the maximum observed ranges between $8 \mathrm{IU}(0.2 \mu \mathrm{~g})$ and 92 IU $(2 \cdot 3 \mu \mathrm{~g}) / 100 \mathrm{ml}^{(32)}$. It is important to interpret these results with caution as the doses administered in this trial are
extreme and would not be feasible to incorporate into the daily management of a dairy herd. Furthermore, little is also known on the safety, efficacy and longer-term effects of prolonged usage 'mega-doses', other than the data available for acute doses used in the treatment of milk fever ${ }^{(33,34)}$.

A research team led by Hollis collected milk samples from two groups of cows ( $4000 \mathrm{IU}(100 \mu \mathrm{~g}) ~ v . ~ 40000$ IU $(1000 \mu \mathrm{~g})$ daily), and found concentrations of vitamin D, 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D in the milk to be greater in those cattle receiving a higher daily dose of vitamin ${ }^{(8)}$. Similar results were noted for 24,25-dihydroxyvitamin D and 25,26-dihydroxyvitamin $D^{(8)}$. This research indicates that the intake of sufficient quantities of dietary vitamin D is enough to increase the vitamin D content of the milk produced.

A cross-over study randomised fourteen Danish Holstein cows based on parity and milk yield to receive a one-off 250 mg dose of vitamin $\mathrm{D}_{2}$, followed by the same dose of vitamin $D_{3}$ in capsule-form or vice versa ${ }^{(6)}$. Plasma samples were obtained and area under the curve was used to determine the impact of the two different doses on the plasma 25 -hydroxyvitamin D status. Results found that the concentrations of plasma 25 -hydroxyvitamin $\mathrm{D}_{2}$ when $\mathrm{D}_{2}$ was administered first was less than half that of $25(\mathrm{OH}) \mathrm{D}_{3}$ when the vitamin $\mathrm{D}_{3}$ dose was given first $(P \leq 0.001)^{(6)}$, suggesting that vitamin $D_{2}$ may impair the utilisation of vitamin $D_{3}$.

McDermott et al. assigned twenty Holstein cows to receive $0 \mathrm{IU}, 10000 \mathrm{IU}(250 \mu \mathrm{~g}), 50000 \mathrm{IU}(1250 \mu \mathrm{~g})$ or $250000 \mathrm{IU}(6250 \mu \mathrm{~g})$ vitamin $\mathrm{D}_{3}$ daily, for 14 weeks starting at 2 weeks pre-partum ${ }^{(35)}$. Vitamin $\mathrm{D}_{3}$ concentrations in the colostrum were significantly higher $(P<0.05)$ in cows receiving $250000 \mathrm{IU} / \mathrm{d}$ compared with the other groups, although this dropped during the transition to normal milk from colostrum, about 1 week post-partum. At the end of the study the vitamin $\mathrm{D}_{3}$ content of the milk was approximately $0.075 \mathrm{ng} / \mathrm{ml}, 0.16 \mathrm{ng} / \mathrm{ml}, 20 \mathrm{ng} /$ ml and $22 \mathrm{ng} / \mathrm{ml}$ for $0,10000,50000$ and 250000 IU , respectively ${ }^{(35)}$. A mean concentration of $0.15 \mathrm{ng} / \mathrm{ml}$ for 25 $(\mathrm{OH}) \mathrm{D}_{3}$ was observed in normal milk ${ }^{(35)}$.

The need to supplement cattle over the summer months with vitamin $\mathrm{D}_{3}$ was investigated in Swedish Holsteins, assigned to receive a mineral feed containing vitamin $D_{3}$ concentrations in accordance with Swedish recommendations (control) or the same feed providing approximately $20000 \mathrm{IU}(500 \mu \mathrm{~g})$ vitamin $\mathrm{D}_{3}$ daily ${ }^{(2)}$. Plasma samples collected over the 2 -year period showed a significant effect of treatment on the cattle's circulating $25(\mathrm{OH}) \mathrm{D}_{3}$ concentrations compared with control $(P \leq 0.001)$ and moreover, the $25(\mathrm{OH}) \mathrm{D}_{3}$ concentrations in both the supplemented and unsupplemented cows increased when the cattle were out at pasture over the summer months ${ }^{(2)}$. The authors concluded that cattle obtain adequate vitamin $\mathrm{D}_{3}$ from dermal synthesis over the summer, but that stores were not adequate to maintain the status and they had to rely on supplemental vitamin $D$ over the winter ${ }^{(2)}$.

Overall the results of the earlier studies provide evidence to suggest that dietary vitamin $\mathrm{D}_{3}$ is adequate to improve the vitamin D content of the milk produced and to help maintain the status in times where dermal synthesis is not feasible, despite the fermentative
environment of the rumen. These findings are of particular importance in relation to recent changes in husbandry practices, which have seen a growing shift to the yearround housing of cattle.

## Genetic factors

## Breed

The variation in the vitamin D content of milk produced by different cattle breeds is supported by evidence conducted across the world (Table 2). The HolsteinFriesian cross has become the most common breed of dairy cow, used for milk production across the world, due to the high production rates ${ }^{(36)}$ and also remains a popular choice within the majority of British herds ${ }^{(37)}$.

The average vitamin D content of milk produced in the UK is currently documented as 'trace' for whole, semi-skimmed and skimmed milk, albeit breed is not specified, with the exception of whole milk from the Channels Islands where Jersey cows are the dominant breed $(0.1 \mu \mathrm{~g} / 100 \mathrm{~g})^{(24)}$. The differences in vitamin D reported in the current Food Composition Tables support the results of Wallis ${ }^{(38)}$ who compared the vitamin D content of the milk from Holsteins and Jerseys in the 1940s. Results from this early work showed that although Holsteins produced vastly greater quantities of milk, the vitamin D content of the Jersey cows was on average 3 -fold higher owing to higher butterfat concentrations ${ }^{(38)}$. Bechtel and Hoppert noted that not only was the vitamin D content of the milk higher in the summer months, but also that the milk fat produced from the Guernsey cattle was higher than the milk of the Holstein cattle ${ }^{(39)}$. A British study involving three cattle breeds (Friesian, Jersey and Ayrshire) commonly milked in the UK observed differences across the three breeds in summer milk, with little difference apparent in winter milk ${ }^{(19)}$. In Portugal, two studies have noted a higher vitamin D content of milk from indigenous dairy breeds (Barrosã and Minhota) when compared that from with Friesians and Holstein-Friesians ${ }^{(36,40)}$.

## Hair coverage and dominant colour

To determine if cattle could synthesis vitamin $\mathrm{D}_{3}$ regardless of hair coverage, Hymeller and Jensen ${ }^{(41)}$ designed a study involving sixteen Danish Holstein cattle, which had been depleted of their vitamin D stores, and randomised based on parity and milk yield to one of the four groups. The treatment groups consisted of different levels of body coverage with a fabric, which prevented vitamin D synthesis for 28 d: a horse blanket; an udder cover; a horse blanket and an udder cover; no coverage ${ }^{(41)}$. The cattle were on pasture for 5 h each day and inside for the remainder of the day, and were fed a vitamin $\mathrm{D}_{3}$-free diet throughout the study ${ }^{(41)}$. Mean plasma $25(\mathrm{OH}) \mathrm{D}_{3}$ concentrations increased from $2.8(0.2) \mathrm{ng} / \mathrm{ml}$ at baseline in all treatment groups, in a dose-dependent manner with the increasing level of body coverage ${ }^{(41)}$ (Table 3).

More recently, Hymøller and Jensen ${ }^{(42)}$ randomised twenty Danish Holstein heifers based on milk yield and
Table 2. Studies investigating the impact of cattle breed on the vitamin D concentrations of milk

| Study | Study design |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Country | Cattle breed | $n$ | Milk sample collected | $n$ | Vitamin D concentrations |
| Bechtel and Hoppert ${ }^{(39)}$ | USA | Guernesy; Holstein | Eight cows; fourteen cows | Fresh milk | Monthly; 1932-1934 | 0.12-1.09 $\mu \mathrm{g} /$ quart; $0.08-0.69 \mu \mathrm{~g} /$ quart |
| Wallis ${ }^{(38)}$ | USA | Jersey; Holstein | Three cows; three cows | Fresh milk - extracted butterfat | Monthly - for up to 13 months | $0.75 \mu \mathrm{~g} /$ quart; $0.25 \mu \mathrm{~g} /$ quart |
| Thompson et al. ${ }^{(19)}$ | England | Aryshire; Jersey; Friesian | Sixty cows; sixty cows; fifty cows | Fresh milk - churned to butter for analysis | Single 15 gallon sample collected from each herd in early March | $0.016 \mu \mathrm{~g} / \mathrm{g}$ of fat*; $0.013 \mu \mathrm{~g} / \mathrm{g} \mathrm{fat}^{*}$; $0.011 \mu \mathrm{~g} / \mathrm{g}$ fat* |
| Pires et al. ${ }^{(40)}$ | Portugal | Barrosã; Friesian | Five cows; five cows | Fresh milk | 1 from each cow | $2.60 \mu \mathrm{~g} / 100 \mathrm{~g} ; 1.21 \mu \mathrm{~g} / 100 \mathrm{~g}$ |
| Ramhola et al. ${ }^{(36)}$ | Portugal | Minhota; HolesteinFriesian | Fifteen cows; fifteen cows | Fresh milk | Monthly: October 2008 September 2009 | $0.11 \mu \mathrm{~g} / \mathrm{g}$ fat $\dagger ; 0.10 \mu \mathrm{~g} / \mathrm{g}$ fat $\dagger$ |
| McCance and Widdowson ${ }^{(24)}$ | UK | Jersey; UK pooled milk (breed not specified) | Not specified | Whole milk; whole, semi-skimmed and skimmed | 6 (3 summer and winter); not specified | $0.01 \mu \mathrm{~g} / 100 \mathrm{~g}$; 'trace' for whole, semi-skimmed and skimmed |

*Summer vitamin D concentration. Winter concentrations for all three breeds were $0.002 \mu \mathrm{~g} / \mathrm{g}$ fat.
$\dagger$ Provitamin $D_{3}$ also measured - mean concentrations higher in the milk of Holstein-Friesian than Minhota cattle ( 0.77 and $0.45 \mu \mathrm{~g} / \mathrm{g}$ fat).
dominant hair colour (black or white) to five different groups, allocated to an increasing length of time on pasture per day $(0,15,30,75,150 \text { or } 300 \mathrm{~min})^{(42)}$. At baseline, the mean plasma $25(\mathrm{OH}) \mathrm{D}_{3}$ concentration for all the heifers was $44.9(2.4) \mathrm{nm} / \mathrm{l}$. Over 28 d , the cattle on pasture for 15,30 or 75 min were unable to maintain their $25(\mathrm{OH}) \mathrm{D}_{3}$ concentrations from that at baseline. A significant increase in mean $25(\mathrm{OH}) \mathrm{D}_{3}$ concentration was observed however in those outside on pasture for 150 or $300 \mathrm{~min}^{(42)}$. In addition, they found that the dominant coat colour (black or white) had no significant effect on the plasma concentrations of $25(\mathrm{OH}) \mathrm{D}_{3}$, illustrating that prominent coat colour does not influence the dermal synthesis of vitamin $D_{3}$ in such cattle ${ }^{(42)}$ (Table 3).

The results of these two unique studies eloquently demonstrate that cattle can synthesis vitamin $D_{3}$ through all areas of their skin and not just in the udders or muzzle, where hair coverage is scarce. The work by Hymøller et al. also illustrates that, unlike human subjects, pigmentation has no effect on the synthesis of vitamin $D_{3}$ following UVB exposure ${ }^{(43)}$. Further work in other cattle breeds is required to further investigate the variance in vitamin D levels in the milk produced. In addition, it may be beneficial to further explore the research by Hymøller and Jensen in other breeds to determine other factors that may prevent the dermal synthesis of vitamin D , such as long haired cattle breeds.

## Other factors

## Age

A German two-series study investigated the impact age has on the metabolism of $25(\mathrm{OH}) \mathrm{D}_{3}^{(44)}$. In the first series, fourteen multiparous cows were supplemented orally with 3 $\mathrm{mg} 25(\mathrm{OH}) \mathrm{D}_{3}$ daily from day 270 of gestation until parturition, with blood samples collected every other day ${ }^{(44)}$. Ninety cows were allocated in the second series to receive $0,4,6 \mathrm{mg} 25(\mathrm{OH}) \mathrm{D}_{3}$ daily through mineral feed additives for the last $8-10 \mathrm{~d}$ of gestation, with blood samples also taken every other day until parturition, and at several intervals thereafter ${ }^{(44)}$. Calculated slopes found the difference in $25(\mathrm{OH}) \mathrm{D}_{3}$ between cattle in their second and third lactation to be significantly higher in the second lactation $(P<0.001)$, suggesting that younger cattle are more efficient at absorbing $25(\mathrm{OH}) \mathrm{D}_{3}$ or that in older cattle the rate of $25(\mathrm{OH}) \mathrm{D}_{3}$ elimination is faster, with 1,25-dihydroxyvitamin $\mathrm{D}_{3}$ increased in cattle in the third lactation or higher ${ }^{(44)}$.

## Stage of lactation

A Japanese study collected milk samples from three Holstein cows at stage points post-partum: 1 d after, colostrum; 2-4 d after, early milk and 15 d after, later milk $^{(45)}$. Similar concentrations of vitamin $D$ were recorded across the three points for two of the cows (33.2 IU/l $\quad(0.83 \mu \mathrm{~g} / \mathrm{l}), 30.9 \mathrm{IU} / 1 \quad(0.77 \mu \mathrm{~g} / \mathrm{l}), 35.6 \mathrm{IU} / \mathrm{l}$ ( $0.89 \mu \mathrm{~g} / \mathrm{l}$ ); and $47.0 \mathrm{IU} / \mathrm{l}(1 \cdot 18 \mu \mathrm{~g} / \mathrm{l}), 47.0 \mathrm{IU} / \mathrm{l}(1 \cdot 18 \mu \mathrm{~g} / \mathrm{l})$, 55.7 IU/l $(1.39 \mu \mathrm{~g} / \mathrm{l})$, respectively), with no trend noted in

Table 3. Studies investigating the impact of hair coverage and dominant hair colour on the vitamin D synthesis

| Study | Study design |  |  |  |  |  | Plasma 25(OH)D concentrations |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Country | Cattle breed | $n$ | Study duration | Treatment groups | $n$ | Mean at baseline | Mean at end-point | $P$ |
| Hymøller and Jensen ${ }^{(41)}$ | Denmark | Holstein | Sixteen cows | 28 d | Allocated to coverage; Horse blanket; Udder cover; Horse blanket and udder cover; Natural | 14 | $\begin{gathered} 2.5(0.2) \\ \mathrm{ng} / \mathrm{ml} \end{gathered}$ | 8.9 (1.8) ng/ml; 23.2 (1.5) $\mathrm{ng} / \mathrm{ml} ; 6.0$ ( 0.5 ) ng/ml; $28.6(3.1) \mathrm{ng} / \mathrm{ml}$ | $\leq 0.01$ |
| Hymøller and Jensen ${ }^{(42)}$ | Denmark | Holstein | Twenty cows | 28 d | Allocated to daily pasture for; $15 \mathrm{~min} ; 30 \mathrm{~min} ; 75 \mathrm{~min} ;$ $150 \mathrm{~min} ; 300 \mathrm{~min}$ | 14 | $\begin{gathered} 44.9(2 \cdot 4) \\ \text { nм/l } \end{gathered}$ | 36.2 (6.4) nм/l; 26.7 (2.8) nм/; 43.9 (8.5) nм/; 67.4 (8.6) nм/l; 95.9 (6.4) nм/l | $\leq 0.001^{*}$ |

25(OH)D, 25-hydroxyvitamin D.
*While no figures were reported it was also determined that dominant hair colour (black or white) had no impact on plasma 25(H)D concentrations.
the third $(77.0 \mathrm{IU} / 1(1.93 \mu \mathrm{~g} / \mathrm{l}), 88.9 \mathrm{IU} / 1(2.22 \mu \mathrm{~g} / \mathrm{l})$ and 47.4 IU/l $(1 \cdot 19 \mu \mathrm{~g} / \mathrm{l}))^{(45)}$.

Further work required to fully elucidate the impact of age and lactation on the vitamin D content of milk, as this has previously been established for other nutrients such as fatty acids ${ }^{(46,47)}$, this is of importance as the cattle milked on a farm will be at various stages of lactation depending on calving dates and parity.

## Conclusion

The present review has identified a number of environmental and genetic factors, which can influence both the vitamin $D$ status of cattle and the vitamin $D$ content of the milk produced. It is worthy to note however that most of the research investigating the factors influencing the composition of cows' milk are, more often than not, concerned only with the macronutrient (namely protein and fat content). Much of the research available with regard to the vitamin D content of cow's milk is in relation to the prevention and treatment of hypocalcaemia and milk fever in dairy herds. Of particular importance to the dairy industry, the present review of the literature indicates that further research is needed to fully elucidate how farmers could manipulate the various factors identified to their advantage with respect to increasing the vitamin D content of milk, and standardising it across the year. Notwithstanding the clear and established health benefits for the animal associated with an improved vitamin D status, this approach potentially could also provide a premium product with an improved vitamin D content for the eventual benefit of the consumer.

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## Conflict of Interest

None.

## Authorship

R. R. W. conducted the literature search and drafted the manuscript. J. J. S., M. J., C. L., A. M. F., S. S. and L. K. P. reviewed and approved the final manuscript.

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