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 ultraviolet (UV)-B 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. This 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. This 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.
**Introduction**

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 humans, 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 ultraviolet (UV)-B emitted from the sun\(^4\). Dietary sources, however, can provide both vitamin D\(_3\) and vitamin 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/day). 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 vs. grazing on pasture, and grass vs. 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\(^7,8,9\).

Cows’ milk provides many nutrients in the human diet (e.g. protein, calcium, riboflavin, vitamin B12, 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\(^12,13,14,15\).

The aims of this review were to provide an overview of (1) the genetic and the environmental factors that influence the vitamin D status of dairy cattle, and; (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 owing to differences in both husbandry and feeding practices between the seasons. Reports dating back as far as the 1920’s 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 2-3-fold increase in the vitamin D content of the milk produced was observed when the cow was out to pasture, compared to 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 vitamin D in the liver and fat tissue 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 – 0.0014µg per gram of fat) across different countries and breeds of cattle (Table 1)\(^{(24, 25, 26, 27)}\). Although seasonal variation in vitamin 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, semi-skimmed and skimmed milk, but was observed in the whole milk samples from the Channel Islands, where mean concentrations for summer and winter were 0.04µg/100g and 0.03µg/100g, 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 0.01µg/100g and trace for whole, semi-skimmed, skimmed and one-percent 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 of vitamin D in milk across the UK and Ireland is warranted.

**UVB Exposure**

In a study by Hymøller, cows from two organic dairy farms in Denmark were selected to determine the effect of sunlight on 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 (November – April)\(^{(25)}\). Results from Farm 1 found no significant effect of treatment allocation on plasma 25(OH)D\(_3\) concentration in March \((P=0.350)\) or April \((P=0.060)\), with mean plasma 25(OH)D\(_3\) concentrations of 7.84ng/ml and
5.85ng/ml for the outdoor and indoor groups, respectively (25). On Farm 2, the outdoor group had a significantly higher 25(OH)D3 concentration in March, compared to the indoor group (5.71ng/ml vs. 3.36ng/ml; *P*<0.05), but the same difference was not reported in April (*P*=0.100) (25). Hymøller and colleagues (25) concluded that the assumption was that supplemental vitamin D3 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 D3 synthesis in 16 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 minutes daily for 24 days, or 60 minutes for 73 days; the length of UVB exposure was designed to be equivalent to 1, 2, 3 and 4 hours of sunlight at pasture at 56°N respectively (9). After 24 days, exposure to supplemental UVB light significantly increased the vitamin D3 and 25(OH)D3 concentrations in the milk in a dose-dependent manner over 30, 90 and 120 minutes (9). In the cattle allocated to receive 60 minutes daily, a significant increase (*P*=0.029) in the vitamin D3 (but not the 25(OH)D3) concentration of the milk produced between day 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 bio-fortification 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 vitamin D2 and D3 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 re-authorisation of a vitamin D2 dossier, and as a result cattle can now only obtain vitamin D2 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 D3 is now the only authorised source of supplemental vitamin D for cattle (27), with maximum permitted levels set at 4,000IU (100µg) per kg of feed (28).
Although cattle are reliant on dietary vitamin D during the winter months, it has been suggested that fat soluble 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 15kg of ruminal contents were removed and mixed with a vitamin D\(_2\) and D\(_3\) (both 250mg) and vitamin E pre-mix\(^{(4)}\). The contents were then returned to the rumen; ruminal and blood samples were then collected over the subsequent 30 hour period\(^{(4)}\). Once collected, ruminal samples were freeze dried (\textit{in vivo} samples), additional ruminal samples were collected at the 1 hour time-point, and stored in plastic bottles which were then placed in a water bath (37°C) (\textit{in vitro} samples). Samples were then removed from the \textit{in vitro} over the 30 hour period and freeze dried for analysis. The concentrations of both vitamin D\(_2\) and D\(_3\) declined over the study period in the \textit{in vivo} samples, with concentrations remaining stable in the \textit{in vitro} samples, suggesting no degradation in the intact ruminal sample\(^{(4)}\). Results showed that the plasma concentrations of both vitamin D\(_2\) and vitamin D\(_3\) increased over the first few hours, from levels below the limit of detection, and reached a maximum concentration after 24 hours (99 ± 15ng/ml and 163 ± 16ng/ml, respectively), with vitamin D\(_3\) concentrations significantly higher than those for vitamin 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(OH)D observed by Hymøller 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 1,000,000 IU (25,000µg) of vitamin D\(_2\) and 1,000,000 IU (25,000µg) of vitamin D\(_3\) mixed in corn oil to 2 dairy cows, collecting milk and blood samples for 10 days after. The results showed that the maximum plasma vitamin D concentrations were observed after 2-3 days, with maximum concentrations in the milk 1-3 days after\(^{(32)}\). At the same time 12 additional cows were allocated to be orally or intravenously administered with vitamin D\(_3\) in doses of 5,000,000 IU (125,000µg) or 10,000,000 IU (250,000µg). Increases in the vitamin D content of the milk produced varied between animal, with maximum levels reached between 3-7 days for the oral doses and up to 10 days for the intravenous doses, with maximum observed ranges between 8 IU (0.2µg) – 92 IU (2.3µg) per 100ml\(^{(32)}\). 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 [4,000 IU (100µg) vs. 40,000 IU (1,000µg) daily], and found concentrations of vitamin D, 25(OH)D and 1,25-
dihydroxyvitamin D (1,25(OH)₂D) in the milk to be greater in those cattle receiving a higher daily dose of vitamin⁸. Similar results were noted for 24,25-dihydroxyvitamin D (24,25(OH)₂D) and 25,26-dihydroxyvitamin D (25,26(OH)₂D)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 14 Danish Holstein cows based on parity and milk yield to receive a one-off 250mg dose of vitamin D₂, followed by the same dose of vitamin D₃ in capsule-form, or vice versa⁶. Plasma samples were obtained and area under the curve was used to determine the impact of the two different doses on plasma 25(OH)D status. Results found that the concentrations of plasma 25(OH)D₂ when D₂ was administered first was less than half that of 25(OH)D₃ when the vitamin D₃ dose was given first (P≤0.001)⁶, suggesting that vitamin D₂ may impair the utilisation of vitamin D₃.

McDermott and colleagues assigned 20 Holstein cows to receive 0 IU, 10,000 IU (250µg), 50,000 IU (1,250µg), or 250,000 IU (6,250µg) of vitamin D₃ daily, for 14-weeks starting at 2 weeks pre-partum⁳⁵. Vitamin D₃ concentrations in the colostrum were significantly higher (P<0.05) in cows receiving 250,000 IU/day compared to the other groups, although this dropped during the transition to normal milk from colostrum, around 1 week post-partum. At the end of the study the vitamin D₃ content of the milk was approximately 0.075ng/ml, 0.16ng/ml, 20ng/l and 22ng/ml for 0, 10,000, 50,000 and 250,000 IU respectively⁳⁵. A mean concentration of 0.15ng/ml for 25(OH)D₃ was observed in normal milk⁳⁵.

The need to supplement cattle over the summer months with vitamin D₃ was investigated in Swedish Holsteins, assigned to receive a mineral feed containing vitamin D₃ concentrations in accordance to Swedish recommendations (control) or the same feed providing approximately 20,000 IU (500µg) of vitamin D₃ daily². Plasma samples collected over the 2-year period showed a significant effect of treatment on the cattle’s circulating 25(OH)D₃ concentrations compared to control (P≤0.001) and moreover, the 25(OH)D₃ concentrations in the both the supplemented and unsupplemented cows increased when the cattle were out at pasture over the summer months². The authors concluded that cattle obtain adequate vitamin D₃ from dermal synthesis over the summer, but that stores were not adequate to maintain status and they had to rely on supplemental vitamin D over the winter².

Overall the results of the above studies provide evidence to suggest that dietary vitamin D₃ is adequate to improve the vitamin D content of the milk produced and to help maintain 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 practises, which has seen a
growing shift to the year round 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 Holstein-Friesian cross has become the most
common breed of dairy cow, used for milk production across the world, owing 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 Channel Islands where Jersey cows are the dominant breed (0.1µg/100g)\(^{(24)}\). 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 1940’s.
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 D\(_3\) regardless of hair coverage, Hymøller and Jensen\(^{(41)}\)
designed a study involving 16 Danish Holstein cattle, which had been depleted of their vitamin D
stores, and randomised based on parity and milk yield to one of four groups. The treatment groups
consisted of different levels of body coverage with a fabric which prevented vitamin D synthesis for
28 days; a horse blanket: an udder cover: a horse blanket and an udder cover: no coverage\(^{(41)}\). The
cattle were on pasture for 5 hours each day and inside for the remainder of the day, and were fed a
vitamin D3 free diet throughout the study\textsuperscript{(41)}. Mean plasma 25(OH)D3 concentrations increased from 2.8 ± 0.2ng/ml at baseline in all treatment groups, in a dose-dependent manner with the increasing level of body coverage\textsuperscript{(41)} (Table 3).

More recently Hymøller & Jensen\textsuperscript{(42)} randomised 20 Danish Holstein heifers based on milk yield and 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 or 300 minutes)\textsuperscript{(42)}. At baseline, the mean plasma 25(OH)D3 concentration for all the heifers was 44.9 ± 2.4nmol/l. Over 28 days, the cattle on pasture for 15, 30 or 75 minutes were unable to maintain their 25(OH)D3 concentrations from that at baseline. A significant increase in mean 25(OH)D3 concentration was observed however in those outside on pasture for 150 or 300 minutes\textsuperscript{(42)}. In addition, they found that the dominant coat colour (black or white) had no significant effect on the plasma concentrations of 25(OH)D3, illustrating that prominent coat colour does not influence the dermal synthesis of vitamin D3 in such cattle\textsuperscript{(42)} (Table 3).

The results of these two unique studies eloquently demonstrate that cattle can synthesis vitamin D3 through all areas of their skin and not just in the udders or muzzle, where hair coverage is scarce. The work by Hymoller and colleagues also illustrates that, unlike humans, pigmentation has no effect on the synthesis of vitamin D3 following UVB exposure\textsuperscript{(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 Hymoller & Jensen in other breeds to determine other factors that may prevent the dermal synthesis of vitamin D, such as longed haired cattle breeds.

Other Factors

Age

A German two series study investigated the impact age has on the metabolism of 25(OH)D3\textsuperscript{(44)}. In the first series, 14 multiparous cows were supplemented orally with 3mg of 25(OH)D3 daily from 270\textsuperscript{th} day of gestation until parturition, with blood samples collected every other day\textsuperscript{(44)}. Ninety cows were allocated in the second series to receive 0, 4, 6 mg of 25(OH)D3 daily through mineral feed additives for the last 8-10 days of gestation, with blood samples also taken every other day until parturition, and at several intervals thereafter\textsuperscript{(44)}. Calculated slopes found the difference in 25(OH)D3 between cattle in their 2\textsuperscript{nd} and 3\textsuperscript{rd} lactation to be significantly higher in the 2\textsuperscript{nd} lactation ($P<0.001$), suggesting that younger cattle are more efficient at absorbing 25(OH)D3 or that in older cattle the rate of 25(OH)D3 elimination is faster, with 1,25(OH)\textsubscript{2}D3 increased in cattle in third lactation or higher\textsuperscript{(44)}. 
**Stage of lactation**

A Japanese study collected milk samples from three Holstein cows at stage points post-partum: 1 day after, colostrum; 2–4 days after, early milk and 15 days after, later milk\(^\text{4(5)}\). Similar concentrations of vitamin D were recorded across the three points for two of the cows [33.2 IU/l (0.83µg/l); 30.9 IU/l (0.77µg/l); 35.6 IU/l (0.89µg/l), respectively and 47.0 IU/l (1.18µg/l); 47.0 IU/l (1.18µg/l); 55.7 IU/l (1.39µg/l), respectively], with no trend noted in the third [77.0 IU/l (1.93µg/l), 88.9 IU/l (2.22µg/l) and 47.4 IU/l (1.19µg/l)]\(^\text{4(5)}\).

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\(^\text{4(6, 4(7)}\), 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 noteworthy, 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 regards 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, this 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

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References


### Table 1: Studies investigating the seasonal variation of vitamin D concentrations in cow’s milk

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Cattle Breed</th>
<th>Collection Date</th>
<th>Milk sample collected</th>
<th>Summer content</th>
<th>Winter content</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bechtel &amp; Hoppert, 1936&lt;sup&gt;(39)&lt;/sup&gt;</td>
<td>United States of America</td>
<td>Guernsey; Holstein</td>
<td>July 1932 - July 1934</td>
<td>Fresh milk</td>
<td>Max - 0.78µg/quart; Max - 0.42µg/quart</td>
<td>Max - 0.12µg/quart; Max - 0.08µg/quart</td>
<td>Not reported</td>
</tr>
<tr>
<td>Thompson et al., 1964&lt;sup&gt;(24,19)&lt;/sup&gt;</td>
<td>England</td>
<td>Aryshire; Jersey; Friesian</td>
<td>Early March 1960</td>
<td>Single 15 gallon sample collected from each herd</td>
<td>0.016µg/g of fat</td>
<td>0.013µg/g of fat</td>
<td>0.011µg/g of fat</td>
</tr>
<tr>
<td>Scott et al., 1984&lt;sup&gt;(20)&lt;/sup&gt;</td>
<td>Great Britain</td>
<td>Non-Channel Island breeds</td>
<td>May 1980 - September 1981</td>
<td>Pasturised milk</td>
<td>0.033µg/100g of milk</td>
<td>0.026µg/100g of milk</td>
<td>Not reported</td>
</tr>
<tr>
<td>Kurmann &amp; Indky, 1994&lt;sup&gt;(21)&lt;/sup&gt;</td>
<td>New Zealand</td>
<td>Friesian and Jersey-cross herds</td>
<td>August 1991 - May 1992</td>
<td>Bulk tank milk</td>
<td>0.006µg/g of fat</td>
<td>0.002µg/g of fat</td>
<td>Not reported</td>
</tr>
<tr>
<td>Lindmark-Månsson et al., 2003&lt;sup&gt;(22)&lt;/sup&gt;</td>
<td>Sweden</td>
<td>Not specified</td>
<td>November 1995 - November 1996</td>
<td>Bulk tank milk</td>
<td>Range 0.01 - 0.12µg/100g*</td>
<td>Mean 0.03µg/100g</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Seasonal means not reported

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### Table 2: Studies investigating the impact of cattle breed on the vitamin D concentrations of milk
<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Cattle Breed</th>
<th>n</th>
<th>Milk sample collected</th>
<th>n</th>
<th>Vitamin D Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bechtel &amp; Hoppert, 1936[39]</td>
<td>United States of America</td>
<td>Guernsey; Holstein</td>
<td>8 cows;</td>
<td>Fresh milk</td>
<td>Monthly; 1932 - 1934</td>
<td>0.12 - 1.09µg/quart; 0.08 - 0.69µg/quart</td>
</tr>
<tr>
<td>Wallis, 1944[38]</td>
<td>United States of America</td>
<td>Jersey; Holstein</td>
<td>3 cows;</td>
<td>Fresh milk</td>
<td>Monthly - for up to 13 months</td>
<td>0.75µg/quart; 0.25µg/quart</td>
</tr>
<tr>
<td>Thompson et al., 1964[19]</td>
<td>England</td>
<td>Arshire; Jersey; Friesian</td>
<td>60 cows;</td>
<td>Fresh milk - churned to butter for analysis</td>
<td>Single 15 gallon sample collected from each herd in early March</td>
<td>0.016µg/g of fat*; 0.013µg/g of fat*; 0.011µg/g of fat*</td>
</tr>
<tr>
<td>Pires et al., 2003[40]</td>
<td>Portugal</td>
<td>Barrosã; Friesian</td>
<td>5 cows;</td>
<td>Fresh milk</td>
<td>1 from each cow</td>
<td>2.60µg/100g; 1.21µg/100g</td>
</tr>
<tr>
<td>Ramhola et al., 2012[36]</td>
<td>Portugal</td>
<td>Minhota; Holestein-Friesian</td>
<td>15 cows;</td>
<td>Fresh milk</td>
<td>Monthly: October 2008 - September 2009</td>
<td>0.11µg/g of fat†; 0.10µg/g of fat†</td>
</tr>
<tr>
<td>McCance &amp; Widdowson, 2014[24]</td>
<td>United Kingdom</td>
<td>Jersey; UK pooled milk</td>
<td>Not specified</td>
<td>Whole milk; whole, semi-skimmed and skimmed</td>
<td>6 (3 summer and winter); not specified</td>
<td>0.01µg/100g; 'trace' for whole, semi-skimmed and skimmed</td>
</tr>
</tbody>
</table>

*Summer vitamin D concentration. Winter concentrations for all three breeds were 0.002µg/g of fat
†Provitamin D₃ also measured - mean concentrations higher in the milk of Holstein-Friesian than Minhota cattle (0.77µg and 0.45µg per gram of fat)
Table 3: Studies investigating the impact of hair coverage and dominant hair colour on the vitamin D synthesis

<table>
<thead>
<tr>
<th>Study Design</th>
<th>Plasma 25(OH)D Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study Country Cattle Breed n Study Duration</td>
<td>Mean at Baseline Mean at End-point P</td>
</tr>
<tr>
<td>Hymøller and Jensen, 2010(41) Denmark Holstein 16 cows 28 days</td>
<td>28 days</td>
</tr>
<tr>
<td>Hymøller and Jensen, 2012(42) Denmark Holstein 20 cows 28 days</td>
<td>28 days</td>
</tr>
</tbody>
</table>

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