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 practises 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)D_3 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 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 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 D_3 and 25(OH)D_3 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 D_3 (but not the 25(OH)D_3) 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 D_2 and 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 re-authorisation of a vitamin D_2 dossier, and as a result cattle can now only obtain vitamin 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 D_3 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 (*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) (*in vitro* samples). Samples were then removed from the 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 *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 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$(OH)_2$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 (24,25$(OH)_2$D) and 25,26-dihydroxyvitamin D (25,26$(OH)_2$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 14 Danish Holstein cows based on parity and milk yield to receive a one-off 250mg dose of vitamin 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 plasma 25(OH)D status. Results found that the concentrations of plasma 25(OH)D_2 when D_2 was administered first was less than half that of 25(OH)D_3 when the vitamin 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 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 D_3 daily, for 14-weeks starting at 2 weeks pre-partum[35]. Vitamin D_3 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 D_3 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[35]. A mean concentration of 0.15ng/ml for 25(OH)D_3 was observed in normal milk[35].

The need to supplement cattle over the summer months with vitamin D_3 was investigated in Swedish Holsteins, assigned to receive a mineral feed containing vitamin D_3 concentrations in accordance to Swedish recommendations (control) or the same feed providing approximately 20,000 IU (500μg) of vitamin D_3 daily[2]. Plasma samples collected over the 2-year period showed a significant effect of treatment on the cattle's circulating 25(OH)D_3 concentrations compared to control ($P \leq 0.001$) and moreover, the 25(OH)D_3 concentrations in the 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 D_3 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 winter[2].

Overall the results of the above studies provide evidence to suggest that dietary vitamin D_3 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 Channels 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 D_3 free diet throughout the study[41]. Mean plasma 25(OH)D_3 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[41] (Table 3).

More recently Hymøller & Jensen[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)[42]. At baseline, the mean plasma 25(OH)D_3 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)D_3 concentrations from that at baseline. A significant increase in mean 25(OH)D_3 concentration was observed however in those outside on pasture for 150 or 300 minutes[42]. In addition, they found that the dominant coat colour (black or white) had no significant effect on the plasma concentrations of 25(OH)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 and colleagues also illustrates that, unlike humans, 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 & 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)D_3[44]. In the first series, 14 multiparous cows were supplemented orally with 3mg of 25(OH)D_3 daily from 270th day 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 mg of 25(OH)D_3 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[44]. Calculated slopes found the difference in 25(OH)D_3 between cattle in their 2nd and 3rd lactation to be significantly higher in the 2nd lactation ($P<0.001$), suggesting that younger cattle are more efficient at absorbing 25(OH)D_3 or that in older cattle the rate of 25(OH)D_3 elimination is faster, with 1,25(OH)$_2D_3$ increased in cattle in third lactation or higher[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)[45]. 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)][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 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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Table 1: Studies investigating the seasonal variation of vitamin D concentrations in cow's milk

Study Design							Vitamin D Concentrations		
Study	Country	Cattle Breed	*n*	Collection Date	Milk sample collected	*n*	Summer content	Winter content	*P*
Bechtel & Hoppert, 1936[39]	United States of America	Guernsey; Holstein	8 cows; 14 cows	July 1932 - July 1934	Fresh milk	Monthly	Max - 0.78µg/quart; Max - 0.42µg/quart	Max - 0.12µg/quart; Max - 0.08µg/quart	Not reported
Thompson *et al*., 1964[2419]	England	Aryshire; Jersey; Friesian	60 cows; 60 cows; 50 cows	Early March 1960	Fresh milk - churned to butter for analysis	Single 15 gallon sample collected from each herd	0.016µg/g of fat 0.013µg/g of fat 0.011µg/g of fat	0.002µg/g of fat 0.002µg/g of fat 0.002µg/g of fat	Not reported
Scott *et al.*, 1984[20]	Great Britain	Non-Channel Islannd breeds	12 dairies	May 1980 - September 1981	Pasturised milk	Every 7 weeks	0.033µg/100g of milk	0.026µg/100g of milk	Not reported
Kurmann & Indky, 1994[21]	New Zealand	Predominantly Friesian and Jersey-cross herds	1 processing site	August 1991 - May 1992	Bulk tank milk	Monthly	0.006µg/g of fat	0.002µg/g of fat	Not reported
Lindmark-Månsson *et al*., 2003[22]	Sweden	Not specified	9 dairies	November 1995 - Novemeber 1996	Bulk tank milk	Every 2 months	Range 0.01 - 0.12µg/100g* Mean 0.03µg/100g		<0.001

*Seasonal means not reported

Table 2: Studies investigating the impact of cattle breed on the vitamin D concentrations of milk

Study Design						
Study	Country	Cattle Breed	*n*	Milk sample collected	*n*	Vitamin D Concentrations
Bechtel & Hoppert, 1936[39]	United States of America	Guernesy; Holstein	8 cows; 14 cows	Fresh milk	Monthly; 1932 - 1934	0.12 - 1.09µg/quart; 0.08 - 0.69µg/quart
Wallis, 1944[38]	United States of America	Jersey; Holstein	3 cows; 3 cows	Fresh milk - extracted butterfat	Monthly - for up to 13 months	0.75µg/quart; 0.25µg/quart
Thompson *et al.*, 1964[19]	England	Aryshire; Jersey; Friesian	60 cows; 60 cows; 50 cows	Fresh milk - churned to butter for analysis	Single 15 gallon sample collected from each herd in early March	0.016µg/g of fat*; 0.013µg/g of fat*; 0.011µg/g of fat*
Pires *et al.*, 2003[40]	Portugal	Barrosã; Friesian	5 cows; 5 cows	Fresh milk	1 from each cow	2.60µg/100g; 1.21µg/100g
Ramhola *et al.*, 2012[36]	Portugal	Minhota; Holestein-Friesian	15 cows; 15 cows	Fresh milk	Monthly: October 2008 - September 2009	0.11µg/g of fat†; 0.10µg/g of fat†
McCance & Widdowson, 2014[24]	United Kingdom	Jersey; UK pooled milk (breed not specified)	Not specified	Whole milk; whole, semi-skimmed and skimmed	6 (3 summer and winter); not specified	0.01µg/100g; 'trace' for whole, semi-skimmed and skimmed

*Summer vitamin D concentration. Winter concentrations for all three breeds were 0.002µg/g of fat

†Provitamin D_3 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

				Study Design			Plasma 25(OH)D Concentrations		
Study	Country	Cattle Breed	*n*	Study Duration	Treatment Groups	*n*	Mean at Baseline	Mean at End-point	*P*
Hymøller and Jensen, 2010[41]	Denmark	Holstein	16 cows	28 days	Allocated to coverage; Horse blanket; Udder cover; Horse blanket and udder cover; Natural	14	2.5 ±0.2ng/ml	8.9±1.8ng/ml; 23.2±1.5ng/ml; 6.0±0.5ng/ml; 28.6±3.1ng/ml	≤0.01
Hymøller and Jensen, 2012[42]	Denmark	Holstein	20 cows	28 days	Allocated to daily pasture for; 15mins; 30 mins; 75 mins; 150 mins; 300 mins	14	44.9±2.4nmol/l	36.2±6.4nmol/l 26.7±2.8nmol/l; 43.9±8.5nmol/l; 67.4±8.6nmol/l; 95.9±6.4nmol/l	≤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