



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

The effect of season, management and endocrinopathies on vitamin D status in horses

Citation for published version:

Dosi, M, McGorum, B, Kirton, RD, Cillan-Garcia, E, Mellanby, R, Keen, J, Hurst, E & Morgan, R 2022, 'The effect of season, management and endocrinopathies on vitamin D status in horses', *Equine Veterinary Journal*, pp. 1-9. <https://doi.org/10.1111/evj.13873>

Digital Object Identifier (DOI):

[10.1111/evj.13873](https://doi.org/10.1111/evj.13873)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

Equine Veterinary Journal

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



ORIGINAL ARTICLE

The effect of season, management and endocrinopathies on vitamin D status in horses

Miranda C. M. C. Dosi¹  | Bruce C. McGorum¹  | Roxane D. Kirton² |
Eugenio Cillán-García¹ | Richard J. Mellanby^{1,3} | John A. Keen¹  |
Emma A. Hurst^{3,4} | Ruth A. Morgan^{1,5,6} 

¹Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, UK

²Redwings Horse Sanctuary, Norwich, UK

³The Roslin Institute, University of Edinburgh Roslin, Midlothian, UK

⁴Mass Spectrometry Core, Edinburgh Clinical Research Facility, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, UK

⁵Queen's Medical Research Institute, The University of Edinburgh, Edinburgh, UK

⁶Scotland's Rural College, Edinburgh, UK

Correspondence

Miranda C. M. C. Dosi, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Roslin, Midlothian, UK.

Email: m.c.m.dosi@sms.ed.ac.uk

Funding information

Fiona and Ian Russell Seed Corn Grant

Abstract

Background: Vitamin D deficiency is common in humans and is increasingly linked to the pathogenesis of a multitude of diseases including obesity and metabolic syndrome. The biology of vitamin D in horses is poorly described; the relative contribution of the diet and skin synthesis to circulating concentrations is unclear and associations with the endocrine disease have not been explored.

Objectives: To determine the relationship between management, season and endocrine disease and vitamin D status in horses.

Study design: Cross-sectional cohort study.

Methods: Plasma concentrations of 25-hydroxyvitamin D₂ (25(OH)D₂) and D₃ (25(OH)D₃) were measured by liquid chromatography-tandem mass spectrometry in 34 healthy unsupplemented grazing ponies and 22 stabled Thoroughbreds receiving supplementary vitamin D₃ in feed. A nested group of 18 grazing ponies were sampled on long and short days (>12 and <12 h of light/day) to determine the effect of sunlight exposure. In addition, the relationships between age, sex, adiposity, serum insulin, adrenocorticotrophic hormone and vitamin D status were assessed in a mixed group of 107 horses using a linear regression model.

Results: All animals had a measurable level of 25(OH)D₂ (median 10.7 nmol/L) whilst 25(OH)D₃ was only detected in Thoroughbreds receiving D₃ supplementation. Thoroughbreds had lower concentrations of 25(OH)D₂ than ponies (7.4 vs. 12.6 nmol/L, $p < 0.01$). In grazing ponies, 25(OH)D₂ concentrations were significantly higher on long days compared to short days (14.4 vs. 8.7 nmol/L, $p < 0.01$), whilst 25(OH)D₃ was undetectable. Measures of increased adiposity, but not basal insulin, were

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2022 The Authors. *Equine Veterinary Journal* published by John Wiley & Sons Ltd on behalf of EVJ Ltd.

associated with higher 25(OH)D₂ concentrations, conversely to humans. Increasing ACTH was associated with lower 25(OH)D₂ ($p < 0.01$).

Main limitations: Vitamin D₂ concentrations were not measured in grass or forage.

Conclusions: In horses 25(OH)D₂ is the predominant vitamin D metabolite, and there is an apparent lack of endogenous vitamin D₃ production. The relationship between vitamin D and endocrine disorders in horses does not reflect that of other species and warrants further investigation.

KEYWORDS

adipose tissue, horse, insulin, obesity, season, vitamin D

1 | INTRODUCTION

Vitamin D is implicated in many pathophysiological processes beyond its well-recognised role in skeletal health, although very little is known about the importance of this hormone in equine health and disease.¹ There are two main forms of vitamin D; vitamin D₂ and vitamin D₃. Previtamin D₃ is endogenously synthesised in the skin of most mammals in response to UVB exposure, whilst previtamin D₂ is produced by fungi growing on plants and is activated following ingestion (Figure 1).² In humans and other domestic herbivores, vitamin D₃,

synthesised in the skin, makes up most of the total vitamin D measured in plasma.³ Horses, like other herbivores, source vitamin D₂ from their forage-based diet but it is unclear whether they can synthesise vitamin D₃.¹ Our understanding of vitamin D biology in horses and ponies is very limited with few equine-specific peer-reviewed publications in the last 30 years.^{1,4-14}

Vitamin D status is most commonly assessed by measuring plasma concentrations of the most abundant circulating metabolites; 25-hydroxyvitamin D₂ (25(OH)D₂) and 25-hydroxyvitamin D₃ (25(OH)D₃). These constitute the largest circulating pool of vitamin D, which then

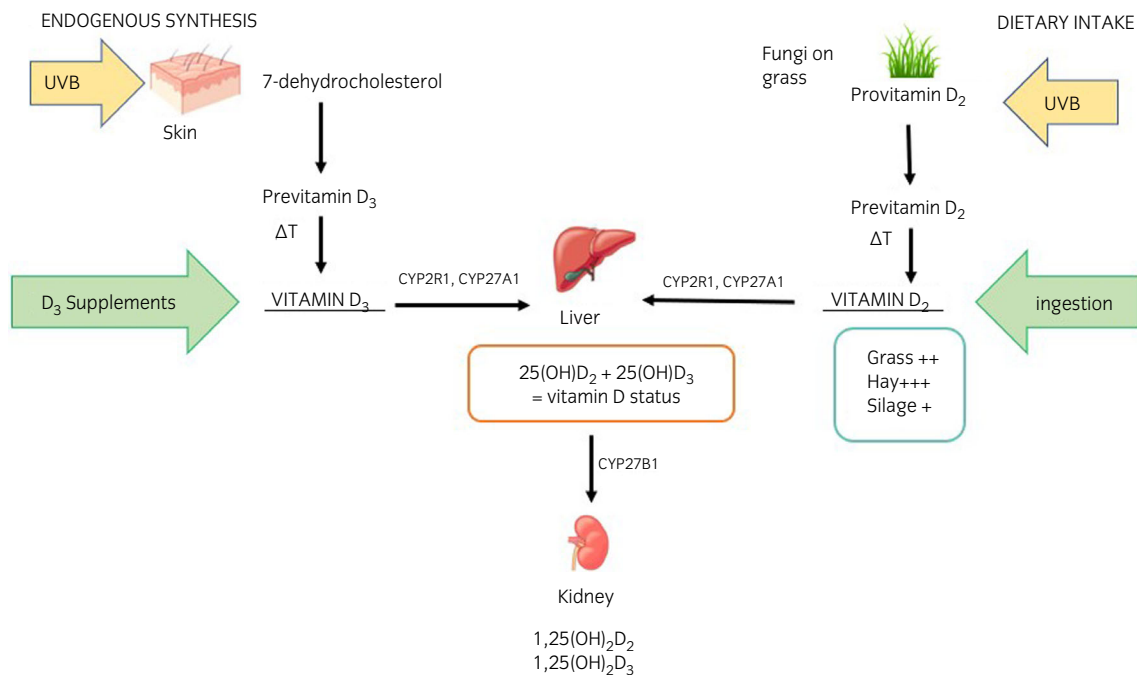


FIGURE 1 Vitamin D₂ and D₃ metabolism (modified with the authors' permission from Hurst et al.³). The precursor 7-dehydrocholesterol is converted to previtamin D₃ following exposure to UVB in the skin of most domestic herbivores. Previtamin D₃ undergoes thermoisomerisation to vitamin D₃. This is the form that is most commonly added to horses' concentrates and supplements. Provitamin D₂ (ergosterol) is produced by fungi colonising pasture herbage. This is converted to previtamin D₂ in response to UVB irradiation. Previtamin D₂ undergoes thermoisomerisation to vitamin D₂. This is the form that is normally consumed by herbivores in grass, forage and silage. Both vitamin D₂ and D₃ undergo hydroxylation first in the liver and then in the kidneys, resulting in the biologically active molecules 1,25-dihydroxyvitamin D₂ and 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₂ and 1,25(OH)₂D₃). The hydroxylation reactions are catalysed by the enzymes CYP2R1, CYP27A1 and CYP27B1. Vitamin D status is conventionally considered to be the sum of the plasma/serum concentrations of 25(OH)D₂ and 25(OH)D₃

undergo further hydroxylations in the kidneys and other peripheral tissues to the metabolically active metabolites 1,25(OH)₂D₂ and 1,25(OH)₂D₃. Concentrations of these metabolites are consistently reported as being lower in horses than in other domestic mammals³ and humans; indeed the concentrations observed in healthy horses would be considered indicative of a severe deficiency in humans (total 25(OH)D below 25 nmol/L).¹⁵ Moreover, recent studies using highly sensitive assays, such as liquid chromatography-tandem mass spectrometry (LC-MS/MS), showed that grazing Thoroughbreds and Standardbreds in New Zealand that were not receiving dietary vitamin D supplementation had very low to undetectable plasma concentrations of 25(OH)D₃ throughout the year.^{10,11}

According to those studies, vitamin D₂-related metabolites were predominant in defining vitamin D status.

Seasonal changes in 25(OH)D in response to sun exposure are well documented in humans and sheep living at UK latitudes.^{16,17} In humans this is generally attributed to the different amount of radiation that reaches the skin and subsequently different rates of endogenous production of 25(OH)D₃. On the other hand, in herbivores, seasonal changes in plasma 25(OH)D₂ are due to variations in the D₂ content of grass, which is also influenced by sunlight exposure.¹⁰ There are no published data on physiological concentrations of 25(OH)D₂ or 25(OH)D₃ in UK horses or ponies measured with modern highly sensitive techniques.

Vitamin D insufficiency/deficiency is common in humans and oral supplementation is often recommended in conditions such as obesity, metabolic syndrome and type 2 diabetes, as it may improve insulin sensitivity of liver, adipose tissue and skeletal muscle.^{2,18} In contrast, Hookey et al.¹⁹ found no association between adiposity and vitamin D status in healthy adult dogs. To date, there are no data available in horses on the relationship between vitamin D status and endocrine conditions such as insulin dysregulation (ID), obesity or pars pituitary intermedia dysfunction (PPID).

The objectives of this study were first to determine circulating concentrations of 25(OH)D₂ and 25(OH)D₃ using ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) in two equine populations representative of the extremes of dietary management in the United Kingdom; a herd of grazing ponies not receiving any supplementary feed and a group of Thoroughbred racehorses receiving supplementary vitamin D₃. Second, we aimed to determine the effect of day length (UVB light exposure) on vitamin D status in nonrugged ponies kept at pasture all year. Finally, we sought to test the hypothesis that, as in humans, vitamin D status is associated with obesity, ID and PPID.

2 | MATERIALS AND METHODS

2.1 | The effect of dietary management and day length on vitamin D status

Blood samples were obtained for clinical reasons from 22 Thoroughbred racehorses (Group 1) stabled in Scotland (56° parallel North), and

34 Welsh and Welsh cross-ponies (Group 2), kept on a ryegrass pasture as a herd in England (52° parallel North).

Horses in Group 1 were predominantly stabled, going outside only for training and for 40–60 min daily turnout wearing a rug. They were fed an average of 8–10 kg of haylage and 6–8 kg of HDF Spillers Power Cubes containing 1100 IU/Kg (of dry matter) of vitamin D₃ daily. Ponies in Group 2 were kept on ryegrass pasture all year round, were not rugged and were not ridden. During winter they were supplemented with hay or a mixture of hay and barley straw. Groups 1 and 2 were sampled once between March and April.

A nested sample of Group 2 ponies (*n* = 18) was also sampled between April and September (long days, >12 h of light per day) and between October and March (short days, <12 h of light per day). Cloud coverage and UV index data for the study period (March 2018 to March 2019) were obtained from the UK Department for Environment, Food and Rural Affairs (<https://uk-air.defra.gov.uk/data/uv-data>).

2.2 | The relationship between vitamin D status and obesity, ID and PPID

This part of the study used 107 animals (Group 3), including 10 of the racehorses in Group 1, all 34 ponies in Group 2 and an additional 63 horses presented to the Royal (Dick) School of Veterinary Studies with and without obesity, PPID and ID. Vitamin D status was assessed as described above. Validated assays (Siemens Immulite 2000 Xpi analyser) were used to measure serum insulin and plasma ACTH concentration in all 107 horses.^{20,21}

Morphometric parameters were recorded by a veterinary surgeon at the time of sampling. These included Body Condition Score (BCS; 1–5), Neck Crest Score (NCS; 1–5), bodyweight (electronic scale) and the presence or absence of regional adiposity elsewhere in the body (i.e., localised adipose accumulation on the rump, lumbar region, shoulder, loin).^{22,23}

Diagnosis of PPID was made if basal ACTH concentration exceeded seasonal cut-off values of 29 pg/ml between November and July and 47 pg/ml between August and October and based on clinical signs (including hirsutism, epaxial muscle wastage, lethargy).²⁴ In 19 horses, diagnosis of PPID was confirmed using a thyrotropin-releasing hormone stimulation test. Diagnosis of ID was made if basal serum insulin concentration exceeded 20 mIU/L. The horses were stabled the night before the sample was obtained and a haynet of soaked hay was offered. They did not receive any highly caloric meal the morning of the testing. In 25 horses, ID was confirmed using a combined glucose-insulin test.²⁵ The presence of laminitis, based on history, clinical examination, postmortem observation of hoof sections and/or radiological assessment, was recorded.

2.3 | Measurement of 25(OH)D₂ and 25(OH)D₃

Plasma concentrations of 25(OH)D₂ and 25(OH)D₃ (concentrations summed to provide total 25(OH)D) were measured by LC-MS/MS by

the Vitamin D Animal Laboratory (VitDAL) using an assay that has been certified as proficient by the international Vitamin D Quality Assessment Scheme (DEQAS) and described in detail previously.²⁶ Briefly, calibrators were prepared by spiking 3% bovine serum albumin with certified reference standards of 25(OH)D₂ and 25(OH)D₃ (Sigma-Aldrich). Samples (200 µl serum) and 200 µl calibrators were spiked with equal amounts of isotopically labelled internal standards (deuterium-labelled 25(OH)D₂ (6,19,19-d₃-25(OH)D₂) and carbon-13-labelled 25(OH)D₃ (23,24,25,26,27-¹³C₅-25(OH)D₃) (Sigma-Aldrich) and prepared by automated supported liquid extraction using 96-well plates (Biotage) on the Biotage Extrahera. Samples and calibrators were then subject to derivatisation with DMEQ-TAD (Abcam). LC-MS/MS analysis was conducted on an Acquity UPLC I-Class system (Waters Corporation) coupled with a Sciex QTrap 6500+ quadrupole mass spectrometer (AB Sciex). Liquid chromatography separation was done using a Raptor FluoroPhenyl column (2.7 µm 100 Å, LC Column 100 × 2.1 mm) (Thames Restek). The mobile phase was 2 mM ammonium formate in water with 0.1% formic acid (A) and 2 mM ammonium formate in methanol with 0.1% formic acid (B). The flow rate was 0.4 ml/min and the total run time was 10 min per sample. For mass spectrometer analysis, ionisation was performed by electrospray ionisation in positive ion mode. Multiple reaction monitoring was used to monitor and quantify derivatised standards and endogenous vitamin D analytes with the following *m/z* transitions: DMEQ-25(OH)D₂ *m/z* 758.4 > 740.2; DMEQ-d₃-25(OH)D₂ *m/z* 761.3 > 743.4; DMEQ-25(OH)D₃ *m/z* 747.3 > 729.3; and DMEQ-¹³C₅-25(OH)D₃ *m/z* 751.5 > 733.5. The results are presented as total 25(OH)D concentration which is the sum of the concentrations of 25(OH)D₂ and 25(OH)D₃. The lower limit of quantitation (LLOQ) was 0.5 nmol/L for 25(OH)D₂ and 4 nmol/L for 25(OH)D₃.

2.4 | Data analysis

Retrospective power calculation indicated that sampling of 34 grazing ponies and 22 Thoroughbreds allowed adequate power (80%) for detecting an intergroup difference as small as 2.5 nmol/L of 25(OH)D. Standard deviations of 2.2 and 3.4 nmol/L were used for Thoroughbreds and grazing ponies, respectively.

Statistical analysis was performed using SPSS 25 (IBM). When data were not normally distributed (Shapiro-Wilk test), logarithmic (ln) transformation was performed. Student's *t*-test for independent and paired samples was used to compare means between groups for normally distributed, parametric data and a Mann-Whitney *U* test for nonparametric data. Results are expressed as mean and standard deviation if normally distributed and as the median and interquartile range (IQR) if nonparametric. One-way analysis of variance was used for normally distributed variables when multiple means were compared.

To investigate the relationship between obesity, ID and PPID and vitamin D, a linear regression analysis was conducted with ln25(OH)D₂ as the outcome, as only 25(OH)D₂ was detectable in all the horses. 25(OH)D₃ was detected only in 10 horses, all of which were receiving oral supplementation, therefore these data were excluded from the linear

regression analysis. The following explanatory variables were tested in a univariate model: age, sex, breed type, BCS, NCS, regional adiposity, morphometric score, diagnosis of laminitis, basal ACTH, basal insulin and diagnosis of PPID or ID. All variables that significantly contributed to a change in outcome with a *p* < 0.25 were put forward to the multivariate analysis. BCS, NCS and the presence or absence of regional adiposity were strongly positively correlated to each other (NCS and BCS *r* = 0.563, NCS and regional adiposity *r* = 0.533, BCS and regional adiposity *r* = 0.676, *p* < 0.001 for all correlation coefficients); therefore, they had to be entered in the analysis individually to avoid multicollinearity. Thus, in the multivariate analysis, a composite score calculated through dimension reduction (factor analysis) has been used. The goodness of fit of the regression models was assessed using Hosmer-Lemeshow test.

3 | RESULTS

3.1 | The effect of dietary management on vitamin D status

Group 1 comprised 22 Thoroughbreds, including 20 geldings and two mares. Group 2 comprised 34 Welsh cobs and crosses, including 24 mares and 10 geldings. Horses in Group 1 were significantly younger than ponies in Group 2 (median 7 years, IQR 3 vs. 16 years, IQR 10, *p* < 0.001).

Total plasma 25(OH)D (i.e., 25(OH)D₂ + 25(OH)D₃) was higher in Group 1 versus Group 2 (Group 1: median 15.8 nmol/L, IQR 6.8 against Group 2 12.6 nmol/L, IQR 5.1, *p* < 0.01; Figure 2A). 25(OH)D₂ was significantly higher in Group 2 (median 12.6 nmol/L, IQR 5.1 against 7.4 nmol/L, IQR 4.5, *p* < 0.001; Figure 2B), whereas 25(OH)D₃ was only detectable above the LLOQ in the vitamin D₃ supplemented Group 1 horses (median 8.5 nmol/L, IQR 4.0; Figure 2C).

3.2 | The effect of season on vitamin D status of grazing ponies

During each month of the long-day season there was significantly more sunny weather than in the short-day season (mean 224.6 ± 55.5 h/month of the sun against 113.4 ± 51.7 h/month). The mean UV index was also significantly higher in long-day months compared to short-day months (<12 h/>12 h of light/day). The concentration of 25(OH)D₂ in grazing ponies was significantly higher on long days compared to short days (median 14.4 nmol/L IQR 4.60 vs. median 8.7 nmol/L IQR 4.5, respectively, *p* < 0.001; Figure 3), whilst 25(OH)D₃ remained undetectable throughout the year.

3.3 | The relationship between vitamin D and obesity, ID and PPID

Data from the 107 horses in Group 3 were used. The age range was 4–28 years (median 15.5, IQR 9); 54 were mares and 53 were

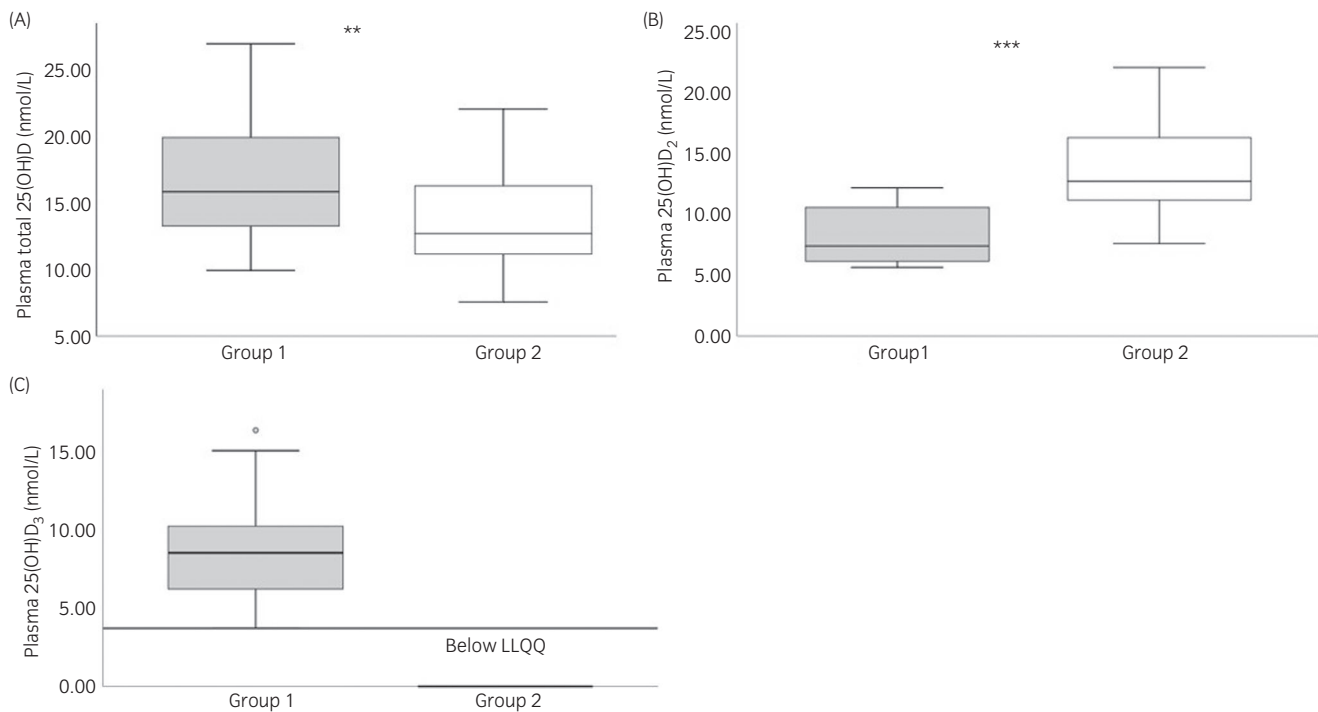


FIGURE 2 Boxplots showing plasma (A) total 25(OH)D, (B) 25(OH)D₂ and (C) 25(OH)D₃ (nmol/L) concentrations for thoroughbreds (Group 1; $n = 22$) and grazing ponies (Group 2; $n = 34$)

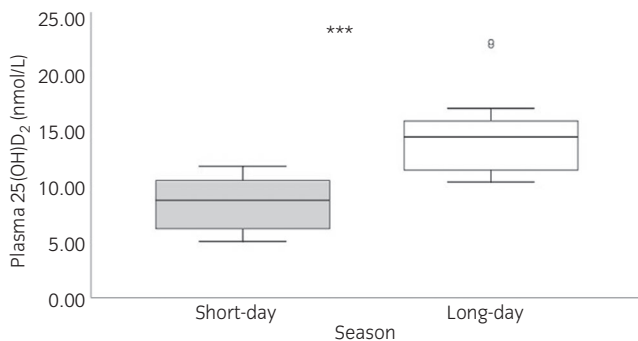


FIGURE 3 Boxplots comparing plasma 25(OH)D₂ concentrations measured in short-day and long-day seasons in a subset ($n = 18$) of ponies kept at pasture. ***Intergroup difference significant at $p < 0.001$

geldings. Breeds were classified into five categories: Welsh cobs and crosses ($n = 58$), Thoroughbreds and Thoroughbred crosses ($n = 21$), Draught breeds (including Percheron, Irish draught, Clydesdale and Friesian; $n = 6$), Native Ponies (including Exmoor, New Forest, Shetland, Connemara, Icelandic, Highland, Dartmoor and British miniature; $n = 13$) and a mixture of other breeds (other: including Quarter Horse, Irish Sport Horse, Trakhener, Arab, Dutch Warmblood, American Paint; $n = 9$).

Of the 107 horses, 32 were diagnosed with PPID, 41 with ID and 14 with both PPID and ID. Of the 107 horses, 40 had a previous diagnosis of laminitis.

Basal insulin measurements were available for all 107 horses, whilst basal ACTH was available for 94 of the 107 horses.

Plasma 25(OH)D₂ was detectable in all 107 horses (median 10.7 nmol/L, IQR 5.33), whilst 25(OH)D₃ was quantifiable in only 10 of the 107 horses, all of which were Thoroughbreds from Group 1 that were receiving dietary supplementation. For this reason, 25(OH)D₂ was therefore the only outcome considered.

In the univariate linear regression (Table 1) BCS ($r = 0.44$, $p < 0.001$), NCS ($r = 0.29$, $p < 0.01$), presence of regional adiposity ($r = 0.35$, $p < 0.001$) and morphometric score ($r = 0.41$, $p < 0.001$) were positively correlated with 25(OH)D₂. Obese horses (BCS > 3.5) had higher 25(OH)D₂ concentrations than nonobese horses (median 9.3 nmol/L IQR 4.48 against 12.5 nmol/L IQR 4.9, $p < 0.001$). There were significant differences in BCS between mares and geldings (mares higher score than geldings $p < 0.01$) and between horses with and without ID (ID higher score than non-ID $p < 0.01$). As such, the effect of these variables on vitamin D status was confounded by BCS and we cannot draw firm conclusions about their relationship as different sex and ID groups were not matched for BCS.

The breed was put forward to the final model as it reached the predefined level of significance, although when ln25(OH)D₂ concentrations amongst breeds were compared, there was a statistically significant difference between Thoroughbred and Welsh cobs only ($F(4,102) = 5.632$, $p < 0.001$). This difference once again was considered to be affected by the significant intergroup difference in BCS ($p < 0.001$). Vitamin D status did not differ in horses with or without laminitis.

TABLE 1 Coefficients for BCS, regional adiposity, NCS, morphometric score, diagnosis of PPID, ln basal ACTH, diagnosis of ID, ln basal insulin, age, sex, breed and diagnosis of laminitis, calculated in the linear univariate regression having as outcome ln25(OH)D₂

Univariate analysis				
Variable	Regression coefficient (β)	Standard error	Pearson's correlation coefficient (<i>r</i>)	<i>p</i> Value
BCS	0.168	0.049	0.444	0.5×10^{-6a}
Regional adiposity Reference category: No	0.300	0.075	0.352	0.1×10^{-3a}
NCS	0.098	0.030	0.299	0.001 ^a
Morphometric Score	0.172	0.037	0.408	0.7×10^{-5a}
Diagnosis of PPID Reference category: No	0.288×10^{-3}	0.088	0.295×10^{-3}	0.9
ln basal ACTH	-0.142	0.049	0.286	0.01 ^a
Diagnosis of ID Reference category: No	0.204	0.081	0.228	0.01 ^a
ln Basal insulin	0.025	0.028	0.088	0.4
Age	0.0004	0.006	0.061	0.5
Sex Reference category: Mare	0.205	0.076	0.241	0.01 ^a
Breed Reference category: Welsh Cobs	-0.051	0.029	0.161	0.08 ^a
Diagnosis of laminitis Reference category: No	0.038	0.083	0.043	0.7

^aVariables put forward to the multivariate logistic regression as $p < 0.25$.

TABLE 2 Coefficients calculated for the variables retained in the multivariate linear regression having as outcome ln25(OH)D₂

Multivariate analysis—Final model				
Variable	Regression coefficient	Standard error	Pearson's correlation coefficient	<i>p</i> Value
ln Basal ACTH	-0.100	0.048	0.454	<0.001 ^{***}
BCS	0.148	0.039		
Intercept	2.204	0.238		

^{***} $p < 0.001$.

Of the predictors put forward to the final model (Table 2), only basal ACTH and BCS were retained, respectively, negatively and positively associated with 25(OH)D₂ concentration.

4 | DISCUSSION

Vitamin D is important for calcium homeostasis but it also has a diverse range of nonskeletal actions which are poorly understood. Vitamin D deficiency is reported in approximately 50% of humans,²⁷ has been associated with several diseases and is an independent risk factor for mortality.^{2,28} Our aim in this study was to determine normal plasma concentrations of 25(OH)D₂ and 25(OH)D₃ in horses representing the extremes of dietary management in the United Kingdom, to determine the effect of day length on vitamin D status and to investigate the relationship between vitamin D and endocrine diseases.

This study demonstrates that management factors are important determinants of a horse's vitamin D status. Consistent with previous

findings,^{10,11} horses managed extensively all year round had undetectable 25(OH)D₃ and had higher 25(OH)D₂ concentrations in summer than in winter. In contrast, stabled horses supplemented with vitamin D₃ had detectable 25(OH)D₃, but concentrations of 25(OH)D₂ were lower than those of grazing ponies.

Previous work has often been hampered by a lack of a suitable assay to accurately measure vitamin D in horses. In this study, 25(OH)D₂ and 25(OH)D₃ were detected at concentrations as low as 0.5 and 4 nmol/L, respectively, using a DEQAS accredited LC-MS/MS which is considered the gold-standard method for vitamin D analysis.²⁹ Our findings confirmed that horses' total 25(OH)D concentrations are exceptionally low compared with those of other species and were similar to those reported by other equine studies which used UHPLC-MS/MS.^{11,14}

The median total vitamin 25(OH)D concentration of all horses in this study (10.7 nmol/L) was considerably below the threshold indicative of a deficiency in humans (insufficiency is <70–75 nmol/L and deficiency is <25–30 nmol/L total 25(OH)D). In man, these concentrations would be associated with a high risk for the

development of nutritional osteomalacia.¹⁵ Similarly, total 25(OH)D concentrations below 25 nmol/L are a risk factor for developing clinical calcium/phosphorous derangement in dairy cows, whilst values below 10 nmol/L are associated with rickets in growing sheep, llamas and alpacas.³⁰ Nutritional osteomalacia has not been reported in horses, whilst developmental rickets has been either anecdotally reported or induced only in experimental settings.^{31,32} None of the horses in our study showed any clinical evidence of osteomalacia. The reason for the interspecies difference in vitamin D biology is not known, but it has been suggested that calcium metabolism in horses is influenced to a lesser extent by vitamin D status than in other species.⁷ In horses, even when hypervitaminosis D is experimentally induced, the plasma calcium concentration increases only moderately.⁸ Azarpeykan et al.¹² suggest that even low concentrations of 25(OH)D metabolites are capable of regulating vitamin D-related gene expression in the equine kidney.

Domestic animals have developed different evolutionary adaptations to maintain physiological levels of vitamin D. Cats and dogs, being carnivores, obtain vitamin D from their diet and therefore do not need to synthesise endogenous vitamin D₃ in their skin.^{26,33} On the other hand both dietary vitamin D₂ and endogenous vitamin D₃ production contribute to vitamin D status in other domestic grazing animals including sheep, goats, camelids, cows and rabbits.^{17,34-36} The undetectable plasma concentrations of 25(OH)D₃ in nonsupplemented horses suggest that endogenous cutaneous production of D₃ in the horse is either absent or minimal compared to other species and that vitamin D status is predominantly influenced by ingestion of vitamin D₂ in herbage or supplemental dietary vitamin D₂ or D₃.

The grazing ponies in Group 2 were not ridden whilst those in Group 1 were racehorses in training that were exercised either the same day or the day before sampling. Exercise could have potentially contributed to the lower 25(OH)D₂ concentrations measured in Group 1, as a reduction in plasma 25(OH)D₂ is observed after a single bout of high-intensity exercise.¹⁴ Further investigations into the effect of exercise on vitamin D metabolism in horses is warranted to provide more specific guidance regarding vitamin D supplementation for equine athletes.

Grazing ponies had increased serum 25(OH)D₂ concentrations during long day length months. In contrast, there was no detectable 25(OH)D₃ even on long days when 25(OH)D₂ concentrations were the highest. The undetectable plasma concentrations of 25(OH)D₃ in nonsupplemented horses suggest that the vitamin D system is predominantly based on D₂ rather than D₃. The amount of UVB radiation that reaches the ground influences both the fungal activity and the rate of conversion from provitamin D₂ (ergosterol) to vitamin D₂ making season and latitude important factors in determining pasture D₂ content.³⁷ A significant seasonal variation of ergosterol in perennial ryegrass hay has been reported.³⁸ The seasonal variation in plasma 25(OH)D₂ observed in grazing ponies likely reflected the seasonal variation of ergosterol content of the pasture herbage.^{10,38} Further investigations into the vitamin D₂ content of the pasture grazed by these ponies are required to confirm this hypothesis. Importantly, despite ponies having a 42% reduction in 25(OH)D₂ and 13 of the 19 of them having 25(OH)D₂ concentrations below 10 nmol/L during the winter months, none had clinical evidence of disease associated with hypovitaminosis D.

In obese humans, vitamin D deficiency is common, and a low 25(OH)D concentration is associated with a higher risk of developing ID and diabetes.³⁹ Whilst direct causality has not been confirmed, vitamin D supplementation is often prescribed as a complementary and preventative therapy.^{40,41}

Vitamin D is a secosteroid lipophilic hormone that is stored within adipose tissue.⁴² Adipose tissue is increased in volume in obese people and theoretically, vitamin D sequestration within these large stores is thought to explain, at least in part, why deficiency is routinely observed in obese humans.⁴³⁻⁴⁵ Unexpectedly, in contrast to observations in humans, obese horses had significantly higher 25(OH)D₂ than nonobese horses, with measures of adiposity being the most predictive factor of vitamin D status. Our measures of adiposity are limited to quantification of subcutaneous adipose tissue and, despite the known correlation between BCS and total body fat, BCS may not accurately reflect the visceral adipose content of these animals.⁴⁶ Quantification of vitamin D metabolites in equine subcutaneous and visceral adipose tissue is needed to determine how these compounds are distributed throughout the body stores in obese and lean horses. One could speculate that the visceral adipose tissue inflammation and dysfunction reported in hyperinsulinaemic and obese horses could impair uptake and utilisation of vitamin D from the fat stores, which might explain the higher circulating 25(OH)D₂ found in obese horses.⁴⁷

We found no association between serum insulin concentration or diagnosis of ID and vitamin D status in our population. In humans, the association between ID and 25(OH)D is mitigated by adjusting for the amount of visceral adipose implying that it is an indirect relationship.⁴⁵

Whilst ACTH level was negatively associated with 25(OH)D₂ concentrations, PPID was not significantly associated with vitamin D status. This observation likely reflects the opposing seasonal changes that occur with vitamin D and ACTH concentrations, rather than reflecting a direct association between PPID and vitamin D status. In this respect, basal ACTH values tend to rise as days are shortening, whilst vitamin D levels fall.⁴⁸ Furthermore, 8 of the 32 horses diagnosed with PPID were treated with pergolide which could have affected basal ACTH concentrations unpredictably. We, therefore, consider the relationship observed between these two hormones secondary to their physiological seasonal pattern rather than being associated with PPID.

A key question facing veterinary surgeons is whether horses require vitamin D supplementation, and, if so, at what level. The National Research Council's recommendation, first published in 1989 states that the daily requirement is around 6.6 IU/kg but does not specify the type of vitamin D.⁴⁹ This recommendation is based on an experimental study conducted in 1979 in which circulating 25(OH)D concentrations were not measured.³² Our data suggest that grazing animals can remain clinically healthy despite having undetectable levels of 25(OH)D₃ because pasture-derived 25(OH)D₂ meets their vitamin D requirements; supplementation of sedentary animals with access to pasture/forage is therefore unlikely to be necessary for optimal health. Further work is necessary however to determine if vitamin D₂ or D₃ supplementation is necessary to maintain optimal health in equine athletes with limited access to pasture or forage.

The main limitation of this study is that only 25(OH)D was measured. Despite this being the analyte most commonly used to determine vitamin D status, the vitamin D system is complex and involves a multitude of others metabolites that were not here considered.

5 | CONCLUSION

This study demonstrates some marked differences in vitamin D biology in the horse compared with that of other species and advocates the need for further research in this field to provide evidence-based advice on nutritional requirements for athletic horses. Finally, the physiological role of vitamin D in horses is poorly understood, therefore 25(OH)D variation in pathological condition remains of difficult interpretation. Despite this, adiposity seems to have a significant contribution to vitamin D status.

AUTHOR CONTRIBUTIONS

Miranda C. M. C. Dosi contributed to study execution, data analysis and preparation of the manuscript. John A. Keen and Bruce C. McGorum contributed to study design and sample collection. Richard J. Mellanby and Emma A. Hurst contributed to sample analysis, whilst Roxane D. Kirton and Eugenio Cillán-García contributed to sample collection. Ruth A. Morgan contributed to study design, sample collection, data analysis and preparation of the manuscript. All authors gave their final approval of the manuscript.

ACKNOWLEDGEMENTS

The authors are grateful to the staff of Redwings Horse Sanctuary for allowing us to enrol the animals under their care in our study. The authors thank Scott Denham and Jo Simpson in the Mass Spectrometry Core for their work on development and validation of the methods. This study received funding from Fiona and Ian Russell Seed Corn Grant.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/evj.13873>.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This study was approved by the University of Edinburgh Veterinary Ethics and research Committee (approval 124.19). Consent was obtained from owners and trainers of all horses included in the study.

ORCID

Miranda C. M. C. Dosi  <https://orcid.org/0000-0002-1094-1184>

Bruce C. McGorum  <https://orcid.org/0000-0002-6977-6101>

John A. Keen  <https://orcid.org/0000-0002-7862-5321>

Ruth A. Morgan  <https://orcid.org/0000-0003-1117-2273>

REFERENCES

- Hymøller L, Jensen SK. We know next to nothing about vitamin D in horses! *J Equine Vet.* 2015;35(10):785–92. <https://doi.org/10.1016/j.jevs.2015.06.010>
- Giustina A, Adler RA, Binkley N, Bollerslev J, Bouillon R, Dawson-Hughes B, et al. Consensus statement from 2nd international conference on controversies in vitamin D. *Rev Endocr Metab Disord.* 2020; 21(1):89–116. <https://doi.org/10.1007/s11554-019-09532-w>
- Hurst EA, Homer NZ, Mellanby RJ. Vitamin D metabolism and profiling in veterinary species. *Metabolites.* 2020;10(9):371. <https://doi.org/10.3390/metabo10090371>
- Pozza ME, Kaewsakhorn T, Trinarong C, Inpanbutr N, Toribio RE. Serum vitamin D, calcium, and phosphorus concentrations in ponies, horses and foals from the United States and Thailand. *Vet J.* 2014; 199(3):451–6. <https://doi.org/10.1016/j.tvjl.2014.01.002>
- Markku S, Johanna J. Influence of dietary supplementation on serum vitamin A and D concentrations and their seasonal variation in horses. *Agric Food Sci.* 1992;1(5):477–82. <https://doi.org/10.23986/afsci.72460>
- Breidenbach A, Schlumbohm C, Harmeyer J. Peculiarities of vitamin D and of the calcium and phosphate homeostatic system in horses. *Vet Res.* 1998;29(2):173–86.
- Rourke KM, Coe S, Kohn CW, Rosol TJ, Mendoza FJ, Toribio RE. Cloning, comparative sequence analysis and mRNA expression of calcium-transporting genes in horses. *Gen Comp Endocrinol.* 2010; 167(1):6–10. <https://doi.org/10.1016/j.ygcen.2010.02.022>
- Harmeyer J, Schlumbohm C. Effects of pharmacological doses of vitamin D₃ on mineral balance and profiles of plasma vitamin D₃ metabolites in horses. *J Steroid Biochem Mol Biol.* 2004;89-90:595–600. <https://doi.org/10.1016/j.jsbmb.2004.03.034>
- Piccione G, Assenza A, Fazio F, Bergero D, Caola G. Daily rhythms of serum Vitamin D-metabolites, calcium and phosphorus in horses. *Acta Vet Brno.* 2008;77:151–7. <https://doi.org/10.2754/avb200877020151>
- Azarpeykan S, Dittmer KE, Gee EK, Marshall JC, Elder P, Acke E, et al. Circadian rhythm of calciotropic hormones, serum calcium, phosphorus and magnesium during the shortest and longest days of the year in horses in New Zealand. *J Anim Physiol Anim Nutr.* 2016;100(6): 1058–66. <https://doi.org/10.1111/jpn.12477>
- Azarpeykan S, Dittmer KE, Gee EK, Marshall JC, Wallace J, Elder P, et al. Influence of blanketing and season on vitamin D and parathyroid hormone, calcium, phosphorus, and magnesium concentrations in horses in New Zealand. *Domest Anim Endocrinol.* 2016;56:75–84. <https://doi.org/10.1016/j.domaniend.2016.03.003>
- Azarpeykan S, Dittmer KE, Marshall JC, Perea KC, Gee EK, Acke E, et al. Evaluation and comparison of vitamin D responsive gene expression in ovine, canine and equine kidney. *PLoS One.* 2016;11(9): e0162598. <https://doi.org/10.1371/journal.pone.0162598>
- Kamr AM, Dembek KA, Reed SM, Slovis NM, Zaghawa AA, Rosol TJ, et al. Vitamin D metabolites and their association with calcium, phosphorus, and PTH concentrations, severity of illness, and mortality in hospitalized equine neonates. *PLoS One.* 2015;10(6):e0127684. <https://doi.org/10.1371/journal.pone.0127684>
- Puangthong C, Sukhong P, Saengnual P, Srikuea R, Chanda M. A single bout of high-intensity exercise modulates the expression of vitamin D receptor and vitamin D-metabolising enzymes in horse skeletal muscle. *Equine Vet J.* 2021;53(4):796–805. <https://doi.org/10.1111/evj.13346>
- Amrein K, Scherkl M, Hoffmann M, Neuwersch-Sommeregger S, Köstenberger M, Tmava Berisha A, et al. Vitamin D deficiency 2.0: an update on the current status worldwide. *Eur J Clin Nutr.* 2020;74(11): 1498–513. <https://doi.org/10.1038/s41430-020-0558-y>

16. Webb AR, Kazantzidis A, Kift RC, Farrar MD, Wilkinson J, Rhodes LE. Colour counts: sunlight and skin type as drivers of vitamin D deficiency at UK latitudes. *Nutrients*. 2018;10(4):457.
17. Nemeth MV, Wilkens MR, Liesegang A. Vitamin D status in growing dairy goats and sheep: influence of ultraviolet B radiation on bone metabolism and calcium homeostasis. *J Dairy Sci*. 2017;100(10):8072–86. <https://doi.org/10.3168/jds.2017-13061>
18. Lontchi-Yimagou E, Kang S, Goyal A, Zhang K, You JY, Carey M, et al. Insulin-sensitizing effects of vitamin D repletion mediated by adipocyte vitamin D receptor: studies in humans and mice. *Mol Metab*. 2020;42:101095. <https://doi.org/10.1016/j.molmet.2020.101095>
19. Hookey TJ, Backus RC, Wara AM. Effects of body fat mass and therapeutic weight loss on vitamin D status in privately owned adult dogs. *J Nutr Sci*. 2018;7:e17. <https://doi.org/10.1017/jns.2018.7>
20. Carslake HB, Pinchbeck GL, McGowan CM. Evaluation of a chemiluminescent immunoassay for measurement of equine insulin. *J Vet Intern Med*. 2017;31(2):568–74. <https://doi.org/10.1111/jvim.14657>
21. Rendle DI, Litchfield E, Heller J, Hughes KJ. Investigation of rhythms of secretion and repeatability of plasma adrenocorticotrophic hormone concentrations in healthy horses and horses with pituitary pars intermedia dysfunction. *Equine Vet J*. 2014;46(1):113–7. <https://doi.org/10.1111/evj.12114>
22. Carroll CL, Huntington PJ. Body condition scoring and weight estimation of horses. *Equine Vet J*. 1988;20(1):41–5. <https://doi.org/10.1111/j.2042-3306.1988.tb01451.x>
23. Carter RA, Geor RJ, Burton Staniar W, Cubitt TA, Harris PA. Apparent adiposity assessed by standardised scoring systems and morphometric measurements in horses and ponies. *Vet J*. 2009;179(2):204–10. <https://doi.org/10.1016/j.tvjl.2008.02.029>
24. Copas VE, Durham AE. Circannual variation in plasma adrenocorticotrophic hormone concentrations in the UK in normal horses and ponies, and those with pituitary pars intermedia dysfunction. *Equine Vet J*. 2012;44(4):440–3. <https://doi.org/10.1111/j.2042-3306.2011.00444.x>
25. Eiler H, Frank N, Andrews FM, Oliver JW, Fecteau KA. Physiologic assessment of blood glucose homeostasis via combined intravenous glucose and insulin testing in horses. *Am J Vet Res*. 2005;66(9):1598–604. <https://doi.org/10.2460/ajvr.2005.66.1598>
26. Hurst EA, Homer NZ, Gow AG, Clements DN, Evans H, Gaylor D, et al. Vitamin D status is seasonally stable in northern European dogs. *Vet Clin Pathol*. 2020;49(2):279–91. <https://doi.org/10.1111/vcp.12859>
27. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357(3):266–81. <https://doi.org/10.1056/NEJMra070553>
28. Melamed ML, Michos ED, Post W, Astor B. 25-Hydroxyvitamin D levels and the risk of mortality in the general population. *Arch Intern Med*. 2008;168(15):1629–37. <https://doi.org/10.1001/archinte.168.15.1629>
29. Carter GD, Berry J, Durazo-Arvizu R, Gunter E, Jones G, Jones J, et al. Hydroxyvitamin D assays: an historical perspective from DEQAS. *J Steroid Biochem Mol Biol*. 2018;177:30–5. <https://doi.org/10.1016/j.jsbmb.2017.07.018>
30. Dittmer KE, Thompson KG. Vitamin D metabolism and rickets in domestic animals: a review. *Vet Pathol*. 2010;48(2):389–407. <https://doi.org/10.1177/0300985810375240>
31. Asin J, Murphy BG, Samol MA, Polanco J, Moore JD, Uzal FA. Rickets in a thoroughbred-cross foal: case report and review of the literature. *J Vet Diagn Invest*. 2021;33(5):987–92. <https://doi.org/10.1177/10406387211025232>
32. El Shorafa WM, Feaster JP, Ott EA, Asquith RL. Effect of vitamin D and sunlight on growth and bone development of young ponies. *J Anim Sci*. 1979;48(4):882–6. <https://doi.org/10.2527/jas1979.484882x>
33. How KL, Hazewinkel HA, Mol JA. Dietary vitamin D dependence of cat and dog due to inadequate cutaneous synthesis of vitamin D. *Gen Comp Endocrinol*. 1994;96(1):12–8. <https://doi.org/10.1006/gcen.1994.1154>
34. Mäkitaipale J, Sievänen H, Sankari S, Laitinen-Vapaavuori O. Diet is a main source of vitamin D in Finnish pet rabbits (*Oryctolagus cuniculus*). *J Anim Physiol Anim Nutr*. 2019;103(5):1564–70. <https://doi.org/10.1111/jpn.13120>
35. Jakobsen J, Jensen SK, Hymøller L, Andersen EW, Kaas P, Burild A, et al. Short communication: artificial ultraviolet B light exposure increases vitamin D levels in cow plasma and milk. *J Dairy Sci*. 2015;98(9):6492–8. <https://doi.org/10.3168/jds.2014-9277>
36. Van Saun RJ, Smith BB, Watrous BJ. Evaluation of vitamin D status of llamas and alpacas with hypophosphatemic rickets. *J Am Vet Med Assoc*. 1996;209(6):1128–33.
37. Jäpelt RB, Jakobsen J. Vitamin D in plants: a review of occurrence, analysis, and biosynthesis. *Front Plant Sci*. 2013;4:136. <https://doi.org/10.3389/fpls.2013.00136>
38. Jäpelt RB, Didion T, Smedsgaard J, Jakobsen J. Seasonal variation of provitamin D₂ and vitamin D₂ in perennial ryegrass (*Lolium perenne* L.). *J Agric Food Chem*. 2011;59(20):10907–12. <https://doi.org/10.1021/jf202503c>
39. Abbasi F, Blasey C, Feldman D, Caulfield MP, Hantash FM, Reaven GM. Low circulating 25-hydroxyvitamin D concentrations are associated with defects in insulin action and insulin secretion in persons with prediabetes. *J Nutr*. 2015;145(4):714–9. <https://doi.org/10.3945/jn.114.209171>
40. Pittas AG, Lau J, Hu FB, Dawson-Hughes B. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. *J Clin Endocrinol Metab*. 2007;92(6):2017–29. <https://doi.org/10.1210/jc.2007-0298>
41. Mitri J, Muraru MD, Pittas AG. Vitamin D and type 2 diabetes: a systematic review. *Eur J Clin Nutr*. 2011;65(9):1005–15. <https://doi.org/10.1038/ejcn.2011.118>
42. Mawer EB, Backhouse J, Holman CA, Lumb GA, Stanbury SW. The distribution and storage of vitamin D and its metabolites in human tissues. *Clin Sci*. 1972;43(3):413–31. <https://doi.org/10.1042/cs0430413>
43. Abbas MA. Physiological functions of vitamin D in adipose tissue. *J Steroid Biochem Mol Biol*. 2017;165(Pt B):369–81. <https://doi.org/10.1016/j.jsbmb.2016.08.004>
44. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr*. 2000;72(3):690–3. <https://doi.org/10.1093/ajcn/72.3.690>
45. Cheng S, Massaro JM, Fox CS, Larson MG, Keyes MJ, McCabe EL, et al. Adiposity, cardiometabolic risk, and vitamin D status: the Framingham heart study. *Diabetes*. 2010;59(1):242–8. <https://doi.org/10.2337/db09-1011>
46. Dugdale AH, Grove-White D, Curtis GC, Harris PA, Argo CM. Body condition scoring as a predictor of body fat in horses and ponies. *Vet J*. 2012;194(2):173–8. <https://doi.org/10.1016/j.tvjl.2012.03.024>
47. Reynolds A, Keen J, Fordham T, Morgan R. Adipose tissue dysfunction in obese horses with equine metabolic syndrome. *Equine Vet J*. 2019;51(6):760–6.
48. Durham AE, Clarke BR, Potier JFN, Hammarstrand R, Malone GL. Clinically and temporally specific diagnostic thresholds for plasma ACTH in the horse. *Equine Vet J*. 2021;53(2):250–60. <https://doi.org/10.1111/evj.13292>
49. National Research Council, Division on Earth and Life Studies, Board on Agriculture and Natural Resources, Committee on Nutrient Requirements of Horse. Nutrition requirements of horses. 6th ed. Washington, DC: The National Academy Press; 2007.

How to cite this article: Dosi MCMC, McGorum BC, Kirton RD, Cillán-García E, Mellanby RJ, Keen JA, et al. The effect of season, management and endocrinopathies on vitamin D status in horses. *Equine Vet J*. 2022. <https://doi.org/10.1111/evj.13873>