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Vitamin D and Calcium Metabolism In Horses in New Zealand

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Abstract

The physiology of vitamin D in horses has not been studied in great depth. Few studies on vitamin D metabolites (25OHD₂, 25OHD₃, and 1,25(OH)₂D) and their relationship to other serum analytes exist. In addition, some studies suggest that equine vitamin D physiology may be different from other species. This thesis aimed to investigate aspects of vitamin D metabolism in horses.

The effect of blanketing on vitamin D synthesis and its relationship with other analytes involved in calcium homeostasis, including vitamin D metabolites (25-hydroxyvitamin D₂ (25OHD₂), and 25-hydroxyvitamin D₃ (25OHD₃), 1,25-dihydroxyvitamin D (1,25(OH)₂D)), ionised calcium (iCa), total calcium (tCa), phosphorus (P), total magnesium (tMg) and parathyroid hormone (PTH) were studied in horses. Regardless of blanketing, 25OHD₃ was undetectable in equine serum and 25OHD₂ was the main form of 25OHD in circulation. A strong seasonal variation in serum 25OHD₂, 1,25(OH)₂D, iCa, tCa, P, tMg and PTH concentrations was detected, although no differences were seen between horses that were blanketed and those that were not. The circadian rhythms of serum vitamin D metabolites, iCa, tCa, P, tMg, and PTH concentrations in horses was studied over 48 h on the summer and winter solstices. A significant difference was seen between the serum concentrations of studied analytes between solstices, with no rhythm detected in winter. An *in vivo* study suggested that equine skin may be unable to convert 7-dehydrocholesterol (7-DHC) to vitamin D₃ after exposure to ultraviolet B (UVB) light. Quantitative PCR was performed on equine kidney to study the expression of vitamin D responsive and calcium transporting genes, which were then compared to genes in sheep and dogs.

The results suggested that TRPV6, calD_{9k} /calD_{28k}, and PMCA were the main calcium transporting pathways in the kidney of these species, and there was a high correlation between VDR and other studied genes. It was concluded that 25OHD₂ is the main metabolic precursor for 1,25(OH)₂D and should be considered the best available index of vitamin D status in unsupplemented horses, and that horses most likely rely on diet as their primary source of vitamin D.

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Glossary

1,25(OH) ₂ D	1,25-dihydroxyvitamin D
18S	ribosomal RNA
25OHD	25-hydroxyvitamin D
25OHD ₂	25-hydroxyvitamin D ₂
25OHD ₃	25-hydroxyvitamin D ₃
28S	ribosomal RNA
7-DHC	7-dehydrocholesterol
B2M	β-2-microglobulin
Ba ²⁺	Barium
BLAST	Basic Local Alignment Search Tool
BMD	bone mineral density
Ca ²⁺	calcium
Calbindin	calcium-binding protein
CalbindinD _{9k}	vitamin-D-dependent 9k, calcium-binding protein
CalbindinD _{28k}	vitamin-D-dependent 28k, calcium-binding protein
cAMP	cyclic 3',5'-adenosine monophosphate
CaSR	calcium sensitive receptors
C cells	parafollicular cells
cDNA	complementary DNA
CGRP	calcitonin gene-related peptide
Cl ⁻	chloride
CNT	connecting tubule
CYP24A1	24-hydroxylase
CYP27A1	cytochrome P450, family 27, subfamily A, polypeptide 1
CYP27B1	1α-hydroxylase
CYP2R1	cytochrome P450, family 2, subfamily R, polypeptide 1
DAG	diacylglycerol
DBP	vitamin D-binding protein
DCAD	dietary cation-anion difference
DCs	dendritic cells

DCT	distal convoluted tubule
DHCR1	7-dehydrocholesterol reductase 1
DNA	deoxyribonucleic acid
Fe ²⁺	iron
FGF23	fibroblast growth factor 23
FE _{Ca}	fractional urinary clearance of calcium
FE _{Mg}	fractional urinary clearance of magnesium
FE _P	fractional urinary clearance of phosphorus
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GC	glucocorticoids
GC content	guanine-cytosine content
GCs	glucocorticoid hormones
GH	growth hormone
GIT	gastrointestinal tract
HKG	housekeeping gene
HMBS	hydroxymethylbilane synthase
HPO ₄ ²⁻ / H ₂ PO ₄ ⁻	phosphorus anions
HPLC	high-performance liquid chromatography
HPRT1	hypoxanthine phosphoribosyltransferase 1
iCa	ionised calcium
CI	confidence interval
CV	coefficient of variation
IGFs	insulin-like growth factors
IL-1	interleukin 1
IL-6	interleukin 6
IVABS	Institute of Veterinary, Animal and Biomedical Sciences
K ⁺	potassium
LC-MS	liquid chromatography–mass spectrometry
MAPK	mitogen-activated protein kinase
Mg ²⁺	magnesium
mgcv	mixed generalized additive models computation vehicle
MS	multiple sclerosis

Na ⁺	sodium
Na ⁺ /Pi	sodium-dependent phosphate
NCBI	national centre for biotechnology information
NCX1	sodium calcium exchanger 1
NH ₄ ⁺	ammonium ions
NH ₄ Cl	ammonium chloride
NIWA	National Institute of Water and Atmospheric research
NRC	nutrient requirements of horses
NZVP	New Zealand Veterinary Pathology
OPG	osteoprotegerin
P	phosphorus
PCR	polymerase chain reaction
PGK1	phosphoglycerate kinase 1
PKC	protein kinase C
PLA2	phospholipase A2
PLC	phospholipase C
PLD	phospholipase D
PMCA	plasma membrane Ca ²⁺ -ATPase
PTH	parathyroid hormone
PTHr1	parathyroid hormone receptor 1
PTHrP	parathyroid hormone -related peptide
RANK	receptor activator of the nuclear factor-kappaB
RANKL	receptor activator of the nuclear factor-kappaB ligand
RNA	ribonucleic acid
RPL13A	ribosomal protein L13a
RPL30	ribosomal protein L30
RPL32	ribosomal protein L32
RPS5	ribosomal protein S5
RPS19	ribosomal protein S19
RT-qPCR	real-time quantitative reverse transcriptase polymerase chain reaction
SCN	suprachiasmatic nucleus

SDHA	succinate dehydrogenase complex
SE	standard error
sFRP-4	secreted frizzled related protein-4
SLC17	type I sodium-phosphate co-transporters
SLC20/PiT2	type III sodium-phosphate co-transporters
SLC34	type II sodium-phosphate co-transporters
SLC34A1	type II sodium-phosphate co-transporters, member 1
SLC32A2	type II sodium-phosphate co-transporters, member 2
SLC34A3	type II sodium-phosphate co-transporters, member 3
SNPs	single nucleotide polymorphisms
SPF	sun protection factor
SST	serum separator tube
tCa	total calcium
TLR	toll-like receptors
tMg	total magnesium
TRPM	transient receptor potential cation channel, subfamily M
TRPM6	transient receptor potential cation channel, subfamily M, member 6
TRPM7	transient receptor potential cation channel, subfamily M, member 7
TRPV5	transient receptor potential cation channel, subfamily V, member 5
TRPV6	transient receptor potential cation channel, subfamily V, member 6
UBB	ubiquitin B
UV	ultraviolet
UVB	ultraviolet B
VDR	vitamin D receptor
VLATU	Veterinary Large Animal Teaching Unit
YWHAZ	zeta polypeptide