

Vitamin D mediated Calcium and Phosphorus metabolism in cattle

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This is for Bob,

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Abstract

Vitamin D metabolites and the major mammalian minerals phosphorus (P) and calcium (Ca) have a complex and detailed relationship. The relationship, especially for Ca, is delicately controlled by a range of hormones and is largely dependent on the animal's physiological state. Studies have identified that Vitamin D metabolites given in supraphysiological doses to cattle can manipulate Ca and P metabolism. Furthermore, large doses of the metabolite 25-hydroxy vitamin D (25-vitD) can replace the actions of active vitamin D, 1,25-dihydroxy vitamin D (1,25-vitD) and initiate increased absorption of both Ca and P from the digestive tract in mice. These findings led to the hypothesis that 25-vitD could be used to favorably manipulate the metabolism of both Ca and P in cattle.

The studies undertaken within this thesis are focused on the use of 25-vitD in both the beef and dairy industries. A group of beef breed steers were recruited and trained to halter, stand in metabolism crates and spend extended periods of time in individual pens. For studies concerning Ca, urine was the most important measurement. An increase in urinary Ca excretion demonstrated that there has been an increase in available Ca, either from the diet or skeletal reserves. With P studies faecal P excretion and plasma P were important guides to changes in P metabolism.

The results of the metabolism studies undertaken in the thesis identify that Ca and P homeostasis is manipulated by 25-vitD and the majority of the increase in availability of both Ca and P originates from the diet. This is further supported by the absence of bone degradation.

Thus, the inclusion of 25-vitD in a typical anionic transition diet will increase Ca absorption prior to parturition, enabling labile bone Ca stores to remain intact and available for immediate use at parturition whilst increasing the amount of Ca available to the animal from both bone and diet. Furthermore, the combination of 25-vitD and anionic salts has physiological implications that allow sufficient generation of extracellular Ca at parturition.

An increase in plasma 25-vitD concentrations, to approximately 375 ng/ml, facilitated an increase in the concentration of plasma P and a reduction in faecal P, which indicates that dietary P absorption was increased.

Detailed Summary

Chapter 1. Introduction

Chapter 2. Abstract of literature review

The potential for increasing dietary P absorption with 25-vitD supplementation in cattle

Research has demonstrated that supplementation of bovine with 25-vitD in therapeutic amounts (ie. Plasma concentration >200 ng/ml), increases the concentration of P and Ca in plasma of cattle. Most studies have concluded that this increase in plasma P and Ca concentration is a consequence of an increase in bone degradation or have not drawn a conclusion. However, endocrine research suggests that bone degradation is only likely when high levels of plasma 25-vitD concentration coincide with increased secretion of PTH. Therefore it is more likely that the increase in mineral concentration originates from the dietary tract.

Studies have concluded that supraphysiological plasma concentrations of 25-vitD can replace many of the roles of 1,25-vitD in mice. Therefore it is likely that supraphysiological concentrations of 25-vitD will increase both P and Ca absorption and may be able to improve P retention on low P concentration diets. The literature suggests that concentrations of 25-vitD in plasma greater than 200 ng/ml will increase P absorption leading to an increase in P retention and plasma P concentration in ruminants.

The effect of 25-vitD and anionic salts on increasing Ca availability in cattle

The combination of anionic salts, to reduce dietary cation anion difference DCAD, and 25-vitD for the mitigation of hypocalcaemia may be a practical option. Neutral to negative DCAD has been demonstrated to improve the sensitivity of hormone receptors, release Ca from bone and increase Ca excretion in urine; all three affects should increase a cow's ability to respond to hypocalcaemia. However, rapidly available bone Ca reserves are finite and it is difficult to feed highly negative DCAD as most forage feed stuffs are cationic and anionic salts result in low feed intake, often attributed to reduced palatability.

The addition of 25-vitD may allow an increase in Ca absorption from the diet as well as providing a readily available source for metabolism of 1,25-vitD when PTH is secreted in response to hypocalcaemia.

Chapter 3

Two studies were conducted, prior to the commencement of the main studies, to ascertain the effectiveness of using steers as models for DCAD influenced mineral metabolism and the use of urinary spot samples for estimation of daily urine Ca excretion. The studies were designed to assess the effectiveness of using steers (n=6) and urine spot samples as techniques for evaluating Vitamin D manipulated Ca and P metabolism in both dairy and beef cattle. The studies demonstrated that steers were very useful for mineral metabolism studies as their urinary pH reacted appropriately to anionic salts and their urinary Ca excretion was similar to dairy cows when calculated on a live weight basis. Furthermore, their feed intake was not affected by the presence of anionic salts. Urine spot sampling was demonstrated to be an effective method of assessing total daily urinary Ca excretion. This enabled metabolism studies to be undertaken without the use of metabolism crates and in extensive situations.

Chapter 4

The influence of feeds containing varying DCAD with and without supplements of 25-vitD on urine pH and excretion of macro minerals was determined in fistulated crossbred steers (n= 18, mean live weight 315±45 kg). A basal forage diet comprised of lucerne hay and wheat chaff was used, to which varying quantities of MgCl₂ or K₂CO₃ were added to achieve four levels of DCAD: -300, 50, 150 or 250 mEq/kg dry matter (DM). Steers were allocated to one of six treatments, one treatment for each diet and a further treatment for both the 50 and 150 mEq/kg DCAD diets, which were supplemented with 25-vitD at a rate of 3 mg/steer per day. Urine pH from steers offered the diets comprising DCADs of 50, 150 and 250 mEq/kg ranged from 8.3 to 8.8. In treatments not containing 25-vitD with DCADs of 50 to 250 mEq/kg there was no significant differences in urine pH or Ca excretion. However, steers offered the diet with a DCAD of -300 mEq/kg DM produced urine with a significantly lower pH (6.5-7.5). Daily output of Ca in urine was also significantly higher from steers given this diet. Supplementation with 25-vitD significantly increased urinary Ca excretion from steers offered diets of DCADs 50 and 150 mEq/kg DM. Estimates of daily urinary Ca excretion, calculated using the ratio of creatinine to Ca in 'spot' urine samples, were less variable than those based on total collection (residual mean square of 0.54 and 0.63, respectively).

Chapter 5

Parturient paresis is a consequence of a cow's failure to activate or respond to hormonal mechanisms that increase Ca availability. Feeding anionic salts prior to parturition has been proven to initiate Ca mobilisation resulting in an increase in urinary Ca. Supplementation of 25-vitD increases the plasma level of 25-vitD which may potentially amplify the Ca mobilising effect of traditional anionic transition diets. Steers (n=18) were allocated to one of two diets with different DCAD with one diet being anionic (DCAD -120) and one cationic (DCAD 150). Steers were randomly allocated rumen boluses that contained either monensin or a combination of monensin and 25-vitD. The capsules contained a mechanism that delayed the release of 25-vitD. Steers had blood and urine samples taken at approximately ten days post 25-vitD release. As seen in previous studies the anionic treatment, DCAD -120, had greater urine Ca excretion than DCAD 150 ($P < 0.05$). Plasma 25-vitD levels greater than 75 ng/ml increased urinary Ca excretion within treatment DCAD -120 ($P < 0.05$). Supplementation of steers in treatment DCAD 150 with 25-vitD did not increase urine Ca excretion. The results demonstrate that Ca mobilisation mechanisms were initiated by anionic salts and enhanced when anionic salts were combined with 25-vitD supplementation. The combination of 25-vitD and anionic salts prior to parturition may provide greater resistance to parturient paresis and sub-clinical hypocalcaemia.

Chapter 6

Supplementing ruminants with Vitamin D or 25-vitD in their feed has been demonstrated to be a reliable method of improving the Vitamin D status of the animal. However, it is not possible to accurately supplement animals when they have access to free choice supplements. Furthermore, in many rangeland conditions it is not practical to supplement animals at all. Intra-ruminal supplementation with 25-vitD has been attempted, and concentrations of plasma 25-vitD were significantly lower than achieved when similar amounts of supplemental 25-vitD were given in the feed. There were several potential causes for this reduced efficacy of absorption of 25-vitD. This study aimed to investigate whether the cause was a consequence of inadequate emission of the bolus contents or an influence of anionic salts in the diet. The study utilised 19 fistulated steers, which were allocated to 5 treatments with two treatments offered a cationic diet and the other three the same forage diet with additional anionic salts. Treatments on both diets were split between 1.25% concentrate

beadlet 25-vitD (+D) and control (-D) with the third anionic diet treatment allocated the contents of a bolus that contained monensin and 90% concentrate crystalline 25-vitD (Mon/+D). The results demonstrated that absorption of 25-vitD from beadlet +D treatments, from both cationic (175 ng/ml) and neutral (200 ng/ml) diets was similar and greater ($P < 0.05$) than both control treatments (~13 ng/ml) and Mon/+D treatments (41 ng/ml). While the Mon/+D treatment did have an increase ($P < 0.05$) in plasma 25-vitD concentration over the control treatments, the difference in efficacy between crystalline 25-vitD combined with monensin in bolus pellet matrix is profoundly less than beadlet 25-vitD. The absorption of 25-vitD from the digestive tract is not influenced by release characteristics of intra-rumen boluses or an interaction with monensin. Therefore, there must be another factor involved in impeding absorption of crystalline 25-vitD in the digestive tract of bovine.

Chapter 7

Active absorption of P from the alimentary tract is promoted by increased concentrations of 1,25-vitD in blood; however, the production of this active vitamin is determined by plasma concentrations of Ca and not P. As a consequence, diets of adequate Ca content, but insufficient P, will not promote active P absorption. Dietary supplements of 25-vitD, the precursor of 1,25-vitD, may stimulate P absorption and P retention of ruminants consuming diets marginally deficient in P. To evaluate this hypothesis, steers ($n = 18$) were fed a pelleted low-quality roughage diet containing an adequate Ca concentration (0.68%). Nine steers received a supplement of 25-vitD mixed into their feed, at a rate of $3.25 \text{ mg/hd.day}^{-1}$, prior to pelleting. The other nine steers were fed the control diet without supplementation. All steers were individually housed for 10 d before being moved into metabolism crates for a further 3d period. The steers which received the diet containing 25-vitD exhibited increased retention of P and Ca, approximately 4 g/d and 3 g/d, respectively. Blood samples were taken prior to the adaptation period and then daily during the period in the metabolism crates. Plasma concentrations of both P and Ca were increased by 25-vitD supplementation throughout the collection period. Addition of 25-vitD to the diet of grazing animals may reduce the need for P supplementation programs and improve productivity.

Chapter 8

Vitamin D and its metabolites are known to play an important role in mineral metabolism and have an effect on the deposition and degradation of the skeleton. This experiment was

designed to test if the treatments undertaken in Chapter 4 and Chapter 6 had any effect on the rate of bone degradation or accretion. Two ELISA test kits were purchased for the estimation of the concentration of markers in plasma that described that rate of bone degradation and bone accretion. A further ELISA kit was purchased for the estimation of 1,25-vitD concentration in plasma. Both the osteocalcin (marker for accretion) and serum crosslaps (marker for degradation) showed no difference between treatments and when compared to previous work they demonstrated that the rate of accretion and degradation was particularly low. This suggested that the 25-vitD treatments were not having an effect on bone metabolism within the time period of the studies. The concentration of 1,25-vitD estimated by the ELISA kit was below the level of recording. This confirmed previous work that suggested that large doses of 25-vitD inhibit the production of 1,25-vitD. Therefore, the physiological changes mediated by 25-vitD must be originating from the 25-vitD not another source.

List of abbreviations

25-vitD	25 hydroxyvitamin D, the Vitamin D (cholecalciferol) molecule after it has been hydroxylated at C-25.
1,25-vitD	1,25 dihydroxyvitamin D, calcitriol. The molecule derived from hydroxylation of 25-vitD at C-1.
24,25-vitD	24, 25 dihydroxyvitamin D. Part of the degradation pathway of Vitamin D.
PTH	Parathyroid hormone
Ca	Calcium
Ca ⁺	Ionised Calcium
P	Phosphorus
iP	Ionised phosphorus
1 α -OHase	1 alpha hydroxylase, the enzyme responsible for conversion of 25-vitD to 1,25-vitD
VDR	Vitamin D receptor, usually within the small intestine. Responsible for activation of cellular responses sensitive to vitamin D.
ATP	Adenosine triphosphate
CTx	Serum Crosslaps, marker of bone degradation.
OC	Osteocalcin, marker of bone accretion
FGF23	Fibroblast growth factor
ARC	Australian Research Council
NRC	National Research Council

List of Publications

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