Skeletal health in the modern dairy cow: Is it a driver of metabolic health?

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Introduction

Calcium and Phosphorus are amongst the most important minerals in the dairy cow with many structural and biochemical functions. They make up much of the structure of the skeleton, play major roles in muscle control, energy production and immunity amongst other roles. However, there has been little focus on total life time Ca status within dairy cows and the implications that this may have on animal health.

Hypocalcaemia (HC) and sub-clinical hypocalcaemia (SHC) are well recognized as one of the key diseases of dairy production systems. HC is a devastating syndrome resulting in morbidity and mortality during the peri partum period. Whereas SHC is a precursor of many of the significant post parturition diseases, such as, ketosis, displaced abomasum, retained placenta, metritis and mastitis (Curtis et al. 1983, Erb et al. 1985).

The contribution of Ca to the HC syndrome is contrary to what one would normally think. When lecturing students, the introduction of HC at parturition is often met with intense confusion. The fact that animals suffering from a Ca deficiency by often consuming too much Ca often takes a little time to understand.

Perhaps, as a consequence, the beneficial role of Ca and P in the HC syndrome has often been overlooked. Wonderful work around the use of Ca deficient diets (Boda and Cole, 1954), the injection of vitamin D (Bar et al. 1985) and the control of DCAD (Ender and Dishington, 1962, Block 1984) for the prevention of HC has, rightfully so, focused attention on enhancing the cow's ability to control her own Ca homeostasis. Thereby improving the animal's ability to secrete PTH for the generation of activated vitamin D, 1,25 dihydroxycholecalciferol (1,25vitD), which enables up regulation of Ca metabolism (Goff et al. 1991). The success of these techniques for controlling the visible signs of HC may have resulted in less focus on other aspects of skeletal health which are integral for Ca and P mediated health.

Skeletal health can be principally considered to be a description of the Ca and P status of the animal (McGrath et al. 2017). The potential for the skeleton to now be considered an endocrine organ firmly puts the Ca status of the modern day dairy cow at the forefront of science (McNeil and Anderson, 2013). Furthermore, studies demonstrating the level of sub clinical hypocalcemia in dairy cows also suggest that the prevention of HC by nutritional techniques may have only solved the acute cases not the disease itself (Reinhardt et al. 2011).

The role of Vitamin D metabolites in the control of Ca and P metabolism in ruminants has been lagging other species. In both swine and poultry, the use of Vitamin D_3 and 25-hydroxycholecalciferol (25vitD) have been shown to improve bone strength, calcification, immunity and muscle content (Chou et al. 2009, Sugiyama et al. 2013). Often resulting in less morbidity and mortality as well as greater productivity. Yet, as an industry we have made very little effort to investigate the Ca status in the dairy cow, which has a similar amount of Ca excretion as a layer or sow.

This paper aims to describe the role 25vitD in the metabolism of Ca and P in lactating and peri parturient dairy cows. As well as demonstrate the integration of vitamin D and skeletal health into pasture based dairy systems. Furthermore, the hypothesis that the high producing TMR based dairy cow is likely to have reduced effects of HC and SHC when there is focus on skeletal health management during the full lactational cycle is presented.

Skeletal health as a driver of health and immunity

HC and SHC at parturition results in productivity losses and animal ill-health. Hypocalcaemia is estimated to cost $334 per incident (Guard 1996) and shortens the productive life of the dairy cow by 3.4 years (Chan *et al.* 2006). Longevity of a dairy cow's productive life is a key driver of efficiency and profitability (Essl 1998).

While the incidence of clinical hypocalcaemia in US herd is now typically below 5% the incidence of sub clinical deficiency is still close to 50% (Reinhardt et al. 2011). Why this is the case is not known. Perhaps it is due to the inability of high producing cows in the US to rebuild their skeletal Ca storage in the previous lactation (Horst 1986) or because of the loss of bone during transition period when consuming an anionic salt diet (Block 1984).

New Zealand's pasture based dairy system has a considerably lower milk production per unit of bodyweight (10L/Kg.LW NZ, 15L/Kg.LW USA, based on data generated by DairyNZ, USDA and USA Holstein association). However, these cows also suffer considerably from HC and SHC with DairyNZ (2012) publishing figures estimating HC at 7% and SHC at 33%.

The incidence rates of SHC in both TMR and pasture based dairy systems demonstrate that most dairy cows will likely face this challenge during one of their lactations. The implications for associated diseases, insulin sensitivity, loss of productivity and potentially fertility are well described elsewhere (McNeill and Anderson, 2012, McGrath et al, 2015). What is important to describe is the fact that the incidence of SHC is not linked simply to high levels of production. Both NZ (Pasture) and USA (TMR) dairy cows suffer from similar levels of SHC.

In both TMR and Pasture production systems the cow faces considerable challenges for maintaining optimal skeletal health. In the pasture based system the severity of poor skeletal health has now increased to the point that osteoporosis and osteomalacia are both clinically evident post peak lactation in the NZ dairy system (Dittmer et al. 2016). However, it is likely that the reasons for this susceptibility are considerably different.

McGrath et al. (2015) presented a case for poor bone repletion in dairy cows grazing pastures. In summary, pastures which are naturally high in K will result in sub-clinical Mg deficiency potentially resulting in sub optimal production of PTH leading to a lack of 1,25vitD and subsequent poor absorption of Ca and P. In specific cases sub optimal ratios of Ca and P would also impede absorption of these minerals. Furthermore, the lack of Vitamin D during the typical calving period would further create an issue for replenishment of skeletal reserves. However, in cows fed in TMR situations poor dietary mineral ratios should not be evident. Mineral concentrations and ratios are easy to manipulate and supplementation of Vitamin D3 is routine.

While the nutritional parameters of TMR fed cows should be optimal, epidemiological surveys would suggest that the incidence of hypocalcaemia is still a major problem (Reinhardt et al. 2011). Could it be that the level of production in TMR dairy cows result in an inability to replace skeletal Ca with standard nutritional parameters? The productivity of US dairy cows continues to increase. However, the nutritional targets set by the NRC are relatively slow moving. To compare in different species, it has now been demonstrated that in broilers optimal skeletal health, demonstrated by tibial strength, is achieved by a combination of vitamin D metabolites. Furthermore, in high producing dairy cows it has been demonstrated that the higher producing cows are more likely to have sub-clinical hypocalcaemia during early lactation than low producing cows (Jawor et al. 2012).

Many studies have demonstrated that dairy cows often experience negative Ca balance during early lactation (Ellenberger *et al*. 1931, Klooster 1976, Beighle 1999, Taylor *et al.* 2009) and that cows will often spend late lactation replacing the Ca lost from the skeleton in early lactation (Horst 1986, Liesegang *et al.* 2000). Furthermore, Ward *et al.* (1972) demonstrated that approximately 50% of the cows in their study were in negative Ca and P balances during the first 5 months of lactation, even when fed above the NRC (2001) Ca recommendations.

Several studies have attempted to clinically investigate the skeletal health of dairy cows with equivocal results (Liesegang et al. 2000; Taylor et al. 2009) This is likely because of the various states of bone, the labile bone Ca required by cows to maintain normal plasma Ca levels would be very hard to define by simple bone biopsy, blood markers of bone accretion and degradation or even x-ray. However, in extreme cases osteoporosis has been able to be demonstrated in dairy cows (Dittmer et al. 2016). Outside of extreme cases, it is still likely that poor skeletal health or put simply, the inability of cows to replace their skeletal Ca reserves during lactation (Horst 1986, NRC 2001) maybe a contributing factor for the increased incidence of sub clinical hypocalcaemia as cows age (Reinhardt et al. 2011).

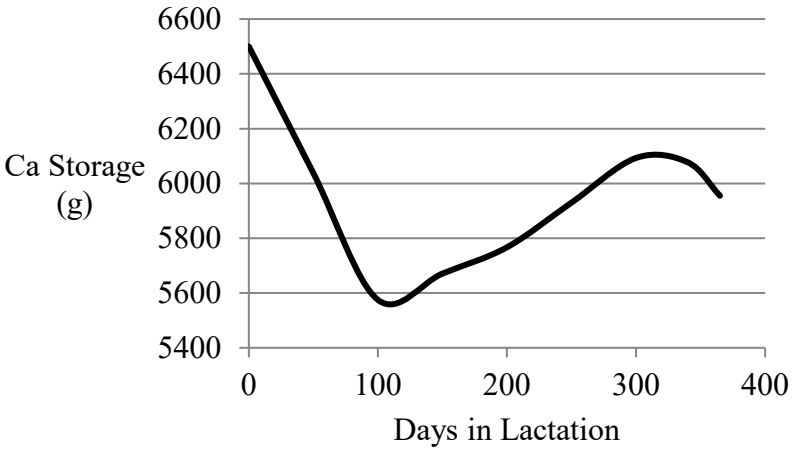

Figure 1. Ca status of a lactating dairy cow under typical Australian dietary and production parameters, mineral levels meeting NRC 2001 guidelines. Negative DCAD prepartum for 21d (McGrath et al. 2015).

Simply providing adequate dietary Ca concentrations may well meet the daily requirements of cows but they may not contribute to replacing the Ca lost during early lactation and the transition period, especially if anionic salts are used during transition. Ellenberger *et al.* (1931) suggested that 800 – 1300g of Ca were lost in early lactation which suggests that an extra 8 g/d of Ca are required in late lactation for replacement purposes (NRC 2001). This is not accounted for in currently published Ca requirements. Furthermore, when a simple model is created utilising NRC (2001) estimates for intake and production and Klooster (1976) absorption efficiency, for lactation and dry period respectively, the amount of Ca lost from the animal is approximately 9 g/d (Fig 1). This simple calculation reinforces the work conducted by Ellenberger *et al.* (1931).

Augmentation of Ca and P metabolism

To improve skeletal health in dairy cows, like what has been achieved in broilers, layers and sows, the animal must be able to generate sufficient supply of Ca and P in the plasma for bone deposition to occur. One likely way of achieving this is with the targeted use of the vitamin D metabolite 25vitD (Fig. 2). Alternatively, increasing bone repletion in dairy cows has also been attempted in pasture based dairy cows by manipulating DCAD by McNeill et al. (2002).

Vitamin D is well recognized as a requirement for the optimum absorption of Ca and P from the diet in mammals and has been demonstrated to improve Ca balance in cows during lactation (Ward et al. 1972). However, to achieve enhanced dietary Ca absorption driven by vitamin D metabolites, and not PTH, greater than normal concentrations 25vitD in plasma are required (McGrath et al. 2012, Oehlschlaeger et al. 2014).

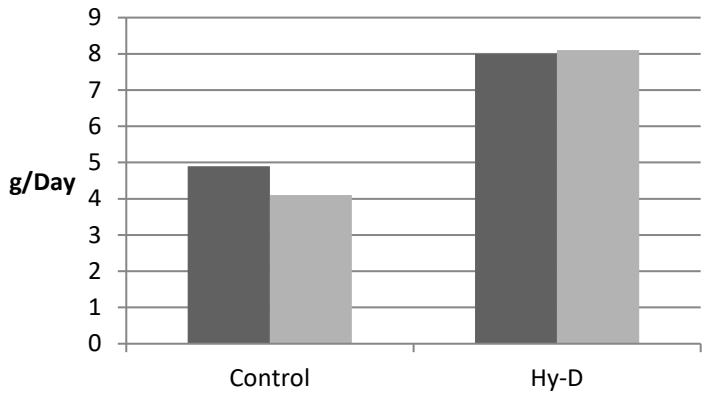

Figure 2 Dietary supplementation of 25vitD in cattle increases the retention of Ca (grey) and P (black) (McGrath et al. 2012)

Vitamin D metabolism is demonstrated in Figure 3 and the process is described in detail elsewhere (Fraser 1980). In summary, the production of 25vitD from Vitamin D3 is limited (Webb et al. 1983) and the risk of toxicity may be with the D3 compound not the metabolite 25vitD (pers comms. D. Fraser).

Recent studies utilizing the vitamin D metabolite 25vitD have demonstrated that Ca nutrition during different stages of the production cycle can be positively influenced. The combination of 25vitD and diets with negative DCAD have been shown to reduce the nadir of Ca in plasma immediately post calving (Wilkens et al. 2012) and reduce the incidence of diseases linked with sub-clinical hypocalcaemia (Martinez et al. in print). There have also been investigations into the use of 25vitD during lactation, with studies demonstrating an increase in absorption efficiency of both Ca and P as well as a reduction in bone degradation and increase in bone formation (Kim et al. 2011, McGrath et al. 2012, Oehlschlager et al. 2014). Furthermore, a recent study also demonstrated a link between 25vitD and energy metabolism (Rodney et al. 2017).

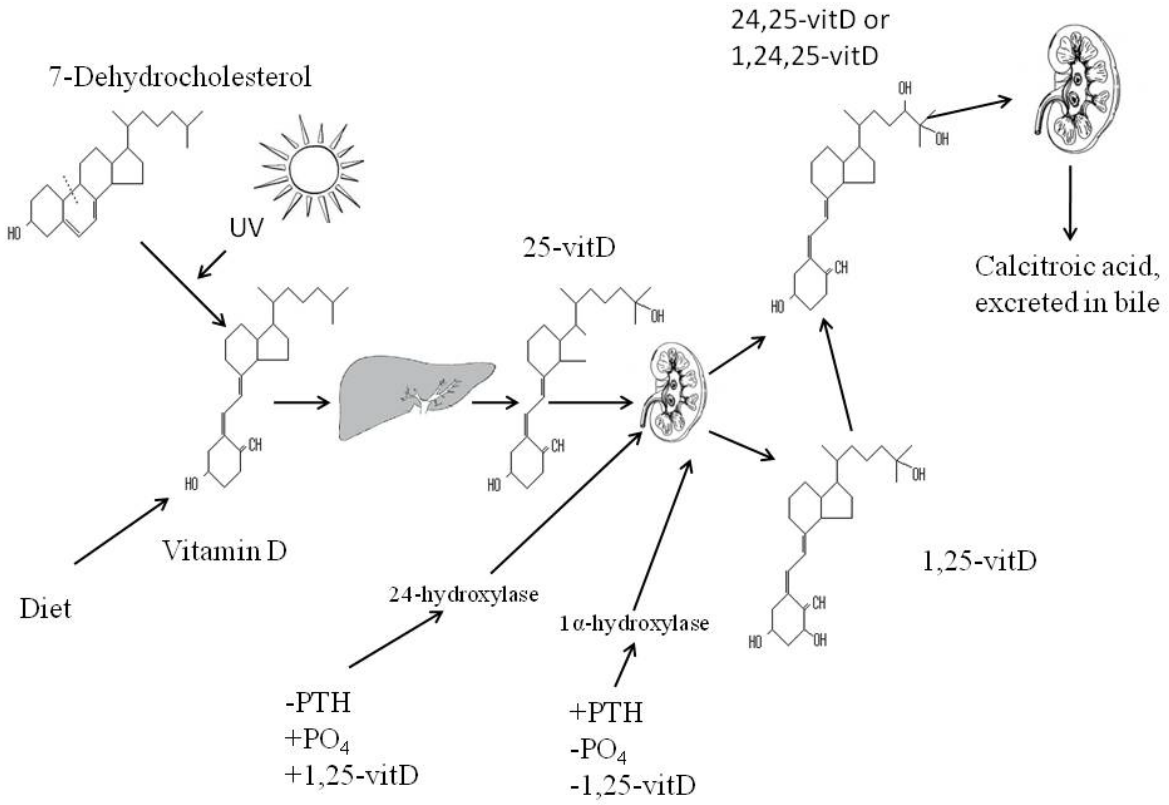

Figure 3 Synthesis and metabolism of vitamin D. Vitamin D is hydroxylated to 25vitD in the liver. Plasma concentrations of PTH, PO_4 and 1,25vitD determine the hydroxylation position of 25vitD in the kidney. Hydroxylation of 25vitD to 1,25vitD utilises the enzyme 1α-hydroxylase, whereas hydroxylation to 24,25vitD requires 24-hydroxylase (adapted from a combination of Cunningham, 1997 and Christakos et al., 2010).

A clear risk, if cows are not replenishing adequate reserves of Ca before parturition, is that the ability of the cow to liberate sufficient Ca at parturition will be compromised as she faces subsequent lactations. Limited reserves of body Ca could also compromise the effectiveness of anionic salts. Perhaps the increased incidence of clinical cases of milk fever in mature cows is not only a factor of slower humoral responses to vitamin D metabolites but also a consequence of reduced Ca status. Hibbs and Conrad (1976) demonstrated that in mature cows with a history of milk fever the incidence of milk fever in subsequent lactations was reduced (60% to 26.1%) when fed continuously with very large doses of vitamin D (~300 000 IU/day) during the lactation and dry period. This suggests that Ca skeletal storage was enhanced in susceptible cows and they could draw on this during parturition more effectively than the non-supplemented cows. The technique had no effect on cows with no milk fever history, suggesting that these animals may have already had adequate skeletal reserves

For the transition cow there is only a limited amount of labile bone Ca (soluble bone Ca), which is understood to be easily exhausted. Through a series of isotope studies the soluble Ca pool was estimated at 2% of a total of 7850g of Ca in a typical cow skeleton (Kleiber and Luick, 1956).

Further Ca release then must result from further osteoclastic degradation. Osteoclastic degradation is less responsive than absorption mechanisms in the alimentary tract and it also results in a release of P from bone stores, which may inhibit 1α-hydroxylase. The transition from labile Ca release to osteoclastic release may be the reason cows consuming transition diets that are anionic (DCAD < 0 mEq/kg) for 30d instead of 20d increase their risk of clinical hypocalcaemia by 42% (Lean *et al.* 2006).

Therefore, improvements in transition feeding should aim to increase Ca availability from diets. If supraphysiological levels of 25vitD can replace the actions of 1,25vitD in cattle, as they can in mice (Rowling et al. 2007), then the absence of PTH prior to parturition ensures that 25vitD will increase Ca absorption from the small intestine. This will benefit the cow at parturition as the labile bone stores of Ca should still be available to be activated by PTH release during parturition.

During a normal lactation cycle cows will spend approximately the last half of pregnancy replacing Ca in the skeleton that is lost during transition and early lactation (Liesegang et al. 2000). Therefore, 25vitD may increase the rate of this activity, potentially making up for the extra Ca lost as a consequence of anionic salt treatments and high milk production. The use of 25vitD during late lactation should enhance absorption of Ca and P from the diet, thereby providing more substrate for bone deposition without the need for increasing dietary concentrations of Ca and P.

Furthermore, 25vitD supplementation may also prove valuable in early lactation. The net loss of Ca is a characteristic of this period (Liesegang *et al.* 2000). Elevated concentrations of 25vitD will increase the availability of Ca and P from the diet in beef steers (McGrath *et al.* 2012) and potentially increase bone deposition, so that net bone loss could either reduced and high production cows may spend less time in SHC state post parturition.

While the direct implications of optimal skeletal Ca reserves on Ca availability at parturition and lactation are easily linked, the implications that Ca status may have on other metabolic factors are less apparent. The skeleton has been recently proposed as being a source of a hormone that may have implications for the energy regulation within cows. A review by McNeill and Anderson (2012) has built upon work undertaken for human nutrition, although using mice as the model.

The review builds on work done by Karsenty (2011) and follows on from the finding that glucose uptake is enhanced by undercarboxylated osteocalcin (gluOC) a hormone released by bone, that acts to increase insulin production and secretion by the pancreas, as well as by causing a diverse range of tissues, such as the adipose, liver, and muscle, to become more sensitive to insulin. Basically, insulin signals osteoblasts to help promote normal glucose homeostasis by inhibiting osteoprotegerin (Simonet *et al.* 1997), an inhibitor of osteoclast differentiation, thus stimulating bone resorption. This in turn stimulates decarboxylation (activation) of osteocalcin (Ferron *et al.* 2010), which in turn stimulates insulin release and sensitivity in muscle, liver and adipose tissue (Lee *et al.* 2007). Studies in both humans and mice have shown that disruption of osteocalcin signaling leads to insulin resistance and glucose

intolerance (Oury *et al.* 2013). This finding links the two largest gate way diseases for dairy cows; hypocalcaemia and ketosis (McNeil and Anderson, 2012). The mechanism of bovine ketosis is not well understood, but involves the intense mobilization of fat and a high glucose demand, with a reduced secretion of insulin (Baird 1982).

The proposition of McNeill and Anderson (2012) is that healthier bone prior to calving will have maximum Ca storage, be more reactive to metabolic Ca demands and improve the cow's capacity to recharge lost Ca in later lactation. Furthermore, the active skeleton will secrete optimum levels of gluOC which in turn may optimize insulin sensitivity resulting in greater control of ketosis and NEB.

The healthy bone hypothesis of McNeill and Anderson (2012) may also help to explain the increased incidence of hypocalcaemia as cows age. Lower skeletal activity, precipitated by lower Ca status prior to parturition will produce less gluOC as a consequence of there being less bone available for rapid degradation during transition and early lactation. The relationship between ketosis and Ca homeostasis is synergistic as hypocalcaemia can initiate ketosis post calving. However, lower feed intake, precipitated by ketosis, exacerbates the negative Ca balance during early lactation and amplifies the challenge of replenishing Ca prior to the subsequent lactation. Further to this, gut motility is compromised by hypocalcaemia (Jorgensen *et al.* 1998) and may also limit food intake and/or digestion and the severity of NEB during early lactation is correlated with a reduction in fertility.

Conclusion

The role of skeletal health in the modern dairy cow is critical to the optimum metabolic health of cows at parturition regardless of the production system the cow is managed in. The involvement of the skeleton in the control of Ca and P status, as a reservoir at parturition and early lactation means that the skeleton plays a key role in resistance to HC. However, a focus on the skeletons status in further research may enable the industry to reduce incidence rates of SHC and its related diseases such as ketosis and metritis. Further work should focus on the skeleton status, which requires analysis of mass, content and structure.

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