# Vitamin $D_3$ metabolism after ultra-high supplementation to beef animals to alleviate the effects of beta-agonist supplementation in feedlot cattle.

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*Abstract*— **To determine vitamin $D_3$ and derivative 25-hydroxyvitamin $D_3$ levels in liver, meat and fat of feedlot animals treated with vitamin $D_3$ to alleviate the effects of beta-agonist supplementation and correlate them with blood parathyroid hormone, calcium and phosphate. Vitamin $D_3$ is metabolised in the liver into 25-vitamin $D_3$ and amongst others it controls calcium and phosphate homeostasis being regulated by parathyroid hormone. This system plays a major role in controlling the level of calcium for proteases (calpains) activation that degrades the meat proteins. It is hypothesised that ultra-high vitamin $D_3$ supplementation should alleviate negative effects of beta-agonist supplementation, but toxic levels of vitamin D for human consumption is possible. In this study, 20 young steers received no beta agonist or vitamin $D_3$ (C), 20 animals each of 100 received zilpaterol hydrochloride (Z) and various levels (*x*M=*x* million units), days fed (*y*D) and withdraw period (*z*N) of vitamin $D_3$, C, Z, Z3D7M, Z6D7M, Z6D7M7N, Z9D1M treatment groups. Samples of blood and liver, meat and fat were analysed for vitamin $D_3$, 25-hydroxyvitamin $D_3$, serum calcium, phosphate and parathyroid hormone. Levels of vitamin $D_3$ and 25-hydroxyvitamin $D_3$ differed corresponding the amount fed and were mostly lower than the RDA for adults. There were high level outliers noticed in the liver of this groups 6D7M and 9D1M (higher than the average RDA for infants and children of 400 IU and 2000 IU for adults). Overall calcium and phosphate correlated positively, and parathyroid hormone negatively with vitamin $D_3$ and 25-hydroxyvitamin $D_3$ in meat and fat.**

*Keywords*— **V**itamin $D_3$, beta-agonist supplementation

## **I**. INTRODUCTION

Beta agonists are fed to feedlot cattle and are known to affect meat tenderness (and other quality traits) negatively due to an increase in calpastatin activity. Various researchers suggest that supplementing extremely high levels of dietary vitamin $D_3$ for a limited time prior to slaughter improved meat tenderness by increasing blood calcium levels which play an important role in activating the calpain protease system [1][2]. Meat quality and more specific meat tenderness is the most important challenging factors to achieve by meat producers to meet consumer satisfaction.

There are various factors that can affect the quality of meat e.g. calcium content of the meat. The relationship between calcium level and vitamin $D_3$ content of the meat has been reported [3]. Vitamin D is one of the groups of fat soluble molecules appearing mainly in two forms being vitamin $D_2$ (ergocalciferol) and vitamin $D_3$ (cholecalciferol) cholesterol derivative. Vitamin $D_3$ is converted to 25-vitamin $D_3$ in the liver by the enzymatic system in the mitochondria [3]. It is further metabolized in the kidney to 1, 25-hydroxyvitamin $D_3$. Metabolism of 25-hydroxyvitamin $D_3$ in the kidney is regulated by hormones and some ions. Parathyroid hormone is one of the hormones which is said to regulate the metabolism of 25-hydroxyvitamin $D_3$ by triggering the activity of 25-hydroxyvitamin $D_3$-1α-hydroxylase. This active metabolite (1α, 25-hydroxy-vitamin $D_3$) exerts its action by binding to the nuclear proteins called vitamin D receptors. After binding they alter the rate of transcription of target genes. The proteins transcribed then carry the real functions of vitamin $D_3$ [4] which is the maintenance of serum calcium and phosphorus homeostasis, [6].

Methods have been developed to extract and quantify the amount of vitamin D in various food sources. One of the methods is by means of high performance liquid chromatography (HPLC) which determines the total amount of vitamin D content of a particular food source.

As any other biomolecule vitamin D taken at high dose can results in vitamin D intoxication. Some symptoms of vitamin D toxicity are a result of hypercalcemia caused by increased intestinal calcium absorption.

To enable the understanding of the metabolism of Vitamin D in beef animals and whether there is an advantage in the treatment of the animals to alleviate the toughening effect of beta-agonist-treatment on the resultant meat product, without being detrimental to health safety, the different levels of Vitamin D metabolites, calcium- and phosphorus levels, parathyroid hormone were quantified.

## **II.** MATERIALS AND METHODS

In this study, 20 young steers received no beta agonist or vitamin $D_3$ (C), 20 animals each of 100 received zilpaterol hydrochloride (Z) and various levels (*x*M=*x* million units), days fed (*y*D) and withdraw period (*z*N) of vitamin $D_3$, i.e. C, Z, Z3D7M, Z6D7M, Z6D7M7N, Z9D1M treatment groups. Samples of blood and liver, meat and fat were analysed for vitamin $D_3$, 25-hydroxyvitamin $D_3$, serum calcium, phosphate and parathyroid hormone. The samples were liver, meat (*M. longissimus lumborum*), fat and blood. The samples were frozen at -20 ˚C till further analysis.

### *A. Vitamin $D_3$ and 25-vitamin $D_3$ analyses*

Vitamin $D_3$ and 25-vitamin $D_3$ were assayed by the HPLC method adapted from [5] with modifications [6]

A 200 µl of vitamin $D_3$ extract was injected in Shimadzu HPLC (JAPAN) equipped with Reservoir Tray, Prominence Diode Array Detector with a thermostatic standard cell according to Moloto et al., (2010) [10 ]. Vitamin $D_3$ was quantified from its peak height using vitamin $D_2$ as an internal standard. Quality control tests were done according to [10]

### *B., Calcium, phosphate and parathyroid hormone analyses.*

Serum calcium and Phosphorus concentrations were analysed by means of colorimetric assay kit (Roche, Mannheim, Germany). Plasma parathyroid hormone levels (PTH) were determined by electrochemiluminescence immunoassay employing a sandwich test principle on a Modular Analytics E 170 (Roche Diagnostic Systems, Nutley, NJ).

### *C., Statistical analysis*

Data was analyzed by Analysis of Variance (ANOVA), for a split-plot design [7]

## **III.** RESULTS AND DISCUSSION

Feeding high levels of vitamin $D_3$ to beef animals may subject them to accumulation of the compound in the liver and meat and other tissue cells. In this study vitamin $D_3$ supplementation exerted different effects in the studied tissues as shown in Table 1. All different Vitamin $D_3$ supplementation showed tremendous increase in liver vitamin $D_3$ and a slight increase in liver 1, 25-vitamin $D_3$. Liver, meat and fat showed differences in metabolism of vitamin $D_3$. Liver is the tissue that has the highest accumulated by vitamin $D_3$. The supplementation of 7 million IU for six days resulted in approximately 74 fold increase of the liver Vitamin $D_3$ and 8 fold increases in the meat when compared to the un-supplemented animals. Before 1997, the recommended dietary allowance of vitamin D (RDA; 3) for infants and children was 10 g (400 IU) and for adults 50 g (2000 IU) and had long been considered safe and effective in preventing rickets [8]. On contrary the recent studies shows that the dose that was first presumed to be toxic was not. Hathcock [9] reviewed the levels (400 IU) that were thought to be upper limit for Vitamin $D_3$ and found that actually newer clinical trial data were sufficient to show that vitamin D is not toxic at intakes much higher than previously considered unsafe. He found that toxicity was absent in healthy adults that used vitamin D dose $\geq$ 250 µg/md (10 000 IU). So depending on the view point taken the high vitamin $D_3$ in the liver and fat could be toxic or not, the Z3D7M and Z6D7M treatment groups exceeded the average RDA of 400 IU which is equivalent to 10 µg. None the less there were high level outliers noticed in the liver for groups 6D7M and 9D1M and therefor the safety of those tissues for human consumption or not should still not be taken lightly and should be investigated.

Vitamin $D_3$ supplementation did not have a significant effect on the different increasing blood calcium levels in the highly supplemented group (7 million IU) that is justified by the low blood parathyroid hormone. Parathyroid hormone is secreted when blood calcium is very low. Even though the liver and the meat of the highly supplemented group (7 million IU) had high vitamin $D_3$ levels but fairly less blood calcium than untreated group, this scenario has been discussed by [10], explaining that most of the circulating vitamin $D_3$ is bound to the vitamin D binding proteins. Protein bound vitamin D metabolites have limited access to target cells and hence increased half-life in circulation. Thus, vitamin D and metabolites have high half-life in circulation. Only free fractions of vitamin D are metabolized and tissue availability of vitamin D is determined by the free fraction like most other hormones. Similarly [11] also found that feeding high Vitamin $D_3$ on lambs did not improve the tenderness or aging characteristics of lamb muscles. He suggested that other factors, such as the hormone calcitonin, may be regulating calcium concentrations and limiting its deposition in muscle tissues. Further research is needed to understand calcium regulation to determine how vitamin $D_3$ can be used as a tool to improve tenderness.

## **IV.** CONCLUSION

The results of supplementation mostly 7 million IU/animal.day in this study shows high accumulation of vitamin $D_3$ in liver and all pose potential toxicological hazards. The same results has been demonstrated by [2] and suggested that liver from supplemented animal specifically the 7 million IU/animal.day should be eliminated from the food chain and producers should be discouraged from using this rate of supplementation. In contrast it was [10] suggested that vitamin D intake should be higher to achieve additional benefits from this vitamin at higher levels than previously recognized

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Table 1. Least square mean values and standard errors of means (SEM) for vitamin $D_3$ and derivative 25-hydroxyvitamin $D_3$ levels in liver, meat and fat of feedlot animals treated with different levels of vitamin $D_3$ to alleviate the effects of beta-agonist supplementation.

| | Vitamin $D_3$ treatment × $10^6$ IU/(animal .day) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Z3D7M | Z6D7M | Z6D7M7N | Z9D1M | Control(C) | Zilmax(Z) | SEM[e] | P-value |
| Liver µg/100g | | | | | | | | |
| Vit-D3 | 48.7[c] | 74.4[d] | 10.7[b] | 16.9[b] | 0.8[a] | 1.7[a] | 3.014 | <0.001 |
| 25-Vit-$D_3$ | 0.086[b] | 0.162[d] | 0.120[c] | 0.080[b] | 0.060[a] | 0.095[b] | 0.006 | <0.001 |
| Meat µg/100g | | | | | | | | |
| Vit-D3 | 4.78[b] | 8.39[c] | 4.23[b] | 3.71[b] | 0.8[a] | 1.74[a] | 0.418 | <0.001 |
| 25-vit-$D_3$ | 0.536[a] | 1.023[b] | 0.702[a] | 1.023[b] | 0.708[a] | 0.570[a] | 0.072 | <0.001 |
| Fat µg/100g | | | | | | | | |
| Vit-$D_3$ | 13.65[bc] | 18.44[c] | 16.61[c] | 11.45[b] | 3.94[a] | 2.31[a] | 1.633 | <0.001 |
| 25-vit-$D_3$ | 0.336[b] | 0.415[c] | 0.404[c] | 0.274[ab] | 2.862[b] | 2.886[b] | 0.023 | <0.001 |

[a,b,c,d] Different superscripts in a row shows significant difference (<0.001)

[e] Standard error of the mean

Table 2 Effect of supplementation on serum calcium, phosphate and parathyroid hormone (PTH)

| Treatment | Z3D7M | Z6D7M | Z6D7M7N | Z9D1M | Control(C) | Zilmax(Z) | SEMe | P-value |
|---|---|---|---|---|---|---|---|---|
| Calcium mg/100ml | 2.522[a] | 2.567[a] | 2.715[b] | 2.781[b] | 2.862[b] | 2.862[b] | 0.063 | <0.001 |
| Phosphate mg/100ml | 2.836 | 2.920 | 2.926 | 2.945 | 2.586 | 2.541 | 0.091 | 0.003 |
| PTH pg/100ml | 18.1[a] | 22.9[a] | 25.4[ab] | 37.1[b] | 61.6[c] | 82.2[d] | 4.36 | <0.001 |

[a,b,c,d] Different superscripts in a row shows significant difference (<0.001)

[e] Standard error of the mean

Table 3. Correlation-matrix showing how vitamin $D_3$ and derivative 25-hydroxyvitamin $D_3$ levels in liver, meat and fat correlate with blood parathyroid hormone (PTH), calcium and phosphate.

| | Vit-$D_3$ liver | Vit-$D_3$ meat | Vit-$D_3$ fat | 25-Vit-$D_3$ liver | 25-Vit-$D_3$ meat | 25-Vit-$D_3$ fat |
|---|---|---|---|---|---|---|
| Calcium | 0.196 | 0.243 | **0.354** | 0.117 | 0.126 | 0.023 |
| Phosphate | -0.135 | 0.082 | -0.099 | -0.007 | **-0.400** | 0.241 |
| PTH | **-0.326** | **-0.477** | **-0.400** | -0.244 | -0.174 | **-0.338** |

Values in bold correlates significantly ($p<0.05$).