Supplementation to improve beta-agonist beef quality - relationship of vitamin D_3 and 25-hydroxivitamin D_3 levels in meat with measured meat tenderness characteristics

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Abstract—**The combination of zilpaterol and vitamin D supplementation is detrimental to calpain proteolytic degradation and subsequent meat tenderness [1]. The relationship of vitamin D_3 ($VitD_3$) and derivative 25-hydroxyvitamin D_3 (25-$VitD_3$) levels in meat with the Warner Bratzler shear force (WBS) and myofibril fragmentation (MFL) at 3d and 14d post slaughter and calpain system levels at 1h post slaughter of feedlot animals treated with vitamin D_3 in combination with beta-agonist supplementation were determined.**

Beta agonists used to improve feed efficiency and yield are known to affect meat tenderness negatively. Procedures like electrical stimulation however, could accelerate rigor and the aging process. The supplementation of very high levels of vitamin D_3 a few days prior to slaughter has also been used to improve meat tenderness. 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. After slaughter carcasses were split, the left side electrically stimulated (ES) and the right side not stimulated (NES). Loin samples were analysed for $VitD_3$, 25-$VitD_3$, calpastatin and µ-calpain enzyme activity. Samples aged for 3 and 14 days post mortem were analysed for WBS and MFL.

$VitD_3$, and 25-$VitD_3$ levels in meat correlated positively with WBS, MFL and calpastatin and negatively with µ-calpain enzyme activity.

The combination of zilpaterol and vitamin D supplementation is detrimental to calpain proteolytic degradation and subsequent meat tenderness.

Keywords— **Tenderness, Vitamin D_3, Zilpaterol, electrical stimulation, calpains.**

I. INTRODUCTION

A large portion of South African feedlot cattle are supplemented with a beta agonist to improve feed efficiency and yield. Beta agonists are known to affect meat tenderness (and other quality traits) negatively due to an increase in calpastatin activity [2]. Hope-Jones, Strydom, Frylinck & Webb (2010) [3] reported that electrical stimulation combined with post mortem aging could improve, but not completely overcome, the negative effect of a beta agonist on beef loin tenderness.

Supplementation of very high levels of vitamin D_3 over the final days before slaughter to activate the calcium-dependent protease system and overcome meat tenderness problems was tested. Unexpectedly vitamin D_3 supplementation did not have the expected improvement effect on meat tenderness in ß-agonist treated beef. Shorter but higher dose (3D7M) and longer but lower dose (9D1M) of vitamin D_3 showed small improvements in tenderness, under conditions of no electrical stimulation [1]. The benefit of using electrical stimulation on its own gave better results on improving ß-agonist treated beef compared to vitamin D_3 with no stimulation. Furthermore, with electrical stimulation, no added advantage of feeding vitamin D_3 is achieved [1]. The relationship of vitamin D_3 ($VitD_{3)}$ and derivative 25-hydroxyvitamin D_3 (25-$VitD_3$) levels in meat with the Warner Bratzler shear force (WBS) and myofibril fragmentation (MFL) at 3d and 14d post slaughter and calpain system levels at 1h post slaughter could help explain these unexpected results.

II. MATERIALS AND METHODS

Young steers (20) 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. *M. longissimus lumborum)* samples were analysed for vitamin D_3, 25-hydroxyvitamin D_3, The samples were frozen at -20 ˚C or -80 ˚C till further analysis. The

following tests were conducted: Meat tenderness, measured by Warner-Bratzler shear force (WBS), and myofibril fragment length (MFL) aged 3 and 14 days post mortem (2 - 4 °C) as described in [1]. Proteinase enzyme system was measured as calpastatin activity: µ-calpain activity at 1 h post mortem as described in [1]. One unit of calpastatin activity was defined as the amount that inhibited one unit of m-calpain activity. One unit of calpain activity was defined as an increase in absorbance at 366 nm of 1.0 per hour, at 25 °C. .

$VitD_{3,}$ and 25-$VitD_3$ levels were assayed by the HPLC method adapted from [4] with modifications [5]. $VitD_{3,}$ and 25-$VitD_3$ were quantified from peak heights using vitamin D_2 as an internal standard. Quality control tests were described [5].

Data was analyzed by Analysis of Variance (ANOVA), for a split-plot design [6].

II. RESULTS

The relationship between $VitD_{3,}$ and derivative 25-VitD levels measured in *M. longissimus lumborum* (LL) (secondary axix) and Warner Bratzler shear force (WBS; $P < 0.001$), calpastatin activity/ µ-calpain activity and myofibril fragmentation (MFL) (primary y-axix) determined for LL of treatment groups (C, 3D7M, 9D1M, 6D7M7N, 6D7M, Z) in electrical stimulation (ES) and non-electrical stimulated (NES) carcasses are presented in Figures 1, 2 and 3 respectively. Treatment had a significant effect ($P < 0.001$) on $VitD_{3,}$ and derivative 25-VitD levels similar to WBS, calpain system and MFL. Both electrical stimulation (ES) and prolonged aging reduced WBS significantly ($P < 0.001$) relative to no stimulation (NES) and aging for three days, respectively. A significant interaction ($P < 0.001$) occurred between treatment and stimulation regarding WBS. ES had very little effect on the tenderness of C, 3D7M and 9D1M but did have a significant effect on 6D7M7N, 6D7M and Z. ES did not however improve WBS of these 3 groups to the level of C but did improve their WBS to the level of the 3D7M and 9D1M groups. The interaction between treatment and aging for WBS ($P < 0.001$) with 6D7M, Z and 6D7M7N groups showing a faster aging rate than the 3D7M and 9D1M groups and C being least affected by aging but overall still being the most tender group (results published in [1].

III. DISCUSSION

Interestingly the $VitD_{3,}$ levels in meat follow the profile of WBS, calpastatin/µ-calpain and MFL measured in the different treatments and also corresponded with the amount of vitamin D_3 fed to the treatment groups initially. The control group that did not receive any additional vitamin D than naturally available was the most tender and electrical stimulation had a small benefit on tenderness. In this study MFL seemed to be slightly negatively affected by ES (a measurement only taking the perpendicular breaks into account). As expected the Zilmax group had higher WBS, higher calpastatin/µ calpain and longer MFL. Naturally available vitamin D_3 was higher in the Zilmax group than control, maybe because metabolic feedback control resulted in higher vitamin D levels where it was “needed”. Although to a certain level, additional vitamin D did seem to benefit Z3D7M and Z6D7M7N groups although still far from that of C, over supplementation such as in Z6D7M seem to have an inhibitory effect on the calpain system which is reflected in the high calpastatin/µ-calpain levels measured in this group.

IV. CONCLUSION

: It seems that there is a fine line between the beneficial availability of vitamin D and the over dose of vitamin D which will rather push the tenderisation mechanism in more tougher direction, keeping in mind that the natural dynamics would probably be in the direction of building the muscle (preservation of life?) vitamin D being an antioxidant.

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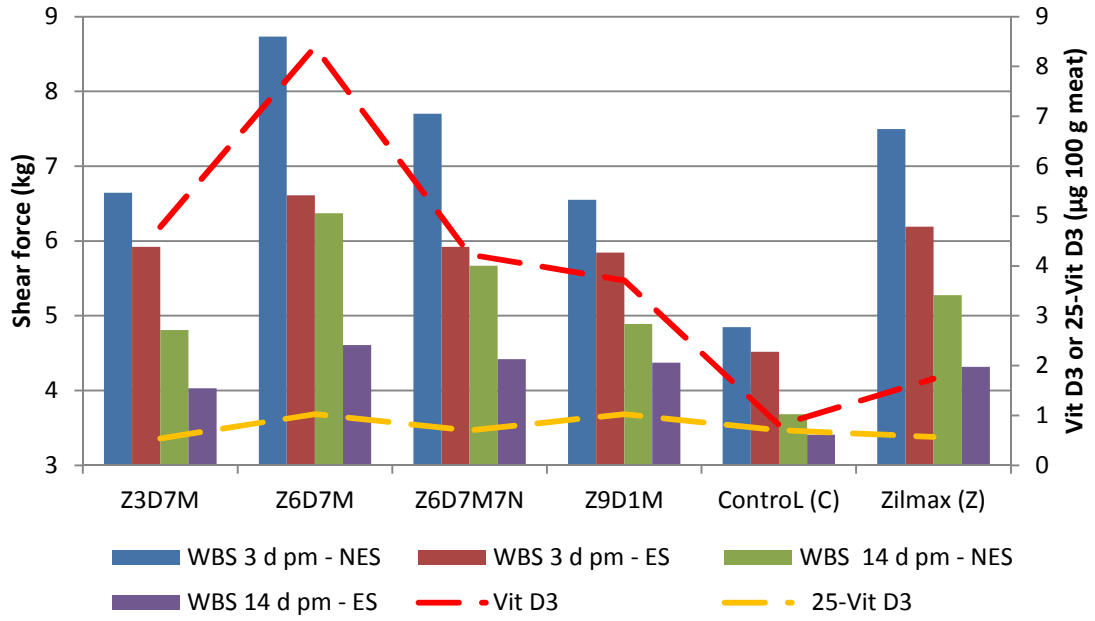

Figure 1. Relationship between vitamin D_3 and derivative 25-hydroxyvitamin D_3 levels determined in meat (*M. longissimus lumborum)* and Warner Bratzler shear force (WBS; $P < 0.001$) (primary y-axix) of treatment groups (C, 3D7M, 9D1M, 6D7M7N, 6D7M, Z) in electrical stimulation (ES) and non-electrical stimulated (NES) carcasses.

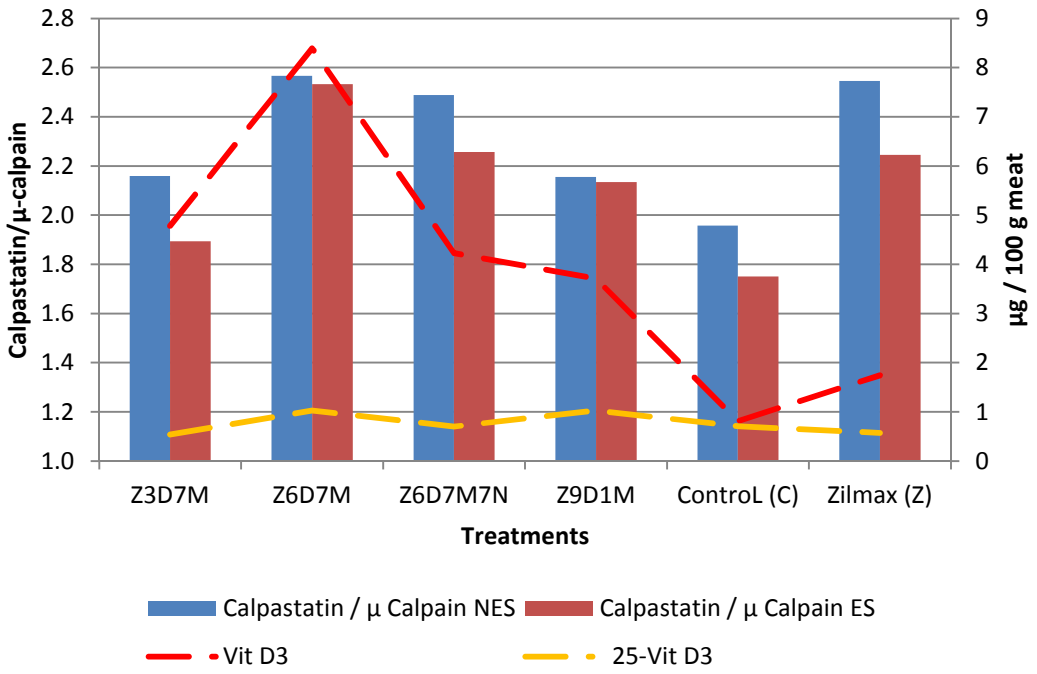

Figure 2. Relationship between vitamin D_3 and derivative 25-hydroxyvitamin D_3 levels determined in meat (*M. longissimus lumborum)* and the ratio between calpastatin activity and calpain activity ($P < 0.001$) (primary y-axix) of treatment groups (C, 3D7M, 9D1M, 6D7M7N, 6D7M, Z) in electrical stimulation (ES) and non-electrical stimulated (NES) carcasses.

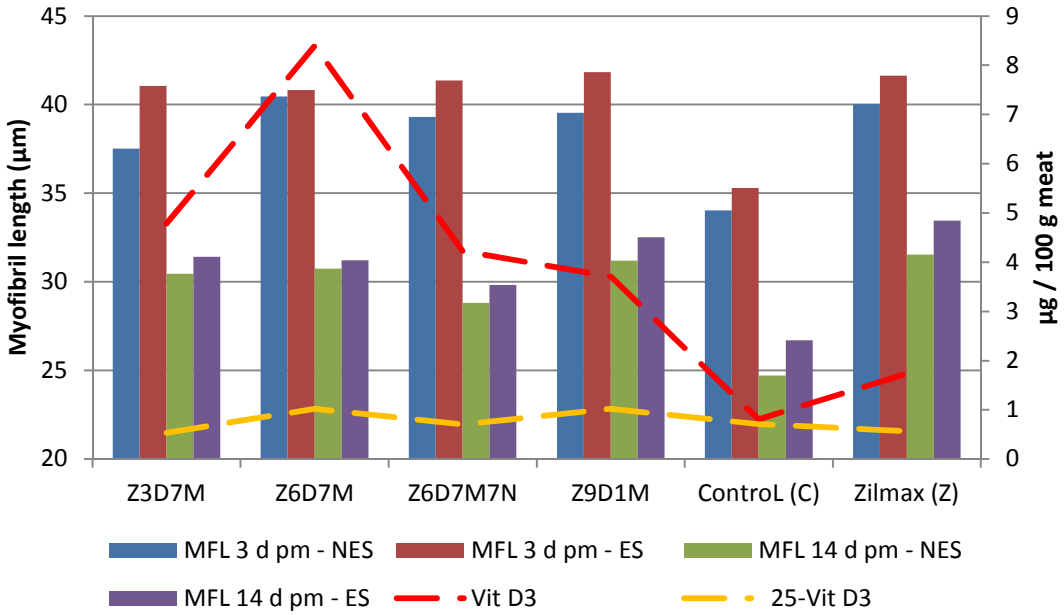

Figure 3. Relationship between vitamin D_3 and derivative 25-hydroxyvitamin D_3 levels determined in meat (*M. longissimus lumborum)* and myofiber fragmentation length (MFL) ($P < 0.001$) (primary y-axix) of treatment groups (C, 3D7M, 9D1M, 6D7M7N, 6D7M, Z) in electrical stimulation (ES) and non-electrical stimulated (NES) carcasses.