

# **The effects of a beta-agonist treatment, Vitamin D<sub>3</sub> supplementation and electrical stimulation on meat quality of feedlot steers**

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## **Abstract**

In this study, 20 young steers received no beta-agonist (C), 100 animals all received zilpaterol hydrochloride (Z), with 1 group only receiving Z while the other 4 groups received zilpaterol and vitamin D<sub>3</sub> at the following levels (IU/animal /day) and durations before slaughter: 7 million for 3 days (3D7M); 7 million for 6 days (6D7M); 7 million for six days with 7 days no supplementation (6D7M7N) and 1 million for 9 days (9D1M). Left carcass sides were electrically stimulated (ES) and the right side not stimulated (NES). Samples were aged for 3 or 14 days post mortem. Parameters included Warner Bratzler shear force (WBSF), myofibril filament length, sarcomere length and calpastatin and calpain enzyme activity.

Both ES and prolonged aging reduced WBSF ( $P < 0.001$ ). 6D7M, 6D7M7N and Z remained significantly tougher than C ( $P < 0.001$ ), while 3D7M) and 9D1M improved WBSF under NES conditions. ES is more effective to alleviate beta-agonist induced toughness than high vitamin D<sub>3</sub> supplements.

**Keywords:** Vitamin D<sub>3</sub>, zilpaterol, electrical stimulation, calpains, meat tenderness

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## 1. 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 (Koochmaraie, Shackelford, Muggli-Cockett, & Stone, 1991). Electrical stimulation (ES) could improve meat tenderness by early activation of the calpain system (calcium-dependent proteases) (Hwang & Thompson 2001a). Hope-Jones, Strydom, Frylinck, and Webb (2010) showed 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.

Various other attempts have been made to activate the calcium-dependent protease system and overcome meat tenderness problems. Wheeler, Koochmaraie, Lansdell, Siragusa, and Miller, (1993), Kerth, Miller, and Ramsey (1995) and others have used CaCl<sub>2</sub> injections in pre- and post-rigor cuts to activate intra-cellular  $\mu$ - and m-calpain (Goll, Thompson, Taylor, & Zalewska., 1992; Koochmaraie & Shackelford, 1991). Koochmaraie and Shackelford (1991) also showed that CaCl<sub>2</sub> infusion was effective in overcoming beta-agonist induced toughness. In more recent years, supplementation of very high levels of vitamin D<sub>3</sub> over the final days before slaughter has been investigated by Montgomery et al. (2002), Montgomery, Blanton, et al. (2004) and others to increase plasma calcium levels by stimulating intestinal calcium absorption, mobilizing calcium from bone mineral and increasing renal re-absorption of calcium (as reviewed by

Swanek et al., 1999). Higher blood and muscle calcium would lead to increased activation of the calpains and improved tenderness (Karges, Brooks, Morgan, Gill, Breazile, & Owens, 2001; Montgomery et al., 2002; Montgomery, King, et al., 2004). Various studies achieved success with the latter method but reports are not consistent with regards to the level and duration of supplementation as well as the magnitude of the effects. In addition, no studies have reported on vitamin D<sub>3</sub> supplementation to overcome the negative effects of beta-agonists. It is also yet to be verified if the effect of electrical stimulation and vitamin D<sub>3</sub> are additive.

In this trial we investigated various levels and durations of vitamin D<sub>3</sub> supplementation in an attempt to establish the best scenario in terms of cost, safety and efficacy. Our choices were based on previous studies with variable outcomes. Karges, Brooks, Morgan, Gill, Breazile, and Owens (2001) used 6 million IU/animal per day for 4 days and 6 million IU/animal for 6 days resulting in increased blood plasma concentrations and improved beef tenderness. Tipton, King, Paschal, Hale and Savell (2007), on the other hand, used 3 million IU/animal per day for 5 days and found an increase in calcium levels but no improvement in WBSF. Tenderness did however improve when vitamin D<sub>3</sub> was withdrawn for 7 days after 5 days supplementation. The withdrawal also resulted in lower levels of vitamin D<sub>3</sub> in the liver, but higher levels of calcium were maintained improving tenderness. Montgomery et al. (2002) reported positive effects for tenderness at a relatively low dose (0.5 IU/animal per day) of vitamin D<sub>3</sub> for 9 days without negatively affecting feedlot performance (feed intake) or tissue residues. We have used these scenarios to design our study and also added a short duration of 3 days at 7 million IU/animal per day in an attempt to take advantage of increased calcium levels before homeostasis could restore blood calcium to normal levels.

## 2. Materials and methods

### 2.1. *Experimental design and feedlot phase*

One hundred and twenty Bonsmara steers of approximately 9 months of age were raised on a commercial feedlot diet for 120 days. The animals were divided into six groups (n = 20) so that the average weight and variation was the same for all groups. The groups represented 6 treatments, namely a control (C), which received the feedlot diet only, while the five remaining groups were supplemented with the beta-agonist, zilpaterol hydrochloride, (Intervet/Schering-Plough Animal Health, South Africa) at 0.15 mg/kg live weight for 30 days during the final weeks of finishing. One of the five groups only received zilpaterol (Z), while the other four groups received zilpaterol and vitamin D<sub>3</sub> (vitamin D<sub>3</sub> 500, Advit Animal Nutrition S.A. (Pty) Ltd, Sebenza, South Africa) at the following levels and durations before slaughter (D = days supplemented; M = million IU; N = days withdrawn): 7 x 10<sup>6</sup> IU/animal /day for 3 days prior to slaughter (3D7M); 7 x 10<sup>6</sup> IU/animal /day for 6 days prior to slaughter (6D7M); 7 x 10<sup>6</sup> IU/animal /day for 6 days followed by 7 days of no supplementation prior to slaughter (6D7M7N) and 1 x 10<sup>6</sup> IU/animal /day for 9 days prior to slaughter (9D1M). In abbreviations of treatments D = days, M = million, N = no supplement. Zilpaterol was withdrawn from feed 4 days prior to slaughter. The vitamin D<sub>3</sub> supplement was mixed with maize to increase the volume and supplied as (1 kg) top dressing on the daily supply of the feedlot diet (10 animals per pen). To ensure equal and complete consumption of the supplement, a clean bunk approach was followed, i.e. all feed was consumed before new feed was given each morning. All animals in each pen therefore approached the bunk at the same time and sufficient bunk space was provided so that all animals per pen could have access to feed at the same time.

## 2.2. Slaughter and sampling procedures

The animals were slaughtered at the abattoir of the Animal Production Institute (Agricultural Research Council, Irene, Gauteng Province). Blood was collected from all steers at exsanguination using sodium EDTA (parathyroid analysis) and lithium heparinised (calcium analysis) containers and stored on ice. After centrifugation, plasma and serum was stored at  $-18\text{ }^{\circ}\text{C}$  until analysed. Carcasses were split and the left sides were electrically stimulated for 30 seconds (400 V peak, 5 ms pulses at 15 pulses per second) within 30 minutes of killing. Carcass sides were then chilled at  $3 \pm 2\text{ }^{\circ}\text{C}$  (chiller temperature at loading). Temperature and pH measurements of the *M. longissimus lumborum* (LL) were taken every hour for 4 h and a final measurement was taken at 18 h post mortem. All samples were collected from the LL on the day of slaughter, or after rigor *mortis*, depending on the purpose of the sample. The following tests were conducted: a) Meat tenderness, measured by Warner-Bratzler shear force (WBSF), and myofibril fragment length (MFL) on LL aged 3 and 14 days post mortem at  $2 \pm 1\text{ }^{\circ}\text{C}$ . b) Sarcomere length measured at 1 day post mortem. c) Proteinase enzyme system measured as  $\mu$  and m-calpain and calpastatin activity at 1 and 24 h post mortem.

## 2.3. Warner-Bratzler shear force measurements

Aged samples for Warner-Bratzler shear force (WBSF) were frozen at  $-20\text{ }^{\circ}\text{C}$  and then processed into 30 mm steaks by means of a band saw. The frozen steaks were thawed at  $2 \pm 1\text{ }^{\circ}\text{C}$  for 24 h and cooked using an oven-broiling (Mielé, model H217, Mielé & Cie, Gütersloh, Germany) method with direct radiant heat (American Meat Science Association (AMSA), 1995). The steaks were broiled at  $260\text{ }^{\circ}\text{C}$  (pre-set) to  $70\text{ }^{\circ}\text{C}$  internal temperature and cooled down to  $18\text{ }^{\circ}\text{C}$ . Six round cores (12.7 mm diameter) were removed from the steaks parallel to the muscle fibers (American Meat Science Association (AMSA), 1995). Each core was sheared once through the center,

perpendicular to the fiber direction, by a Warner-Bratzler shear device mounted on an Universal Instron apparatus (Model 4301, Instron Ltd, Buckinghamshire, England; cross head speed = 20 mm/min) and the mean value of the 6 recordings were used as a shear value.

#### *2.4. Histological measurements*

Samples for sarcomere lengths of a fresh LL samples (24 h post mortem), were prepared according the method of Hegarty and Naudé (1970), by using distilled water instead of Ringer Locke solution (Dreyer, Van Rensburg, Naudé, Gouws, & Stiemie, 1979). Fifty sarcomeres per sample were measured by means of a video image analyses (VIA) using an Olympus B x 40 system microscope at a 1 000 x magnification equipped with CC12 video camera (Olympus, Tokyo, Japan). AnalySIS Life Science software package (Soft Imaging Systems GmbH, Münster, Germany) was used to process and quantify measurements.

Myofibril fragment lengths (MFL) of LL aged for 3 and 14 days post mortem were measured by means of VIA. Myofibrils were extracted according to Culler, Parrish, Smith and Cross (1978) as modified by Heinze and Bruggemann (1994). One hundred myofibril fragments per sample were examined and measured with an Olympus B x 40 system microscope at a 400 x magnification.

#### *2.5. Muscle biochemistry*

Samples collected for enzyme studies (1 and 24 h post mortem) were snap-frozen in liquid nitrogen and preserved at -70 °C. Calpastatin,  $\mu$ -calpain and m-calpain were extracted from 5 g of the LL frozen samples as described by Dransfield (1996) and separated by means of the two-step gradient ion-exchange chromatography-method according to Geesink and Koochmaraie (1999a). Calpain assays were determined by

using azo-casein as substrate according to Dransfield (1996). The use of azo-casein eliminates the problem of background absorbance of non-specific proteins in the extracts. One unit of calpain activity was defined as an increase in absorbance at 366 nm of 1.0 per h, at 25 °C. One unit of calpastatin activity was defined as the amount that inhibited 1 unit of m-calpain activity. Data were expressed as units per gram of muscle.

#### *2.6. Serum calcium and parathyroid analyses*

Serum calcium concentrations were analyzed using a 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 ).

#### *2.7. Statistical analyses*

Data of WBSF, MFL, calpastatin,  $\mu$ -calpain and m-calpain activities were subjected to analysis of variance for a split-plot design (GenStat® VSN International, Hemel Hempstead, UK; Payne, Murray, Harding, Baird, & Soutar, 2007) with the 6 treatment groups (C, Z, 3D1M, 9D1M, 6D7M7N, 6D7M) as whole plots and the two stimulation sub-treatments (ES and NES) and the two ageing periods (3 and 14 days post mortem) or time of analyses of enzyme activity (1 h and 24 h) as sub-plots. Data of sarcomere length was subjected to a similar analyses, six treatment groups (C, Z, 3D1M, 9D1M, 6D7M7N, 6D7M) as whole plots and only the two stimulation sub-treatments (ES and NES) as sub-plots. Means for the interactions between the whole plot and sub-plots were separated using Fisher's protected t-test least significant difference (LSD) at the 5% level (Snedecor & Cochran, 1980).

### 3. Results

#### 3.1 *Effect of treatment, stimulation and aging on physical and histological characteristics.*

General statistics for the effects of treatment (C, 3D7M, 6D7M, 6D7M7N, 9D1M, Z), stimulation and aging on WBSF, MFL and sarcomere length are presented in Table 1. Treatment had a significant effect ( $P < 0.001$ ) on WBSF. Both electrical stimulation (ES) and prolonged aging reduced WBSF significantly ( $P < 0.001$ ) relative to no stimulation (NES) and aging for 3 days, respectively. A significant interaction ( $P < 0.001$ ) occurred between treatment and stimulation ( $P < 0.001$ ), treatment and aging ( $P < 0.001$ ) and stimulation and aging ( $P = 0.053$ ) for WBSF. Stimulation had very little effect on the tenderness of the control, but did have a significant effect on all other treatments in particular the zilpaterol group, 6D7M7N and 6D7M (Fig. 1a). However, the variation (standard deviation) within non-stimulated and 3 days aged treatment groups were higher than within stimulated and 14 days aged groups (data not shown). Furthermore, stimulation also reduced the variation between mean values of all zilpaterol treated groups (zilpaterol and vitamin D<sub>3</sub> treatments). Nevertheless, the control still had a significant ( $P < 0.05$ ) advantage over other treatments even after 14 days aging (Fig. 1a).

The control recorded shorter ( $P < 0.001$ ) MFL's (more myofibrillar degradation) than the other treatment groups under any aging and stimulation scenario (Fig. 1b). Aging reduced MFL ( $P < 0.001$ ) indicating myofibrillar breakdown through proteolyses. The longer MFL's (or no change) of stimulated samples (Fig. 1b), indicating less myofibrillar breakdown, were not expected as it contrasts the lower WBSF (more tender meat) of stimulated samples (Fig. 1a). This suggests the involvement of other



tenderizing mechanisms. Neither treatment ( $P = 0.732$ ) nor stimulation ( $P = 0.626$ ) had a significant effect on sarcomere length.

### 3.2 *Effect of treatment, stimulation and aging on the calcium dependant proteinase system.*

General statistics for the effects of treatment (C, 3D7M, 6D7M, 6D7M7N, 9D1M, Z), stimulation and time of measurement on the calcium dependent proteinase system (CDP) are presented in Table 2. Control samples had lower calpastatin values than zilpaterol and zilpaterol combined with vitamin D<sub>3</sub> samples independent of time of measurement (Fig. 1c). Stimulation reduced  $\mu$ -calpain and calpastatin activity measured at 24 h in contrast to 1h measurements (Fig. 1c and 1d) (Interaction:  $P < 0.001$ ). Available  $\mu$ -calpain activity was also lower at 24 h compared with 1 h recording, although the effect was much more pronounced for the control and 3D7M than for the other treatments (interaction:  $P < 0.001$ ; Fig. 1d). Stimulation tended to increase m-calpain activity at 1 h and reduce activity at 24 h.

### 3.3 *Effect of treatment on blood parameters.*

Table 3 shows the effect of treatment on calcium (Ca) and parathyroid hormone (PTH) levels in the blood. All the vitamin D<sub>3</sub> supplemented groups showed higher blood Ca levels ( $P < 0.001$ ) than the control and zilpaterol groups with the exception of 6D7M that did not differ from zilpaterol. PTH levels were higher for the control than for all vitamin D<sub>3</sub> treatments and zilpaterol, and zilpaterol alone had higher levels than all vitamin D<sub>3</sub> treatments.

#### 4. Discussion

The expectation that control samples would be more tender than zilpaterol samples was confirmed and this is in agreement with other studies showing the negative effects of beta-agonists on tenderness (Hilton et al., 2009; Rathman et al., 2009; Strydom, Frylinck, Montgomery, & Smith, 2009). However considering the reported improvement in tenderness with high vitamin D<sub>3</sub> supplements in other studies (Swanek et al., 1999; Vargas, Down, Webb, Han, Morgan, & Dolezal, 1999; Montgomery, Parrish, Beitz, Horst, Huff-Lonergan, & Trenkle, 2000; Montgomery, King, et al., 2004) the poor response of improvement in tenderness by vitamin D<sub>3</sub> in our study was unexpected. Closer examination of the various studies show an inconsistent response to vitamin D<sub>3</sub> supplement in any event related to dose or withdrawal times (Foote, Horst, Huff-Lonergan, Trenkle, Parrish, & Beitz, 2004; Lawrence et al., 2006, Wertz et al., 2004). In some studies even negative results were reported (sheep: Boleman, McKenna, Ramsey, Peel, & Savell, 2004; Berry, Gill & Ball, 2000). The main difference between the present study and others is that it had a beta-agonist in the protocol that is known to affect tenderness and it is clear that vitamin D<sub>3</sub>-supplement has very little effect on increasing tenderness under these conditions.

The argument behind supplementing super levels of vitamin D<sub>3</sub> would be to rise Ca levels above the blood Ca homeostasis level of 8 to 12 mg/dL (in cattle) but more particularly to increase free cytosolic Ca during early rigor and binding of this excess Ca at strategic places in the muscle cell that would increase calpain activity and increase myofibrillar proteolysis (Boehm, Kendall, Thompson, & Goll 1998; Montgomery, Blanton, et al., 2004). Swanek et al. (1999) and Montgomery et al. (2002) speculated that increased Ca recorded with high vitamin D<sub>3</sub> supplements may even be sufficient to activate m-calpain. Although the activities of Ca dependent proteases (CDP) and their relation to tenderness measurements have been inconsistent among studies it is

suggested that the activation and subsequent proteolytic action of  $\mu$ -calpain starts soon after killing (within the first 3 hours post mortem) and become undetectable after 3 days (Veiseth, Shackelford, Wheeler, & Koohmaraie, 2004). They also found that m-calpain remained constant throughout aging and calpastatin activity remained stable over the first 12 h and then declined. Dransfield (1996) regarded the reduced recovered activity of calpains during rigor *mortis* and subsequent storage as unknown but important in relation to tenderization, while he accounted the reduction to either autolysis of activated calpain, a low extractability due to post mortem protein aggregation, or post rigor degradation. Several studies have demonstrated a negative relationship between calpastatin activity and proteolysis in post mortem muscle (Geesink & Koohmaraie, 1999a,b; Koohmaraie et al., 1991) which corresponds with the increased calpastatin activities and reduced tenderness of zilpaterol treated steers in our study and our previous study involving zilpaterol and other beta-agonists (Strydom, Frylinck, Montgomery, & Smith, 2009). Following this line of reasoning, any CDP mediated effect of supplemented vitamin D<sub>3</sub> on tenderness of zilpaterol treated samples would therefore show through increased action of calpains (possibly lower 24 h activities) and a consequent reduced activity of calpastatin (Doumit & Koohmaraie, 1999) at 24 h as was reported by Swanek et al. (1999; 7.5 million IU per day for 10 days). In our study, the best positive effect on calpastatin was recorded for 6D7M7N at 1 h, yet in all scenarios the calpastatin activities for vitamin D treatments were still significantly higher than for the control (Fig. 1c). Mu-calpain showed some variation at 1 h for stimulated samples but apart from a higher activity recorded for 3D7M, the variation in enzyme activity among all treatments did not agree with the response to vitamin D<sub>3</sub> reported by Swanek et al. (1999) nor did they relate to variation in tenderness among treatments as presented in Fig. 1a. Only the study of Tipton, King, Paschal, Hale, and Savell (2007) investigated the effect of vitamin D under conditions tenderness could have been

compromised (*Bos indicus*) and reported positive results on tenderness when vitamin D<sub>3</sub> was supplemented for 5 days and then withdrawn for 7 days. No effect was recorded when cattle were slaughtered directly after the 5 days supplement period. No CDP related tests were performed but serum and tissue Ca was significantly elevated when vitamin D<sub>3</sub> was withdrawn for 7, but not directly after the 5 day supplement. Despite elevated levels in serum Ca for 3D7M, 6D7M7N and 9D1M, it probably had little effect on free cytosolic Ca to activate sufficient  $\mu$ -calpain to neutralize the effect of raised calpastatin activities caused by beta-agonists. M-calpain is generally regarded as having a minor or no part in proteolysis due to the unavailability of sufficient free Ca in post mortem muscle to activate the enzyme, and a lack of evidence of autolyzation (measured as decline in post mortem activity) and therefore proteolyses (Boehm, Kendal, Thompson, & Goll, 1998; Veiseth, Shackelford, Wheeler, & Koohmaraie, 2001; 2004). M-calpain activity increased between 1 h and 24 h in our study which is strange but not uncommon (Veiseth, Shackelford, Wheeler & Koohmaraie, 2001; 2004). Swanek, et al. (1999) reported lower 24 h for  $\mu$ - and m-calpain activities with associated improvement in tenderness for vitamin D<sub>3</sub> treated loins. They also argued that the levels of water-extractable muscle Ca recorded in their treated samples were sufficient to activate both  $\mu$ - and m-calpain activities. We could show no evidence that vitamin D<sub>3</sub> supplements increased Ca levels to affect m-calpain activity and subsequently WBSF tenderness.

In relation to increased Ca levels, 3D7M and 9D1M showed the best response in tenderness during early stages of aging and when samples were not stimulated, while 6D7M7N and 6D7M recorded tenderness values similar and higher than to zilpaterol samples, respectively. A plausible explanation for the extreme effect of 6D7M (and to a lesser extent 6D7M7N) on WBSF could be that the high levels of vitamin D<sub>3</sub> maintained for relatively long periods could have caused an over compensation and therefore a

relative shortage of available Ca due to the reaction of regulating hormones. Decreased PTH levels in all vitamin D<sub>3</sub> groups, but particularly 6D7M and 6D7M7N, support this argument as this hormone decreases in response to increased serum Ca levels (Aranda-Osorio, 2002; Littledike & Goff, 1987).

In general, other reports showed very little consistent results with regard to dosages and duration of vitamin D<sub>3</sub> in relation to Ca levels and tenderness. Karges et al. (2001) tended to find better WBSF results with longer (6 vs. 4 days) supplement periods, while Scanga, Belk, Tatum and Smith (2001) reported elevated Ca levels with no response in tenderness improvement similar to our study. Montgomery et al. (2002) showed a positive dose response to levels of vitamin D<sub>3</sub> for plasma Ca but trends for improved WBSF in the opposite direction, i.e. lower supplement levels gave numerically better WBSF results than increasing dosages.

Electrical stimulation (ES) had an overall positive effect on WBSF (lowered values) which coincided with lower 24 h calpastatin activity in general (Fig. 1c) and a larger reduction in available  $\mu$ -calpain activity in ES samples between 1 and 24 h (Fig. 1d). This is in agreement with previous reports by Rosenvold et al. (2007), Dransfield, Etherington, and Taylor (1992) and Hwang and Thompson (2001a) who found that ES advances the onset of rigor (pH = 6.1, Dransfield et al., 1992) where the activation of  $\mu$ -calpain is initiated due to the release of Ca<sup>+</sup> ions which causes proteolysis and tenderization (Ducastaing, Valin, Schollmeyer, & Cross, 1985). Changes in calpastatin and  $\mu$ -calpain activities due to ES was accompanied by reduced variation in WBSF among all treatments groups and had the greatest effect on those treatments that were most compromised under NES conditions, such as 6D7M, 6D7M7N and zilpaterol (Fig. 1a). Similar results were reported by Ferguson, Jiang, Hearnshaw, Rymill, and Thompson (2000) and Wheeler, Savell, Cross, Lunt, and Smith (1990) when tenderness potential facilitated by the CDP system was compromised by breed (*Bos indicus*) effect. The

negative breed effect was substantially reduced by ES on *Bos indicus* samples and the total effect was higher than for the non-*indicus* group. The combined effect of ES and aging in our study reduced the difference between the control and the other treatments to between 1.3 kg (6D7M) and 0.6 kg (3D7M) (Fig. 1a) but could not completely overcome the effect of zilpaterol on WBSF toughness. Furthermore none of the vitamin D<sub>3</sub> treatments differed from zilpaterol, although the 3D7M group recorded lower WBSF values than 6D7M ( $P < 0.05$ ). Only two other studies used electrical inputs in combination with either vitamin D<sub>3</sub> or their metabolites (Lawrence et al., 2006; electrical inputs such as rigidity probe and immobiliser; Tipton et al., 2007; dual system, 150 and 300 V, 28 s) but only Tipton et al. (2007) recorded a slight advantage recorded an advantage for vitamin D<sub>3</sub> treatments of 3 to 4 N (0.3 to 0.4 kg) above control samples. This agrees with our results that stimulation generally reduce variation in tenderness.

## 5. Conclusion

High vitamin D<sub>3</sub> supplement levels does not seem to be a viable option for improving meat tenderness in beta-agonist treated beef. Only a shorter but higher dose (3D7M) and a longer but lower dose (9D1M) of vitamin D<sub>3</sub> showed small but significant improvements in tenderness, under conditions of no electrical stimulation. The benefit of using electrical stimulation on its own should be less costly and show better results on improving beta-agonist treated beef compared to any vitamin D<sub>3</sub> treatment with no stimulation. Furthermore, with electrical stimulation, no added advantage of feeding vitamin D<sub>3</sub> is achieved. Even though stimulation is effective in reducing the effect of a  $\beta$ -agonist on tenderness, the beta-agonist treated meat remains tougher than non-treated meat and should be avoided if meat quality is the primary objective.

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## References

- American Meat Science Association (AMSA) (1995). Research guidelines for cookery, sensory evaluation and instrumental tenderness of fresh meat. National Livestock and Meat Board, Chicago, IL, USA.
- Aranda-Osorio, G. (2002). Effect of vitamin D<sub>3</sub> on Ca homeostasis of beef steers. PhD thesis, University of Saskatchewan, Saskatchewan, Canada.
- Berry, B. A., Gill, D. R., & Ball, R. (2000). Effects of feeding vitamin D on feedlot performance, carcass traits, and meat tenderness of finishing steers. In: *Animal Science Research Report*, (pp. 98-103). Department of Animal Science, Ohio State University, USA.
- Boehm, M. L., Kendall, T. L., Thompson, V. F., & Goll, D. E. (1998). Changes in the Calpains and Calpastatin During post mortem Storage of Bovine Muscle. *Journal of Animal Science*, 76, 2415-2434.
- Boleman, C. T., McKenna, D. R., Ramsey, W. S., Peel, R. K., & Savell, J. W. (2004). Influence of feeding vitamin D<sub>3</sub> and aging on the tenderness of four lamb muscles. *Meat Science*, 67, 185-190.

- Culler, R. D., Parrish, F. C., Smith, G. C., & Cross, H. R. (1978). Relationship of myofibril fragmentation index to certain chemical, physical and sensory characteristics of bovine longissimus muscle. *Journal of Food Science*, 43, 1177-1180.
- Doumit, M. E., & Koohmaraie, M. (1999). Immunoblot analysis of calpastatin degradation: Evidence for cleavage by calpain in postmortem muscle. *Journal of Animal Science*, 77, 1467–1473.
- Dransfield, E., Etherington, D. J., & Taylor, M. A. J. (1992). Modelling post mortem tenderisation –II: Enzyme changes during storage of electrically stimulated and non-stimulated beef. *Meat Science*, 31, 75-84.
- Dransfield, E. (1996). Calpains from thaw rigor muscle. *Meat Science*, 43, 311-320.
- Dreyer, J. H., Van Rensburg, A. J. J., Naudé, R. T., Gouws, P. J., & Stiemie, S. (1979). The effect of chilling temperatures and mode of suspension of beef carcasses on sarcomere length and meat tenderness. *South African Journal of Animal Science* 9, 1-9.
- Ducastaing, A., Valin, C., Schollmeyer, J., & Cross, R. (1985). Effects of electrical stimulation on post mortem changes in the activities of two Ca dependent neutral proteinases and their inhibitor in beef muscle. *Meat Science*, 15, 193-202.
- Ferguson, D. M., Jiang, S-T., Hearnshaw, H., Rymill, S. R., & Thompson, J. M. (2000). Effect of electrical stimulation on protease activity and tenderness of *M. longissimus* from cattle with different proportions of *Bos indicus* content. *Meat Science*, 55, 265-272.
- Foote, M. R., Horst, R. L., Huff-Lonergan, E. J., Trenkle, A. H., Parrish, F. C., Jr., & Beitz, D. C. (2004). The use of vitamin D<sub>3</sub> and its metabolites to improve beef tenderness. *Journal of Animal Science*, 82, 242-249.



- Geesink, G. H., & Koohmaraie, M. (1999a). Effect of calpastatin on degradation of myofibrillar proteins by  $\mu$ -calpain under post mortem conditions. *Journal of Animal Science*, 77, 2685-2692.
- Geesink, G. H., & Koohmaraie, M. (1999b). Postmortem proteolysis and calpain/calpastatin activity in callipyge and normal lamb biceps femoris during extended postmortem storage. *Journal of Animal Science*. 77, 1490–1501.
- Goll, D. E., Thompson, V. F., Taylor, R. G., & Zalewska, T. (1992). Is calpain activity regulated by membranes and autolysis or by calcium and calpastatin? *Bioessays* 14, 549-556.
- Hegarty, P. V. J., & Naudé, R. T. (1970). The accuracy of measurement of individual skeletal muscle fibres separated by a rapid technique. *Laboratory Practice* 19, 161-164.
- Heinze, P. H., & Brueggemann, D. (1994). Ageing of beef: Influence of two ageing methods on sensory properties and myofibrillar proteins. *Sciences Des Aliments* 14, 387-391.
- Hilton, G. G., Montgomery, J. L., Krehbiel, C. R., Yates, D. A., Hutcheson, J. P., Nichols, W. T., et al. (2009). Effects of feeding zilpaterol hydrochloride with and without monensin and tylosin on carcass cutability and meat palatability of beef steers. *Journal of Animal Science*, 87, 1394-1406.
- Hope-Jones, M., Strydom, P. E., Frylinck, L., & Webb E. C. (2010). The efficiency of electrical stimulation to counteract the negative effects of beta-agonists on meat tenderness of feedlot cattle. *Meat Science*, 86, 699-705.
- Hwang, I. H., & Thompson, J. M. (2001a). The effect of time and type of electrical stimulation on the calpain system and meat tenderness in beef longissimus dorsi muscle. *Meat Science*, 58, 135-144.

- Karges, K., Brooks, J. C., Morgan, J. B., Gill, D. R., Breazile, J. E., Owens, F. N. & Morgan, J. B. (2001). Effects of supplemental vitamin D<sub>3</sub> on feed intake, carcass characteristics, tenderness, and muscle properties of beef steers. *Journal of Animal Science*, 79, 2844-2850.
- Kerth, C. R., Miller, M. F., & Ramsey, C. B. (1995). Improvement of beef tenderness and quality traits with calcium chloride injection in beef loins 48 hours post mortem. *Journal of Animal Science*, 73, 750-756.
- Koohmaraie, M., & Shackelford, S. (1991). Effect of calcium chloride infusion on the tenderness of lambs fed a  $\beta$ -adrenergic agonist. *Journal of Animal Science*, 69, 2463-2471.
- Koohmaraie, M., Shackelford, S. D., Muggli-Cockett, N. E., & Stone, R. T. (1991). Effect of the  $\beta$ -adrenergic agonist L<sub>644,969</sub> on muscle growth, endogenous proteinase activities, and post mortem proteolysis in wether lambs. *Journal of Animal Science*, 69, 4823-4835.
- Lawrence, R. W., Doyle, J., Elliot, R., Loxton, I., McMeniman, J. P., Norton, B. W., et al. (2006). The efficacy of a vitamin D<sub>3</sub> metabolite for improving the myofibrillar tenderness of meat from *Bos indicus* cattle. *Meat Science*, 72, 69-78.
- Littledike, E. T., & Goff, J. (1987). Interactions of calcium, phosphorus, magnesium and vitamin D that influence their status in domestic meat animals. *Journal of Animal Science*, 65, 1727-1743.
- Montgomery, J. L., Parrish, F. C., Jr., Beitz, D. C., Horst, R. L., Huff-Lonergan, E. J., & Trenkle, A. H. (2000). The use of vitamin D<sub>3</sub> to improve beef tenderness. *Journal of Animal Science*, 78, 2615-2621.
- Montgomery, J. L., Carr, M. A., Kerth, C. R., Hilton, G. G., Price, B. P., Galyean, M. L., et al. (2002). Effect of vitamin D<sub>3</sub> supplementation level on the post mortem tenderization of beef from steers. *Journal of Animal Science*, 80, 971-981.

- Montgomery, J. L., King, M. B., Gentry, J. G., Barham, A. R., Barham, B. L., Hilton, G. G., et al. (2004). Supplemental vitamin D<sub>3</sub> concentration and biological type of steers. II. Tenderness, quality, and residues of beef. *Journal of Animal Science*, 82, 2092-2104.
- Montgomery, J. L., Blanton, J. R., Horst, R. L., Galyean, M. L., Morrow, Jr., K. J., Allen, V. G., et al. (2004). Effect of supplemental vitamin D<sub>3</sub> concentration on concentrations of calcium, phosphorus, and magnesium relative to protein in subcellular components of the longissimus and the distribution of calcium within longissimus muscle of beef steers. *Journal of Animal Science*, 82, 2742-2749.
- Payne, R. W., Murray, D. A., Harding, S. A., Baird, D. B., & Soutar, D. M. (2007). GenStat for Windows® (10<sup>th</sup> Edition) Introduction. VSN International, Hemel Hempstead, UK.
- Rathmann, R. J., Mehaffey, J. M., Baxa, T. J., Nichols, W. T., Yates, D. A., Hutcheson, J. P., et al. (2009). Effects of duration of zilpaterol hydrochloride and days on the finishing diet on carcass cutability, composition, tenderness, and skeletal muscle gene expression in feedlot steers. *Journal of Animal Science*, 87, 3686-3701.
- Rosenvold, K., North, M., Devine, C. E., Micklander, E. P. Hansen, P, Dobbie, P., et al. (2007). The protective effect of electrical stimulation and wrapping on beef tenderness at high pre rigor temperatures. *Meat Science*, 79, 299-306.
- Scanga, J. A., Belk, K. E., Tatum, J. D., & Smith, G. C. (2001). Supranutritional oral supplementation with vitamin D<sub>3</sub> and calcium and the effects on beef tenderness. *Journal of Animal Science*, 79, 912-918.
- Snedecor, G. W., & Cochran, W. G. (1980). In: *Statistical methods* 7<sup>th</sup> Edition, 507, Iowa State University Press.

- Strydom, P. E., Frylinck, L., Montgomery, J. L., & Smith, M. F. (2009). The comparison of three beta-agonists for growth performance, carcass characteristics and meat quality of feedlot cattle. *Meat Science*, 81, 557-564.
- Swanek, S. S., Morgan, J. B., Owens, F. N., Gill, D. R., Strasia, C. A., Dolezal, H. G., et al. (1999). Vitamin D<sub>3</sub> supplementation of beef steers increases longissimus tenderness. *Journal of Animal Science*, 77, 874-881.
- Tipton, N. C., King, D. A., Paschal, J. C., Hale, D. ., & Savall, J. W. (2007). Effects of oral vitamin D<sub>3</sub> supplementation and supplement withdrawal on the accumulation of magnesium, calcium, and vitamin D in the serum, liver and muscle tissue and subsequent carcass and meat quality of *Bos indicus* influenced cattle. *Meat Science*, 75, 150-158.
- Vargas, D. N., Down, A. E., Webb, D. S., Han, H., Morgan, J. B., & Dolezal, H. G. (1999). Effects of dietary supplementation of feedlot steers with vitamins E and D<sub>3</sub> on live performance, carcass traits, shelf-life attributes and longissimus muscle tenderness. Oklahoma State University, Department Animal Science, Research report, 59-66. [www.ansi.okstate.edu/research/research-reports-1/1999](http://www.ansi.okstate.edu/research/research-reports-1/1999).
- Veiseth, E., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2001). Effect of post mortem storage on  $\mu$ -calpain and m-calpain in ovine skeletal muscle. *Journal of Animal Science*, 79, 1502-1508.
- Veiseth, E., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2004). Indicators of tenderization are detectable by 12 h post mortem in ovine longissimus. *Journal of Animal Science*, 82, 1428-1436.
- Wertz, A. E., Knight, J. J., Trenkle, A., Sonon, R., Horst, R. L., Huff-Lonergan, E. J., et al. (2004). Feeding 25-hydroxyvitamin D<sub>3</sub> to improve beef tenderness. *Journal of Animal Science*, 82, 1410-1418.

Wheeler, T. L., Savell, J. W., Cross, H. R., Lunt, D. K., & Smith, S. B. (1990).

Mechanisms associated with the variation on tenderness of meat from Brahman and Hereford cattle. *Journal of Animal Science*, 68, 4206-4220.

Wheeler, T. L. M., Koochmarai, M., Lansdell, J. L., Siragusa, G. R., & Miller, M. F.

(1993). Effects of post mortem injection time, injection level, and concentration of calcium chloride on beef quality traits. *Journal of Animal Science*, 71, 2965-2974.

**Table 1**

General statistics for the effects of treatment, stimulation and aging and their first order interactions on Warner-Bratzler shear force (WBSF) and histological characteristics of *M. longissimus lumborum* (Data pooled for treatment, stimulation and *post mortem* aging; degrees of freedom parenthesized)

Effect	WBSF		Myofibrillar fragment length		Sarcomere length	
	Significance	F ratio	Significance	F ratio	Significance	F ratio
<i>Main effect</i>						
Treatment	$P < 0.001$	15.53 (5,54)	$P < 0.001$	6.81 (5,54)	$P = 0.732$	0.56 (5,114)
Stimulation	$P < 0.001$	118.37 (1,54)	$P < 0.001$	34.84 (1,54)	$P = 0.626$	0.24 (1,113)
Aging	$P < 0.001$	897.3 (1,108)	$P < 0.001$	933.9 (1,108)		
<i>Interactions</i>						
T x S	$P < 0.001$	6.89 (5,54)	$P = 0.394$	1.06 (5,54)		
T x A	$P < 0.001$	7.08 (5,108)	$P = 0.071$	2.10 (5,108)		
S x A	$P = 0.056$	3.74 (1,108)	$P = 0.518$	0.42 (1,108)		
T x S x A	$P = 0.703$	0.60 (5,108)	$P = 0.375$	1.08 (5,108)		

T: Treatment (control, zilpaterol and 4 vitamin D<sub>3</sub> supplemented groups)

S: Stimulation (stimulated and non-stimulated)

A: Aging (3 and 14 days post mortem)

**Table 2**

General statistics for the effects of treatment, stimulation, time of measurement and their first order interactions on calpastatin and calpain activity of *M. longissimus lumborum* (degrees of freedom parenthesized)

Effect	Calpastatin activity <sup>a</sup>		μ-calpain activity <sup>b</sup>		m-calpain activity <sup>b</sup>	
	Significance	F ratio	Significance	F ratio	Significance	F ratio
<i>Main effect</i>						
Treatment	$P < 0.001$	13.09 (5,54)	$P = 0.005$	3.80 (5,54)	$P < 0.001$	4.94 (5,54)
Stimulation	$P < 0.001$	75.33 (1,54)	$P < 0.001$	89.32 (1,54)	$P = 0.018$	5094 (1,54)
Time of measurement	$P = 0.608$	0.26 (1,108)	$P < 0.001$	346.0 (1,108)	$P < 0.001$	328.1 (1,108)
<i>Interactions</i>						
T x S	$P = 0.489$	0.90 (5,54)	$P = 0.102$	1.94 (5,54)	$P = 0.346$	1.15 (5,54)
T x M	$P = 0.412$	1.02 (1,108)	$P < 0.001$	7.42 (5,108)	$P = 0.006$	3.49 (5,108)
S x M	$P < 0.001$	34.1 (1,108)	$P < 0.001$	83.86 (1,108)	$P < 0.001$	92.43 (1,108)
T x S x M	$P = 0.697$	0.60 (5,108)	$P = 0.977$	83.86 (1,108)	$P = 0.261$	92.43 (1,108)

<sup>a</sup> One unit of calpastatin activity is defined as the amount that inhibited one unit of m-calpain activity.

<sup>b</sup> One unit of calpain activity is defined as an increase in absorbance at 366 nm of 1.0 absorbance unit per g of muscle per h, at 25 °C.

T: Treatment (control, zilpaterol and 4 vitamin D<sub>3</sub> supplemented groups)

S: Stimulation (stimulated and non-stimulated)

M: Time of measurement 1 and 24 h post mortem)

**Table 3:**

Effect of treatment on serum calcium and parathyroid hormone (PTH).

Treatment	Control	Zilpaterol	Vitamin D <sub>3</sub> treatments				SEM <sup>e</sup>	P value
			3D7M	9D1M	6D7M7N	6D7M		
Calcium (mg/100 ml)	9.39 <sup>a</sup>	9.57 <sup>ab</sup>	10.85 <sup>c</sup>	10.43 <sup>c</sup>	10.76 <sup>c</sup>	10.17 <sup>bc</sup>	0.255	<0.001
PTH (pg/mL)	70.0 <sup>d</sup>	49.4 <sup>c</sup>	24.9 <sup>b</sup>	13.2 <sup>ab</sup>	5.9 <sup>a</sup>	10.7 <sup>a</sup>	4.36	<0.001

<sup>a,b,c,d</sup> Different superscripts in a row shows significant difference ( $P < 0.05$ )

<sup>e</sup> Standard error of means.

Control: no zilpaterol and no vitamin D<sub>3</sub> supplement.

Zilpaterol: 0.15 mg zilpaterol hydrochloride per kg live weight for 30 days during the final weeks of finishing.

Vitamin D<sub>3</sub> treatments: D = number of days supplemented; M = x million IU vitamin D<sub>3</sub>/animal/day; N = number of days vitamin D<sub>3</sub> was withdrawn; all vitamin D<sub>3</sub> received 0.15 mg zilpaterol hydrochloride per kg live weight for 30 days during the final weeks of finishing.



## Figure caption

**Fig. 1.** Interaction between treatment (Control, 3D7M, 9D1M, 6D7M7N, 6D7M, Zilpaterol), electrical stimulation and post mortem aging (3 and 14 days) or time of measurement (1 h and 24 h) for (a) Warner Bratzler shear force, (b) Myofibril fragment length, (c) Calpastatin activity, (d)  $\mu$ -calpain activity and (e) m-calpain activity.

*ES and NES = stimulated and non-stimulated.*

*Control: no zilpaterol and no vitamin D<sub>3</sub> supplement.*

*Zilpaterol: 0.15 mg zilpaterol hydrochloride per kg live weight for 30 days during the final weeks of finishing.*

*Vitamin D<sub>3</sub> treatments: 3D7M, 9D1M, 6D7M 6D7M7N – D = number of days supplemented; N = number of days vitamin D<sub>3</sub> was withdrawn; M = millions IU vitamin D<sub>3</sub>/animal/day, all vitamin D<sub>3</sub> received 0.15 mg zilpaterol hydrochloride per kg live weight for 30 days during the final weeks of finishing.*

### Key to symbols:

■ - 3D7M

▲ – 9D1M

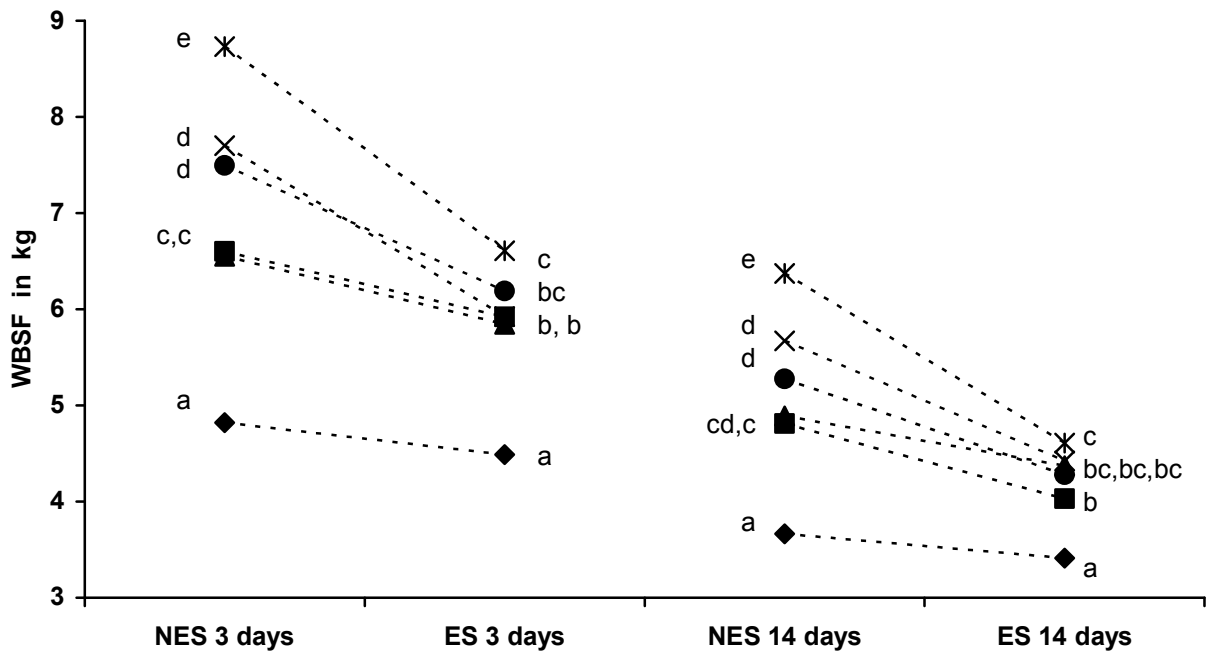
● - Zilpaterol

◆ - Control

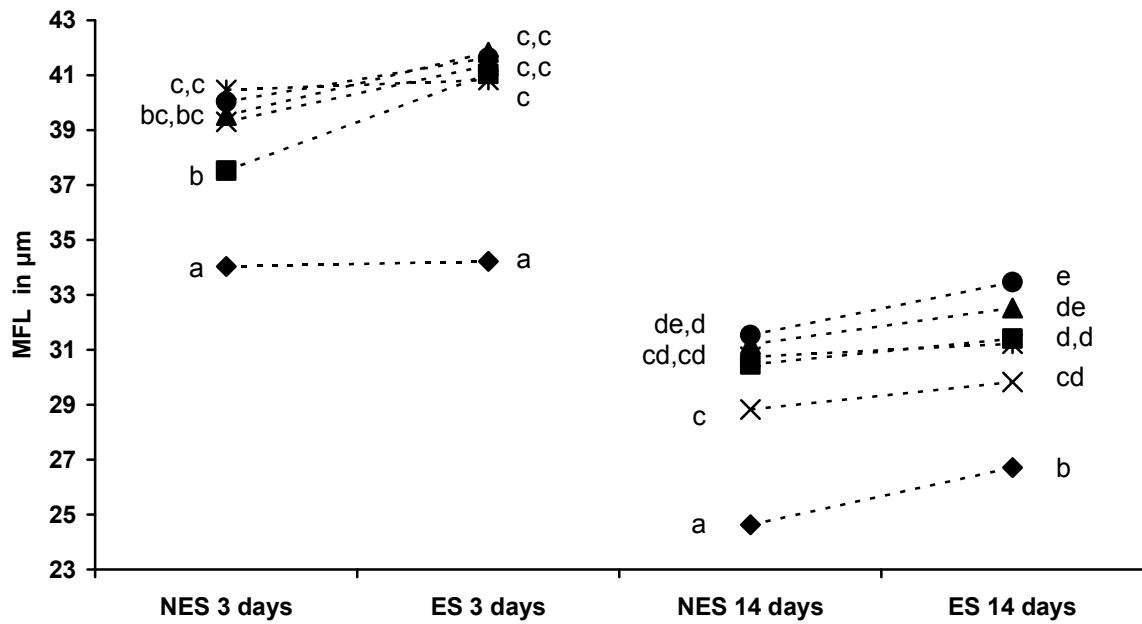
X – 6D7M7N

✕ – 6D7M

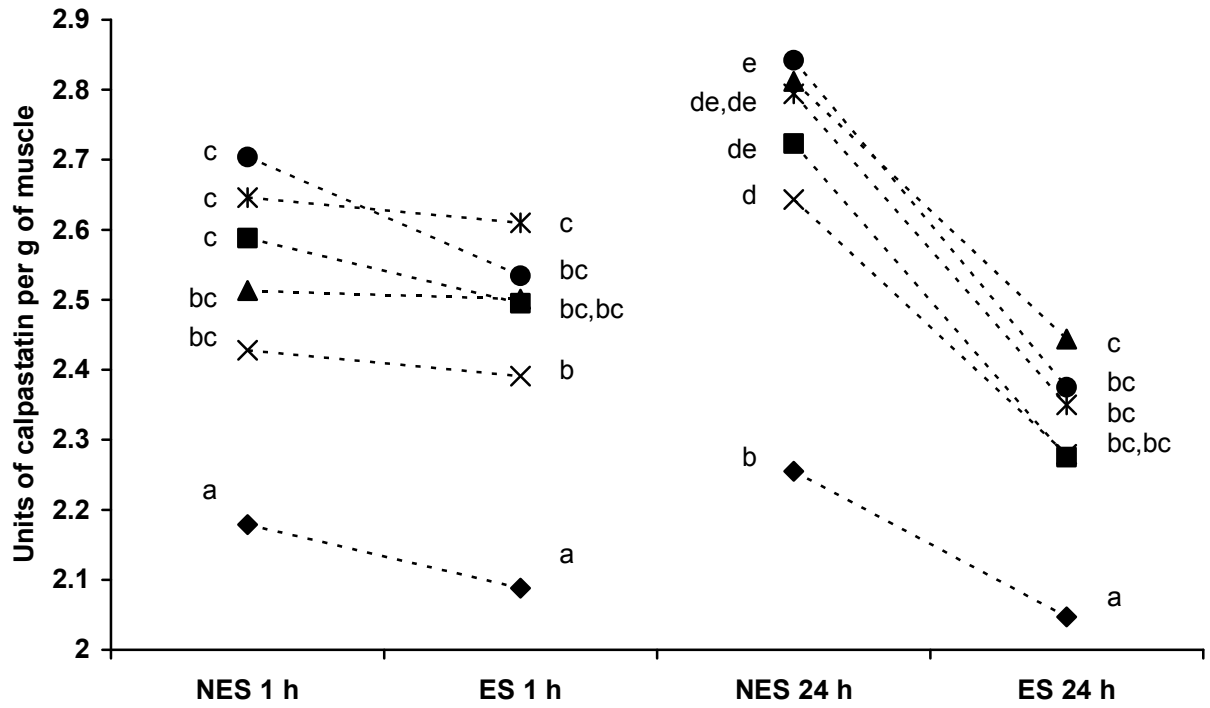
a)



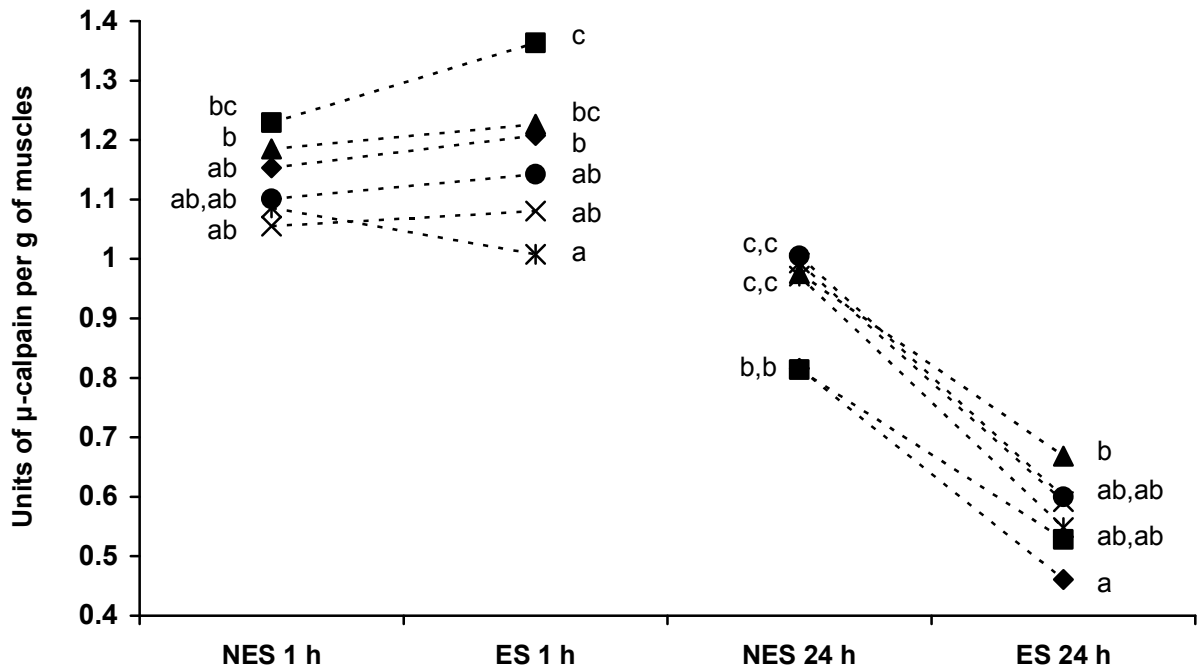
b)



c)



d)



e)

