Feeding 25-hydroxyvitamin D₃ to improve beef tenderness
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J ANIM SCI 2004, 82:1410-1418.

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Feeding 25-hydroxyvitamin D₃ to improve beef tenderness


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ABSTRACT: The objective of this trial was to determine if a single oral bolus of 25-hydroxyvitamin D₃ (25-OH D₃) given at various times before slaughter would enhance the tenderness of beef loin steaks. One hundred eight crossbred steers were allotted to 18 pens so that the mean weight of the cattle in each pen was similar. Treatments (25-OH D₃ dose [62.5 or 125 mg]) and time of administration of the single oral bolus (4, 7, 21, or 35 d before slaughter) were assigned randomly to each pen of steers. Serial plasma samples were collected at each bolus administration time for control animals. For steers assigned to a treatment group, a baseline blood sample was collected before bolus administration and at each subsequent administration when other treatment groups received their bolus. Plasma samples were assayed for 25-OH D₃ and calcium concentrations. Troponin-T degradation and Warner-Bratzler shear force were measured as indicators of tenderness for loin steaks collected at slaughter and aged for 6 or 14 d postmortem. Muscle samples, collected concurrently, were assayed for 25-OH D₃ and calcium concentrations. A single oral bolus of 25-OH D₃ was sufficient to increase plasma 25-OH D₃ concentrations (P < 0.001) through slaughter, regardless of dose or time of bolus administration. The single oral bolus of 25-OH D₃, however, did not increase plasma calcium concentrations (P > 0.05). As a result, neither troponin-T degradation nor Warner-Bratzler shear force was improved (P > 0.05) by treatment. Muscle 25-OH D₃ concentrations were increased (P > 0.001) by treatment with 25-OH D₃. Although sustained plasma 25-OH D₃ concentrations did not increase plasma or muscle calcium at slaughter nor influence tenderness, the use of 25-OH D₃ as a nutritional means of improving beef tenderness is in its infancy, and more research to delineate an effective dose and the potential interaction of seasonal exposure to ultraviolet light is warranted.

Key Words: Beef, 25-Hydroxyvitamin D₃, Tenderness, Troponin-T

Introduction

Although inconsistencies exist as to the efficacy of dietary vitamin D₃ and beef tenderness, vitamin D₃, in general, is a nutritional means of elevating muscle calcium concentrations and improving beef tenderness (Swanek et al., 1999; Montgomery et al., 2000, 2002). Elevated muscle calcium concentration enhances the calcium-dependent myofibrillar protein degradation postmortem to improve tenderness (Kooohmaraei, 1988; Kooohmaraei et al., 1991).

Feeding excess vitamin D₃ close to slaughter results in higher concentrations of vitamin D₃ and 25-hydroxyvitamin D₃ (25-OH D₃) in muscle (Montgomery et al., 2002; Foote et al., 2004). Calcification of soft tissues has resulted from excessive (80,000 IU/mo) dietary vitamin D, and concerns regarding the health risk of foods high in vitamin D content have been raised (Rajasree et al., 2002). Feeding 25-OH D₃, however, results in muscle vitamin D₃ concentrations similar to those of control steers, and 25-OH D₃ concentrations one-third the concentration for vitamin D₃-treated steers (Foote et al., 2004). Foote et al. (2004) reported that a single oral bolus of 25-OH D₃ increased plasma Ca for up to 20 d and resulted in LM Warner-Bratzler shear force (WBSF) and troponin-T degradation values that were intermediate to those of control and vitamin D₃-treated steers. We hypothesize that the length of time between the administration of a single oral bolus of 25-OH D₃ and slaughter can vary and may result in elevated plasma and muscle Ca at slaughter and improved tenderness. A variable length of time between 25-OH D₃...
treatment and slaughter would allow beef producers greater flexibility in marketing their cattle. Therefore, the objectives of this trial were 1) to evaluate the effects of a single oral bolus of 25-OH D3 on plasma and muscle Ca and on 25-OH D3 concentrations and 2) to determine the effects of a single oral bolus of 25-OH D3 given 4, 7, 21, or 35 d before slaughter on beef tenderness.

Materials and Methods

Animals and Treatments

Steers used in this experiment were cared for under the guidelines set forth by the Iowa State University Committee on Animal Care. British × Continental beef steers (n = 108) designated for this project were transported to the Iowa State University Beef Cattle Nutrition and Management Research Farm, where they were allowed 10 d to adapt to the new environment before the initiation of the trial. Following the adaptation period, steers were allotted to pens (six steers per pen). Then, pens of steers were assigned randomly to one of nine treatments (two pens per treatment) arranged in a 2 × 4 factorial design with untreated control. Treatments were two doses of 25-OH D3 (62.5 or 125 mg) and four preslaughter administration times (4, 7, 21, or 35 d before slaughter). Gelatin capsules used to supply the 25-OH D3 bolus were prepared by dissolving 10 g of 25-OH D3 in 80 mL of ethanol to yield a concentration of 125 mg/mL. One milliliter of the solution (125 g of 25-OH D3) was pipetted into each of 48 gelatin capsules containing approximately 2 g of finely ground alfalfa hay. Likewise, 62.5-mg boluses were prepared by pipetting 0.5 mL of the solution into gelatin capsules containing ground alfalfa hay. Gelatin capsules were capped and stored at ≥−20°C for subsequent analyses of vitamin D3 metabolites and calcium. A baseline blood sample was collected from each steer before bolus administration on the respective treatment day. Intermediate blood samples were collected on each subsequent day when other treatment groups received a bolus. A final blood sample from each steer before bolus administration on the respective treatment day. Intermediate blood samples were collected on each subsequent day when other treatment groups received a bolus. A final blood sample was collected from a single pen of steers at a time was worked through the chute (without the use of electric cattle prods); boluses were administered by the same experienced animal caregiver at each bolusing period.

Feedlot Performance Data Collection

Steers were allowed ad libitum access to a common finishing diet throughout the adaptation period and the subsequent trial. The finishing diet was balanced to meet or exceed NRC (1996) requirements (Table 1). Dry matter intake and feed refusals were monitored on a daily basis. Steers were weighed on two consecutive days after trial initiation and before trial termination, and these data were used to calculate average DMI, ADG, and G:F.

Blood Sample Collection

Baseline and intermediate blood samples were collected starting at 0800 on each of the collection days.

<table>
<thead>
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<th>Ingredient</th>
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</tr>
</thead>
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<td>78.88</td>
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<tr>
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<tr>
<td>Soybean meal</td>
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<tr>
<td>Steep liquor</td>
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<td>Urea</td>
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<td>Limestone</td>
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<td>Rumensin</td>
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On sampling and bolus administration days, steers were not fed until after blood samples were collected. Steers were restrained using a hydraulic squeeze chute, and a rope halter was used to restrain the head of the steer. Blood samples were collected by trained personnel using a syringe fitted with an 18-gauge needle. During these collection times, 20 mL of blood was collected via jugular venipuncture into sodium heparinized Vacutainers (Becton-Dickinson, Franklin Lakes, NJ). Blood samples were centrifuged immediately at 2,200 × g for 20 min. Plasma was stored in duplicate aliquots at −20°C for subsequent analyses of vitamin D3 metabolites and calcium. A baseline blood sample was collected from each steer before bolus administration on the respective treatment day. Intermediate blood samples were collected on each subsequent day when other treatment groups received a bolus. A final blood sample was collected into sodium-heparinized Vacutainers at the abattoir during exsanguination. Samples were stored on ice and transported back to Iowa State University, where blood was centrifuged and stored as described above. A blood sample was collected from control steers each time of treatment administration for other treatment groups and at slaughter.

Plasma Vitamin D3 Metabolite and Calcium Analysis

The 25-OH D3 was extracted from plasma with acetonitrile and quantified by RIA with an I125 tracer as described by Hollis et al. (1993). Plasma 1,25-dihydroxyvitamin D3 (1,25-(OH)2 D3) was extracted with acetonitrile, and the supernatant was treated with sodium periodate (Hollis et al., 1996). Plasma 1,25-(OH)2 D3 was then purified from the supernatant by solid-phase chromatography and quantified by RIA with an I125 tracer according to the procedures of Hollis et al. (1996). Plasma samples were prepared for quantification of calcium concentration by adding 5 mL of 0.1%
La$_2$O$_3$ solution to duplicate 100-µL aliquots of plasma. Plasma calcium was quantified by atomic absorption spectrometry (Perkin-Elmer Corp., Norwalk, CT) using a standard curve calibrated for 0, 5, 10, and 15 mg/dL of CaCl$_2$.

**Carcass Data and Muscle Sample Collection**

On the morning of d 36, steers were transported 363 km to a commercial abattoir (PM Windom, Windom, MN) for slaughter that afternoon. Steers were stunned via captive-bolt pistol and exsanguinated. Hot carcass weights were recorded for all steers on the day of slaughter, and quality and yield grade data (USDA, 1996) were collected after a 48-h chilling period at 4°C.

Seventy-two hours after slaughter, carcasses were shipped to a beef fabrication plant (Iowa Beef Specialties, Hartley, IA). A 10-cm section of the LM from each carcass was vacuum-packaged and transported to the Iowa State University Meats Laboratory. Then, sections were hand-sliced into two 2.54-cm-thick LM steaks, individually vacuum-packaged, and aged at 4°C for either 6 or 14 d postmortem. After the respective aging time, LM steaks were frozen at −20°C for subsequent WBSF analysis.

The remaining portion of the LM was divided further into three sections that were individually vacuum-packaged. One portion was stored immediately at −20°C for subsequent analysis of calcium and vitamin D$_3$ metabolite concentrations. The remaining portions were aged at 4°C for either 6 or 14 d postmortem and, after the designated aging time, these LM portions were frozen at −20°C for subsequent analysis of troponin-T degradation.

**Muscle Vitamin D$_3$ Metabolite and Calcium Analyses**

Concentrations of 25-OH D$_3$ and 1,25-(OH)$_2$ D$_3$ in muscle were quantified by the methodology of Horst et al. (1981). Briefly, 1 g of fresh LM was homogenized in 4 mL of PBS (0.14 M NaCl and 0.01 M K$_2$HPO$_4$; pH 7.0) with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY). Radiolabeled [³H]-25-OH D$_3$ and [³H]-1,25-(OH)$_2$ D$_3$ (Amersham Life Sciences, Arlington Heights, IL), approximately 1,000 counts/min each, were added to the homogenate for use in establishing recovery estimates. Muscle lipids were extracted with chloroform and methanol. Extracted volumes were dried under vacuum using a Savant SpeedVac concentrator, and the residue was resuspended in 1 mL of hexane.

The 25-OH D$_3$ fraction was dried under vacuum (Savant SpeedVac concentrator) and the residue was re-suspended in 150 µL of running solvent (hexane: methylene chloride:isopropanol; 88:10:2) for HPLC purification. The 25-OH D$_3$ fraction was injected onto a Dupont Zorbax NH$_2$ 4.6 × 250-mm HPLC column (Mac-Mod Analytical, Chadds Ford, CA). The running solvent transgressed the column at 2 mL/min, and the purified 25-OH D$_3$ fraction collection was based on the elution time for the 25-OH D$_3$ standard in hexane:methylene chloride:isopropanol (88:10:2). The concentration of 25-OH D$_3$ was quantified by using an RIA as described by Hollis et al. (1993).

The 1,25-(OH)$_2$ D$_3$ fraction was dried under vacuum (Savant SpeedVac concentrator) and re-suspended in 150 µL of hexane:isopropanol (92:8). The fraction was purified by using a Zorbax SiI HPLC column (4.6 × 250 mm; Mac-Mod Analytical) with hexane:isopropanol (92:8) transgressing the column at 2 mL/min. Collection of the purified 1,25-(OH)$_2$ D$_3$ fraction was based on the elution time for the 1,25-(OH)$_2$ D$_3$ standard in hexane:isopropanol (92:8). The concentration of 1,25-(OH)$_2$ D$_3$ was quantified using an RIA as described by Hollis et al. (1996).

Water-soluble muscle calcium concentration was determined using a modified technique of Nakamura (1973). Five grams of fresh LM were homogenized in 20 mL of monooiodoacetic acid (2 mg%) with a Polytron homogenizer. An additional 10 mL of the monooiodoacetic acid solution was added, and samples were centrifuged at 10,000 × g for 20 min. Supernatant (25 mL) was transferred to 100-mL Pyrex beakers and evaporated. The residue that remained following evaporation was ashed using a modified HNO$_3$-H$_2$SO$_4$ wet combustion method (NMAM, 1994). All glassware used in the preparation of muscle samples for Ca analysis was washed with 2 N HCl and rinsed with distilled-deionized water. Ten milliliters of concentrated (16.5 N) HNO$_3$ was added to the beakers containing the evaporated supernatant. The beaker was covered with a watch glass and swirled, and samples were allowed to stand overnight in HNO$_3$. The following morning, 3 mL of concentrated (36 N) H$_2$SO$_4$ was added to the contents and heated at 150°C for 1 h (or until brownish fumes disappeared). Once brownish fumes had cleared, samples were heated to 400°C until white fumes evolved. At this point, the watch glass was removed, and the acid solution was condensed to approximately 3 mL. If white fumes did not evolve and the beaker contents turned dark, 5-mL volumes of HNO$_3$ were added to the beaker until white fumes resulted and the liquid became clear. Beaker contents then were condensed to approximately 3 mL and transferred to a 25-mL volumetric flask. Samples were brought to volume with 1% LaCl$_3$. Calcium was quantified using atomic absorption spectrometry (Perkin-Elmer Corp.), and a standard curve was calibrated for 0, 5, 10, and 15 mg/dL of CaCl$_2$. 

Plasma calcium was quantified by atomic absorption spectrometry (Perkin-Elmer Corp.) using a standard curve calibrated for 0, 5, 10, and 15 mg/dL of CaCl$_2$. 

The 25-OH D$_3$ fraction was dried under vacuum (Savant SpeedVac concentrator), and the residue was re-suspended in 150 µL of running solvent (hexane: methylene chloride:isopropanol; 88:10:2) for HPLC purification. The 25-OH D$_3$ fraction was injected onto a Dupont Zorbax NH$_2$ 4.6 × 250-mm HPLC column (Mac-Mod Analytical, Chadds Ford, CA). The running solvent transgressed the column at 2 mL/min, and the purified 25-OH D$_3$ fraction collection was based on the elution time for the 25-OH D$_3$ standard in hexane:methylene chloride:isopropanol (88:10:2). The concentration of 25-OH D$_3$ was quantified by using an RIA as described by Hollis et al. (1993).

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Warner-Bratzler Shear Force Determination of Tenderness

Steaks were thawed for 24 h at 4°C, broiled with an industrial broiler (model CNO2; General Electric, Chicago Heights, IL) to an internal temperature of 35°C, turned, and broiled to an internal temperature of 71°C. Steaks were wrapped individually in Saran Wrap (S.C. Johnson & Son, Inc., Racine WI,) and chilled overnight at 4°C. The following morning, six 1.27-cm diameter cores were removed parallel to the muscle fiber orientation (AMSA, 1995), and cores were sheared perpendicular to the muscle fibers with a texture analyzer (model TA-XTi; Texture Technologies Corp., Scarsdale, NY) fitted with a Warner-Bratzler cutting blade that adhered to the specifications described by Wheeler et al. (1997). The test was run at a penetration speed of 3.3 mm/s, and peak shear force for the six cores was averaged for statistical analysis.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis and Western Blotting Determination of Troponin-T Degradation

Samples used to quantify the 30-kDa proteolytic degradation component of troponin-T were prepared according to the procedures of Huff-Lonergan et al. (1996b). Samples were loaded onto a polyacrylamide gel along side a molecular weight standard to assure that the protein concentration loaded onto the gel was similar for all samples and for band identification. Proteins were transferred from the gel to a membrane for Western blotting according to the procedures of Huff-Lonergan et al. (1996a). An internal standard for beef troponin-T degradation was loaded in duplicate onto each gel. Labeled protein bands were detected using the ECL chemiluminescent system (Amersham Pharmacia Biotech, Piscataway, NJ) as described in Huff-Lonergan et al. (1996a). An internal standard for beef troponin-T data were analyzed as a repeated measure with carcass/steer as the experimental unit and postmortem aging day as the repeated variable. The statistical model included the 25-OH D3 treatments and postmortem aging time as fixed effects, and pen nested within treatments and carcass/animal nested within the pen × treatment interaction as random effects. Least squares means were computed for all fixed effects and separated using pair-wise t-tests (PDIF option) when a significant F-test ($P < 0.05$) was detected.

Results and Discussion

Feedlot Performance

Feedlot performance results are reported in Table 2. Average initial weights were similar ($P > 0.05$) for 25-OH D3-treated and control steers. Average daily gain during the final 35 d before slaughter did not differ ($P > 0.05$) between 25-OH D3-treated and control steers, and as a result, final live weights were similar ($P > 0.05$) for all groups of steers. Additionally, average DMI was similar ($P > 0.05$) for 25-OH D3-treated and control steers.

Results of Foote (2001) indicated that administration of a single oral bolus of 25-OH D3 decreased ADFI from 9 kg/d to a nadir of 6.5 kg/d by 2 d following treatment; however, feed intake gradually increased thereafter to an amount similar to that of controls. Hypercalcemia achieved by Foote (2001) with the administration of 125 mg of 25-OH D3 most likely led to anorexia in these cattle. Likewise, Littledike and Horst (1982) demonstrated that vitamin D3 toxicity in dairy cattle results in elevated plasma calcium concentrations, as well as a 50% decrease in feed intake. Moreover, these results were substantiated by the corresponding decrease in feed intake associated with elevated plasma calcium concentration (Karges et al., 2001; Scanga et al., 2001; Montgomery et al., 2002). Blood samples collected in the current trial did not ($P > 0.05$) indicate hypercalcemia as a result of 25-OH D3 treatment; therefore, decreased feed intake would not be expected in the current trial because hypercalcemia was not achieved. Thus, oral administration of a single bolus of 62.5 or 125 mg of 25-OH D3 did not adversely influence feedlot performance.

Plasma Vitamin D Metabolites and Calcium

A single oral bolus of 25-OH D3 given between 35 and 4 d before slaughter resulted in elevated ($P < 0.001$)
plasma 25-OH D$_3$ concentrations, which remained elevated through slaughter ($P < 0.001$) when compared with concentrations for untreated control steers (Figure 1). Steers that received 125 mg of 25-OH D$_3$ 4 d before slaughter had higher ($P = 0.02$) plasma 25-OH D$_3$ concentrations at slaughter than did steers given the 62.5-mg dose 4 d before slaughter. Additionally, plasma 25-OH D$_3$ concentrations at slaughter were lower ($P = 0.03$) for steers given 125 mg of 25-OH D$_3$ 35 d before slaughter compared with steers given the same dose 4 d before slaughter. However, with these exceptions, plasma 25-OH D$_3$ concentrations at slaughter did not differ ($P > 0.05$) as a result of bolus dose or time of administration relative to slaughter. These results indicate that the amount of 25-OH D$_3$ given will influence plasma 25-OH D$_3$ concentration at slaughter when the bolus is given in close proximity to slaughter (within 4 d), and that plasma 25-OH D$_3$ concentrations at slaughter decrease as length of time between bolus administration and slaughter increases for steers given 125 mg of 25-OH D$_3$. Plasma 25-OH D$_3$ concentrations measured at slaughter for steers assigned to the 25-OH D$_3$ treatments were similar to those reported by Foote et al. (2004). In contrast, plasma 25-OH D$_3$ concentration measured for control steers in this trial was nearly twice that of control steers reported by Foote et al. (2004). 25-Hydroxyvitamin D$_3$ is the plasma metabolite that most accurately predicts vitamin D status (Combs, 1992). The current trial was conducted in September and October, whereas the work of Foote et al. (2004) was conducted in December and January. Differences in plasma 25-OH D$_3$ concentrations are likely attributable to seasonal influence on vitamin D status. Hidiroglou et al. (1979) reported that plasma 25-OH D$_3$ concentrations were more than double for cattle during the summer months when compared with those for winter months. This seasonal fluctuation in plasma 25-OH D$_3$ concentration was substantiated by recently reported results with human subjects (Barger-Lux and Heaney, 2002). Because baseline 25-OH D$_3$ concentrations were higher in this trial compared with those of Foote et al. (2004), the difference between baseline and plasma 25-OH D$_3$ concentrations at slaughter was not as great.

Combs et al. (1992) indicated that 25-OH D$_3$ at 100 times the physiological requirement competes for the intracellular 1,25(OH)$_2$ D$_3$ receptor and enhances calcium absorption and resorption. Foote et al. (2004) demonstrated hypercalcemia in response to elevated plasma 25-OH D$_3$ concentration that peaked at 4 d after bolus administration and persisted for 20 d after bolus administration. In the current trial, hypercalcemia was not detected ($P > 0.05$) for steers despite a similar peak plasma 25-OH D$_3$ concentration. An exact explanation for this discrepancy is largely unknown, but may be attributed to less of a difference between baseline and peak plasma 25-OH D$_3$ concentrations.

Vitamin D hypervitaminosis in mammals rarely results from overexposure to UV light, primarily because bodily mechanisms exist to metabolize excess 25-OH D$_3$ to an inactive 24,25-dihydroxyvitamin D$_3$ (Combs, 1992). During periods of limited ultraviolet light exposure, cattle may be more responsive to a given dose of 25-OH D$_3$ because feedback mechanisms are not currently capable of accommodating environmental excesses of 25-OH D$_3$, whereas a higher dose of 25-OH D$_3$ may be necessary during seasons of high UV light exposure because the feedback mechanism has adjusted to accommodating high amounts of 25-OH D$_3$. Plasma 1,25(OH)$_2$ D$_3$ concentrations in this trial were similar ($P > 0.05$), regardless of treatment and, although lower than those reported by Foote et al. (2004), were consistent with the normal range indicated by Combs (1992). 1,25-Dihydroxyvitamin D$_3$ is the biological form of vitamin D$_3$ that elicits absorption of calcium from the small intestine and calcium resorption from bone and kidney (Combs, 1992). Exogenous administration of 62.5 or 125 mg of 25-OH D$_3$ did not elevate plasma 1,25(OH)$_2$ D$_3$ concentrations, and it is likely that the excess 25-OH D$_3$ was converted to inactive 24,25-dihydroxyvitamin D$_3$.

### Carcass Characteristics and Muscle

#### Vitamin D Metabolites and Calcium

Standard carcass measures including hot carcass weight, LM area, 12th-rib fat thickness, quality grade, and yield grade are presented in Table 3. No carcass trait was affected ($P > 0.05$) by 25-OH D$_3$ treatment. A single oral bolus of 25-OH D$_3$, regardless of bolus dose or time of bolus administration relative to slaughter, increased ($P \leq 0.001$) 25-OH D$_3$ concentrations in

<table>
<thead>
<tr>
<th>Item</th>
<th>62.5 mg of 25-OH D$_3$</th>
<th>125 mg of 25-OH D$_3$</th>
<th>Control SEM</th>
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<tbody>
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<td>G:F</td>
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</table>

*Means represent the average for a pen of six steers and two pens per treatment.

Table 2. Feedlot performance for steers given a single oral bolus of 25-hydroxyvitamin D$_3$ (25-OH D$_3$) at various times before slaughter
Figure 1. Plasma 25-hydroxyvitamin D3 (25-OH D3) concentrations for steers given a single oral bolus of 25-OH D3 at 4 (horizontal hatch bars), 7 (solid black bars), 21 (dotted bars), and 35 d (diagonal hatch bars) preslaughter, as well as untreated control (open bars). An asterisk (*) indicates plasma 25-OH D3 concentrations were increased ($P \leq 0.001$) with a single oral bolus of 25-OH D3 and remained increased through slaughter ($P \leq 0.001$) compared with those of untreated control steers. Among steers treated 4 d preslaughter, those given 125 mg of 25-OH D3 had higher ($P = 0.02$) plasma 25-OH D3 concentrations at slaughter than did steers given 62.5 mg of 25-OH D3.

Muscle 25-OH D3 concentrations reported for the LM of 25-OH D3-treated steers in this trial were similar to those reported by Foote et al. (2004). Furthermore, Foote et al. (2004) reported the concentration of vitamin D3 in muscle to be similar between controls and 25-OH D3-treated steers; thus, muscle vitamin D3 concentration was not measured in this trial. Additionally, steers that received a 25-OH D3 bolus 4 or 7 d before slaughter had muscle 25-OH D3 concentrations that were higher ($P \leq 0.03$) than those found in steers that received a bolus 35 d before slaughter, indicating that increased time between bolus administration and slaughter results in lower concentrations of 25-OH D3 in the LM of 25-OH D3-treated steers. In agreement with Foote et al. (2004), 1,25(OH)2 D3 concentration of LM was unchanged ($P > 0.05$) by 25-OH D3 treatment. The 1,25-(OH)2 D3 concentrations for LM in the current trial, however, were lower than those reported by Foote et al. (2004). This difference in muscle 1,25-(OH)2 D3 concentration is reflective of the difference that was reported for plasma 1,25(OH)2 D3 concentration in this trial.

Nakamura (1973) suggested that water-soluble Ca was a good indication of Ca available for use by the calpains for postmortem proteolysis. Water-soluble Ca concentrations in the LM were similar ($P > 0.05$) for 25-OH D3-treated and control steers, averaging 7.11 μg/g (Table 3). This concentration was intermediate to those reported for beef by Swanek et al. (1999) and for
Table 3. Carcass and muscle characteristics for steers given a single oral bolus of 25-hydroxyvitamin D3 (25-OH D3) at various times before slaughter

<table>
<thead>
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Carcass characteristics

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<td>Quality gradea</td>
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<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Yield grade</td>
<td>2.1</td>
<td>2.2</td>
<td>1.7</td>
<td>2.0</td>
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</tr>
</tbody>
</table>

Vitamin D metabolites and calcium in muscleb

<table>
<thead>
<tr>
<th>Item</th>
<th>25-OH D₃, ng/g</th>
<th>1,25-(OH)₂D₃, pg/g</th>
<th>Calcium, μg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>−4</td>
<td>3.1ddef</td>
<td>24.5</td>
<td>8.4</td>
</tr>
<tr>
<td>−7</td>
<td>2.8g</td>
<td>19.1</td>
<td>5.3</td>
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<tr>
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<td>3.0f</td>
<td>14.4</td>
<td>6.3</td>
</tr>
<tr>
<td>−35</td>
<td>2.5h</td>
<td>21.3</td>
<td>9.4</td>
</tr>
<tr>
<td>Control</td>
<td>3.5d</td>
<td>20.3</td>
<td>6.2</td>
</tr>
</tbody>
</table>

a1 = Prime; 2 = Choice; 3 = select; 4 = Standard.
bConcentrations of metabolites and calcium are expressed on a wet-weight basis.
c1,25-dihydroxyvitamin D₃.
d,e,f,g,h,iWithin a row, least squares means without a common superscript letter differ (P ≤ 0.001).

poultry meat by Nakamura (1973). Swanek et al. (1999) reported that feeding 5 million IU of vitamin D3 for 7 d before slaughter resulted in water-soluble muscle calcium concentrations of 19.9 μg/g compared with 13.9 μg/g for control steers at 7 d postmortem.

Tenderness

Warner-Bratzler shear force (Figure 2) and troponin-T degradation (Figure 3) were similar (P > 0.05) for steaks from control and 25-OH D₃-treated steers. Although length of aging postmortem improved steak tenderness, as indicated by WBSF (P ≤ 0.001) and troponin-

Figure 2. Effects of a single oral bolus of 25-hydroxyvitamin D₃ (25-OH D₃) on Warner-Bratzler shear force (WBSF) values of LM steaks aged 6 or 14 d postmortem. Longissimus muscle steaks aged for 14 d postmortem (open bars) had lower (P < 0.001) WBSF values than did LM steaks aged for 6 d postmortem (solid black bars). Pooled SEM = 0.10 for effects of postmortem aging period on WBSF. Warner-Bratzler shear force did not differ (P > 0.05) for steaks from 25-OH D₃-treated and control steers, regardless of bolus dose or time of administration relative to slaughter (pooled SEM = 0.27).
Figure 3. Effects of a single oral bolus of 25-hydroxyvitamin D$_3$ (25-OH D$_3$) on 30-kDa protein band intensity as an indicator of troponin-T degradation in LM steaks aged 6 or 14 d postmortem (pooled SEM = 0.07). Longissimus muscle steaks aged for 14 d postmortem (open bars) had greater ($P \leq 0.001$) troponin-T degradation than did LM steaks aged for 6 d postmortem (solid black bars). Troponin-T degradation did not differ ($P > 0.05$) for steaks from 25-OH D$_3$-treated and control steers, regardless of bolus dose or time of administration relative to slaughter (pooled SEM = 0.17).

Montgomery et al., 2002). Karges et al. (2001) summarized the effects of vitamin D$_3$ on beef tenderness by concluding that 53% of steaks from untreated beef cattle had WBSF values greater than 3.86 kg, whereas 23% of vitamin-D$_3$ treated cattle reached this threshold. More recently, Platter et al. (2003) concluded that the threshold at which tenderness influences the acceptability of beef is 4.5 kg of shear force. Average WBSF for LM steaks and gluteus medius steaks from vitamin D$_3$-treated and control steers did not reach this threshold (Karges et al., 2001). Swanek et al. (1999) demonstrated a tendency for the percentage of LM steaks with a shear force greater than 4.6 kg to be decreased at d 7 and 21 postmortem when 7.5 million IU of vitamin D was supplemented for 10 d before slaughter. However, when the vitamin D$_3$ dose was 5 million IU administered 7 d before slaughter, the percentage of steaks with a shear force greater than 4.6 kg was decreased for steaks aged 7 d postmortem but not for steaks aged 14 or 21 d postmortem. Thus, vitamin D$_3$ dose and duration of treatment may affect the efficacy of vitamin D$_3$ on tenderness, and the same may be true for 25-OH D$_3$ treatment. Montgomery et al. (2000) demonstrated an improvement in WSBF for top round steaks and LM steaks from steers supplemented with five and 7.5 million IU of vitamin D$_3$ and aged for 14 d postmortem; however, no difference was noted for steaks aged for 3, 7, or 21 d. Additionally, Montgomery et al. (2002) reported that supplementing 5 or 7.5 million IU of vitamin D$_3$ improved WSBF of LM steaks aged 7 d postmortem, but did not improve WSBF at 10, 14, or 21 d postmortem. Troponin-T is a major myofibrillar protein, and its degradation is indicative of postmortem tenderization (Huff-Lonergan et al., 1996a). Both Montgomery et al. (2000) and Foote et al. (2004) report that oral administration of vitamin D$_3$ enhanced troponin-T degradation in LM steaks of steers supplemented with 5 million IU of vitamin D$_3$.

Although inconsistencies exist concerning the efficacy of dietary vitamin D$_3$ and beef tenderness, vitamin D$_3$ has been indicated as a nutritional means of improving beef tenderness. If the mechanism by which vitamin D$_3$ improves beef tenderness is via elevated muscle Ca and enhanced Ca-activated proteolysis, then vitamin D$_3$ must be converted to 25-OH D$_3$ and then to 1,25-(OH)$_2$ D$_3$ before it is biologically active and able to enhance Ca absorption from the small intestine and resorption from the kidney and muscle. Following this logic, orally administered 25-OH D$_3$ should be hydroxylated to 1,25-(OH)$_2$ D$_3$ to elicit the same enhancement of calcium absorption and resorption. Additionally, Combs et al. (1992) indicated that 25-OH D$_3$, at 100 times the physiological requirement, competes for the intracellular 1,25-(OH)$_2$ D$_3$ receptor and elicits the same effects on Ca as 1,25-(OH)$_2$ D$_3$. These results suggest that our chosen doses of 62.5 or 125 mg of 25-OH D$_3$ were not sufficient to enhance calcium absorption and resorption. This response may be the result of an inappropriate dose or interfering seasonal differences in sunlight exposure. Regardless, vitamin D and its metabolites are highly regulated within the mammalian system, therefore leading to contradictory results concerning the effects of exogenous vitamin D or its metabolites to enhance Ca-activated proteolysis and improve beef tenderness.

Implications

These results demonstrate that plasma 25-hydroxyvitamin D$_3$ remains elevated for up to 35 d after a single oral bolus. However, neither 62.5 nor 125 mg of 25-hydroxyvitamin D$_3$ was sufficient to increase plasma
or longissimus muscle calcium concentrations at slaughter or influence tenderness positively. Little research has been conducted to demonstrate the effects of supplemental 25-hydroxyvitamin D₃ on beef tenderness. As a result, further research is needed to investigate the proper dose of 25-hydroxyvitamin D₃, ideal time of administration relative to slaughter, and potential environmental factors that result in inconsistencies.

Literature Cited


USDA. 1996. United States Standards of Slaughter Cattle. USDA, Washington, DC.
