Supplemental vitamin D$_3$ and zilpaterol hydrochloride. I. Effect on performance, carcass traits, tenderness, and vitamin D metabolites of feedlot steers

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ABSTRACT: Angus × Simmental steers ($n = 210$; initial BW $314 \pm 11$ kg) were separated into heavy and light BW blocks and allotted evenly by BW to 6 treatments (3 heavy and 2 light pens per treatment) to determine the effect of supplemental vitamin D$_3$: 0 IU (no D), 250,000 IU for 165 d (long-term D), or $5 \times 10^6$ IU for 10 d (short-term D) on performance, carcass traits, vitamin D metabolites, and meat tenderness in steers fed either 0 (NZ) or 8.38 mg/kg zilpaterol hydrochloride (ZH) daily for 21 d. Placebo or ZH was added to the diet 24 d, and short-term D was added 13 d before slaughter. Vitamin D$_3$, ZH, and placebo were all removed from the diet 3 d before slaughter. Steers fed ZH tended to have improved overall G:F compared with steers not fed ZH ($P < 0.09$). Overall performance was not affected by long-term D, with or without ZH ($P = 0.11$) compared with no D, with or without ZH. Short-term D decreased final BW, ADG, and G:F ($P = 0.04$) compared with no D, when ZH was not fed. Zilpaterol hydrochloride increased HCW, dressing percentage, and LM area ($P < 0.01$); and decreased fat thickness, yield grade, and marbling ($P < 0.03$). Carcass traits were not impacted by long-term D without ZH ($P > 0.13$), but long-term D with ZH decreased percentage KPH ($P < 0.02$). Compared with no D, short-term D tended to decrease HCW ($P < 0.07$), decreased fat thickness ($P < 0.01$), and tended to increase dressing percentage ($P < 0.10$) when ZH was not fed, yet did not impact carcass traits when ZH was fed ($P < 0.13$). Feeding ZH tended to decrease ($P < 0.09$) LM 1,25-dihydroxyvitamin D$_3$ [1,25(OH)$_2$D$_3$]. The long-term D treatment increased LM vitamin D$_3$ and 25-hydroxyvitamin D$_3$ (25OHD$_3$) 18- and 5-fold, respectively, when ZH was not fed ($P < 0.04$) and increased LM 25OHD$_3$ by 4-fold when ZH was fed ($P < 0.01$). Short-term D increased LM vitamin D$_3$ and 25OHD$_3$ by 52- and 9-fold, respectively, when ZH was not fed ($P < 0.01$), and by 24- and 9-fold, respectively, when ZH was fed ($P < 0.01$). Also, short-term D increased LM 25OHD$_3$ by 2-fold ($P < 0.04$) when ZH was fed. Warner-Bratzler shear force (WBSF) was greater for ZH steaks than non-ZH steaks at 7, 14, and 21 d postmortem aging ($P < 0.01$). Vitamin D did not reduce WBSF ($P = 0.18$). When ZH was fed, long-term D tended to increase WBSF in steaks aged 21 d ($P = 0.06$). In conclusion, ZH improved carcass leanness and decreased tenderness, and vitamin D feeding increased vitamin D$_3$ metabolites in LM, but did not improve tenderness in steers fed ZH.

Key words: beef, tenderness, vitamin D, zilpaterol hydrochloride


INTRODUCTION

Zilpaterol hydrochloride (ZH) is a is a category 2 β-adrenergic agonist (β-AA) that improves growth performance and increases muscle mass in beef cattle through increased protein synthesis and decreased protein degradation (Mersmann, 1998; Moody et al., 2000). Though ZH improves feedlot performance and carcass leanness (Leheska et al., 2009), decreased tenderness is a concern (Garmyn et al., 2010).

Supplementation of supranutritional levels of vitamin D$_3$ (D$_3$) at ≥1 million IU/d for 7 to 10 d before slaughter have been reported to increase plasma and muscle Ca concentrations, increase activation of calpains, and improve beef tenderness (Montgomery et al., 2000). Therefore, supplementation of supranutritional D$_3$ has the potential to overcome increased toughness in steers fed ZH. In fact, Koohmaraie and Shackelford (1991)
demonstrated that CaCl₂ infusion into meat was effective in overcoming β-AA-induced toughness. However, supplementation of supranutritional D₃ can decrease DMI and BW gain, and can cause D₃ metabolites in muscle, liver, and kidney to reach excessive concentrations (Montgomery et al., 2000); thus, alternative D₃ feeding strategies are needed. DePra et al. (2004) demonstrated that feeding 50,000 or 100,000 IU of D₃ daily for 175 d increased plasma and kidney D₃ metabolite concentrations, but did not increase liver and muscle D₃ metabolite concentrations or decrease DMI and BW gain. However, the impact of long-term D₃ supplementation on tenderness was not measured, and to our knowledge, has not been investigated. Thus, our hypothesis was that including supplemental D₃ at a rate of 5 × 10⁶ IU/d for the last 10 d before slaughter or at a rate of 250,000 IU/d for the entire feeding period would enhance tenderness in steers fed ZH. Our objectives were to determine the effects of different D₃ feeding strategies on performance, carcass traits, and LM tenderness, as well as concentration of D₃ metabolites in the LM, liver, and kidney in steers fed ZH or not fed ZH.

MATERIALS AND METHODS

Research protocols using animals followed guidelines in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1998) and were approved by the Purdue Animal Care and Use Committee.

Animals and Diets

Angus × Simmental steers (n = 210; initial BW 314 ± 11 kg) from 2 Purdue University Agricultural Centers were allotted evenly by BW and sire to 6 treatments to determine the effect of supplemental vitamin D₃: 0 IU (ND), 250,000 IU for 165 d (LD), or 5 × 10⁶ IU for 10 d (SD) on performance, carcass traits, vitamin D metabolites, and meat tenderness in steers fed either 0 (NZ) or 8.38 mg/kg ZH (Zilmax; Merck Animal Health, Summit, NJ) daily for 21 d before slaughter.

Upon arrival at the Purdue University Animal Science Research and Education Center (ASREC, West Lafayette, IN), cattle were placed in outdoor lots and adapted to a common growing phase diet (30% corn silage, 20% distillers grain; Table 1) over a 3-wk period. Steers were vaccinated against bovine rhinotracheitis, bovine viral diarrhea, parainfluenza-3, and bovine respiratory syncytial virus (Bovi-Shield GOLD FP 5; Pfizer Animal Health, Kalamazoo, MI), against Haemophilus somnus, Pasteurella, and Clostridia (Vision-7 Somnus; Merck Animal Health, Summit, NJ), and treated with an anthelmintic (Valbazen; Pfizer Animal Health) for internal and external parasites at weaning and on arrival at the ASREC feedlot. At initiation of the study, cattle were weighed, implanted with Revalor-XS (provided courtesy of Merck Animal Health), blocked by BW (3 heavy pens and 2 light pens per treatment), and allotted to pens and treatments. Steers were randomly assigned to pen (7 steers per pen) and housed in curtain-sided, slatted-floor finishing barn in 6.1 × 3.3 m pens. Initial and final BW were determined by weighing steers on 2 consecutive days. Body weight was determined monthly as well as at the initiation of ZH feeding. Two steers were removed from the study for reasons unrelated to the treatments.

Steers were fed Zilmax premix 3 – – – 2.1
Non-Zilmax premix 3 – – 2.1 –
Vitamin/mineral premix 2 2.6 1.7 1.7 1.7

Table 1. Composition of basal diets fed to steers during the growing and finishing phases

<table>
<thead>
<tr>
<th>Item</th>
<th>Growing</th>
<th>Finishing</th>
<th>Finishing diet, Non-Zilmax</th>
<th>Finishing diet, Zilmax³</th>
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<tr>
<td>Ingredient, % (DM basis)</td>
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<tr>
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<td>–</td>
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<tr>
<td>Nutrient composition, %</td>
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<tr>
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<td>Phosphorus⁴</td>
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<tr>
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<td>2.14</td>
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<td>NEm, Meal/kg⁶</td>
<td>1.33</td>
<td>1.39</td>
<td>1.36</td>
<td>1.36</td>
</tr>
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</table>

¹Merck Animal Health, Summit, NJ.
²Vitamin/Mineral premix contains (DM basis): 16.41% Ca, 0.60% Mg, 0.32% K, 0.46% S, 11.64 ppm Co, 582 ppm Cu, 10.2 ppm Se, 0.16% Zn, 176 IU/g vitamin D, 440 IU/kg vitamin E, 1.32% Rumensin (176.4 g/kg, Elanco Animal Health, Indianapolis, IN), 0.40% Tylan (88.2 g/kg, Elanco Animal Health).
³The non-Zilmax supplement contains (DM basis): 80% wheat midds, 7.5% wheat flour, 7.5% DDGS, 5% CaCO₃ (Kalmbach Feeds, Upper Sandusky, OH). The Zilmax supplement was identical, but contained (DM basis): 396.5 mg/kg Zilmax.
⁴Calculated composition.
⁵The NDNZ (0 IU vitamin D₃ without zilpaterol hydrochloride) and NDZ (0 IU vitamin D₃ with zilpaterol hydrochloride) contained no additional vitamin D₃. SDNZ (5 × 10⁶ IU vitamin D₃ for 10 d without zilpaterol hydrochloride) and SDZ (5 × 10⁶ IU vitamin D₃ with zilpaterol hydrochloride) steers were fed 0.454 kg of corn that contained 5 million IU of supplemental vitamin D₃ (DSM Nutritional Products, Parsippany, NJ) for 10 d. The LDNZ (250,000 IU vitamin D₃ for 165 d without zilpaterol hydrochloride) and LDZ (250,000 IU for 165 d with zilpaterol hydrochloride) steers were fed 0.454 kg of corn that contained 250,000 IU of supplemental vitamin D₃ (DSM Nutritional Products, Parsippany, NJ) from 0 until 3 d before slaughter.
⁶Calculated composition.
Daily feed calls were adjusted to allow for ad libitum feed intake with little or no accumulation of unconsumed feed. Feed refusals were weighed, recorded, and discarded daily. Feed samples were taken every other week, dried in a forced air oven at 60°C for 48 h and composited for analysis of DM, CP, ADF, and NDF. Supplemental vitamin D$_3$ (provided courtesy of DSM Nutritional Products, Parsippany, NJ) was provided daily in 0.454 kg of a ground corn supplement in the amounts of 0 IU (ND), 250,000 IU for the length of the study (LD), or $5 \times 10^6$ IU vitamin D$_3$ for 165 d without ZH (SD). Beginning 24 d before slaughter, one-half the pens from each vitamin D treatment received ZH in pellet for a total diet ZH content of 8.38 mg/kg and the other one-half received a pellet without ZH (placebo). Because Tipton et al. (2007) demonstrated that feeding 3 million IU/d of vitamin D for 5 d followed by a 7 d withdrawal before slaughter decreased LM vitamin D concentrations, increased LM Ca, and still improved tenderness, vitamin D$_3$ in the current study was removed 3 d before slaughter along with ZH and the placebo. The 6 treatments were 0 IU vitamin D$_3$ without ZH (NDNZ), 0 IU vitamin D$_3$ with ZH (NDZ), 250,000 IU vitamin D$_3$ for 165 d without ZH (LDNZ), 250,000 IU for 165 d with ZH (LDZ), $5 \times 10^6$ IU vitamin D$_3$ for 10 d without ZH (SDNZ), and $5 \times 10^6$ IU vitamin D$_3$ with ZH (SDZ). Zilpaterol hydrochloride feeding was initiated when cattle in each block achieved an average BW of 569 kg.

**Carcass Data Collection**

Cattle were slaughtered at Tyson Foods (Joslin, IL) after 151 d (heavy block) and 186 d (light block) on feed. The total time that ZH was fed was 21 d, and the total time short-term D was fed was 10 d. Hot carcass weights were determined after slaughter and before chilling, and fat thickness, KPH, LM area, and USDA quality and USDA yield grades were determined for all cattle by qualified University personnel 24 h after slaughter. Longissimus muscle samples (12th rib to third lumbar vertebra) were collected from the right and left sides of each steer. Due to circumstances unrelated to treatment, LM samples could only be obtained for 20 steers in the NZ heavy treatment; therefore, LM vitamin D metabolite concentrations were only measured in the light block (14 steers per treatment). Longissimus muscle samples were collected 24 h after slaughter from all carcasses in the light block and ZH heavy treatments. Longissimus muscle samples were transported on ice to the Purdue University Meat Laboratory where they were cut 2.54 cm thick, vacuum packaged, and aged for 7, 14, or 21 d before freezing at −20°C for subsequent Warner-Bratzler shear force (WBSF) determinations. Carcass side and anterior or posterior position of the steak were evenly allotted among aging treatments.

**Warner-Bratzler Shear Force Determination**

Warner-Bratzler shear force was determined according to standards set by the American Meat Science Association (AMSA, 1995). Steaks were thawed and cooked on an electric clam shell grill (George Foreman Indoor/Outdoor grill, model GRP99; Miramar, FL) to an internal temperature of 40°C, turned over, and removed from the heat when an internal temperature of 71°C was reached. Cooking temperature was measured using a hand-held digital thermometer with probe (Model SH66A, Cooper-Atkins Corp., Middlefield, CT) placed in the geometric center of the steak. After cooking, steaks were chilled at 2 to 5°C overnight. From each steak, six to eight 1.27-cm-diam. cores were removed parallel to the muscle fiber orientation and sheared once in the center perpendicular to the muscle fibers. A texture analyzer (TA. HD plus Texture Analyser, Stable Micro Systems LTD.; Godalming, Surrey, UK) with a WBSF attachment was used at a crosshead speed of 20 cm/min to collect WBSF measurements. Peak measurements were averaged to obtain a single WBSF value for each steak.

**Vitamin D Analysis**

Liver samples were collected immediately after evisceration, and kidney and subsamples of LM were collected from the carcass 24 h after slaughter. Liver, kidney, and LM samples were transported to Purdue, frozen 48 h after slaughter, and stored at −20°C for later determination of vitamin D metabolites. Vitamin D$_3$, 25-hydroxyvitamin D$_3$, 1,25-dihydroxyvitamin D$_3$, vitamin D$_2$, and 25-hydroxyvitamin D$_2$ concentrations were determined by a modification of the methods explained in Ding et al. (2010). Briefly, tissues were ground in a Rival food chopper (Jarden Consumer Solutions, Boca Raton, FL), and 2-g samples were weighed into 15-mL centrifuge tubes (BD Falcon, Franklin Lakes, NJ). Samples were then spiked with 10 μL of 1 ng/μL deuterated vitamin D$_3$ (vitamin D$_3$d$_3$). Two milliliters of PBS and 6-mL tert-Butyl methyl ether were added to the samples. Samples were then placed in a sonicator for 10 min, followed by a 10-min centrifugation at 22°C and 1500 × g. The upper phase was collected and dried under nitrogen gas. Two hundred microliters of acetonitrile followed by 800-μL water were added to the dried samples. The samples were then solid phase extracted using Water’s Oasis HLB (hydrophilic-lipophilic-balanced reversed-phase sorbent for acids, bases and neutrals, 30 mg) cartridges (Milford, MA). The samples were dry eluted in a speedvac and reacted with 50-μL of 4-Phenyl-1,2,4-triazoline-3,5-dione (PTAD) solution (1-mg/mL acetonitrile) at room temperature for 1 h. Samples were analyzed on LC-MS/MS (Liquid Chromatography—Tandem Mass
Zilpaterol hydrochloride and vitamin D$_3$ 3325

Statistical Analysis

Data were analyzed using the MIXED procedures (SAS Inst. Inc., Cary, NC). For performance, carcass, WBSF, and vitamin D concentration data, pen was the experimental unit with block and pen nested within treatment included in the model as random effects. Block was removed if not significant. Treatment and day (for repeated measures) were included as fixed effects. Performance and WBSF were analyzed as repeated measures by comparing 4 covariance structures for each variable (compound symmetric, autoregressive order one, heterogeneous autoregressive order 1, and unstructured) and the covariance structure that yielded the smallest Bayesian information criterion was used for the results presented. Carcass data and vitamin D metabolites were analyzed as a completely randomized design. The Shapiro-Wilk test was performed to test for normality. Vitamin D$_3$ and vitamin D$_2$ concentrations of LM, liver, and kidney were determined via standard curve.

RESULTS

Performance Traits

Feedlot performance data collected before ZH inclusion, during ZH inclusion, and over the entire length of the experiment are presented in Table 2. For the contrasts tested there were no significant differences in BW, ADG, total DMI, daily DMI, or feed efficiency before the start of ZH ($P > 0.52$). During the ZH inclusion period, ZH tended to increase ADG ($P = 0.10$), decreased ($P < 0.01$) total and daily DMI, improved feed efficiency ($P < 0.03$), but did not impact final BW ($P > 0.26$). When measured over the length of the study, ZH did not impact ADG, total DMI, or daily DMI ($P > 0.31$) and tended to improve feed efficiency ($P = 0.09$). When steers were not fed ZH, long-term D had no effect on ADG, DMI, or feed efficiency ($P > 0.68$). When steers were fed ZH, long-term D increased ADG ($P < 0.04$), and decreased total and daily DMI ($P < 0.02$) during the ZH inclusion period, but long-term D had no effect when examined over the entire study ($P = 0.11$). Short-term D decreased slaughter weights, ADG, total DMI, daily DMI, and feed efficiency ($P < 0.03$) during the ZH inclusion period when ZH was not fed, resulting in an overall decrease in ADG and feed efficiency ($P < 0.04$). However, when ZH was fed, short-term D had no effect ($P = 0.41$) on performance during the ZH inclusion period or overall ($P = 0.17$) when compared with no supplemental D.

Carcass Traits

Carcass data are presented in Table 3. Steers that were fed ZH had increased ($P < 0.01$) HCW, dressing percentage, and LM area, and decreased ($P < 0.03$) fat thickness, yield grade, marbling score, and percentage of carcasses grading USDA Choice or greater compared with steers that were not fed ZH. Long-term D had no effect on carcass traits when ZH was not in the diet ($P = 0.13$). However, when ZH was included, long-term D decreased KPH compared with carcasses from NDZ steers ($P < 0.02$). When ZH was not in the diet, short-term D tended ($P = 0.07$) to decrease HCW and decreased ($P < 0.01$) fat thickness. However, when ZH was in the diet, short-term D did not alter carcass characteristics compared with when no D was fed ($P > 0.13$).

Vitamin D in Beef, Kidney, and Liver

Vitamin D metabolite concentrations of LM, liver, and kidney are presented in Table 4. Zilpaterol hydrochloride tended ($P = 0.09$) to decrease LM concentrations of 1,25(OH)$_2$D$_3$, but had no effect ($P > 0.13$) on any other vitamin D metabolite. Long-term D increased LM concentrations of 25-hydroxyvitamin D$_3$ (25OHD$_3$) and 24,25-dihydroxyvitamin D$_3$ [24,25(OH)$_2$D$_3$], increased liver concentrations of vitamin D$_3$ and 25-hydroxyvitamin D$_3$ (25OHD$_3$), and increased kidney concentrations of vitamin D$_3$, 25OHD$_3$, 24,25(OH)$_2$D$_3$, and 25OHD$_2$ regardless of ZH treatment ($P < 0.05$). Long-term D did, however, have a variable effect on other vitamin D metabolites depending on whether ZH was fed or not. When ZH was fed, long-term D increased liver concentrations of 25-OHD$_3$, 1,25-dihydroxyvitamin D$_3$ [1,25(OH)$_2$D$_3$], and 24,25(OH)$_2$D$_3$, ($P < 0.04$), but did not affect these metabolites in the liver when ZH was not fed ($P = 0.13$). In contrast, when ZH was not fed, long-term D increased LM concentrations of vitamin D$_3$ and D$_2$ ($P < 0.04$), yet these metabolites were not increased by long-term D when ZH was fed ($P = 0.11$). Short-term D increased LM concentrations of vitamin D$_3$, 25OHD$_3$, 25OHD$_2$.
and 24, 25(OH)₂D₃, increased liver concentrations of vitamin D₃, 25OHD₃, 24,25(OH)₂D₃, and 25OHD₂, and increased kidney concentrations vitamin D₃, 25OHD₃, 24,25(OH)₂D₃, and 25OHD₂ regardless of ZH treatment ($P < 0.05$). However, LM concentrations of 1,25(OH)₂D₃ were only increased by short-term D when ZH was fed ($P < 0.04$) and kidney concentrations of 1,25(OH)₂D₃ were only increased by short-term D when ZH was not fed ($P < 0.04$).

**Warner-Bratzler Shear Force**

Vitamin D₃ and ZH treatment effects on WBSF are shown in Fig. 1. There was a treatment by day postmortem effect ($P < 0.01$) indicating that WBSF of steaks from ZH-fed steers decreased at faster rate than WBSF of steaks from NZ steers. Steers fed ZH produced LM steaks with 1.63-kg greater WBSF by 7 d postmortem aging, 1.17-kg greater WBSF at 14 d postmortem aging, and 0.99-kg greater WBSF at 21 d postmortem aging compared with steers not fed ZH ($P < 0.01$). When steers were not fed ZH, long-term D supplementation did not affect WBSF of LM steaks aged for 7, 14, or 21 d compared with steaks from steers not supplemented with long-term D ($P > 0.18$). However, when steers were fed ZH, long-term D supplementation tended ($P = 0.06$) to increase WBSF of LM steaks aged for 21 d. Warner-Bratzler shear force values of LM steaks from steers supplemented with short-term D were not affected ($P > 0.20$) at 7, 14, or 21 d postmortem aging whether or not the steers were fed ZH.

**DISCUSSION**

Vitamin D₃ is a secosteroid and must be converted to its biologically active form, 1,25-dihydroxyvitamin D₃. Previous studies have demonstrated that feeding increased doses of vitamin D₃ can result in prolonged...
hypercalcemia, decreased feed intake, and decreased BW gain (Karges et al., 2001; Montgomery et al., 2002, 2004a). Therefore, it is not surprising that the $5 \times 10^6$ dose in the current study decreased DMI and final BW, ADG, and feed efficiency. However, this negative effect was offset when steers were fed ZH. Furthermore, ZH interacted in a positive manner on performance with long-term vitamin D$_3$ supplementation. When ZH was fed to steers that received supplemental vitamin D$_3$ at a dose of 250,000 IU/steer daily for 165 d, ADG and DMI during the ZH inclusion period were increased compared with steers that received ZH but no long-term vitamin D$_3$. This data suggests that a low dose of supplemental vitamin D$_3$ fed over a long-term period may have an additive effect with ZH and could provide even greater benefits in feedlot performance and carcass traits that warrants further research. It may also indicate that ZH-fed steers have a greater vitamin D requirement. The additive effect of long-term vitamin D and ZH could be particularly beneficial for cattle fed indoors that may not get adequate sunlight for formation of vitamin D$_3$. Strydom et al. (2011) reported that ZH decreased parathyroid hormone (PTH), a major controller of whole-body calcium homeostasis. Decreased PTH has the potential to decrease the conversion of 25-hydroxyvitamin D$_3$ to 1,25-dihydroxyvitamin D$_3$, decrease gut absorption of calcium, decrease bone resorption of calcium, and decrease calcium entry into muscle cells (Potts and Gardella, 2007). The effects of decreased PTH on calcium homeostasis is consistent with our finding that ZH increased plasma total Ca$_3$ d before slaughter and decreased LM ionic calcium (Korn et al., 2013). The biologically active form of vitamin D can induce rapid skeletal muscle uptake of calcium by causing modulation of the calcium channel through the cAMP pathway (Ball, 2004; Vazquez et al., 1995). It is possible that the decrease in concentration of 1,25(OH)$_2$D$_3$ observed in LM of steers fed ZH in the current study plays a role in the decreased tenderness of steaks from steers fed ZH.

Vitamin D$_3$ supplemented at $5 \times 10^6$ IU/d has been reported to increase vitamin D$_3$ concentrations in liver, kidney, and uncooked beef, and increase 25OHD$_3$ residues in uncooked beef and liver (Montgomery et al., 2000). Montgomery et al. (2004b) reported an increase in 25OHD$_3$ in kidney as well as in beef and liver when $5 \times 10^6$ IU vitamin D/steer was fed daily the last 8 d before slaughter. Furthermore, Montgomery et al. (2002) demonstrated that both vitamin D$_3$ and 25OHD$_3$ further decreased, suggesting an additive effect of long-term D on nutrient repartitioning.

The fact that ZH decreased LM concentrations of 1,25-dihydroxyvitamin D indicates that ZH can impact calcium homeostasis and vitamin D metabolism. Strydom et al. (2011) reported that ZH decreased parathyroid hormone (PTH), a major controller of whole-body calcium homeostasis. Decreased PTH has the potential to decrease the conversion of 25-hydroxyvitamin D$_3$ to 1,25-dihydroxyvitamin D$_3$, decrease gut absorption of calcium, decrease bone resorption of calcium, and decrease calcium entry into muscle cells (Potts and Gardella, 2007). The effects of decreased PTH on calcium homeostasis is consistent with our finding that ZH increased plasma total Ca$_3$ d before slaughter and decreased LM ionic calcium (Korn et al., 2013). The biologically active form of vitamin D can induce rapid skeletal muscle uptake of calcium by causing modulation of the calcium channel through the cAMP pathway (Ball, 2004; Vazquez et al., 1995). It is possible that the decrease in concentration of 1,25(OH)$_2$D$_3$ observed in LM of steers fed ZH in the current study plays a role in the decreased tenderness of steaks from steers fed ZH.

### Table 3. The effect on carcass characteristics of feeding vitamin D$_3$ for 165 or 10 consecutive d to feedlot steers fed zilpaterol hydrochloride

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<th>No. of animals</th>
<th>HCW, kg</th>
<th>Dressing percent</th>
<th>Fat thickness, cm</th>
<th>LM area, cm$^2$</th>
<th>KPH %</th>
<th>USDA Yield Grade</th>
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<td>363.1</td>
<td>62.9</td>
<td>82.7</td>
<td>2.01</td>
<td>3.04</td>
<td>350</td>
<td>73.1</td>
</tr>
<tr>
<td>SDZ</td>
<td>35</td>
<td>383.4</td>
<td>64.2</td>
<td>88.9</td>
<td>1.97</td>
<td>2.85</td>
<td>323</td>
<td>56.1</td>
</tr>
</tbody>
</table>

Contrasts

- ZH vs. NZ
- LNDZ vs. SDNZ
- SDNZ vs. NDNZ
- SDZ vs. NDNZ

SEM$^1$

<table>
<thead>
<tr>
<th>SEM$^1$</th>
<th>Contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>ZH vs. NZ</td>
</tr>
<tr>
<td>0.07</td>
<td>LNDZ vs. SDNZ</td>
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<tr>
<td>0.01</td>
<td>SDNZ vs. NDNZ</td>
</tr>
<tr>
<td>0.01</td>
<td>SDZ vs. NDNZ</td>
</tr>
</tbody>
</table>

$^1n = 5$ pens/treatment.

$^2$Zilpaterol hydrochloride (ZH) vs. no ZH (NZ).

$^3$Long-term vitamin D$_3$ without ZH (LNDZ) vs. no supplemental D$_3$ without ZH (NDNZ)

$^4$Short-term vitamin D$_3$ without ZH (SDNZ) vs. NDNZ

$^5$Long-term vitamin D$_3$ with ZH (LDZ) vs. no supplemental vitamin D$_3$ with ZH (NDZ)

$^6$Short-term vitamin D$_3$ with ZH (SDZ) vs. NDZ.

$^7$USDA marbling scores: 200 to 299 = Slight; 300 to 399 = Small; 400 to 499 = Modest; 500 to 599 = Moderate.

$^8$Includes USDA “quality” grades: Choice°, >, Choice+, Choice++, and Prime.
were increased in cooked liver (75°C) when steers were supplemented with 5 × 10⁶ IU vitamin D/steer the last 9 d of feeding. Previous studies did not detect a difference in 1,25(OH)₂D₃ concentration when steers were supplemented with 5 × 10⁶ IU vitamin D₃ (Montgomery et al., 2000, 2002, 2004b). The current study also resulted in increased concentrations of vitamin D₃ and 25OHD₃ in LM, liver, and kidney of steers supplemented with 5 × 10⁶ IU vitamin D₃ regardless of ZH treatment; however, the current study also demonstrated an increase in 1,25(OH)₂D₃ in LM of SDZ steers, in liver of LDZ steers, and in kidney of SDNZ steers. It is possible that LCMS is better able to detect 1,25(OH)₂D₃ than is RIA, which was used in previous papers (Foote et al., 2004; Montgomery et al., 2004b; Snellman et al., 2010).

Short-term D and long-term D had no effect on liver and kidney vitamin D₂ concentrations, but liver and kidney concentrations of 25OHD₂ were decreased by short- and long-term D regardless of ZH, indicating that supplemental vitamin D₃ may decrease the metabolism of vitamin D₂ in these organs. However, LM concentrations of 25OHD₂ did not differ, and LDNZ actually increased LM concentration of vitamin D₂. The role of vitamin D₂ in meat tenderness is unknown, as this is the first report to our knowledge that has reported vitamin D₂ concentrations in tissues.

In terms of dose response to vitamin D₃ treatment, concentrations of vitamin D₃ and 25OHD₃ in LM, liver, and kidney followed a pattern similar to previous reports (Montgomery et al., 2002, 2004b); as dose increased, concentrations of vitamin D₃ and 25OHD₃ increased. Long-term D appeared to increase vitamin D metabolites to a much lesser extent than short-term D in the current study. However, because long-term and short-term D combined dosage and time, differences between short-term D and long-term D were not statistically compared. Longissimus muscle, liver, and kidney from steers fed short-term D contained 3, 7.5, and 2 times more

### Table 4. Vitamin D metabolites in feedlot cattle fed zilpaterol hydrochloride (Merck Animal Health, Summit, NJ) for 21 d and supplemental vitamin D₃ for 165 or 10 consecutive days

<table>
<thead>
<tr>
<th>Muscle⁸</th>
<th>No Vitamin D</th>
<th>250,000 IU for 165 d</th>
<th>5 million IU for 10 d</th>
<th>ZH vs. NZ³</th>
<th>LDNZ vs. SDNZ vs. NDNZ⁴</th>
<th>LDZ vs. NDZ⁵</th>
<th>SDZ vs. NDZ⁷</th>
</tr>
</thead>
<tbody>
<tr>
<td>D₃, ng/g</td>
<td>0.5 0.7 8.6 6.8</td>
<td>24.5 17.4 2.26</td>
<td>0.17 0.04 0.01 0.11 0.01 0.01 0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25OHD₃, ng/g</td>
<td>3.1 2.9 15.1 12.3</td>
<td>27.1 26.6 1.63</td>
<td>0.40 0.01 0.01 0.01 0.01 0.01 0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,25(OH)₂D₃, pg/g</td>
<td>172.1 77.9 113.7 91.6</td>
<td>186.1 171.1 26.00</td>
<td>0.09 0.16 0.72 0.72 0.04 0.04 0.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24,25(OH)₂D₃, ng/g</td>
<td>1.6 1.4 7.5 10.4</td>
<td>15.9 17.7 1.75</td>
<td>0.33 0.05 0.01 0.01 0.01 0.01 0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D₂, ng/g</td>
<td>0.1 0.1 0.3 0.2</td>
<td>0.1 0.2 0.04</td>
<td>0.04 0.65 0.01 0.65 0.22 0.17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25OHD₂, ng/g</td>
<td>0.9 1.4 0.8 0.3</td>
<td>0.3 2.5 0.51</td>
<td>0.13 0.85 0.44 0.16 0.20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver⁸</td>
<td>D₃, ng/g</td>
<td>0.5 0.5 3.5 4.3</td>
<td>28.4 32.1 4.63 to 9.49</td>
<td>0.41 0.01 0.01 0.01 0.01 0.01 0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25OHD₃, ng/g</td>
<td>6.7 7.5 18.5 29.3</td>
<td>54.3 58.0 13.34</td>
<td>0.32 0.19 0.01 0.02 0.01 0.01 0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,25(OH)₂D₃, pg/g</td>
<td>276.6 148.7 344.2 385.1</td>
<td>303.1 327.0 163.61</td>
<td>0.74 0.54 0.81 0.04 0.12</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>24,25(OH)₂D₃, ng/g</td>
<td>3.3 3.2 12.4 19.5</td>
<td>41.0 44.4 7.46</td>
<td>0.31 0.13 0.01 0.01 0.01</td>
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</tr>
<tr>
<td>D₂, ng/g</td>
<td>0.2 0.2 0.2 0.2</td>
<td>0.3 0.3 0.16 to 0.33</td>
<td>0.70 0.30 0.67 0.92 0.68</td>
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<tr>
<td>25OHD₂, ng/g</td>
<td>0.8 0.7 0.3 0.4</td>
<td>0.5 0.4 0.16</td>
<td>0.52 0.01 0.01 0.01 0.01 0.01</td>
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<tr>
<td>Kidney⁸</td>
<td>D₃, ng/g</td>
<td>0.6 0.8 6.0 7.7</td>
<td>14.1 12.0 2.01</td>
<td>0.99 0.04 0.01 0.01 0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25OHD₃, ng/g</td>
<td>6.7 7.7 22.3 28.2</td>
<td>69.9 47.4 8.11</td>
<td>0.25 0.05 0.01 0.01 0.01</td>
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<td></td>
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</tr>
<tr>
<td>1,25(OH)₂D₃, pg/g</td>
<td>356.5 429.1 384.8 234.3</td>
<td>783.8 434.3 146.29</td>
<td>0.21 0.89 0.04 0.33 0.98</td>
<td></td>
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</tr>
<tr>
<td>24,25(OH)₂D₃, ng/g</td>
<td>2.7 3.0 16.3 23.3</td>
<td>80.1 56.5 11.10 to 16.05</td>
<td>0.72 0.01 0.01 0.01 0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D₂, ng/g</td>
<td>0.2 0.2 0.1 0.1</td>
<td>0.1 0.2 0.10 to 0.20</td>
<td>0.69 0.18 0.58 0.62 0.88</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25OHD₂, ng/g</td>
<td>1.0 1.0 0.2 0.4</td>
<td>0.7 0.4 0.16</td>
<td>0.99 0.01 0.05 0.01 0.01 0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1Vitamin D₃ (D₃), 25-hydroxyvitamin D₃ (25OHD₃), 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), 24,25-dihydroxyvitamin D₃ (24,25(OH)₂D₃), vitamin D₂ (D₂), 25-hydroxyvitamin D₂ (25OHD₂).
2SEM for normally distributed data. Liver vitamin D₃ was square root transformed and liver and kidney vitamin D₂ and kidney 24,25-dihydroxyvitamin D₃ were log transformed and are presented as confidence intervals.
3Zilpaterol hydrochloride (ZH) vs. no ZH (NZ).
4Long-term vitamin D₃ without ZH (LDNZ) vs. no supplemental D₃ without ZH (NDNZ).
5Short-term vitamin D₃ without ZH (SDNZ) vs. NDNZ.
6Long-term vitamin D₃ with ZH (LDZ) vs. no supplemental vitamin D₃ with ZH (NDZ).
7Short-term vitamin D₃ with ZH (SDZ) vs. NDZ.
8Wet tissue basis.
Zilpaterol hydrochloride and vitamin D3, respectively, than steers fed long-term D. Additionally, 25OHD3 concentration was doubled in tissues from steers fed short-term D compared with long-term D. The concentration of 24,25(OH)2D3 also increased as vitamin D dose increased. This result is not surprising, as 24,25(OH)2D3, a metabolite that does not have hormonal activity, is produced when the body has enough 1,25(OH)2D3 to meet demands.

According to the Institute of Medicine (2011), a person between the ages of 1 and 70 yr has a vitamin D recommended dietary allowance (RDA) of 600 IU/d and an adult over 70 has a RDA of 800 IU/d. The upper level intake of vitamin D for a child 1 to 3 yr of age is 2500 IU/d and 3000 IU/d for a child 4 to 8 yr of age. People aged 9 to 71+ yr have an upper level intake of 4000 IU/d vitamin D (Institute of Medicine, 2011). One IU of vitamin D is defined as 0.025 µg of vitamin D3 or its equivalent; therefore, 1-µg vitamin D3 is equivalent to 40 IU and 1-µg 25-OH D3 is equivalent to 200 IU. According to Ovesen et al. (2003), 1,25- and 24,25-dihydroxyvitamin D3 in animal products account for very little of the biological vitamin D activity passed to humans consuming the animal product; therefore, these metabolites were not included in the calculation for IU of vitamin D in tissues.

The Federal Drug Administration (FDA) set consumption values of 300-g muscle, 100-g liver, and 50-g kidney to use for toxicology testing (FDA, 2006). Using these consumption values, vitamin D metabolites of steak, liver, and kidney from steers supplemented with short-term D are equivalent to approximately 1,863, 1,244, and 613 IU, respectively. All of these vitamin D concentrations are well below the 4,000 IU/d upper level intake for vitamin D. Furthermore, vitamin D deficiency is widespread in the United States. Approximately 40% of the US population aged 20 yr or over was vitamin D deficient (serum 25-hydroxyvitamin D3 concentration ≤ 20 ng/mL) in 2005 to 2006 according to data from the National Health and Nutrition Examination Survey (Forrest and Stuhldreher, 2011). Meat from steers supplemented with vitamin D3 could be beneficial in combating vitamin D deficiency. However, promoting meat from steers fed an increased dose of vitamin D3 for a short-time period, such as 5 × 106 IU for 10 d, as a good source of vitamin D for humans requires further research, as some studies have reported vitamin D3 concentrations in liver that approach the upper level intake for vitamin D (Montgomery et al., 2004b).

Zilpaterol hydrochloride is known to increase WBSF (Rathmann et al., 2009; Claus et al., 2010; Scramlin et al., 2010). In our study, WBSF was increased by ZH at 7, 14, and 21 d postmortem. In disagreement with Montgomery et al. (2000) and Foote et al. (2004), we found that supplementing steers with 5 × 106 IU vitamin

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**Figure 1.** Effect of zilpaterol hydrochloride (ZH; Merck Animal Health, Summit, NJ) and vitamin D on Warner-Bratzler shear force (WBSF). Treatments consisted of i) no supplemental vitamin D3 without ZH fed for 21 d (NDNZ; n = 14), ii) supplemental vitamin D3 at 250,000 IU for 165 d without ZH for 21 d (LDNZ; n = 14), iii) supplemental vitamin D3 at 5 × 106 IU for 10 d without ZH for 21 d (SDNZ; n = 14), iv) no supplemental vitamin D3 with ZH fed for 21 d (NDZ; n = 35), v) supplemental vitamin D3 at 250,000 IU for 165 d with ZH for 21 d (LDZ; n = 34), and vi) supplemental vitamin D3 at 5 × 106 IU for 10 d with ZH for 21 d (SDZ; n = 35). Contrasts included ZH vs. no ZH, NDNZ vs. LDNZ, NDNZ vs. SDNZ, NDZ vs. LDZ, and NDZ vs. SDZ. SEM = 0.129. A * indicates that steers fed ZH produced greater WBSF values at all aging time points (P < 0.01; Panel A). A + indicates that steers fed LDZ tended (P = 0.06) to produce greater WBSF values after 21 d of aging (Panel C).


D₃ did not improve tenderness at 14 d postmortem compared with steers that did not receive supplemental vitamin D₃. Our results were similar to those reported by Strydom et al. (2011), who noted that vitamin D₃ supplemented at various doses (1 to 7 million IU/d) and lengths of feeding (3 to 6 d), helped very little in improving tenderness of beef from steers fed ZH. Similar to the present study, Strydom et al. (2011) also observed that feeding 7 million IU vitamin D₃ for 6 d resulted in tougher meat at 3 and 14 d postmortem aging when carcasses were not electrically stimulated. In our study, short-term supplemental vitamin D₃ at an increased dose did not improve tenderness in steers fed ZH and the long-term low dose of vitamin D₃ actually tended to make steaks from ZH-fed steers tougher at 21 d postmortem. However, when using a 5-category scale for tenderness of very tender (<3.36 kg), tender (3.36 to 4.36 kg), intermediate (4.37 to 5.37 kg), tough (5.38 to 6.38 kg), and very tough (>6.38 kg; Destefanis et al., 2008), on average by 14 d of postmortem aging, steaks from steers not fed ZH were classified as very tender and steaks from steers fed ZH were classified as tender; therefore, the effects of vitamin D₃ supplementation may have been too small to detect.

Consumer impressions of beef tenderness can be affected by changes in WBSF of 0.5 kg. Miller et al. (2001) reported that WBSF values of 3.0, 3.4, 4.0, 4.3, and >4.9 resulted in consumer acceptance ratings of 100, 99, 94, 86, and 25%, respectively. Even though our steaks were 1-kg greater in ZH treatments, 99% of consumers would still find them satisfactory at 21 d postmortem and 94% at 14 d postmortem. Warner-Bratzler shear force values from our steaks were low compared with previously reported data (Swanek et al., 1999; Montgomery et al., 2000; Montgomery 2004b). However, our steers were all Angus × Simmental crosses and Montgomery et al. (2004b) included Bos indicus cattle, which might have increased WBSF values in that study. Furthermore, genetics of the U.S. beef herd in regard to tenderness has improved dramatically, as seen in the trend for improved tenderness of all beef cuts from the National Beef Tenderness Survey of 1998 (Brooks et al., 2000) compared with the National Beef Tenderness Survey of 2006 (Voges et al., 2007).

Feeding ZH is beneficial for improving feedlot performance and generating greater muscle mass; unfortunately, it also increases toughness of steaks. Although supplemental vitamin D₃ provided at 5 × 10⁶ IU/steer daily did not improve tenderness of steaks from steers fed ZH or not fed ZH in this study, it has been shown to improve tenderness in other studies and should not be dismissed as a method to improve beef tenderness from steers not fed ZH. Furthermore, long-term low-dose supplemental vitamin D₃ seems to interact with ZH and could be a topic of further research to examine how long and what dose benefits feedlot performance and carcass traits and minimizes the negative effect on tenderness.

**LITERATURE CITED**


