Use of 25-Hydroxyvitamin D₃ to Improve Beef Tenderness

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Summary

Feeding the 25-hydroxyvitamin D₃ (25-OH D₃) metabolite of vitamin D₃ has been reported to improve beef tenderness and result in lower vitamin D₃ metabolite concentrations in meat. Because 25-OH D₃ remains elevated in plasma for at least 8 d subsequent to feeding, we believe that 25-OH D₃ can be fed as a onetime oral bolus and allow a flexible time frame for harvest with the same improvement in postmortem calcium-dependent proteolysis and beef tenderness. To test this hypothesis, 108 crossbred steers were allotted, six steers per pen to 18 pens and treatments were assigned randomly to pen. Treatments were 25-OH D₃ dosage (62.5 or 125 mg) and time of administration of the one-time oral bolus (4, 7, 21, or 35 d before harvest). Control steers received no 25-OH D₃. Regardless of time of bolusing relative to harvest, the one-time oral bolus elevated plasma 25-OH D₃ concentration and it remained elevated through harvest for steers assigned to either dosages of 25-OHD₃. Plasma calcium concentration, however, remained unchanged compared with that of controls, regardless of dosage or time of bolusing relative to harvest. The one-time oral bolus of 25-OH D₃ did not result in an improvement in tenderness as determined by Warner-Bratzler shear force or an improvement postmortem proteolysis as determined by troponin-T degradation. We conclude that a one-time oral bolus of 62.5 or 125 mg of 25-OH D₃ was sufficient to elevate plasma 25-OH D₃ concentration and maintain an elevated plasma 25-OH D₃ concentration for up to 35 d. The dosage of 25-OH D₃, however, was insufficient to result in elevated plasma calcium and therefore did not enhance calciumdependent proteolysis postmortem to result in beef that is more tender.

Introduction

Feeding 0.5 to 7.5 million IU of vitamin D_3 to cattle for 7 to 10 d before harvest results in beef that is more tender and in elevated concentrations of calcium in plasma and

muscle (Swanek et al., 1999; Montgomery et al., 2000; Montgomery et al., 2002). Elevated muscle calcium concentration is believed to enhance the calcium-dependent protease system of myofibrilliar (troponin-T) protein degradation postmortem and result in improved beef tenderness.

The disadvantage of feeding a supernatural dosage of vitamin D₃ close to the time of harvest is that it results in a high concentration of vitamin D₃ and 25-OH D₃ in the meat (Montgomery et al., 2002 and Foote, 2001). However, feeding 25-OH D₃ resulted in meat vitamin D₃ concentrations near that of control steers and 25-OH D₃ concentration in the meat intermediate to control steers and steers treated with vitamin D₃ (Foote, 2001). Feeding 25-OH D₃ tended to result in lower Warner–Bratzler shear force and enhanced troponin-T degradation in steaks aged 14 d postmortem but did not elevate plasma and muscle calcium concentrations (Foote, 2001). Additionally, Foote (2001) reported that a one-time oral bolus of 25-OH D_3 resulted in plasma 25-OH D₃ remaining elevated at harvest 8 d later. Therefore, we hypothesized, because 25-OH D₃ remains elevated in plasma for several days following the one-time oral bolus of 25-OH D₃, that a variable length of time exists between treatment with 25-OH D₃ and harvest of the animal for an improvement in tenderness. If length of time between 25-OH D₃ treatment and harvest can be varied but have a similar influence on tenderness, then producers would have greater flexibility in marketing their cattle. This trial was designed with the following objectives: 1) to evaluate the effects of a one-time oral bolus of 25-OH D₃ on plasma calcium and 25-OH D₃ concentration when given 4, 7, 21, or 35 d before harvest and 2) to determine the effects of a one-time oral bolus of 25-OH D₃ on beef tenderness as indicated by Warner-Bratzler shear force and postmortem calcium-dependent proteolysis as indicated by troponin-T degradation.

Materials and Methods

One hundred eight crossbred finishing steers, predominantly of Continental x British breed crosses, were assigned randomly to nine treatment groups. All steers were offered a high-energy diet *ad libitum* throughout the trial. Each treatment group was housed in two separate pens with six steers per pen (total of 12 steers per treatment). One group was used as a control group and received no 25-OH D₃. Four treatment groups were fed a one-time dosage of 62.5 mg of 25-OH D₃, and the other four treatment groups were fed a one-time dosage of 125 mg of 25-OH D₃. Cattle were administered the 25-OH D₃ by gelatin capsule bolus 35, 21, 7, or 4 d before harvest for both dosages. The experimental design is illustrated as follows:

		Administration of 25-OH D ₃ (days before harvest)					
	35	21	7 4				
Dosage of 25-OH D ₃	62.5 mg	12 steers	12 steers	12 steers	12 steers		
	125 mg	12 steers	12 steers	12 steers	12 steers		

Steers were weighed on two consecutive days at trial initiation and termination. Average daily gain was calculated based on the difference of these weights. Daily feed intake was monitored for each pen, and feed efficiency was calculated as the ratio of average daily gain to average dry matter intake. Jugular blood samples were collected from the steers at regular intervals before harvest and again at time of harvest. The trial was terminated 35 d after the first treatment groups received their 25-OH D₃ bolus, and cattle were transported to a commercial plant for harvest. Standard carcass measurements were collected, and the loin muscle was sampled. Steaks were aged for 6 or 14 days postmortem and stored at -20°C for subsequent analysis. At a later time, these loin steaks were evaluated for tenderness by Warner-Bratzler shear force (AMSA, 1995) and by polyacrylamide gel electrophoresis and western blot analysis for the 30-kDa protein component of troponin-T degradation (Huff-Lonergan et al., 1996a and 1996b). Plasma samples were assayed for concentrations of calcium (AOAC, 1975). Plasma 25-OH D₃ and 1,25dihydroxyvitamin D_3 (1,25-(OH)₂ D_3) concentrations were quantified by using the procedures of Hollis et al. (1993) and Hollis et al. (1996), respectively. Muscle 25-OH D₃ and $1,25-(OH)_2$ D₃ concentrations were quantified by using the procedures of Horst et al. (1981).

Results and Discussion

Table 1 illustrates that a one-time oral bolus of 25-OH D_3 can be given at various times before harvest without deleterious effects on feedlot performance or carcass characteristics. Muscle 25-OH D_3 concentration, although higher ($P \le 0.05$) than that of control steers, was lower than that reported for steers fed vitamin D_3 (Foote, 2001). Muscle 1,25-(OH)₂ D_3 concentrations did not differ between control and treated steers. These data confirm the results of Foote (2001) that suggested feeding 25-OH D_3 as an alternative to vitamin D_3 resulted in lower concentrations of the vitamin D_3 metabolites in muscle.

Figure 1a represents the effect of a one-time oral bolus of 25-OH D₃ on plasma 25-OH D₃ concentration at various times throughout the trial. Plasma 25-OH D₃ increased relative to baseline concentration ($P \le 0.01$) and remained elevated through time of harvest. The relative change from

baseline was similar regardless of 25-OH D_3 dosage or timing of bolus relative to harvest. Figure 1b represents the effects of a one-time oral bolus of 25-OHD₃ on plasma calcium concentration. Plasma calcium concentration was not significantly different as a result of dosage or timing of 25-OH D_3 bolus relative to harvest. These results indicate that cattle can be given a one-time oral bolus of 25-OH D_3 up to 35 d before harvest and that plasma 25-OH D_3 concentration will be elevated relative to baseline and remain elevated through harvest. However, the dosages of 25-OH D_3 used in this trial were not sufficient to elevate plasma calcium.

Because the one-time oral bolus of 25-OH D₃ was not sufficient to elevate the concentration of calcium in plasma, postmortem calcium-dependent proteolysis and ultimately beef tenderness were not altered by 25-OH D₃ treatment, regardless of dosage or time of bolusing relative to harvest. Figure 2 represents the effects of a one-time oral bolus of 25-OH D₃ on Warner-Bratzler shear force of loin steaks aged 6 or 14 d postmortem. Likewise, Figure 3 represents the effects of a one-time oral bolus of 25-OH D₃ on postmortem troponin-T degradation. A one-time oral bolus of 25-OH D₃ did not significantly alter troponin-T degradation in loin steaks aged 6 or 14 d postmortem. Although we were unsuccessful in elevating plasma calcium and as a result did not enhance postmortem calciumdependent troponin-T degradation, we can conclude from these data that the oral administration of supernatural doses of 25-OH D₃ did not have deleterious interaction with aging on beef tenderness. Both Warner-Bratzler shear force and troponin-T degradation were enhanced by the aging process.

Because data reported previously by Foote (2001) indicated an oral bolus of 125 mg of 25-OH D_3 was sufficient to elevate plasma calcium concentrations, we are confident that the ideal dosage of 25-OH D_3 that will elevate calcium concentrations in plasma can be identified. We suspect that 125 mg of 25-OH D_3 borders on the critical dosage that is needed to elicit a calcium response in plasma. Subsequent analysis of preliminary plasma samples collected by Foote (2001) indicated that plasma 25-OH D_3 concentrations (425 ng/mL) were higher than those measured in this trial. Furthermore, data reported in this trial indicate a dose response in plasma 25-OH D_3 concentration. Although we cannot identify a specific reason or reasons why 125 mg of 25-OH D_3 was efficacious in one trial but not the other, perhaps environmental variation, breed differences, or dietary differences can be attributed to the variation in response. These data, however, imply that the dose of 25-OH D_3 supplied by the gelatin capsule needs to be higher than 125 mg to invoke a calcium response.

Implications Once the dosage of 25-OH D₃ that effectively results in elevated plasma calcium is identified, producers will have some flexibility in the

marketing of cattle relative to time of treatment because a one-time oral bolus of 25-OH D₃ results in elevated 25-OH D₃ concentrations in the plasma that remain elevated for up to 35 d.

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Table 1. Feedlot performance and Meat characteristics for steers given a one-time oral bolus of 25-hydroxyvitamin D₃ (25-OH D₃) at various times before slaughter.

	62.5 mg 25-OH D ₃				125 mg 25-OH D ₃					
	Bolus administration relative to harvest (d)									
-	-4	-7	-21	-35	-4	-7	-21	-35	Control	SE
Feedlot Performance										
Initial wt., kg	505	505	507	509	506	505	507	507	509	1.76
Final wt., kg	574	574	579	579	577	577	578	577	580	2.95
ADMI, kg/d^{a}	10.6	10.6	10.6	10.6	10.6	10.6	10.5	10.6	10.6	0.03
ADG, kg/d	1.9	1.9	2.0	1.9	2.0	2.0	1.9	1.9	2.0	0.10
Gain:feed	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.009
Carcass Characteristics										
Carcass wt., kg	342	342	346	349	346	342	351	345	350	6.9
Loin area, cm ²	80.0	78.6	84.5	83.1	84.0	83.6	86.7	79.3	85.4	2.75
Rib fat, mm	9.9	8.9	8.1	9.2	8.8	7.7	8.9	10.0	8.1	1.21
Quality grade ^b	2.8	2.7	2.7	2.6	2.8	3.1	2.8	2.8	3.0	0.19
Yield grade	2.1	2.2	1.7	2.0	2.0	1.8	1.9	2.3	1.8	0.18
Vitamin D Metabolites in Muscle										
25-OH D ₃ ,										
ng/mL	2.9^{cd}	2.8^{cd}	3.2 ^{cd}	2.5 ^{cd}	3.2 ^{cd}	3.3 ^d	2.8^{cd}	2.7 ^{cd}	0.9 ^e	0.28
1,25(OH) ₂ D ₃ ,										
pg/mL ^f	27.3	19.1	14.5	25.3	21.3	20.3	29.5	19.3	16.5	4.76
Calcium, mg/g										

^a Average dry matter intake.

^b Quality grade 1=prime, 2 = choice, 3 = select, 4 = standard.

^{cd e} Means within a row having different superscripts differ (P < 0.05).

^f 1,25-dihydroxyvitamin D₃.



Figure 1. Plasma 25-hydroxyvitamin D₃ (25-OH D₃), calcium, and 1,25-dihydroxyvitamin D₃ (1,25-(OH)₂ D₃) concentration of steers given a one-time oral bolus of 25-OH D3 at various times before harvest.

Effects of one-time oral bolus of 25-hydroxyvitamin D₃ (25-OH D₃) on plasma 25-OH D₃ and calcium concentrations. 1a. Plasma 25-OH D₃ concentration was elevated (P < 0.01) with a one-time oral bolus of 25-OH D₃ and remained elevated (P < 0.01) relative to controls through harvest, regardless of bolus dose or time of bolus administration. When averaged across all time points, the average increase in plasma 25-OH D₃ concentration from baseline

concentration was similar (P > 0.05) regardless of dosage or time of administration of 25-OH D₃ bolus. 1b. Plasma calcium concentrations did not differ (P > 0.05) as a result of 25-OH D3 treatment, regardless of bolus dosage or time of bolus administration. 1c. Plasma 1,25-dihydroxyvitamin D₃ concentration did not differ (P > 0.05) as a result of 25-OH D₃, regardless of bolus dosage or time of bolus administration.





Loin steaks aged for 14 d postmortem had lower (P < 0.01) Warner-Bratzler shear force measurements (more tender) than loin steaks aged for 7 d postmortem. Warner-Bratzler shear force was similar (P > 0.05) for steaks from 25-OH D₃ treated and control steers, regardless of dosage of the onetime treatment with 25-OH D_3 or time of bolus administration.

Figure 3. Effects of a one-time oral bolus of 25-hydroxyvitamin D₃ (25-OH D₃) on 30 kDa protein band intensity as an indicator of troponin-T degradation in loin steaks aged 6 or 14 d postmortem.



Loin steaks aged for 14 d postmortem had greater (P < 0.01) troponin-T degradation (more tender) than loin steaks aged for 7 d postmortem. Troponin-T degradation was similar (P

> 0.05) for steaks from 25-OH D₃ treated and control steers, regardless of dosage of the one-time treatment with 25-OH D₃ or time of bolus administration.

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