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Feeding high levels of vitamin D3 does not improve tenderness of callipyge lamb loin chops

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Abstract

The objective of this study was to determine whether feeding high doses of vitamin D3 7 d before slaughter would increase muscle Ca++ levels and result in more tender loin chops. Market lambs (n = 4 callipyge and 4 normal in Exp. 1, and n = 16 calipyge and 16 normal in Exp. 2) were randomly and equally assigned to feeding groups based on callipyge genotype and experimental diet, (vitamin D3 or control). Serum Ca++, muscle Ca++, Warner-Bratzler shear force, and troponin-T degradation data were analyzed. In Exp. 1, vitamin D3 was supplemented at 1 or 2 x 10(6) IU/d. The 2 x 10(6) IU dose resulted in the greatest serum Ca++ reponse and was chosen for Exp. 2. In Exp. 2, serum Ca++ concentration was higher ($P < 0.05$) for normal and callipyge lambs fed the vitamin D3 diet than for the control diet fed lambs. Muscle Ca++ concentrations, however, were not higher ($P = 0.28$) for the vitamin D3-fed lambs. Warner-Bratzler shear values were higher ($P < 0.05$) for callipyge than for normal lambs, but no differences were observed with vitamin D3 supplementation. These data were supported by results from Western blot analysis of troponin-T degradation, in which no differences were observed for vitamin D3 vs control diet lambs at 14 d postmortem. This experiment showed that feeding 2 x 10(6) IU/d of vitamin D3 to market lambs, callipyge or normal, raised serum Ca++ concentration, but did not increase muscle Ca++ concentration. This lack of response in muscle Ca++ was likely the reason that no differences were observed for Warner-Bratzler shear force values or troponin-T degradation data between the vitamin D3 and control loin chops. A higher dose of vitamin D3 may be required to improve tenderness.

Disciplines

Agriculture | Animal Sciences | Meat Science

Comments

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Feeding high levels of vitamin D_3 does not improve tenderness of callipyge lamb loin chops[1]

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ABSTRACT: The objective of this study was to determine whether feeding high doses of vitamin D_3 7 d before slaughter would increase muscle Ca^{++} levels and result in more tender loin chops. Market lambs (n = 4 callipyge and 4 normal in Exp. 1, and n = 16 calipyge and 16 normal in Exp. 2) were randomly and equally assigned to feeding groups based on callipyge genotype and experimental diet, (vitamin D_3 or control). Serum Ca^{++}, muscle Ca^{++}, Warner-Bratzler shear force, and troponin-T degradation data were analyzed. In Exp. 1, vitamin D_3 was supplemented at 1 or 2×10^6 IU/d. The 2 10^6 IU dose resulted in the greatest serum Ca^{++} reponse and was chosen for Exp. 2. In Exp. 2, serum Ca^{++} concentration was higher ($P < 0.05$) for normal and callipyge lambs fed the vitamin D_3 diet than for the control diet fed lambs. Muscle Ca^{++} concentrations, however, were not higher ($P = 0.28$) for the vitamin D_3-fed lambs. Warner-Bratzler shear values were higher ($P < 0.05$) for callipyge than for normal lambs, but no differences were observed with vitamin D_3 supplementation. These data were supported by results from Western blot analysis of troponin-T degradation, in which no differences were observed for vitamin D_3 vs control diet lambs at 14 d postmortem. This experiment showed that feeding 2×10^6 IU/d of vitamin D_3 to market lambs, callipyge or normal, raised serum Ca^{++} concentration, but did not increase muscle Ca^{++} concentration. This lack of response in muscle Ca^{++} was likely the reason that no differences were observed for Warner-Bratzler shear force values or troponin-T degradation data between the vitamin D_3 and control loin chops. A higher dose of vitamin D_3 may be required to improve tenderness.

Key Words: Sheep Breeds, Tenderness, Vitamin D_3

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Introduction

Recent research has focused on improving lean yield and consumer acceptability of market lambs in the United States. Lambs exhibiting the callipyge condition express extreme muscle hypertrophy (Carpenter et al., 1996) and higher retail yield compared with their normally muscled contemporaries (Koohmaraie et al., 1995). Callipyge lambs also have increased feed:gain ratios and increased average daily gains than normally muscled lambs resulting in improved performance (Jackson and Green, 1993). These advantages, however, are offset by consistently higher shear force of the longissimus muscle (Shackelford et al., 1997). Thus, the challenge is to take advantage of the increased performance and retail product yield from callipyge lambs while marketing a product with acceptable tenderness.

Evidence supports the use of $CaCl_2$ infusion or injection for increasing the tenderness of fresh meat products (Koohmaraie et al., 1990; Whipple and Koohmaraie, 1992), presumably by increasing calcium availability and stimulating calpain activity. If one could increase free calcium in the muscle, the possibility exists that calpain activity and subsequent proteolysis and tenderness would be accelerated. One way to increase circulating Ca^{++} in the animal and in muscle (Jones et al., 1998) is by feeding vitamin D_3 in excess of nutritional requirements. The biologically active form of vitamin D, 1,25-$(OH)_2$ vitamin D, increases serum calcium concentrations in the body by liberating calcium stores from the skeleton and by promoting intestinal absorption of dietary calcium (Jones et al., 1998). Consequently, the hypothesis to be tested was that vitamin D_3 would increase calcium concentrations in the muscle, and consequently enhance postmortem calpain activity and improve tenderness. The objective of this study was to determine whether feeding high levels of vitamin D_3 for 7 d before slaughter improves tenderness.

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Materials and Methods

The project consisted of two experiments and was carried out under the guidelines of the Iowa State University Animal Care and Use Committee. All market lambs were obtained from the McNay Research Unit, McNay, IA. All parent and progeny genotypes were inferred with a gene marker method at Utah State University, Logan (Cockett et al., 1996), to detect the presence or absence of the callipyge genotype. Lambs were sorted into feeding groups according to genotype.

Diets

All control lambs were limit-fed 1.14 kg of shelled corn and a commercial pelleted protein supplement each day. Lambs on vitamin D_3-treated diets received 0.11 kg of a corn-based, ground vitamin D_3 supplement with the appropriate level of D_3. The vitamin D_3 supplement replaced corn by weight in the control diet and was administered as a top dressing to the control diet. Limit feeding was utilized to ensure intake of the treated diet.

Exp. 1

An initial experiment was done in order to determine a dose-time relationship for significantly increasing serum Ca^{++} concentrations with vitamin D_3. Market lambs (n = 8) averaging 47.7 kg of body weight were used. Normal (n = 4) and callipyge (n = 4) lambs were individually fed. Two lambs from each genotype received 1×10^6 IU of vitamin D_3 and two lambs received 2×10^6 IU of vitamin D_3. Lambs were fed for 2 d in order to determine maximum vitamin D_3 response at each dose. A blood sample was collected on each of the 3 d preceding the feeding trial to determine a baseline serum Ca^{++} concentration for each lamb. Blood samples were then drawn from each lamb at 12, 24, and 48 h during the feeding trial. Based on the most consistent increase in serum Ca^{++} response, the level of 2×10^6 IU of vitamin D_3/per day was selected as the dose for Exp. 2.

Exp. 2

Market lambs (n = 32) were penned in groups of eight lambs. Pens included eight normal lambs fed a control diet, eight normal lambs fed a control diet plus 2×10^6 IU of vitamin D_3, eight callipyge lambs fed a control diet, and eight callipyge lambs fed a control diet plus 2×10^6 IU vitamin D_3. Lambs were fed their respective diets for 7 d. Blood samples were collected each morning before feeding at d 1, 3, and 5 of the trial. At d 7, lambs were transported to Iowa Lamb Corporation in Hawarden, IA, for processing. Hot carcass weight and dressing percentage were calculated. After a 24-h chill, all carcasses were ribbed between the 12th and 13th ribs, and ribeye area and 12th-rib fat were recorded. After carcass measurements were made, the wholesale loin was removed from the carcass and vacuum packaged. The loin section was transported back to the ISU Meat Laboratory, where the boneless loin was removed and 2.54-cm chops were cut from each loin. Chops were packaged in pairs and held for 1, 7, 14, and 21 d of aging at 2°C. At the appropriate day of storage, chops were frozen until needed for Warner-Bratzler shear determination. A 1.27-cm chop was removed from each loin for SDS-PAGE and Western blotting determination of troponin-T protein degradation. Blood samples were collected via jugular puncture, transferred to heparinized collection tubes, and placed in an ice-filled cooler. Samples were transported to the lab and spun down in a clinical centrifuge (Model CL IEC/Damon, Needham, MA) at $1{,}500 \times g$ for 15 min. Serum was pipetted into glass vials and frozen at –30°C. At time of serum calcium analysis, samples were thawed in a warm water bath (Isotemp, Fisher Scientific, Chicago, IL) and vortexed to ensure a homogeneous sample. One hundred microliters of blood serum was pipetted into a cuvette with 4 mL of lanthium oxide (Sigma-Aldrich Co., St. Louis, MO). This sample was measured with an atomic absorption spectrophotometer (Analyst 100, Perkin Elmer, Foster City, CA) at 422.7 nm. Muscle calcium was determined by pulverizing 5-g samples of longissimus muscle from the 12th to 13th rib section in liquid nitrogen. The samples were then ashed in a muffle furnace and resuspended in 6 *N* HCl to dissociate the bound Ca^{++}. The Ca^{++} concentrations were determined with the same procedure as the serum samples. Serum and muscle calcium concentrations are reported as milligrams of Ca^{++} per 100 mL.

Warner-Bratzler Shear Force

At the appropriate day of postmortem aging, 2.54-cm chops were cooked to an internal temperature of 35°C and then turned and cooked to a final internal temperature of 71°C in a broiler oven set at 176.7°C. Chops were cooled at 4°C overnight, and three 1.27-cm round cores were removed from each chop for Warner-Bratzler shear determination using an Instron Universal Testing Machine (Model 4502, Canton, MA). Cores were sheared perpendicular to the muscle fibers at a crosshead speed of 250 mm/min.

Troponin-T Degradation Determination

Sample Preparation. Whole muscle samples were prepared according to Huff-Lonergan et al. (1996b). A 0.2-g sample from the longissimus muscle was homogenized with 10 mL of a solution containing 2% (wt/vol) SDS and 10 m*M* sodium phosphate buffer, pH 7.0. The sample was centrifuged at $1{,}500 \times g$ for 15 min to precipitate insoluble components within the sample. The protein concentration of the supernate was determined by using a DC Protein Assay kit, (Bio-Rad Laboratories, Hercules, CA) (Lowry et al., 1951). Samples were diluted with water to 6.4 mg/mL and prepared for SDS-PAGE.

Table 1. Serum Ca^{++} concentration(mg/100 mL) over feeding time for Exp. 1 (dose-time relationship)

Genotype	Dose (IU × 10^6)	Baseline[a]	12 h	24 h	48 h	% change over time
Normal	1	9.75	9.56	9.63	9.81	+0.6
Normal	1	9.11	9.51	9.06	9.95	+9.2
Callipyge	1	9.22	9.50	9.86	9.77	+5.9
Callipyge	1	9.42	9.07	9.60	9.34	–0.8
Normal	2	9.72	9.53	9.47	9.96	+2.4
Normal	2	10.20	10.22	10.37	10.46	+2.5
Callipyge	2	9.03	9.24	9.27	9.62	+6.5
Callipyge	2	9.03	9.20	9.60	9.21	+2.0

[a]Mean serum Ca^{++} 3 d before feeding trial.

One volume of tracking dye (60 m*M* Tris-HCl [pH 6.8], 30% glycerol, 2% SDS, 1% bromophenol blue) and 0.1 vol 2-mercaptoethanol was added to each sample (1 vol = 1 mL of protein, 1 mL of tracking dye, and 0.1 mL of mercaptoethanol). Final concentration of the sample was 4 mg/mL. Samples were heated at 50°C for 20 min.

Gel System. Polyacrylamide gels (15% acrylamide:N,N′-bis-methylene acrylamide of 100:1 [wt/wt]) were used. Each gel consisted of 0.1% (wt/vol) SDS, 0.67% (vol/vol) N′N′N′N′-tetramethylethylenediamine, 0.1% (wt/vol) ammonium persulfate,and 0.375 *M* Tris-HCl (pH 8.8). Additionally, a 4% polyacrylamide stacking gel (acrylamide:N,N′-bis-methylene acrylamide of 100:1 [wt/wt]) was used over the 15% gel. Gel dimensions were 8 cm wide × 9 cm tall × 1.5 mm thick. Gels were electrophoresed using a Hoefer SE260 unit (Pharmacia Biotech, Piscataway, NJ). The upper and lower chamber running buffer consisted of 25 m*M* Tris, 192 m*M* glycine, 2 m*M* EDTA, and 1% (wt/vol) SDS. A 60-μg protein sample was loaded into each well and run at a constant voltage of 120 V at 25°C. Gels were transferred by electroelution to polyvinylidene difluoride membranes.

Transfer Conditions. Gels were equilibrated in 25 m*M* Tris, 192 m*M* glycine, and 15% (vol/vol) methanol. Gels were transferred with a Hoefer TE22 Mighty Small Transphor unit at a constant voltage of 90 V for 90 min.

Table 2. Serum Ca^{++} concentration(mg/100 mL) changes over feeding time at 2 × 10^6 IU vitamin D_3 for Exp. 2 (primary feeding trial)

Genotype	Diet	1 d	3 d	5 d[a]	% change over time
Normal	Control	9.30	9.28	9.44[b]	+1.5
Normal	Vitamin D_3	8.74	9.34	10.27c	+14.9
Callipyge	Control	9.24	9.01	9.29b	+0.5
Callipyge	Vitamin D_3	9.11	9.26	9.89c	+7.8
SE		0.21	0.21	0.21	

[a]Values within a column with different letters significant at $P < 0.05$.

Western Blotting

Western blotting was done with slight modifications to the method of Huff-Lonergan et al. (1996b). The primary antibody was an anti-troponin-T antibody (JLT-12 Sigma Chemical Co.) diluted at 1:15,000 in solution (80 m*M* disodium hydrogen orthophosphate,anhydrous, 20 m*M* sodium dihydrogen orthophosphate, 100 m*M* sodium chloride, 0.1% [vol/vol] polyoxyethylene sorbitan monolaurate [Tween-20]). The secondary antibody was an IgG horseradish peroxidase-conjugated antibody (A2554, Sigma Chemical Co.), diluted 1:5,000 in solution (Tween-20). Gels were incubated (25°C) in the blocking solution for 1 h, in the primary antibody for 1 h, and in the secondary antibody for 1.5 h. Gels were rinsed three times for 10 min per wash at each incubation step. Chemiluminescence was used to detect labeled bands according to the manufacturer's directions (ECL, Amersham, Arlington Heights, IL).

Statistical Analysis

Data were analyzed using the general linear model of SAS (SAS Inst Inc., Cary, NC) in a 2 × 2 factorial design for each experiment. The model included fixed effects of genotype, diet, and pen, where appropriate. Additionally, repeated measures analysis was used for the postmortem aging portion of the study, in which chops were removed from each loin and analyzed over days of vacuum storage.

Table 3. Least squares means and standard errors for longissimus muscle Ca^{++} concentrations

Genotype	Diet	Muscle Ca^{++} (mg/100 mL)	Standard error
Normal	Control	5.38	1.71
Normal	Vitamin D_3	7.15	1.60
Callipyge	Control	4.24	1.60
Callipyge	Vitamin D_3	4.14	1.60

Table 4. Least squares means and standard errors for carcass values by genotype

Genotype	Carcass wt, kg	12th-rib Fat, cm	Ribeye area, cm^2
Normal	26.69	0.85	16.89
Callipyge	31.76	0.60	23.41
SE	0.55	0.07	2.48
P-value	0.03	0.01	0.001

Results and Discussion

In Exp. 1, serum Ca^{++} increased by small percentages for both callipyge and normal lambs over the 7-d trial at both 1 and 2×10^6 IU vitamin D_3/d (Table 1). Montgomery et al. (1997) reported a 30% increase in serum Ca^{++} for beef cattle when feeding at a lower dose:body weight treatment. These differences may have been due to the vehicle of delivery in each of these studies, for which Montgomery et al. (1997) used a vitamin D_3 bolus and our study used a vitamin D_3 premix in the feed. Additionally, Swanek et al. (1999) used a corn and vitamin D_3 pellet for beef cattle and observed a 34% serum Ca^{++} increase over a 7-d feed trial. One possible explanation is that the methods of Montgomery et al. (1997) and Swanek et al. (1999) may have resulted in improved intake of the vitamin D_3 due to more direct delivery to the digestive system than our method.

Calcium plays an important role in the postmortem degradation of muscle proteins (Goll et al., 1983; Koohmaraie et al., 1988). Calcium has been shown to activate the muscle proteases calpains, which have been shown to degrade troponin-T, and as a result the simultaneous production of the 30-kDa component (Olson and Parrish., 1977). This degradation of troponin-T to the 30-kDa component has been linked to meat tenderness (MacBride and Parrish, 1977; Huff-Lonergan et al., 1996a). Given the relationship between calcium and meat tenderness, it seems logical that efforts to increase cellular Ca^{++} before slaughter might also increase tenderness through further stimulating the action of the calpain proteases.

Experiment 2 resulted in greater ($P < 0.05$) serum Ca^{++} increases when control and vitamin D_3 diets were compared at 5 d (Table 2). Additionally, normal lambs fed vitamin D_3 exhibited higher ($P < 0.05$) serum Ca^{++} at 5 d than vitamin D_3 fed lambs. Although these increases were greater in Exp. 2 than in Exp. 1, the magnitude of change was not nearly as great as reported for beef cattle, in which increases of 20 to 30% were observed (Montgomery et. al. 1997; Swanek et al., 1999). Additionally, muscle Ca^{++} data were not different between the genotype or diet groups (Table 3). These data suggest that the high levels of vitamin D_3 used in this study did not result in increases in muscle Ca^{++} concentrations.

Carcass data are presented in Table 4 to characterize the differences between the normal and callipyge carcasses. As expected, callipyge lamb carcasses exhibited heavier ($P < 0.01$) hot carcass weights, greater ($P < 0.03$) ribeye areas, and less ($P < 0.001$) 12th rib fat than normal lamb carcasses. Similar data have been reported (Jackson and Green, 1993; Koohmaraie et al., 1995; Carpenter et al., 1996). An explanation for ribeye area differences between callipyge and normal lambs has been reported by Koohmaraie et al. (1995) and Carpenter et al. (1996). They reported a significantly greater percentage of white, fast-twitch, glycolytic fibers as well as increased muscle fiber diameter for callipyge longissimus muscle than for fibers from normal lambs.

In our study, 1-d shear force values were higher ($P < 0.05$) for callipyge than for normal control and vitamin D_3 fed lamb longissimus, regardless of diet (Table 5). The same differences existed at 21 d of postmortem aging. Shear force values are higher for longissimus muscle from callipyge lambs than those from normal lambs (Koohmaraie et al., 1995; Wiegand et al., 1998). Also, Shackelford et al. (1997) reported significant 123% greater shear values for longissumus muscle from callipyge lambs than from normal lambs. Given these shear force results, it seems that the 21-d aging period was more responsible for the tenderization process than the vitamin D_3 treatment.

Western blot data (Figure 1) showed evidence of troponin-T degradation for each treatment group regardless of diet or genotype by 14 d of aging. The highest and lowest Warner-Bratzler shear values are represented within Figure 1 for each genotype and diet combination. The Western blot results indicated no differences in the

Table 5. Warner-Bratzler shear force values (kg) of loin chops by genotype and diet over 21 d postmortem aging

		Warner-Bratzler Shear Force[a]			
Genotype	Diet	1 d	7 d	14 d	21 d
Normal	Control	3.41^{bc}	3.56^{c}	3.35^{bc}	3.08^{bc}
Callipyge	Control	5.85^{d}	5.44^{d}	5.07^{d}	4.35^{d}
Normal	Vitamin D_3	3.16^{b}	2.82^{b}	2.86^{b}	2.34^{b}
Callipyge	Vitamin D_3	4.75^{cd}	4.63^{cd}	4.39^{cd}	3.70^{cd}
Standard Error		0.48	0.50	0.46	0.42

[a,b,c,d]Means within a column with different letters are significant at $P < 0.05$.

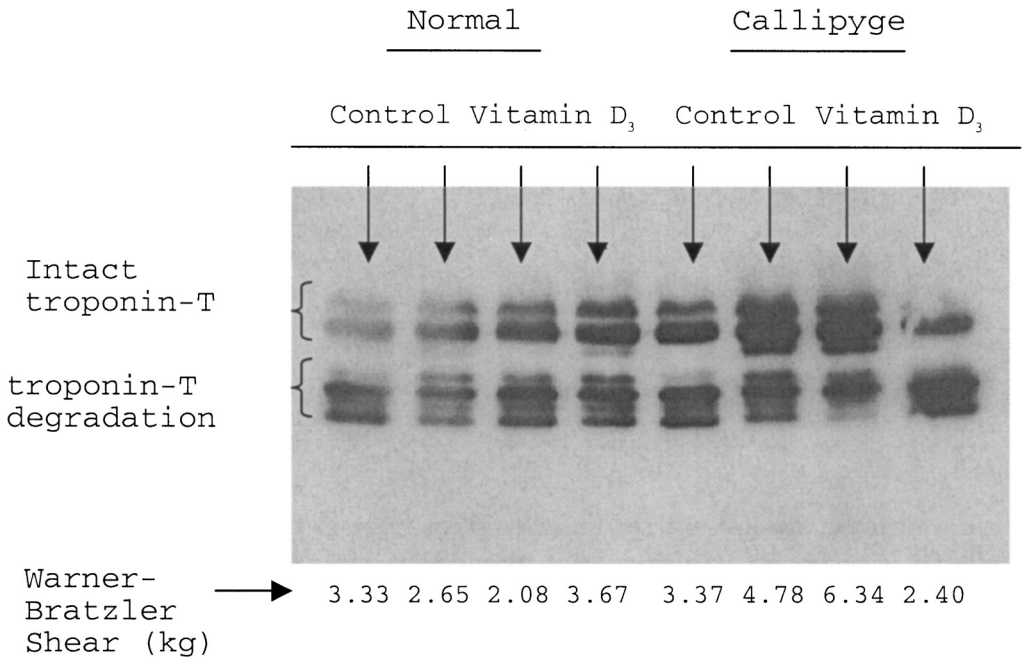

Figure 1. Western blot showing troponin-T degradation of longissimus at 14 d of postmortem aging by genotype and diet.

production of the troponin-T degradation component at 14 d of postmortem aging regardless of Warner-Bratzler shear force levels. Additionally, the rate of protein degradation was equivalent between the various genotype and diet combinations at 14 d of postmortem aging. Koohmaraie et al. (1995), however, reported slower protein degradation at d 7 and 21 for callipyge longissimus.

Implications

Market lambs, callipyge and normal, responded with nominal serum Ca^{++} increases with high levels of vitamin D_3. Callipyge lambs exhibited larger ribeye area and less 12th rib fat than normal lamb carcasses. Supplementation of vitamin D_3 did not improve tenderness as shown by Warner-Bratzler shear force values of longissimus muscle. Longissimus muscle from both normal and callipyge lambs exhibited evidence of troponin-T degradation as detected by Western blotting at 14 d of postmortem aging, but was not different between vitamin D_3 and control diet groups. There were likely no differences in Warner-Bratzler shear force values and troponin-T degradation because muscle Ca^{++} levels were not increased with vitamin D_3 supplementation. Increased dosage or improved delivery of the vitamin D_3 may be required in future studies.

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