Influence of feeding vitamin D₃ and aging on the tenderness of four lamb muscles

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Abstract

In Trial 1, rams (n = 26) were fed different levels (0, 250,000, 500,000 or 750,000 IU) of vitamin D₃ for 4 days to determine the most effective dose to increase blood calcium concentrations. Trial 2 consisted of feeding feedlot lambs (n = 40) different levels (0 or 750,000 IU) of vitamin D₃ for 14 days to determine if vitamin D₃ could improve the tenderness of lamb muscles. Lambs were slaughtered and the M. longissimus lumborum, M. biceps femoris, M. semitendinosus, and M. semimembranosus were removed after chilling, cut into chops, and assigned to an aging period (5, 10 or 15 days) for Warner–Bratzler shear force (WBS) determination. In Trial 1, feed intake and weight gain were lower for rams supplemented with 500,000 IU of vitamin D₃ compared to all other groups. Blood calcium concentrations were not different between groups, although the 750,000 IU group tended (P < 0.10) to have higher blood calcium concentrations on day 5 of the trial compared to controls. In Trial 2, blood calcium concentrations were not different between the treated and control groups, however, treated lambs had higher (P < 0.01) calcium concentrations in both the liver and kidneys. Control chops from the M. longissimus lumborum had lower (P < 0.05) WBS values than chops from vitamin D₃ fed lambs, but no other muscles were affected by vitamin D₃ feeding. An interaction between treatment and aging was observed for the M. biceps femoris, with chops from vitamin D₃ fed lambs having lower WBS values at 5 days aging, but chops from control lambs having lower WBS values at 15 days aging. WBS values decreased for the M. longissimus lumborum, M. semitendinosus, and M. semimembranosus with increasing aging time. Vitamin D₃ supplementation was not an effective means of improving the tenderness characteristics of lamb muscles.

Keywords: Carcass; Lamb; Meat tenderness; Vitamin D₃

1. Introduction

Several researchers have reported accelerated post-mortem tenderization and increased tenderness in lamb primals injected with calcium chloride after slaughter (Clare, Jackson, Miller, Elliot, & Ramsey, 1997; Koohmaraie, Crouse, & Mersmann, 1989; Koohmaraie & Shackelford, 1991; Koohmaraie, Whipple, & Crouse, 1990). They concluded that calcium chloride enhanced the activity of the endogenous calcium-dependent proteases (m- and μ-calpain), ultimately increasing tenderness.

DeLuca and Schnoes (1976) reported that vitamin D₃ functions as a regulator of calcium and is required for calcium absorption. It has been hypothesized that by increasing dietary vitamin D₃ a greater quantity of calcium will be sequestered in muscle, which could enhance calpain activity in postmortem muscle. Hibbs, Krauss, Pounden, Monroe, and Sutton (1946), Hibbs and Pounden (1955), and Hibbs, Pounden, and Krauss (1951) reported elevated calcium levels in blood samples from prepartum dairy cows supplemented with 500,000 or 750,000 IU of vitamin D₃ for 7 or 14 days. Recent investigations into the effects of vitamin D₃ supplementation on beef tenderness have yielded conflicting results. Montgomery et al. (1997) and Swanek et al. (1999) reported increased tenderness in cattle supplemented with vitamin D₃ and speculated that increased
tenderness was related to the activation of calpains. In contrast, Hill, Brito, Pringle, and Williams (1999) and Scanga, Belk, Tatum, Koohmaraie, and Smith (1999) reported no differences in the tenderness of steaks from cattle supplemented with vitamin D₃ compared to steaks from control cattle.

The objective of this study was to determine if supplementing feedlot lambs with vitamin D₃ is an effective means of improving postmortem meat tenderness.

2. Materials and methods

2.1. Trial 1

Yearling rams (n = 26) weighing approximately 40 kg were used to determine the effectiveness of different levels of vitamin D₃ (0, 250,000, 500,000 or 750,000 IU) and the best means of administering doses (bolus or feed-supplementation). Sixteen rams were selected to receive vitamin D₃ doses via bolus and the remainder received doses via feed-supplementation. Rams were housed in 1.83 m × 2.13 m pens. Rams were placed in pens 3 day before initiation of the vitamin D₃ doses and were gradually weaned from a pelleted and textured feed mixture to a completely textured feed. Rovimix D₃ 500 (Roche Vitamins, Inc., Parippany, NJ) was used as the source of vitamin D₃ for boluses and feed-supplementation. For feed supplementation, corn meal was hand-mixed with vitamin D₃ at a ratio of 2:1 to serve as a carrier and to add volume to the ration. Vitamin D₃ doses were administered on day 1, 2, 3, and 4 of the trial.

Blood samples were obtained immediately before feeding by jugular venipuncture using a vacuum tube (Fisherbrand, Pittsburgh, PA) and a vacutainer blood collection needle (Precision Glide, Franklin Lakes, NJ). Saline was injected into the ram to replace the volume of the removed blood. Blood samples were collected on day 1, 2, 3, 4, 5, 6, and 7. Vacutainers were stored on ice until processed. Blood samples were centrifuged at 3000 g for 30 min using a Sorvall OmniSpin R (Newton, CT). Blood serum was removed from each centrifuge tube and transferred to a 12 × 75 mm polypropylene culture test tube, sealed, and stored at 0 °C. Blood serum samples were sent to the Texas Veterinary Medical Diagnostic Laboratory at Texas A&M University for determination of ionized calcium and are reported as mg/dl.

2.2. Trial 2

A mixture of ewe and wether fine-wool and fine-wool crossbred sheep (n = 40) were purchased from a commercial feedlot and transported to the Texas A&M University Sheep Center. Lambs were approximately 8-months old and weighed 40 kg. Lambs were assigned randomly to one of eight pens with a total of five animals per pen. Four pens were designated as controls and the remaining four pens received a treatment of 750,000 IU vitamin D₃.

Lambs were fed in a mock-feedlot environment. A commercial feed ration identical to the ration they were fed at their previous feedlot was used throughout the duration of this study. Pens assigned to receive vitamin D₃ supplementation were fed a mixture of the commercial feed ration and Rovimax D₃ 500 (Roche Vitamins, Inc., Parippany, NJ). Lambs were given free access to a hay supplement for 1 week to allow for adjustment to the new environment. After adjustment, pens were fed 4.54 kg of feed each day for a total of 14 days. Before new feed was given each day, refused feed was cleaned from feeders and weighed to calculate percent intake.

2.2.1. Slaughter and bleeding procedures

Once lambs reached market weight (approximately 50 kg), they were transported to the Rosenthal Meat Science and Technology Center at Texas A&M University and slaughtered according to standard industry practices. Blood samples were collected before animals were slaughtered and processed as described in Trial 1. Liver and kidney samples were collected after evisceration, frozen, and shipped to the USDA – National Animal Disease Center (Ames, IA) for determination of calcium levels. Calcium concentrations in livers and kidneys were reported as ng/g. Carcasses were chilled for 24 h, ribbed between the 12th and 13th rib, and grade data were collected by trained Texas A&M University personnel according to USDA (1992) guidelines. Lamb loins (IMPS #232; NAMP, 1997; USDA, 1996) and legs (IMPS #233A; NAMP, 1997; USDA, 1996) were removed from each carcass and the right sides were separated into individual muscles. The M. longissimus lumborum was separated from the loin and the M. biceps femoris, M. semimembranosus, and M. semitendinosus were removed from the leg. All muscles were trimmed free of external fat and cut into 3.18 cm thick chops by cutting perpendicular to the muscle fiber orientation. Two chops from the M. longissimus lumborum and one chop from each of the M. biceps femoris, M. semimembranosus, and M. semitendinosus were assigned to an aging period of 5, 10 or 15 days and aged at 2 ± 2 °C for that duration.

2.2.2. Warner–Bratzler shear force evaluations

All chops were cooked on Farberware Open-Hearth Broilers (Farberware, Inc., Corning, NY) to an internal temperature of 70 °C (AMSA, 1995). Chops were turned once during cooking when their internal temperature reached 35 °C. Internal temperature was monitored using a digital, hand-held thermometer (Model 91100-50, Cole-Parmer Instrument Co., Vernon Hills, IL) with a
type-K thermocouple (Model KTSS-HH, Omega Engineering, Inc., Stamford, CT). After cooking, chops were covered with Saran® wrap and placed in a 4 ± 2 °C cooler for 24 h. Chops were allowed to equilibrate to room temperature (approximately 21 °C) and cores (1.27 cm diameter) were removed parallel with the muscle fiber orientation from each chop. Cores were sheared using a universal testing machine (Model SSTM-500, United Calibration Corp., Huntington Beach, CA) equipped with a 50 kg compression load cell and a Warner–Bratzler v-notch blade with the crosshead speed set at 200 mm/min. Warner–Bratzler shear (WBS) values for each muscle were determined by averaging the WBS values of the cores from each muscle. WBS values are reported as Newtons.

2.3. Statistical analysis

Data were analyzed using the GLM procedure of SAS (SAS Version 8, SAS Inst., Cary, NC). For Trial 1, treatment level and type of dose administration and their interaction were tested as main effects. Trial 2 was setup as a randomized complete block design with a factorial arrangement containing four replications, two treatments, three aging periods, and four muscles. Main effects of interest were treatment, aging period, muscle and their interactions. Only significant ($P < 0.05$) interactions were retained in the model. When main effects were significant at the $P < 0.05$ level, least squares means were generated and separated using a pairwise t test.

3. Results and discussion

3.1. Trial 1

No interactions were found between treatment and the type of method used to administer doses so rams administered vitamin D$_3$ via bolus and rams fed vitamin D$_3$ were pooled for analysis. No significant differences were noted in blood calcium level between all treatment levels and controls. However, rams administered 750,000 IU vitamin D$_3$ tended ($P = 0.0916$) to have higher blood calcium levels at day 5 than control rams (Fig. 1). Wiegand, Parrish, Morrical, and Huff-Loner-gan (2001) reported small (nonsignificant) increases in serum calcium levels in lambs fed 1 or 2 × 10$^6$ IU of vitamin D$_3$ for 7 days, but levels were not increased 20–30% as observed in beef cattle (Montgomery et al., 1997; Swanek et al., 1999). For feed intake, no differences were observed between the control group, 250,000 IU group or 750,000 IU group, however, the 500,000 IU group had much lower feed intake (Table 1). Weight gain followed a similar trend as feed intake with rams administered 500,000 IU of vitamin D$_3$ having much lower weight gains compared to all other groups. It is unclear why rams administered 500,000 IU of vitamin D$_3$ had such low feed intake and weight gain.

3.2. Trial 1

From the results in Trial 1, it was determined to feed 750,000 IU of vitamin D$_3$. No differences were found in blood calcium levels between treated and control lambs (Table 2), however, analysis of calcium levels in livers and kidneys showed that lambs fed vitamin D$_3$ had much higher calcium concentrations in both the liver (505.54 ng/g in treated lambs vs. 27.13 ng/g in control lambs) and kidneys (1523.20 ng/g in treated lambs vs. 21.18 ng/g in control lambs). McDowell (1989) reported that the hormone calcitonin is secreted when blood calcium levels are elevated, and calcitonin functions by sequestering calcium from the blood and storing it in the liver. This may explain why no differences were observed in the blood calcium concentrations of treated and control lambs, but differences were found in the liver samples.

Carcass characteristics from the study are summarized in Table 3. Adjusted fat thickness and overall conformation score were different between the two

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Feed intake (%)</th>
<th>Weight gain (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>98.52a</td>
<td>3.47</td>
</tr>
<tr>
<td>250,000 IU vitamin D$_3$</td>
<td>89.69a</td>
<td>2.94</td>
</tr>
<tr>
<td>500,000 IU vitamin D$_3$</td>
<td>66.50b</td>
<td>1.31</td>
</tr>
<tr>
<td>750,000 IU vitamin D$_3$</td>
<td>89.41a</td>
<td>3.26</td>
</tr>
<tr>
<td>SEM$^a$</td>
<td>7.12</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Means within a column lacking common letters (a, b) differ ($P < 0.05$).

$^a$ SEM is the standard error of the least squares means.
groups with the group fed vitamin D₃ having a lower adjusted fat thickness and a more muscular conformation. Differences in these characteristics were relatively minor with mean fat thicknesses being different by 1.5 mm. Additionally, differences in overall conformation may have been exacerbated by the trimness of the lamb carcasses. Vitamin D₃ feeding trials in beef have not reported any differences in carcass characteristics (Duckett, Klein, Andrae, & Sanchez, 1998; Hill et al., 1999; Scanga et al., 1999).

Vitamin D₃ supplementation did not decrease WBS values of muscles (Table 4). No differences were observed between WBS values of control and vitamin D₃ chops from the *M. semimembranosus* and *M. semitendinosus*, however, control chops from the *M. longissimus lumborum* had lower WBS values than chops from animals fed vitamin D₃. Koohmaraie, Kent, Shackelford, Veiseth, and Wheeler (2002) described the *M. longissimus lumborum* as having very high calpain activity. Because of its high postmortem calpain activity, we expected vitamin D₃ could enhance the aging response in the *M. longissimus lumborum*, however, no interaction was observed between treatment and aging. This may indicate that the calpain system was already functioning maximally to promote postmortem proteolysis, but this seems unlikely because control *M. longissimus lumborum* chops had lower WBS values than chops from vitamin D₃ fed lambs. An interaction was observed between treatment and aging period for the *M. biceps femoris* (Fig. 2). Chops from lambs fed vitamin D₃ had lower WBS values than control chops after a 5 day aging period, however, no subsequent changes in WBS values were found in treated chops with additional aging, whereas WBS values of control chops were lower as aging time increased. Swaneck et al. (1999) reported *M. longissimus lumborum* steaks from cattle fed vitamin D had lower WBS shear values after 7 days of aging compared to control steaks, but no differences in WBS values were observed after 14 or 21 days of aging.

As expected, there were differences in WBS values between muscles with the *M. longissimus lumborum* having the lowest WBS values, followed by the *M. biceps femoris*, *M. semitendinosus*, and *M. semimembranosus* (Table 5). Belew, Brooks, McKenna, and Savell (2003) and Ramsbottom, Strandine, and Koonz (1945) reported the same stratification pattern for WBS values of four lamb muscles (*M. semimembranosus*, *M. semitendinosus*, and *M. longissimus lumborum*) described by Veiseth and Wheeler (2002) for *M. longissimus lumborum* muscles with the lowest WBS values, followed by *M. biceps femoris*, *M. semitendinosus*, and *M. semimembranosus*. Belew et al. (2003) and Ramsbottom et al. (1945) reported the same stratification pattern for WBS values of four lamb muscles. However, no interaction was observed between treatment and aging.
tenderness assessments of the same beef muscles. WBS values were much lower than the 4.42 kg (43.3 N) of force reported for lamb rib chops by Carpenter and King (1965) and the values reported by Jeremiah, Smith, and Carpenter (1971) for the *M. biceps femoris* (8.00 kg or 78.5 N) and the *M. semimembranosus* (7.73 kg or 75.8 N). Aging reduced the WBS values of the *M. longissimus lumborum*, *M. semimembranosus* and *M. semitendinosus* (Table 6). Aging time required to maximize tenderness appears to be muscle dependent as WBS values for the *M. longissimus lumborum* and *M. semimembranosus* declined incrementally with increasing aging time whereas the *M. semitendinosus* showed no decrease in WBS until after 10 days of aging. Wheeler and Koohmaraie (1999) reported that WBS values for lamb longissimus muscle declined more rapidly during aging than they did for *M. psoas major*.

### 4. Conclusions

Feeding high levels of vitamin D₃ to lambs did not improve the tenderness or aging characteristics of lamb muscles. Other factors, such as the hormone calcitonin, may be regulating calcium concentrations and limiting its deposition in muscle tissues. Further research is needed to understand calcium regulation to determine how vitamin D₃ can be used as a tool to improve tenderness.

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### References


