

## EFFECT OF SHORT-TERM SUPPLEMENTATION WITH VITAMIN D<sub>3</sub> ON BEEF TENDERNESS

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### ABSTRACT

This experiment was conducted to study the effect of dietary supplementation of vitamin D<sub>3</sub> to Baladi cattle bulls before slaughter on meat tenderness. Twelve Baladi bulls were randomly allotted into 3 equal groups each consisted of 4 animals. Group one served as a control, group 2 received a dietary supplementation of vitamin D<sub>3</sub> at a rate of  $5 \times 10^6$  IU/day, while bulls of group 3 were supplemented with  $7.5 \times 10^6$  IU/day. Dietary supplementation of vitamin D<sub>3</sub> was instituted for the last 10 days before slaughter. All bulls were slaughtered with an average final live weight of  $413 \pm 7.2$  kg. Steaks from the Longissimus dorsi muscle of the 9, 10 and 11<sup>th</sup> ribs were separated after chilling and aged at 1°C for 14 days to measure calcium content and eating quality attributes. Plasma calcium level was significantly higher ( $P < 0.05$ ) in group 3 than the control group. Meat calcium concentration in group 2 was significantly ( $P < 0.05$ ) higher than both of the control group and group 3. The lowest Warner-Bratzler shear force value of Longissimus dorsi muscle was recorded for group 3 compared with the control and group 2, without significant differences among the three groups. However, group 2 had better scores for tenderness and juiciness and flavor, these scores were not significantly different from the control and group 3 ( $P > 0.05$ ). Results indicate that oral supplementation with vitamin D<sub>3</sub> for 10 days before slaughter increased plasma and muscle calcium levels but did not significantly improve cooked meat tenderness.

**Keywords:** Beef, Calcium, Tenderness, Vitamin D<sub>3</sub>

### INTRODUCTION

Beef tenderness is one of the most important factor affecting consumers' satisfaction and perception of taste (Morgan *et al.*, 1991; Savell *et al.*, 1991; George *et al.*, 1999; Brooks *et al.*, 2000). Myofibrillar proteolysis from intracellular calcium-dependent proteases,  $\mu$ -calpain and m-calpain, has enhanced meat tenderization (Koochmaraie, 1992; Huff-Lonergan *et al.*, 1996a). Thus, increasing muscle calcium antemortem may activate calpains and improve beef tenderness. Few attempts were studied to enhance meat tenderness; the use of CaCl<sub>2</sub> infusion or injection (Koochmaraie *et al.*, 1990; Whipple and Koochmaraie, 1992), presumably by increasing calcium availability and stimulating caplain activity. Vitamin D<sub>3</sub> plays a vital role in maintaining blood concentrations of calcium (Horst, 1986; Hurwitz, 1996). Early studies with vitamin D indicated that as low as  $1 \times 10^6$  IU/d increased blood calcium and decreased the incidence of milk fever in dairy cows (Hibbs *et al.*, 1946 and 1951).

Dietary supplementation of beef cattle with supranutritional levels of vitamin D<sub>3</sub> before slaughter has been adopted to increase beef tenderness (Montgomery *et al.*, 2000 and Montgomery *et al.*, 2002). Steers given oral boluses of 5 or 7.5 × 10<sup>6</sup> IU/d from vitamin D<sub>3</sub> for 8 days before slaughter had greater concentrations of plasma calcium and produced steaks with lower Warner-Bratzler shear values than did untreated cattle (Montgomery *et al.*, 2000). Swanek *et al.* (1999) reported that cooked beef tenderness was improved through dietary supplementation of cattle with vitamin D<sub>3</sub> at a rate of 5 × 10<sup>6</sup> IU/d for 7 days or 7.5 × 10<sup>6</sup> IU/d for 10 days. On the other hand oral supplementation with vitamin D<sub>3</sub> (at low or high doses) for 2 to 8 days before slaughter increased serum calcium concentration but did not improve cooked longissimus tenderness (Scanga *et al.*, 2001). In lambs, Wiegand *et al.* (2001) also found that vitamin D<sub>3</sub> supplementation at a rate of 1 or 2 × 10<sup>6</sup> IU/day did not improve loin chops tenderness and indicated that a higher dose of vitamin D<sub>3</sub> may be required to improve tenderness. Montgomery *et al.* (2002) found that vitamin D<sub>3</sub> treatment will effectively improve tenderness when cattle tend to be tough and have no impact on cattle that produce tender beef. The previous authors added that feeding steers 0.5 × 10<sup>6</sup> IU of vitamin D daily for 9 days improved tenderness in two muscles without negatively affecting feedlot performance or tissue residues. Recently, Rider Sell *et al.* (2004) reported that vitamin D<sub>3</sub> supplementation at the rate of 5 million IU, or 7.5 million IU daily for 7 days provided little benefit to muscle tenderness of beef from cull cows. While Montgomery *et al.* (2004) found that supplementation with vitamin D<sub>3</sub> at 0.5 million IU/steer daily for 8 consecutive days before slaughter improved tenderness in steaks from different subprimal cuts by affecting muscle Ca concentrations, micro-calpain activities, muscle proteolysis, with only small effect on tissue residues of vitamin D<sub>3</sub>. The objective of the present study was to examine the effect of dietary supplementation of vitamin D<sub>3</sub> to intact males of Egyptian native breed on meat tenderness.

## **MATERIALS AND METHODS**

This study was carried out at the Agricultural Experimental Station and laboratories of the Faculty of Agriculture, Cairo University, Giza, Egypt. Twelve Baladi (local Egyptian cattle) bulls initially weighed 278±9.7 kg were fattened for 231 days. All bulls were fed a concentrate mixture (14% crude protein and 72.8 TDN) as 2% of their live body weight. Concentrates were fed once a day at 8:00. Ingredients and its chemical composition of the concentrate mixture are presented in Table 1. Rice straw was fed ad libitum. Bulls were watered twice a day at 9:00 and 14:30.

Ten days before slaughter, bulls were allotted randomly to one of three groups, 4 animals in each with an overall average weight 400.6±0.92 kg, orally supplemented with 0, 5 and 7.5 × 10<sup>6</sup> IU of vitamin D<sub>3</sub> per day. Blood samples were obtained from the jugular vein into heparinized tubes every two days during the supplementation period. Blood plasma was stored at -18°C for subsequent analysis. Blood collection was done before morning feeding

every 48-h intervals and at slaughter during exsanguination. Concentration of plasma calcium was measured on spectrophotometer according to the method of Gindler and King (1972).

**Table 1. Composition of the concentrate mixture fed to animals during the experiment on DM basis**

| Ingredient      | %    | Nutrient    | %     |
|-----------------|------|-------------|-------|
| Corn            | 40   | TDN         | 74.98 |
| Cottonseed cake | 11.4 | CP          | 13.96 |
| Wheat bran      | 25   | Crude Fiber | 9     |
| Rice Bran       | 15   | Ca          | 1.4   |
| Molasses        | 5    | P           | 0.7   |
| Limestone       | 3    |             |       |
| Vitamin & Trace |      |             |       |
| Minerals Premix | 0.30 |             |       |
| Common salt     | 0.30 |             |       |

Bulls were slaughtered with an average final live body weight of 413±7.2 kg. Hot carcass weights were recorded and dressing percentages were calculated. After chilling of carcasses for one day, steaks from the longissimus dorsi muscle of the 9, 10 and 11<sup>th</sup> ribs were cut 2.5 cm thick, individually packed and aged at 1°C for 14 days. After postmortem aging, steaks were frozen at -20°C until determining the total muscle calcium concentration, Warner-Bratzler shear force and sensory evaluation.

Total extractable calcium concentration of muscle samples was measured according to Nakamura (1973). Upon completion of the digestion process, the amount of Ca<sup>2+</sup> was measured with an atomic absorption spectrophotometer (Unicam, Solaar, UK).

Steaks for Warner-Bratzler shear force were thawed to 2°C then broiled to an internal temperature of 70°C. Warner-Bratzler shear force determination was conducted as described by Wheeler *et al.* (1997). The cooked steaks were cooled to the room temperature and then six cores 1 cm in diameter were removed parallel to the muscle fiber orientation. Cores were sheared through the centre of the core by using a shear head of the device. Peak shear force values were recorded as kilograms per 1 cm. The six shear values per steaks were averaged, and the means for treatments were analyzed for statistical significance.

Sensory evaluation of the Longissimus dorsi steaks was carried out by a 6 –members panel trained. Steaks were broiled as described for Warner-Bratzler shear. Each panelist was served one 1x1x2 cm samples. A 6-point descriptive scale (1 = extremely tough, 6 = extremely tender; 1 = extremely dry, 6 = extremely juicy; 1 = extremely bland, 6 = extremely flavorful) was used by the panelists to evaluate tenderness, juiciness, and flavor, respectively.

The collected data were statistically analyzed as a completely randomized design with individual bulls serving as the experiment unit. The GLM procedure of SAS (1994) was used to determine means and standard errors of means. A probability of less than 0.05 was considered significant.

## RESULTS

Among the three treatment groups, there were no significant differences in the initial live body weight (10 days before slaughter), final live body weight, gain, hot carcass weight, and dressing percentage (Table 2).

**Table 2: Feedlot performance and dressing percentage during the whole experiment**

| Item                                       | Control | Vitamin D <sub>3</sub>   |                            | SE   |
|--|---------|--------------------------|----------------------------|------|
|  |         | 5 x 10 <sup>6</sup> IU/d | 7.5 x 10 <sup>6</sup> IU/d |      |
| N  | 4       | 4                        | 4                          |      |
| Initial wt., kg (10 days before slaughter) | 399.5   | 400.5                    | 401.8                      | 0.9  |
| Final wt., kg                              | 407     | 413                      | 419                        | 7.2  |
| Hot carcass wt., kg                        | 246     | 242                      | 238                        | 10.8 |
| Dressing, %                                | 60.4    | 58.7                     | 57.0                       | 2.9  |

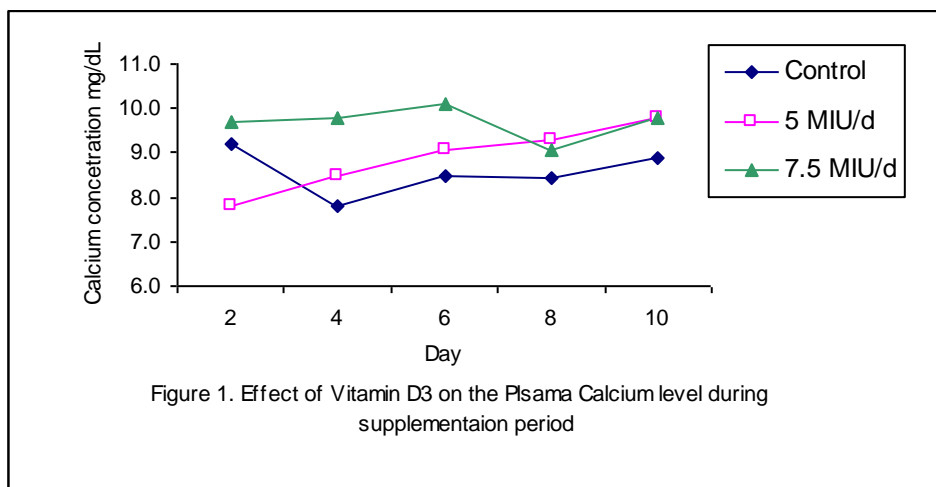
Blood plasma results indicated that vitamin D<sub>3</sub> supplementations increased calcium concentration (Table 3). Plasma calcium concentrations were higher for the supplemented groups compared with the control one during the whole supplementation period (Figure 1). It should be highlighted that approximately 4 and 13% increases in blood calcium concentration were observed when 5 or 7.5 × 10<sup>6</sup> IU of vitamin D<sub>3</sub>, respectively were given to finishing bulls 10 days before their scheduled slaughter date. Significant differences were observed between the control and 7.5 × 10<sup>6</sup> IU of vitamin D<sub>3</sub> supplemented group. No differences in plasma calcium level were detected between the two supplemented groups or between control group and 5 × 10<sup>6</sup> IU of vitamin D<sub>3</sub> supplemented group.

**Table 3: Effects of vitamin D3 supplementation on plasma calcium concentration, meat calcium concentration, shear force and sensory evaluation of the longissimus dorsi steaks**

| Item                            | Control           | Vitamin D <sub>3</sub>   |                            | SE   |
|---------------------------------|-------------------|--------------------------|----------------------------|------|
|                                 |                   | 5 x 10 <sup>6</sup> IU/d | 7.5 x 10 <sup>6</sup> IU/d |      |
| N                               | 4                 | 4                        | 4                          |      |
| Plasma calcium, mg/dL           | 8.58 <sup>b</sup> | 8.90 <sup>ab</sup>       | 9.68 <sup>a</sup>          | 0.32 |
| Muscle calcium, mg %            | 2.55 <sup>b</sup> | 5.00 <sup>a</sup>        | 2.64 <sup>b</sup>          | 0.56 |
| Shear force, kg/cm <sup>2</sup> | 3.78              | 3.47                     | 3.61                       | 0.38 |
| Tenderness <sup>c</sup>         | 4.00              | 4.25                     | 3.75                       | 0.39 |
| Juiciness <sup>c</sup>          | 3.75              | 4.25                     | 4.00                       | 0.39 |
| Flavour <sup>c</sup>            | 4.25              | 4.50                     | 3.75                       | 0.42 |

<sup>a,b</sup> Means in the same row with different superscripts significantly differ ( $p < 0.05$ ).

° Panel scores are based on an 6-point descriptive scale as follows: tenderness, 1 = extremely tough, 6 = extremely tender; juiciness, 1 = extremely dry, 6 = extremely juicy; flavor 1 = extremely bland, 6 = extremely flavorful.



Supplementation of vitamin D<sub>3</sub> increased muscle calcium concentration. On the contrary of blood plasma calcium level,  $5 \times 10^6$  IU of vitamin D<sub>3</sub> supplemented group had significantly higher muscle calcium concentration (5.00 mg %) compared with the control group (2.55 mg %) or  $7.5 \times 10^6$  IU of vitamin D<sub>3</sub> supplemented group (2.64 mg %). No significant differences between control and  $7.5 \times 10^6$  IU of vitamin D<sub>3</sub> supplemented group.

Steaks from bulls orally administered vitamin D<sub>3</sub> preceding slaughter had numerically lower Warner-Bratzler shear values compared with cuts from control carcasses (Table 2). However, no significant differences in shear force values were noticed among the three treatment groups. The steaks from  $7.5 \times 10^6$  IU of vitamin D<sub>3</sub> group seem to be more tender (the lowest shear value) than the other two groups.

No significant differences were detected in sensory scores related to tenderness, juiciness and flavor between steaks from vitamin D<sub>3</sub>-supplemented groups and those from control group. Concerning with the tenderness of longissimus dorsi steaks,  $5 \times 10^6$  IU of vitamin D<sub>3</sub> group rated, numerically, to be more tender compared to control or  $7.5 \times 10^6$  IU of vitamin D<sub>3</sub> groups.

## DISCUSSION

Vitamin D<sub>3</sub> has long been known to have an essential role in vertebrates. Vitamin D<sub>3</sub> helps mediate calcium and phosphorous metabolism to target tissues including, intestine, bone, kidney, and even muscle. Vitamin D<sub>3</sub> from dietary sources is taken up by the blood stream in the intestine or is produced within the body by ultraviolet light conversion of 7-

dehydrocholesterol. Vitamin D<sub>3</sub> is then hydroxylated within the liver to 25-hydroxyvitamin D<sub>3</sub>. Further processing occurs in the kidneys, where 25-hydroxyvitamin D<sub>3</sub>-1 $\alpha$ -hydroxylase introduces a hydroxyl group at the 1 $\alpha$ -position of carbon 1 of the A ring, producing the active steroid 1, 25-dihydroxyvitamin D<sub>3</sub>. This final reaction yields an active metabolism that has biological activity 500-1000-fold higher than its precursor 25-dihydroxyvitamin D<sub>3</sub>. Then 1, 25-dihydroxyvitamin D<sub>3</sub> plays a vital role in regulating calcium and phosphorous and calcium homeostasis by forming a steroid-receptor complex in target cells, to initiate the synthesis of specific RNA-encoding proteins that mediate calcium-binding responses (Montgomery *et al.* 2002). Supplemental dietary vitamin D<sub>3</sub> increases blood calcium markedly via actions of additional 1, 25-dihydroxyvitamin D<sub>3</sub>. Skeletal muscle is an important target organ for vitamin D<sub>3</sub>. Indeed, Swanek *et al.* (1999) found higher calcium concentrations in plasma and in longissimus muscle from steers fed diets containing vitamin D. Also, loin steaks from steers fed vitamin D<sub>3</sub> were tenderer. Montgomery *et al.* (2000) and Scanga *et al.* (2001) found that short-term oral administration of vitamin D<sub>3</sub> to steers or heifers effectively increases blood calcium and has been hypothesized to increase muscle calcium content, the activity of  $\mu$ -calpain, m-calpain, calpastatin and muscle proteases and thus tenderness of cooked meat.

Regarding the effect of vitamin D<sub>3</sub> supplementation on the feedlot performance this study confirmed the findings of Scanga *et al.* (2001) and Montgomery *et al.* (2002) who found that the short-term oral administration of dietary vitamin D<sub>3</sub> to beef cattle before slaughter had no effect on the feedlot performance.

Previous researches (Swanek *et al.*, 1999; Montgomery *et al.*, 2000; Scanga *et al.*, 2001 and Montgomery *et al.*, 2002) demonstrated that vitamin D<sub>3</sub> increased plasma calcium concentration by stimulating intestinal calcium absorption by mobilizing calcium from previously formed bone mineral, and through 1,25-dihydroxyvitamin D<sub>3</sub>, which increases renal reabsorption of calcium from the kidney. In addition to mobilizing calcium, vitamin D<sub>3</sub> stimulates the influx of calcium in skeletal muscle cells through the activation of calcium channels. In agreement with obtained results, both Swanek *et al.* (1999) and Montgomery *et al.* (2002) indicated that vitamin D<sub>3</sub> supplementation increased plasma and total muscle calcium. Acceleration postmortem aging of steaks by vitamin D<sub>3</sub> treatment explained by Montgomery *et al.* (2002) as a result of elevated muscle calcium concentrations and increased proteolysis during postmortem aging.

Regarding the effect of vitamin D<sub>3</sub> on meat tenderness which evaluated mechanically by Warner-Bratzler shear force value, Swanek *et al.* (1999) showed that Longissimus muscle steaks from cattle fed 5 or 7.5  $\times 10^6$  IU of vitamin D<sub>3</sub> daily for 7 days immediately before slaughter had lower Warner-Bratzler shear values at both 7 and 14 days, but not at 21 days postmortem. The same trend was observed by Montgomery *et al.* (2000) who found that giving supplemental daily doses of 5 or 7.5  $\times 10^6$  IU of vitamin D<sub>3</sub> for 9 days to feedlot cattle decreased Warner-Bratzler shear force of beef strip loin and top round steaks that were postmortem aged for 14 days. These findings beside the slight low values of Warner-Bratzler shear force in this study

support our hypothesis that vitamin D<sub>3</sub> may offer an effective way to improve tenderness (decreased Warner-Bratzler shear values) within 14 days of postmortem aging. The link between postmortem aging, myofibrillar degradation, and calcium has long been known. The effect of postmortem tenderization of beef seems to be the results of degradation of key myofibrillar proteins. The calcium-activated protease  $\mu$ -calpain has been shown to proteolytically degrade five skeletal muscle proteins, titin, nebulin, filamin, desmin, and troponin T, during postmortem storage (Huff-Lonergan *et al.*, 1996 a, b). Sensory panel evaluation of longissimus steaks at 14 days postmortem in our study could not detect any significant difference in tenderness which agrees with Montgomery *et al.* (2000).

### Conclusion

Feeding of vitamin D<sub>3</sub> before slaughter could be implemented in a commercial feedlot system to improve tenderness. Elevation of muscle calcium concentration and increased proteolysis seem to be the mechanism of tenderization of Longissimus steaks within 14 days postmortem. Consequently, ante-mortem feeding of supplemental vitamin D<sub>3</sub> may hold the potential of increasing consumer acceptance of beef. However, further studies need to address the optimal doses and length of time to feed vitamin D<sub>3</sub> for maximal tenderization to the Egyptian cattle breeds.

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## تأثير إضافة فيتامين د ٣ إلى علائق عجول التسمين لفترة قصيرة قبل الذبح على طراوة اللحم

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أجريت هذه التجربة لدراسة تأثير إضافة فيتامين د ٣ إلى علائق عجول الإبقار البلدية المسمنة لفترة قصيرة قبل الذبح على طراوة اللحم الناتج. قسم عدد ١٢ عجل بلدى عشوانيا إلى ٣ مجاميع متساوية، أربع عجول فى كل مجموعة . المجموعة الاولى مثلت الحيوانات غير المعاملة (كنترول) أما المجموعة الثانية فتم إضافة ١٠ x ٦ وحدة دولية / يوم أما المجموعة الثالثة فتم إضافة ٧,٥ x ١٠ وحدة دولية / يوم إلى علائق كلا منهما لمدة عشرة أيام متتالية قبل الذبح. تم سحب عينات دم من الوريد الوداجى كل يومين لقياس مستوى الكالسيوم فى بلازما الدم. ذبحت كل العجول بمتوسط وزن ذبح نهائى ٤١٣ كجم. فصلت العضلة العينية من الضلوع ٩، ١٠، ١١ بعد التبريد و عتقت بالحفظ على ٥١ م لمدة ١٤ يوم ثم قيس محتوى العضلات من الكالسيوم وكذلك الصفات الحسية للحم. أظهرت النتائج ارتفاع مستوى الكالسيوم فى بلازما دم عجول المجموعة ٣ مقارنة بالمجموعة الكنترول. بينما كان تركيز الكالسيوم فى لحم المجموعة الثانية أعلى معنويا من مجموعة الكنترول والمجموعة الثالثة. سجلت المجموعة الثالثة أقل قيمة قطع بجهاز الفارنر براتزلر مقارنة بمجموعة الكنترول والمجموعة الثانية لكن بدون أى فروق معنوية بين المجاميع الثلاث. ومع ذلك كانت الطراوة والعصيرية والطعم المقاسة من قبل المتذوقين أعلى فى لحم المجموعة الثانية مقارنة بالكنترول والمجموعة الثالثة ولكن ايضا بدون أى فروق معنوية بينهم . دلت النتائج أن إضافة فيتامين د ٣ لمدة ١٠ أيام قبل الذبح زاد من مستوى الكالسيوم فى بلازما الدم والعضلات لكنه لم يؤثر معنويا على تحسين طراوة اللحوم المطهية.