

## QUALITY CHARACTERISTICS OF *Longissimus dorsi* MUSCLE FROM *Bos indicus* ANIMALS TREATED WITH VITAMIN D<sub>3</sub>

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**ABSTRACT:** Among several techniques to improve beef tenderness, vitamin D<sub>3</sub>, important for calcium mobilization, has recently been developed as an alternative. It acts on the intracellular calcium-dependent proteases ( $\mu$ - and m-calpain). Ten days prior to slaughter, 36 Nelore steers were fed 0, 3, 6 and 9 million IU of supplemental vitamin D<sub>3</sub> (D<sub>3</sub>) per animal per day ( $\text{an}^{-1} \text{d}^{-1}$ ). Animals were slaughtered and tenderness (shear force), cooking losses (1, 8 and 15 days of aging), sensory evaluation, and minerals in blood plasma and muscle (*Longissimus dorsi*) were measured. There were no differences ( $P > 0.05$ ) among treatments for blood plasma and muscle mineral concentration, evaporation losses, and sensory juiciness. For drip and total loss, the smallest losses were for the  $6 \times 10^6$  IU  $\text{an}^{-1} \text{d}^{-1}$  treatment. The control treatment resulted in lowest shear force and aging also tended to lower resistance to shearing. The  $3 \times 10^6$  IU  $\text{an}^{-1} \text{d}^{-1}$  treatment had a positive effect on tenderness, flavor and overall palatability. High levels of supplemental D<sub>3</sub> did not improve the quality characteristics of *Longissimus dorsi* muscle from *Bos indicus* animals.

**Key words:** Nelore cattle, meat tenderness, carcass characteristics

## CARACTERÍSTICAS QUALITATIVAS DO MÚSCULO *Longissimus dorsi* DE ANIMAIS *Bos indicus* TRATADOS COM VITAMINA D<sub>3</sub>

**RESUMO:** Muitas técnicas são empregadas para melhorar a maciez da carne de bovinos. A mais recente usa a vitamina D<sub>3</sub>, que tem importância na mobilização do cálcio e na ativação das proteases cálcio-dependentes ( $\mu$ - e m-calpaína). Neste estudo, 36 machos castrados (Nelore) foram suplementados por via oral com quatro níveis de vitamina D<sub>3</sub> (0, 3, 6 e 9 milhões de UI de vitamina D<sub>3</sub> animal<sup>-1</sup> dia<sup>-1</sup>) durante os 10 dias que antecederam o abate. Após o abate foram medidos a força de cisalhamento e perdas por cozimento (aos dias 1, 8 e 15 de maturação), pH, concentração de minerais no plasma sanguíneo e no músculo *Longissimus dorsi*, além de análise sensorial de amostras. Não houve ( $P > 0.05$ ) efeito da dose de vitamina D<sub>3</sub> na concentração de minerais no plasma sanguíneo e no músculo, nas perdas por evaporação, e na suculência estimada pela análise sensorial. As menores perdas (totais e por gotejamento) foram em resposta à dose  $6 \times 10^6$  UI  $\text{an}^{-1} \text{dia}^{-1}$ . A dose controle resultou na menor força de cisalhamento (FC), que tendeu a ser menor quanto maior o tempo de maturação. A dose  $3 \times 10^6$  UI  $\text{an}^{-1} \text{dia}^{-1}$  afetou positivamente as características de maciez, sabor e aceitação global. A suplementação com elevadas doses de vitamina D<sub>3</sub> não melhorou as características qualitativas do músculo *Longissimus dorsi* (contra-filé) de animais Nelore (*Bos indicus*).

**Palavras-chave:** gado Nelore, maciez, carne, características da carcaça

### INTRODUCTION

In recent years, many techniques have been used to tenderize meat, including delayed chilling, temperature monitoring during cold storage, carcass suspension by the pelvis, electrical stimulation, aging, and calcium-based compounds (calcium chloride and calcium propionate). These techniques have been studied and utilized individually or in a variety of combinations, based on the general principle that, to different extents, all these treatments are capable of increasing meat tenderness by increasing

sarcomere length, enzyme proteolytic activity, and the tension over several leg and loin muscles. Similarly, such techniques are known to minimize protein denaturation and loss of tension in the myofibril component of muscle cells, a result of Z band disintegration, which breaks muscle fibers and supplies exogenous calcium to calcium-dependent proteases ( $\mu$ - and m-calpains). This results in an acceleration of the tenderizing process by increasing m-calpain activity, which, under normal postmortem conditions, is not very active, since it needs a higher concentration of calcium ions to become activated.

Vitamin D<sub>3</sub> is routinely utilized in ruminant diets to prevent milk fever in lactating dairy cows, as it increases calcium concentration in the blood (Morgan, 1998a; 1998b). The use of D<sub>3</sub> to tenderize meat is somewhat recent and consists of supplying the vitamin orally at medium to high levels, making enough calcium available to activate calcium-dependent proteases ( $\mu$ - and m-calpains) and accelerating the meat tenderizing process (Montgomery et al., 2000a; 2000b; 2000c; Morgan, 1998a; Swanek et al., 1999a; 1999b; 1999c).

The present study was targeted at evaluating the quality characteristics (shear force and cooking losses at 1, 8 and 15 days of aging; sensory evaluation - tenderness, juiciness, flavor and overall acceptance - and the concentration of calcium, magnesium, sodium and potassium) in the *Longissimus dorsi* muscle from *Bos indicus* (Nelore) animals supplemented orally with four levels of vitamin D<sub>3</sub> (0, 3, 6 and 9 million IU an<sup>-1</sup> d<sup>-1</sup>) for a 10-day period prior to slaughter. Evaluations were also made for calcium, magnesium, phosphorus, sodium and potassium concentrations in the blood plasma of those animals, to determine which of the four levels would positively affect meat quality and what effect would they have on blood plasma mineral concentrations.

## MATERIAL AND METHODS

Trial was set up with thirty six Nelore steers, (511 kg liveweight; 2.5 to 3 years old), taken from a private herd and kept in 18 (7,000-m<sup>2</sup> each) paddocks with two animals per paddock, for a 20-day period prior to slaughter in Brotas, SP, Brazil (22°17'03"S and 48°07'36"W). Animals were given 0, 3, 6 and 9 million IU of D<sub>3</sub> an<sup>-1</sup> d<sup>-1</sup>, for of 10 days prior to slaughter, supplied orally, mixed with the concentrate (mineral salt + corn flour).

Blood samples were drawn from the jugular vein on slaughter day and centrifuged at 13,000 g for 15 min. Blood serum was removed, frozen at 20°C, and stored for subsequent determination of the concentrations of calcium, magnesium, phosphorus, sodium and potassium. The concentrations of minerals in the plasma and in the meat were determined by of atomic absorption.

Carcasses were stored cold (4°C) for 24 h and than three, 2.5-cm thick steaks were cut from the ribeye (between the 12<sup>th</sup> rib and the 5<sup>th</sup> lumbar vertebra) of each carcass, for utilization in tenderness evaluation, cooking tests, sensory evaluation and to quantify the concentrations of calcium, magnesium, phosphorus, sodium and potassium. Shear force and cooking losses evaluations followed procedures recommended by AMSA (1978). Steaks were broiled (four samples per batch maximum) in an electric oven, pre-heated to 170°C, until their internal temperature reached 71°C (temperature monitored with thermometers placed in the geometric center of each steak); samples steaks they were weighed before and af-

ter cooking to determine cooking losses. After broiling, steaks were cooled to room temperature, kept in refrigerator at 2-5°C overnight and to eight cylindrical samples, 1.27 cm in diameter, were removed parallel to muscle fiber orientation and kept in the refrigerator for shear force measurements in a Warner-Bratzler Shear Force Device.

The sensory evaluation also followed AMSA's (1978) procedures set on a panel of 50 people (samplers). Each person was given an evaluation card, which consisted of an 8-point hedonic scale for tenderness, juiciness, flavor and overall acceptance (8= extremely tough, dry, poor, I disliked it extremely; 1= extremely tender, juicy, intense, I liked it extremely, respectively).

The statistical analysis for sensory evaluation data was done using the SAS software (SAS, 1990), according to a completely randomized design, with four treatments and 50 replicates (samplers). Analysis of variance was used to compare treatment means, since the assumptions for use of the ANOVA technique were satisfied. The experimental design was completely randomized, with four treatments and nine replications. A split-plot arrangement was used for shear force, percentage of evaporation losses, drip losses, total losses and sensory analysis, using the GLM procedure of SAS (SAS, 1990) with plots being the four D<sub>3</sub> levels and subplots being the three aging periods (1, 8, and 15 days). The 'SLICED' statement was used when the interaction was significant, to study the partitioning of the interaction of a factor within each level of the other.

## RESULTS AND DISCUSSION

No differences ( $P > 0.05$ ) were found among D<sub>3</sub> supplementation levels for plasma concentrations of calcium, sodium, potassium and magnesium (Table 1). These results contradict those presented by Karges et al. (2001), Kotrla et al. (2001), Scanga et al. (2001), Rentfrow et al. (2001), Berry et al. (2000), Enright et al. (2000a; 2000b), Morgan & Gill (2000), and Rider et al. (2000), who reported increased plasma calcium concentration in response to D<sub>3</sub>. In a study by Swanek (1999a; 1999b), the concentration of plasma calcium increased between 8 and 48% as a result of D<sub>3</sub> supplementation, mainly because of the effect of the vitamin on calcium metabolism, increasing intestinal reabsorption and calcium loss from bone deposits.

The abundance of vitamin D<sub>3</sub> supplied before slaughter may cause reduction in calcium absorption and in the concentrations of serum calcium, because of the inhibition of calcium synthesis and absorption (as an effect of enzymes Ca<sup>+2</sup>-25 hydroxylase and 1 $\alpha$ -hydroxylase), which are essential for the formation of 1,25(OH)<sub>2</sub>-vitamin D<sub>3</sub> (Scanga et al., 2001), or because the vitamin is not converted to the metabolic forms (25-hydroxyvitamin D<sub>3</sub> and 1,25(OH)<sub>2</sub>-vitamin D<sub>3</sub>). Another

issue, raised by Wiegand et al. (1998; 2001), concerns the form in which  $D_3$  is supplied to the animals. According to this hypothesis, supplying vitamins as gelatin capsules (*bolus*) is a more efficient method than mixed with concentrate, since it is passed directly to the digestive tract. According to Scanga et al. (2001), supplying  $D_3$  orally by means of *bolus* was an effective technique, resulting in increased plasma calcium concentration and improving meat tenderness.

It was expected reduction magnesium concentration in response to increased  $D_3$  levels. However, it did not happen, probably because no differences were found among the  $D_3$  levels used in this study. This result conflicts with those reported by Swanek et al. (1997), who measured a reduction in plasma magnesium concentration (26.6%), and by Karges et al. (1999a; 1999b; 1999c; 1999d), who also found such a reduction, with the lowest concentration at  $6 \times 10^6$  IU  $D_3$   $an^{-1} d^{-1}$ . On the other hand, according to Hill et al. (1999), steers treated with  $7.5 \times 10^6$  IU of  $D_3$  for a period of ten days prior to slaughter showed little difference in the plasmatic concentrations of calcium, magnesium and phosphorus. No differences ( $P > 0.05$ ) were found between treatments for muscle concentrations of calcium, magnesium, phosphorus, sodium and potassium (Table 2). For potassium, all levels were higher than the control. The same was found for the concentration of muscle magnesium.

These results differ from those reported by Montgomery et al. (2002), Morgan & Gill (2000), Rider et al. (2000), Hill et al. (1999), and Swanek et al. (1999a; 1999b; 1999c), who observed increases in the concentra-

tion of muscle calcium with increasing  $D_3$  levels. Swanek et al. (1999b) observed that the concentration of calcium in the *Longissimus* muscle of animals treated with  $D_3$  increased between 43 and 50%. This increase could improve the capacity of calcium-dependent proteases to degrade Troponin-T into a 30 kDa component at the 14-day aging period, resulting in tender meat.

The lowest total cooking loss (TL), were recorded for the  $6 \times 10^6$  IU  $an^{-1} d^{-1}$  level and 1-d aging time, while the highest loss was for the control  $D_3$  level and 8-d aging time (Table 3). For the dose  $\times$  aging time interaction (sliced effect) there were differences among  $D_3$  levels within aging times ( $P < 0.05$ ), which resulted in smaller TL for the  $6 \times 10^6$  IU  $an^{-1} d^{-1}$  treatment, and an effect ( $P < 0.05$ ) was detected for aging time within the control and the  $6 \times 10^6$  IU  $an^{-1} d^{-1}$  levels, resulting in the smallest and greatest TL values, respectively.

The lowest shear force (SF) value was recorded for the control treatment and 15-d aging time, whereas the highest value corresponded to the  $9 \times 10^6$  IU  $an^{-1} d^{-1}$  level and 1-d aging time (Table 4). For the dose  $\times$  aging time interaction (sliced effect) there were differences among  $D_3$  levels within aging times ( $P < 0.01$ ), which resulted in the greatest SF for the  $9 \times 10^6$  IU  $an^{-1} d^{-1}$  level, and there was an effect ( $P < 0.01$ ) of aging time within  $D_3$  levels, with smallest SF values corresponding to longer times.

In this study,  $D_3$  was not effective in improving meat tenderness, which responded more markedly to aging time. These results are similar to those found by Scanga et al. (2001), Rentfrow et al. (2001), Wiegand et

Table 1 - Least squares mean, overall mean, coefficient of correlation and significance of concentrations of plasma minerals in *Bos indicus* animals supplemented with vitamin  $D_3$  for 10 consecutive days prior to slaughter.

Mineral	$D_3$ level ( $\times 10^6$ IU $an^{-1} d^{-1}$ )				Mean	CV	Pr > F
	0	3	6	9			
	----- mg $dL^{-1}$ -----					%	
Calcium	10.56	11.30	10.79	11.28	10.99	6.65	0.11
Sodium	196.10	203.11	194.31	203.80	199.30	5.22	0.13
Potassium	22.18	24.48	22.54	22.19	22.79	20.47	0.71

Table 2 - Least squares means, overall mean, coefficient of variation and significance of concentrations of muscle minerals in *Bos indicus* animals supplemented with vitamin  $D_3$  for 10 consecutive days prior to slaughter.

Mineral	$D_3$ level ( $\times 10^6$ IU $an^{-1} d^{-1}$ )				Mean	CV	Pr > F
	0	3	6	9			
	----- $\mu g g^{-1}$ -----					%	
Calcium	122.75	122.75	121.20	125.40	123.06	9.58	0.88
Sodium	974.13	986.88	1,008.10	1,027.30	1,001.17	7.72	0.49
	----- g (100 g) $^{-1}$ -----					%	
Phosphorus	0.66	0.67	0.67	0.71	0.68	7.74	0.21
Sodium	0.16	0.15	0.14	0.13	0.14	16.70	0.08
Potassium	1.17	1.22	1.26	1.25	1.23	8.40	0.32

Table 3 - Least squares means of cooking total losses and sliced effect in relation to treatment, aging time and interaction treatment  $\times$  aging time in the *Longissimus dorsi* muscle of *Bos indicus* animals supplemented with vitamin D<sub>3</sub> for 10 consecutive days prior to slaughter.

Aging (days)	D <sub>3</sub> level ( $\times 10^6$ IU an <sup>-1</sup> d <sup>-1</sup> )				Mean	Sliced Effect Pr > F <sup>1</sup>
	0	3	6	9		
	----- % -----					
1	22.39	21.57	17.46	22.67	21.02	*
8	27.38	23.29	22.57	22.99	24.06	ns
15	21.74	19.07	18.50	23.84	20.78	*
Mean	23.83	21.31	19.51	23.17		
Sliced Effect Pr > F <sup>2</sup>	*	ns	*	ns		

Overall mean= 21.89; CV= 18.58%; Pr > F=0.0123

<sup>1</sup>Effect of D<sub>3</sub> level within aging time

<sup>2</sup>Effect of aging time within D<sub>3</sub> level

ns= non significant ( $P > 0.05$ ); \* ( $P < 0.05$ ); \*\* ( $P < 0.01$ )

Table 4 - Least squares means of shear force and sliced effect in relation to treatment, aging time and interaction treatment  $\times$  aging time in the *Longissimus dorsi* muscle of *Bos indicus* animals supplemented with vitamin D<sub>3</sub> for 10 consecutive days prior to slaughter.

Aging (days)	D <sub>3</sub> level ( $\times 10^6$ IU an <sup>-1</sup> d <sup>-1</sup> )				Mean	Sliced Effect Pr > F <sup>1</sup>
	0	3	6	9		
	----- % -----					
1	4.92	5.56	4.98	5.69	5.29	**
8	3.67	4.14	4.03	4.19	4.01	ns
15	2.87	3.11	3.02	3.38	3.09	ns
Mean	3.82	4.27	4.01	4.42		
Sliced Effect Pr > F <sup>2</sup>	**	**	**	**		

Overall mean= 4.14; CV= 13.22%; Pr > F=0.0001

<sup>1</sup>Effect of D<sub>3</sub> level within aging time

<sup>2</sup>Effect of aging time within D<sub>3</sub> level

ns= non significant ( $P > 0.05$ ); \* ( $P < 0.05$ ); \*\* ( $P < 0.01$ )

al. (2001), Berry et al. (2000) and Ribeiro (2000), who did not find a decrease in shear force, even when the concentration of plasma calcium was increased by D<sub>3</sub> supplementation. Other studies, however, show meat tenderizing (6.6-50%) with the use of vitamin D<sub>3</sub> supplementation, as reported by Karges et al. (2001), Kotrla et al. (2001), Foote et al. (2001), Montgomery et al. (2001a; 2001b; 2000a; 2000b), Boleman et al. (2000), Morgan & Gill (2000), and Rider et al. (2000). Vitamin D<sub>3</sub> supplementation should be effective to tenderize the meat of animals that produce tough meat, although it would probably have little or no impact on animals that produce tender meat (Montgomery et al., 2002; Karges et al., 1999a).

The ranking utilized to measure shear force is quite variable and the acceptable range is between 4.5 and 6 kg (McKeith et al., 1985; Koohmaraie, 1994). A maximum acceptable limit of 5 kg was used in this work. According to Miller et al. (1993), meat with shear force values above those is considered tough, while those below 5 kg are considered tender. In the present study, D<sub>3</sub> supplementation did not result in improved meat tenderness.

Regarding the responses of sensory evaluation (Table 5), only juiciness did not respond ( $P > 0.05$ ) to D<sub>3</sub> levels. The  $9 \times 10^6$  IU an<sup>-1</sup> d<sup>-1</sup> treatment showed the highest value for sensory tenderness (least tender, or toughest), while the  $3 \times 10^6$  IU an<sup>-1</sup> d<sup>-1</sup> treatment showed the lowest value (most tender). The  $3 \times 10^6$  IU an<sup>-1</sup> d<sup>-1</sup> treatment resulted in the best flavor, whereas the other D<sub>3</sub> levels showed similar results among themselves. Regarding overall acceptance, the poorest evaluation was given to the control treatment and the best was given to the  $3 \times 10^6$  IU an<sup>-1</sup> d<sup>-1</sup> level.

In general, D<sub>3</sub> supplementation improved the meat's sensory characteristics and the best sensory evaluation results were observed for the  $3 \times 10^6$  IU an<sup>-1</sup> d<sup>-1</sup> level. These results are similar to those found by Swanek et al. (1999a; 1999b), who demonstrated a positive effect of vitamin D<sub>3</sub> supplementation on sensory characteristics. Other researchers, such as Karges et al. (2001), Montgomery et al. (2000a; 2000b) and Rider et al. (2000), did not detect effects of supplementation on sensory evaluation.

Table 5 - Mean scores for flavor, juiciness, tenderness and overall acceptance characteristics in sensory evaluation performed for *Longissimus dorsi* muscle of *Bos indicus* animals supplemented with vitamin D<sub>3</sub> for 10 consecutive days prior to slaughter

Characteristic <sup>1</sup>	D <sub>3</sub> level (× 10 <sup>6</sup> IU an <sup>-1</sup> d <sup>-1</sup> )				Overall Mean	CV
	0	3	6	9		
	----- Score -----					%
Tenderness	5.38 <sup>ab</sup>	4.88 <sup>b</sup>	5.10 <sup>ab</sup>	5.86 <sup>a</sup>	5.31	29.18
Flavor	5.44 <sup>a</sup>	4.32 <sup>b</sup>	5.40 <sup>a</sup>	5.18 <sup>a</sup>	5.09	27.90
Juiciness	4.72 <sup>a</sup>	4.14 <sup>a</sup>	4.76 <sup>a</sup>	4.96 <sup>a</sup>	4.65	34.42
Overall	5.44 <sup>a</sup>	4.56 <sup>b</sup>	5.32 <sup>a</sup>	5.26 <sup>ab</sup>	5.15	26.53

<sup>1</sup>8= extremely tough, dry, poor, I disliked it extremely; 1= extremely tender, juicy, intense, I liked it extremely.

<sup>a,b</sup>Means followed by a common letter in a row are not different by Tukey test ( $P > 0.05$ ).

High levels of supplemental D<sub>3</sub> did not improve the quality characteristics of *Longissimus dorsi* muscle from *Bos indicus* animals. Further research is needed in order to assess the effects of D<sub>3</sub> at levels between 0 and 3 × 10<sup>6</sup> IU an<sup>-1</sup> d<sup>-1</sup>, as there may be a differential response among levels within this range. In addition, the method of administration of D<sub>3</sub> may have an impact on meat tenderness and this also deserves further attention.

## ACKNOWLEDGEMENTS

To Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Brazil, for the scholarship granted to the senior author as a graduate student.

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Received October 22, 2002

Accepted August 26, 2003