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Interaction of dietary vitamin D_3 and sunlight exposure on *B. indicus* cattle: Animal performance, carcass traits, and meat quality

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ABSTRACT

Attempts to improve beef tenderness through supplementation with dietary vitamin D_3 have been challenged by null results and negative impacts on animal performance and carcass traits. Because vitamin D_3 is also synthesised by the animal via ultraviolet radiation from sunlight, the effectiveness of supplementation with dietary vitamin D_3 may be modulated by the degree of exposure of the animal to sunlight. Hence, this work aimed to verify whether dietary vitamin D_3 modifies meat quality without negatively affecting animal performance and carcass traits in *B. indicus* beef cattle that were either exposed to or protected from natural sunlight. Forty-two (411 ± 38 kg) Nellore-type castrated males were fed a high-concentrate diet for 45 days after assignment to a treatment group. The treatments comprised combinations of three levels of vitamin D_3 [ViTD — none (V0) or 2×10^6 IU of vitamin D_3 administered for either 2 (V2) or 8 (V8) consecutive days pre-slaughter] and two shading conditions (SHADE — unshaded or shaded). The post-mortem (pm) measurements were taken in the *Longissimus thoracis et lumborum* muscle. The animal performance and carcass traits were unaffected by ViTD or SHADE. The V2 treatment increased the Myofibrillar Fragmentation Index in shaded cle calcium concen- The L^* values were animals in the ViTD or SHADE groups. Higher a^* values were observed among animals in the V8 group than in the V0 group, and higher b^* values were observed among animals in the V8 group than in the V2 or V0 groups, which were not different. In conclusion, ViTD and SHADE did not affect animal performance, carcass traits or shear force, whereas animals receiving a lower ViTD dosage and SHADE exhibited altered myofibrillar fragmentation. ViTD affected the colour parameters, and changes in the lightness of the beef related to the time pm were found in meat from animals under SHADE.

1. Introduction

Vitamin D_3 supplementation has been reported to improve the tenderisation rate (Foote et al., 2004; Karges et al., 2001; Montgomery et al., 2000; Swanek et al., 1999) and colour (Hansen et al., 2011; Strydom et al., 2007) of meat by increasing the concentration of calcium in the plasma and muscle as well as the anti-oxidative capacity. However, high doses of vitamin D_3 have a negative effect on feed intake (Montgomery et al., 2002; Scanga et al., 2001), average daily gain (Montgomery et al., 2002; Reiling and Johnson, 2003), final body weight, hot carcass weight, and fat thickness (Karges et al., 2001; Reiling and Johnson, 2003). Those effects could eventually be related to vitamin residues in the liver, kidneys, and muscle (Foote et al., 2004; Montgomery et al., 2000, 2002).

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In contrast to the results mentioned above, other researchers found no benefits of supplementation with vitamin D_3 or its metabolites regarding the tenderness (Carnagey et al., 2008; Cho et al., 2006; Pedreira et al., 2003; Reiling and Johnson, 2003; Tipton et al., 2007) or colour (Lawrence et al., 2006; Reiling and Johnson, 2003; Tipton et al., 2007) of the meat. Moreover, results have been reported showing no changes in carcass traits as a result of vitamin D_3 supplementation (Montgomery et al., 2002; Swanek et al., 1999).

Although previous studies have investigated the effects of different vitamin dosages, the influence of ultraviolet (UV) radiation from sunlight on the animals has not been considered. This influence may explain inconsistencies in the results regarding vitamin D_3 supplementation, especially at lower levels. The 25-hydroxy-vitamin D_3 [$25(OH)D_3$] concentrations in sheep are affected as the result of the interaction between intravenously administered $25(OH)D_3$ and sunlight exposure (Hidiroglou, 1987). Excessive sunlight exposure could result in conditioning of the animal to control elevated levels of the active metabolite 1,25-di-hydroxy-vitamin D_3 [$1,25(OH)_2D_3$]. Negative feedback generated by $1,25(OH)_2D_3$ through renal metabolism has been observed in mice, where any excess of $25(OH)D_3$ is converted to the inactive molecule 24,25-di-hydroxy-vitamin D_3 [$24,25(OH)_2D_3$] (Omdahl et al., 2002).

This work aimed to verify whether an interaction between levels of vitamin D_3 supplementation and sunlight exposure conditions could improve the meat tenderisation rate and colour without causing negative effects on animal performance and carcass traits of *B. indicus* beef cattle.

2. Materials and methods

2.1. Animals

Forty (412 ± 38 kg) and 41 (411 ± 38 kg) Nellore-type castrated males that originated from a commercial herd with an average age of over 30 months (over four permanent incisors) were used to generate the performance and carcass trait data and the meat quality data, respectively. After adaptation to a high-concentrate diet, the animals were divided into three weight groups (light $= 376 \pm 29$ kg, intermediate $= 403 \pm 16$ kg, and heavy $= 457 \pm 17$ kg), which represented the different growth potentials before the beginning of the experimental feedlot period. The animals within each weight group were randomly assigned to the six treatments (three levels of vitamin D_3 supplementation $\times$ two sunlight exposure conditions). The unshaded and shaded pens occupied different sides of the feedlot; one side of the feedlot had naturally lower insolation than other side (see Section 2.4).

2.2. Treatments

The animals were allocated to individual pens to allow for measurement of feed intake and to assure vitamin D_3 ingestion. The following experimental treatments were tested: 1) no vitamin D_3 supplementation (V0) and no shade ($n = 7$); 2) V0 with shade (50% UV filtration ratio) ($n = 7$); 3) 2×10^6 IU of vitamin D_3 for 2 consecutive days pre-slaughter (V2) and no shade ($n = 7$); 4) V2 with shade ($n = 6$ or 7); 5) 2×10^6 IU of vitamin D_3 for 8 consecutive days pre-slaughter (V8) and no shade ($n = 6$); and 6) V8 with shade ($n = 7$).

2.3. Vitamin D_3 supplementation

The vitamin supplement was donated by the DSM Produtos Nutricionais Brasil Ltda. (São Paulo, SP, Brazil). Vitamin D_3 ($4\ g = 2 \times 10^6$ IU) was mixed with 1 kg of the total diet and provided in a small trough. The vitamin D_3 intake was verified through visual observation. After the vitamin D_3 intake, the animals were fed the whole ration once a day (morning). The diet was composed of 21% roughage (sugarcane bagasse) and 79% concentrate (60.0% grain corn, 15.2% soybean, 3.8% mineral, and vitamin trace/NC Bov Nutron TMR containing 5636 IU vitamin D_3) with the following composition: 80.0% dry matter, 72.1% total digestible nutrients, 13.7% crude protein, 3.0% ether extract, 0.1% calcium, and 0.3% phosphorus.

2.4. Shade and feedlot

Sunshade from Nova Plast Indústria e Comércio Ltda. (Nova Odessa, SP, Brazil) with a UV filtration ratio of 50% was set on the northeast side of the feedlot. This side had naturally lower insolation during the experimental period due to orientation of the roof (length) that covers a central corridor between the pens. The sunshade of 50×9 m was enough to cover the back and sides of the pens in the feedlot. To block the reflected sunlight that reached the front part of the pens, one area covered by sunshade (50×1.5 m) above the feeding trough was used.

The feedlot is composed of two rows of side-by-side pens separated by a central corridor that is 3 m in width, with individual pen areas of 8 m^2. In the corridor, the individual troughs are covered by a roof with a height of 4.5 m and a width of 8 m.

2.5. Adaptation to treatments

After a 56-day period of adaptation to a high-concentrate diet, the animals were confined for 45 days with different sunlight exposure conditions. Twenty steers were held in pens without sunshade (the pens on the southwest side), and the other 21 steers were held in shaded pens (the pens on the northeast side). On the 25th day of exposure to different sunlight exposure conditions, all the animals were conditioned to receive part of their diet in a small trough to simulate the vitamin D_3 supplementation procedure, before receiving the remaining portion of the diet. On the 37th or 43rd day at the feedlot, the animals began supplementation with vitamin D_3 for 8 or 2 days.

2.6. Experimental period and location

The experiment was conducted from 30th November 2007 to 14th January 2008 (summer) in an experimental feedlot located in Andradina (São Paulo state — SP). Andradina is located at the coordinates 20°53′45″ South and 51°22′44″ West and has an altitude of 405 m.

2.7. Global and ultraviolet radiation and climate data

The global radiation (GR) data were obtained using a LI200X LI-COR pyranometer (Campbell Scientific Inc., North Logan, UT, USA) located at the Meteorology Centre (São Paulo State University/UNESP) in the city of Ilha Solteira, SP. The data are available at http://www.agr.feis.unesp.br/clima/ilha_dez07.htm and http://www.agr.feis.unesp.br/clima/ilha_jan08.htm. The data obtained at Ilha Solteira were used because of the small distance between the cities of Andradina and Ilha Solteira (only 72 km) and the similar climate characteristics (Ilha Solteira is located at the coordinates 20°25′58″ South and 51°20′33″ West and has an altitude of 335 m). The equation used to estimate the UV radiation (UVR) in the pens that were naturally exposed to sunlight was "UVR = 0.04155 × GR" (Escobedo et al., 2006). The UVR values estimated for the pens without sunshade were divided in half to estimate the UVR for the pens with sunshade (50% UV radiation filtration). The GR, UVR, and air temperature and humidity (recorded in the pens during the day at 9 am, 12 pm, 3 pm, and 6 pm) confirm the fair weather observed at the feedlot during the experiment (Table 1).

2.8. Animal performance

To study the animal performance, the following variables were recorded: the average feed intake (AFI), the initial (IBW) and final (FBW) body weight, the average daily gain (ADG), and the feed:gain ratio (F:G).

The ingested and refused feed were weighed daily. Additionally, samples of the diet (roughage and concentrate) that was fed and the refused portion were taken at intervals of 20 days to determine the dry matter (DM) content according to AOAC (1990). Both diet and refusal presented the same DM content, and the feed intake (DM basis) was calculated daily using the equation: (offered diet − refusal) × DM.

The following variables were obtained: AFI = DM intake in 45 days ÷ 45 days; ADG = gain (FBW − IBW) in 45 days ÷ 45 days; and F:G = DM intake in 45 days ÷ gain in 45 days. The IBW and FBW were obtained by weighing the animals before and after the experimental period, respectively. All the animals were submitted to an 18-hour fast prior to each weighing.

Table 1
Global and ultraviolet (UV) radiation data, and local air temperature and humidity during the experimental feedlot period and days of vitamin D_3 supplementation for the pens without and with sunshade.

Day	Global radiation (MJ/m^2)		UV radiation[a] (MJ/m^2)		Air temperature (°C)		Air humidity (%)	
	NS[b]	WS[c]	NS	WS	NS	WS	NS	WS
EP[d]	24.5	12.2	1.0	0.5	nd[e]	nd	nd	nd
Day 1	24.8	12.4	1.0	0.5	36.1	34.9	41.0	46.0
Day 2	30.1	15.1	1.3	0.6	36.5	33.8	39.0	46.0
Day 3	31.1	15.6	1.3	0.7	36.3	33.9	38.0	46.0
Day 4	30.7	15.4	1.3	0.6	38.7	35.2	31.0	39.0
Day 5	20.6	10.3	0.9	0.4	32.8	30.6	49.0	59.0
Day 6	24.3	12.2	1.0	0.5	36.3	33.7	41.0	50.0
Day 7	21.5	10.8	0.9	0.5	34.1	31.7	46.0	53.0
Day 8[f]	3.3	1.7	0.1	0.1	36.3	31.9	41.0	55.0

Legend: NS (no shade) = pens without sunshade in which animals were allocated; WS (with shade) = pens with sunshade (50% ultraviolet filtration ratio) in which animals were allocated; and Day 1 to Day 8 = days of vitamin D_3 supplementation. [a] Data obtained from the equation: UV radiation = 0.04155 × global radiation (Escobedo et al., 2006). [b] Data obtained from the Meteorology Centre of the UNESP (Source: http://www.agr.feis.unesp.br/clima/ilha_dez07.htm and http://www.agr.feis.unesp.br/clima/ilha_jan08.htm). [c] Data obtained from the division of global radiation by two, considering the sunshade filtration ratio of 50%. [d] EP — Experimental period before the vitamin D_3 supplementation. [e] nd — not determined. [f] Partially cloudy sky.

2.9. Slaughter and carcass evaluation

Animal slaughter was carried out at a commercial plant under federal inspection from the Ministry of Agriculture. After the slaughter, the carcasses were weighed to obtain the hot carcass weight (HCW). In turn, the hot carcass yield (HCY) was calculated as follows: HCY = (HCW ÷ FBW) × 100.

The ribeye area (REA) between the 12th and 13th thoracic vertebrae was recorded using vegetal paper to trace the perimeter of the *Longissimus thoracis* muscle. The area within the traced perimeter was measured using an LI-3100 Area Meter (LI-COR Inc., Lincoln, NE, USA). This instrument is generally used to measure the leaf area of plants. The fat thickness (FT) was measured perpendicularly to the surface, between the 12th and 13th thoracic vertebrae, at a lateral position from the animal midline at a point 3/4th of the distance from the medial end of the *Longissimus thoracis* muscle, as measured using a graded ruler. The REA and FT adjusted to the average HCW were also included in the analysis.

2.10. Meat samples

After the slaughter, a portion from the *Longissimus thoracis* muscle between the 12th and 13th thoracic vertebrae was immediately collected to determine total muscle calcium concentration and meat colour [0 and 1 h post-mortem (pm)]. At 24 h pm, the *Longissimus thoracis et lumborum* muscle was removed between the 12th thoracic and 5th lumbar vertebrae and cut into nine steaks (2.54 cm in thickness), which were vacuum packaged into 3 packages (3 steaks/package). The Sulpravac-VC1 packages (Unipac Brasil, SP, Brazil) had medium density (52 g/m^2) barrier pouches with 55 micron of thickness and oxygen permeability lower than 10 $cm^3\ m^{-2}\ d^{-1}\ atm^{-1}$ at 0% relative humidity and 23 °C. Following, the steaks were stored at ±2 °C for either 1, 7 or 21 days of ageing. These steaks were used to evaluate the Myofibrillar Fragmentation Index and shear force. One of nine of the steaks that would be aged was randomly selected to record the colour values at 24 h pm.

2.11. Carcass pH and temperature

The carcass pH data were recorded at 0, 3, and 24 h pm, and carcass temperature data were recorded at 0, 3, 6, and 24 h pm. Both measures were taken in the *Longissimus thoracis* muscle at the left carcass side using a Sentron portable pH and temperature meter (Gig Harbor, WA, USA).

2.12. Meat colour

To determine the beef colour, a CR-400 Minolta Chroma Meter (Minolta Corporation/ISD, Ramsey, NJ, USA) with a 0.8-cm aperture, a D65 light source, and a 2° observer was used to measure the lightness (L^*), redness (a^*), and yellowness (b^*) according to the CIELAB system. The readings were taken at the surface of the *Longissimus thoracis* muscle at 0, 1, and 24 h pm.

2.13. Plasma ionised and total muscle calcium

The blood was collected before and after the period of vitamin D_3 supplementation. Plasma ionised calcium concentrations were determined using an i-STAT portable apparatus (Abbot Point of Care Inc., East Windsor, NJ, USA) attached to a $CG8^+$ cartridge. The total muscle calcium concentration was determined at Analytical Chemistry Laboratory by atomic absorption according to Nakamura (1973).

2.14. Warner–Bratzler shear force

The procedure used to analyse the shear force was conducted according to the recommendations of the AMSA (1995). Briefly, steaks with 2.54 cm of thickness were cooked on an electric grill (EDANCA®) until reaching internal temperature of 40 °C, when they were turned over and cooked to reach internal temperature of 71 °C (monitored by thermometers). The steaks were cooled at room temperature and stored overnight at ±2 °C. The next day, six to eight cores with a diameter of 1.25 cm were removed from each steak parallel to the direction of muscle fibres. Then, cores were analysed using the Warner–Bratzler equipment (G-R Manufacturing Co., Manhattan, KS, USA) to measure the force necessary to shear the cores. The results are expressed in kgf.

2.15. Myofibrillar Fragmentation Index (MFI)

Analysis of the MFI was performed with samples that were taken from steaks at 1 and 21 days of ageing and frozen in liquid nitrogen. The homogenisation of 4 g of tissue into MFI buffer using a Waring blender was performed according to the procedure described by Culler et al. (1978). The MFI readings were performed using a spectrophotometer (Coleman Instruments Division, Oak Brook, IL, USA) at 540 nm.

2.16. Statistical analysis

The experimental design was based on randomised complete blocks, where the three different weight groups after adaptation to a high-concentrate diet represented the blocks. An approach was used to analyse two experimental conditions (different sunlight exposure conditions) and three different levels of vitamin D_3 supplementation. To perform this combined analysis, a linear mixed model was used, in which the effect of vitamin D_3 supplementation, sunlight exposure conditions, and their interactions were considered to be fixed, while the effect of blocks nested within the effect of the sunlight exposure conditions was considered to be random. For the initial (before vitamin D_3 supplementation) plasma ionised calcium data, the effect of levels of vitamin D_3 supplementation and their interaction with sunlight exposure conditions were not considered in the model. For the final (after vitamin D_3 supplementation) plasma ionised calcium data analysis, the initial plasma ionised calcium data were included in the model as a covariate. To account for the correlation that exists among the measurements taken over time in the same experimental unit (animal), the dependent variables, such as the carcass pH and temperature, MFI, shear force, and meat colour, were analysed as repeated measures with respect to time. The analyses met the assumptions of the model, where residuals were normally distributed and independent of the data. Each of the six treatments was conducted with seven repetitions, except for two treatments (animal performance and carcass traits) or one (meat quality traits) treatment that presented missing data (making a total of 40 or 41 animals) due to problems during the trait measurements. The data were analysed using PROC MIXED in a statistical package from SAS (2000) using the Tukey–Kramer test for least squares means contrasts.

3. Results

3.1. Animal performance and carcass traits

There was no effect ($P \geq 0.23$) of the sunlight exposure condition and/or level of vitamin D_3 supplementation regarding the animal performance or the carcass traits (Table 2). An effect of time pm was detected ($P<0.01$, data not shown) for carcass pH and temperature decline as expected. The pH values between 0 h [6.61 (0.054)] and 3 h [6.47 (0.035)] decreased slightly ($P=0.06$), while the pH values between either 0 h or 3 h and 24 h [5.71 (0.028)] decreased significantly ($P<0.01$) to reach a pH value close to the normal average. Carcass temperatures significantly dropped ($P<0.01$) across the first 24 h pm [0 h = 27.5 (0.44) °C, 3 h = 19.4 (0.38) °C, 6 h = 13.6 (0.16) °C, and 24 h = 1.2 (0.20) °C].

3.2. Plasma and muscle calcium

The plasma ionised calcium concentration of the animals was not influenced ($P \geq 0.30$) by the sunlight exposure conditions and/or levels of vitamin D_3 supplementation, while the total muscle calcium concentration was altered, showing higher values ($P=0.07$) in the animals that were protected by sunshade (Table 3).

3.3. Meat quality

An interaction between the levels of vitamin D_3 supplementation and sunlight exposure was detected ($P=0.03$) for MFI values determined with frozen samples at days 1 and 21 pm (pooled data, Fig. 1), which would reflect fragmentation extension. Within the animals exposed to sunlight or under sunshade, no differences were observed ($P \geq 0.15$) due to the levels of vitamin D_3 supplementation. However, higher MFI values were found ($P=0.03$) in meat from animals under sunshade compared to those exposed to sunlight within the V2 group.

Table 2
Least squares means of the animal performance and carcass trait data for different levels of vitamin D_3 supplementation and sunlight exposure conditions during the feedlot period.

Sunlight exposure condition	Level of vitamin D_3 supplementation		
	V0	V2	V8
Animal performance			
Initial body weight (kg)			
No shade	416 (25.2)[£]	422 (25.2)	411 (25.4)
With shade	402 (25.4)	413 (25.5)	406 (25.4)
Final body weight (kg)			
No shade	477 (24.4)	487 (24.4)	489 (24.7)
With shade	464 (24.6)	473 (24.8)	466 (24.6)
Average daily gain (kg/day)			
No shade	1.3 (0.17)	1.4 (0.17)	1.7 (0.18)
With shade	1.4 (0.15)	1.3 (0.16)	1.4 (0.15)
Average feed intake (kg DM/day)			
No shade	9.7 (0.54)	9.0 (0.54)	8.9 (0.57)
With shade	8.4 (0.57)	9.0 (0.60)	8.7 (0.57)
Feed:gain ratio (kg DM/kg BWG)			
No shade	7.9 (0.85)	6.7 (0.85)	5.8 (0.92)
With shade	6.3 (0.82)	7.3 (0.89)	7.0 (0.82)
Carcass traits			
Hot carcass weight (kg)			
No shade	246 (13.4)	249 (13.4)	249 (13.6)
With shade	242 (13.3)	243 (13.4)	237 (13.3)
Hot carcass yield (%)			
No shade	51.6 (0.63)	51.3 (0.63)	51.2 (0.68)
With shade	52.2 (0.44)	51.4 (0.47)	50.8 (0.44)
Ribeye area (cm^2)			
No shade	62.2 (1.79)	65.7 (1.79)	64.0 (1.93)
With shade	67.7 (2.40)	65.2 (2.59)	64.8 (2.40)
Adjusted ribeye area (cm^2)			
No shade	62.1 (2.45)	64.8 (2.45)	62.8 (2.57)
With shade	68.3 (2.54)	65.5 (2.64)	66.5 (2.54)
Fat thickness (mm)			
No shade	2.8 (0.58)	2.6 (0.58)	3.4 (0.62)
With shade	3.1 (0.56)	3.3 (0.60)	1.9 (0.56)
Adjusted fat thickness (mm)			
No shade	2.8 (0.51)	2.3 (0.51)	3.0 (0.55)
With shade	3.3 (0.61)	3.4 (0.66)	2.3 (0.61)

Legend: V0 = no supplementation; V2 = with vitamin D_3 supplementation at a dose of 2×10^6 IU for 2 consecutive days pre-slaughter; V8 = with vitamin D_3 supplementation at a dose of 2×10^6 IU for 8 consecutive days pre-slaughter; no shade = animals allocated into pens without sunshade; with shade = animals allocated into pens with sunshade (50% ultraviolet filtration ratio); DM = dry matter; and BWG = body weight gain. [£] Least squares means (standard error).

Table 3
Least squares means of the plasma ionised (mg/dL) and total muscle (μg/g wet muscle) calcium concentrations for different levels of vitamin D_3 supplementation and sunlight exposure conditions.

Level of vitamin D_3 supplementation	Sunlight exposure condition		Means
	No shade	With shade	
Initial plasma ionised calcium concentration (mg/dL)			
Means	4.3 (0.04)[£]	4.3 (0.05)	–
Final plasma ionised calcium concentration (mg/dL)			
V0	4.4 (0.07)	4.4 (0.07)	4.4 (0.05)
V2	4.3 (0.06)	4.4 (0.07)	4.3 (0.05)
V8	4.3 (0.07)	4.3 (0.07)	4.3 (0.05)
Means	4.3 (0.04)	4.4 (0.04)	–
Total muscle calcium concentration (μg/g wet muscle)			
V0	54.0 (5.03)	63.2 (10.09)	58.6 (5.64)
V2	50.4 (5.03)	67.8 (10.09)	59.1 (5.64)
V8	55.7 (5.36)	73.6 (10.09)	64.6 (5.71)
Means*	53.4 (3.72)[b]	68.2 (6.24)[a]	–

Legend: no shade = animals allocated into pens without sunshade; with shade = animals allocated into pens with sunshade (50% ultraviolet filtration ratio); V0 = no supplementation; V2 = with vitamin D_3 supplementation at a dose of 2×10^6 IU for 2 consecutive days pre-slaughter; V8 = with vitamin D_3 supplementation at a dose of 2×10^6 IU for 8 consecutive days pre-slaughter; initial plasma ionised calcium concentration = plasma ionised calcium concentration measured before vitamin D_3 supplementation; and final plasma ionised calcium concentration = plasma ionised calcium concentration measured after vitamin D_3 supplementation. [£]Least squares means (standard error). *$P=0.07$ for the main effect of sunlight exposure conditions. [a,b]Different lowercase letters between the sunlight exposure conditions indicate a significant difference ($P=0.07$).

No effect associated with the levels of vitamin D_3 supplementation and/or sunlight exposure was found during ageing of the meat ($P\geq0.15$) on the MFI (data not shown) and Warner–Bratzler shear force values (Table 4). An effect of the duration of ageing showing the progress of myofibrillar weakening and a decline in the shear force was observed ($P<0.01$).

As expected, the meat colour was affected by the levels of vitamin D_3 supplementation (a^*, $P=0.06$ and b^*, $P<0.01$) and the time pm (L^*, a^*, and b^*, $P<0.01$) (Table 5). The a^* values were higher ($P=0.06$) for the group that received a higher level of vitamin D_3 supplementation than for those that received no vitamin D_3 supplementation, while both groups had similar a^* values ($P\geq0.16$) as the group that received the lower level of vitamin D_3 supplementation. On the other hand, the b^* values were higher ($P<0.01$) for the group that received the higher level of vitamin D_3 supplementation than for those that received the lower level of vitamin D_3 supplementation or no vitamin D_3 supplementation, which were similar ($P=0.85$).

An interaction ($P=0.03$) between sunlight exposure conditions and the time pm was verified for the L^* values (Fig. 2). In animals exposed to sunlight and animals under sunshade, the L^* values were similar ($P\geq0.79$) between 0 and 1 h pm, and they were the highest ($P<0.01$) at 24 h pm. No differences were observed ($P\geq0.16$) due to the sunlight exposure conditions with respect to the length of time pm. On the other hand, the sunlight exposure conditions alone affected ($P=0.03$, data not shown) the b^* values, where meat from animals that were unprotected from sunlight [4.1 (0.17)] had increased ($P=0.03$) yellowness compared to meat from animals that were shaded [3.5 (0.17)].

4. Discussion

4.1. Animal performance and carcass traits

The lack of a difference between the unshaded and shaded animals regarding the animal performance and the carcass traits may be attributed to the beef cattle biological type and intake behaviour. A reduction in animal performance and a decline in the carcass traits among animals exposed to sunlight would be expected due to thermal discomfort (Mitlöhner et al., 2002), however *B. indicus* cattle are tolerant of a hot climate (Gaughan et al., 1999), and therefore, they may not respond to the benefits of sunshade (Mader et al., 1999). In some cases, it has been observed that animals

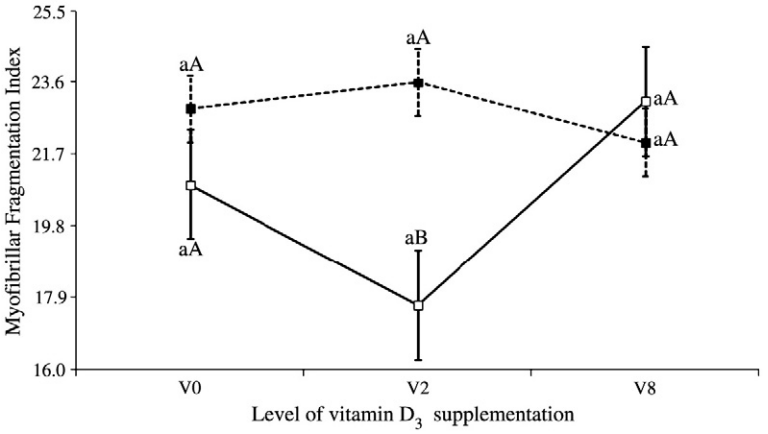

Fig. 1. Myofibrillar Fragmentation Index (MFI) from meat frozen in liquid nitrogen at days 1 and 21 post-mortem (pooled data) from animals that received different levels of vitamin D_3 supplementation and were subjected to different sunlight exposure conditions (interaction — $P=0.03$). Legend: □ no shade = animals allocated into pens without sunshade; ■ with shade = animals allocated into pens with sunshade (50% ultraviolet filtration ratio); V0 = no supplementation; V2 = with vitamin D_3 supplementation at a dose of 2×10^6 IU for 2 consecutive days pre-slaughter; and V8 = with vitamin D_3 supplementation at a dose of 2×10^6 IU for 8 consecutive days pre-slaughter. [a]Similar lowercase letters among the levels of vitamin D_3 supplementation within the sunlight exposure conditions do not significantly differ ($P\geq0.15$). [A,B]Different uppercase letters between the sunlight exposure conditions within the levels of vitamin D_3 supplementation significantly differ ($P=0.03$).

exposed to sunlight compensate by ingesting lower amounts of feed during the day but displaying a higher feed intake during the night (Gaughan et al., 2004). The lack of an effect of sunlight exposure conditions on animal performance was previously reported for cattle with *B. taurus* predominance (Brosh et al., 1998; Mader et al., 1999).

Furthermore, the lack of a difference in the animal performance and the carcass traits among animals that received vitamin D_3 supplementation suggests that vitamin D_3 given as powder mixed into the diet at a low dosage seems to be not detrimental. Only when vitamin D_3 was given via a bolus through a protected gelatinous capsule at the same dosage used in this experiment (Scanga et al., 2001) or fed as a mixed powder at a higher dosage of vitamin D_3 (Karges et al., 2001; Montgomery et al., 2002) was there a decrease in feed intake, average daily gain, final body weight, hot carcass weight, and fat thickness.

The levels of vitamin D_3 supplementation and sunlight exposure conditions did not influence the *Longissimus thoracis* muscle pH or temperature at the tested time points. These results corroborate those of another study reporting no changes in the pH values at 3 and 24 h pm and in the temperature at 3 h pm after supplementation with several different doses of vitamin D_3 (Montgomery et al., 2002). However, the latter study suggested that vitamin D_3 supplementation was related to lower carcass temperatures at 24 h pm.

4.2. Plasma and muscle calcium levels

The lack of an effect of vitamin D_3 supplementation in different sunlight exposure conditions on the plasma ionised calcium concentrations might be explained by the strict control of calcium homeostasis in animals due to its central role in a variety of functions, such as signal transduction and neuromuscular activities (Littledike and Goff, 1987).

Several other reports also have shown no effect of vitamin D_3 and/or its metabolic derivatives on the plasma calcium concentration (Cho et al., 2006; Lawrence et al., 2006; Pedreira et al., 2003; Tipton et al., 2007). In its ionised form, the concentration of calcium in plasma was not changed in

Table 4
Least squares means of the shear force (kgf) of samples from animals that received different levels of vitamin D_3 supplementation and were exposed to different sunlight exposure conditions taken after different durations of post-mortem ageing.

Level of vitamin D_3 supplementation	Sunlight exposure condition					
	NS	WS	NS	WS	NS	WS
	Day 1		Day 7		Day 21	
V0	10.0 (0.88)[£]	10.9 (0.99)	8.7 (0.88)	9.0 (0.99)	7.0 (0.88)	7.6 (0.99)
V2	11.6 (0.88)	10.9 (0.99)	10.6 (0.88)	9.3 (0.99)	7.6 (0.88)	7.3 (0.99)
V8	9.7 (0.92)	10.6 (0.99)	7.6 (0.92)	9.2 (0.99)	6.0 (0.92)	7.0 (0.99)
Means*	10.6 (0.38)[a]		9.1 (0.38)[b]		7.1 (0.38)[c]	

Legend: NS (no shade) = animals allocated into pens without sunshade; WS (with shade) = animals allocated into pens with sunshade (50% ultraviolet filtration ratio); V0 = no supplementation; V2 = with a vitamin D_3 supplementation at a dose of 2×10^6 IU for 2 consecutive days pre-slaughter; and V8 = with vitamin D_3 supplementation at a dose of 2×10^6 IU for 8 consecutive days pre-slaughter. [£]Least squares means (standard error); *$P<0.01$ for main effect of post-mortem ageing periods. [a,b,c]Different lowercase letters among the times of ageing significantly differ ($P<0.01$).

Table 5
Least squares means of the L^*, a^*, and b^* values for samples from animals submitted to different levels of vitamin D_3 supplementation determined at different times post-mortem.

Time post-mortem	Level of vitamin D_3 supplementation			Means**
	V0	V2	V8	
L^* values				
0 h	30.7 (0.68)[£]	29.8 (0.68)	31.2 (0.71)	30.6 (0.40)[B]
1 h	30.5 (0.68)	29.2 (0.68)	30.6 (0.71)	30.1 (0.40)[B]
24 h	33.5 (0.68)	32.7 (0.68)	34.5 (0.71)	33.6 (0.40)[A]
Means	31.6 (0.52)	30.6 (0.52)	32.1 (0.54)	–
a^* values				
0 h	14.9 (0.86)	15.5 (0.86)	17.1 (0.91)	15.8 (0.51)[B]
1 h	14.5 (0.70)	14.6 (0.70)	15.2 (0.72)	14.8 (0.41)[B]
24 h	17.1 (0.52)	17.5 (0.52)	18.6 (0.54)	17.7 (0.31)[A]
Means*	15.5 (0.39)[b]	15.8 (0.39)[ab]	17.0 (0.41)[a]	–
b^* values				
0 h	2.5 (0.39)	2.9 (0.39)	4.1 (0.41)	3.2 (0.23)[B]
1 h	2.9 (0.39)	2.8 (0.39)	3.6 (0.41)	3.1 (0.23)[B]
24 h	4.7 (0.39)	4.8 (0.39)	5.9 (0.41)	5.2 (0.23)[A]
Means*	3.4 (0.20)[b]	3.5 (0.20)[b]	4.5 (0.21)[a]	–

Legend: V0 = no supplementation; V2 = with vitamin D_3 supplementation at a dose of 2×10^6 IU for 2 consecutive days pre-slaughter; V8 = with vitamin D_3 supplementation at a dose of 2×10^6 IU for 8 consecutive days pre-slaughter; 0 h = 0 hour post-mortem; 1 h = 1 hour post-mortem; 24 h = 24 hours post-mortem; L^* = lightness, range from dark to pale; a^* = chroma, range from green to red ($-a^*$ to $+a^*$); and b^* = chroma, range from blue to yellow ($-b^*$ to $+b^*$). [£]Least squares means (standard error). *$P = 0.06$ and $P < 0.01$ for main effect of levels of vitamin D_3 supplementation in a^* and b^* values, respectively. [a,b]Different lowercase letters among the levels of vitamin D_3 supplementation significantly differ for a^* ($P = 0.06$) and b^* ($P < 0.01$) values. **$P < 0.01$ for main effect of time post-mortem in either L^*, a^* or b^* values. [A,B]Different uppercase letters among the times post-mortem significantly differ ($P \leq 0.02$).

lambs (Boleman et al., 2004), although it was increased in cattle (Swanek et al., 1999) supplemented orally with vitamin D_3. In both cases, the supplementation doses used were higher than those used in the present study.

The higher total muscle calcium concentration in the shaded animals may be a result of changes in plasma $25(OH)D_3$. Those animals were not only deprived of sunlight by the sunshade but also by the feedlot roof (made of asbestos-cement), which had an orientation that would favour the reflectance of a large proportion of the UV radiation (49.2%, Roma-Jr et al., 2008) from sunlight for most of the day. Low plasma $25(OH)D_3$ concentrations have been reported in cattle and sheep protected from UV radiation (Hidiroglou, 1987; Hidiroglou et al., 1979). This scenario is linked to increased parathyroid hormone (PTH) secretion, leading to secondary hyperparathyroidism (Zittermann et al., 2007), which is associated with mobilisation of calcium from the bone matrix. In hyperparathyroidism models, which could result from low plasma $1,25(OH)_2D_3$ concentration, calcium accumulation in the soft tissues has been documented in severe cases (Tamagaki et al., 2006).

4.3. Meat quality

Calcium immobilisation in the muscle taken from shaded animals may have favoured a higher myofibrillar fragmentation in the presence of vitamin D_3 supplementation at a low dosage (V2). The majority of published studies have explained the improvement in myofibrillar fragmentation and/or shear force through the increased free calcium concentration in the muscle (Montgomery et al., 2002, 2004; Swanek et al., 1999).

On the other hand, the lower immobilised calcium concentration found in muscle isolated from animals exposed to unlimited sunlight may have been offset by the effects of vitamin D_3 supplementation at a high dosage (V8). It has been reported that $1,25(OH)_2D_3$ effectively increased the intracellular calcium concentration in chicken skeletal muscle cell culture (Capiati et al., 2000). The increased intracellular calcium concentration would be important for the stimulation of calcium-dependent proteases, which have a major impact on MFI (Koohmaraie, 1992). In this scenario, a higher muscular concentration of vitamin D_3 metabolites could have indirectly enhanced the muscle calcium-dependent protease activities.

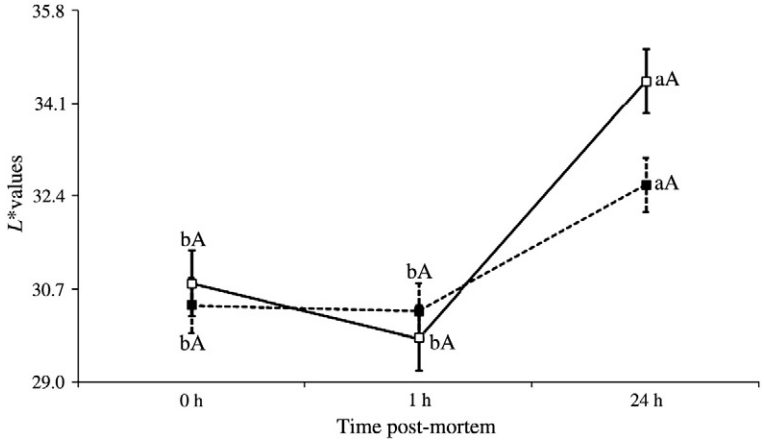

Fig. 2. L^* values in samples of animals submitted to different sunlight exposure conditions and times post-mortem (interaction — $P = 0.03$). Legend: □ no shade = Animals allocated into pens without sunshade; ■ with shade = animals allocated into pens with sunshade (50% ultraviolet filtration ratio); 0 h = 0 hour post-mortem; 1 h = 1 hour post-mortem; and 24 h = 24 hours post-mortem. [a,b]Different lowercase letters among the times post-mortem within the sunlight exposure conditions significantly differ ($P < 0.01$). [A]Similar uppercase letters between the sunlight exposure conditions within the times post-mortem do not significantly differ ($P \geq 0.16$).

The ability of vitamin D_3 supplementation to weaken the myofibrillar structure and improve tenderness in beef cattle has been observed under conditions that increase the concentrations of muscle vitamin D_3 and its metabolites (Foote et al., 2004; Montgomery et al., 2002), especially $1,25(OH)_2D_3$ (Montgomery et al., 2000). Furthermore, supranutritional vitamin D_3 supplementation has been associated with increases in the protease mRNAs and activities (Cho et al., 2006; Swanek et al., 1999). In fact, the V8 condition was found to alter the abundance of mRNA encoding one of the calpastatin isoforms, which may reveal a response to higher calpain activity in the hydrolysis of the calpastatin molecule (Rezende, 2011).

It is important to consider the hypothesis that a high $1,25(OH)_2D_3$ concentration in the muscle and regulation of calcium influx in muscle cells may explain the similar myofibrillar fragmentation levels observed in animals supplemented with the higher level of vitamin D_3.

The lack of differences in the MFI and shear force values within each of the ageing times in response to the level of vitamin D_3 and sunlight exposure may be a result of limitations in the calpain system. The inhibited calpain proteolysis has been described for the biological type (*B. indicus*) of the animals used in this study (Shackelford et al., 1991; Whipple et al., 1990). On the other hand, the background tenderness dictated by the collagen content and solubility might have played a relevant role in the lack of improvement in the shear force values in the animals that received vitamin D_3 supplementation because the animals were over 30 months of age. The animal age or stage of maturity is also an important factor in shear force measurements due the decrease in collagen solubility that occurs with age (Smith and Judge, 1991; Smith et al., 1988).

Vitamin D_3 supplementation has been reported to improve tenderness in *B. taurus* cattle (Karges et al., 2001; Montgomery et al., 2000; Swanek et al., 1999). However, in experiments using *B. indicus* cattle (Lawrence et al., 2006; Pedreira et al., 2003; Tipton et al., 2007) or old animals such as cull cows (Carnagey et al., 2008; Cho et al., 2006), vitamin D_3 supplementation has not been shown to improve tenderness.

No post-mortem effect was noted for beef lightness (L^* values) among the animals that received different levels of vitamin D_3 supplementation or between animals that were exposed to different sunlight conditions, which may be attributed to the similar pH values between the groups. The correlation values ($r = -0.55$ at $P<0.01$) between pH and L^* values from samples collected at 0 and 24 h pm (pooled data) confirm that beef lightness depends on the pH values, as has been previously observed (Page et al., 2001; Wulf and Wise, 1999). At a higher muscle pH, the meat will be darker in colour because there is less free water to reflect light because proteins bind water more strongly (Ledward et al., 1992; Page et al., 2001). In cattle supplemented with vitamin D_3, a lighter colour (Strydom et al., 2007) and minimal lightness variation (Lawrence et al., 2006; Tipton et al., 2007) have been reported.

The higher values for the chroma parameters (a^* and b^*) in samples from animals that received a higher level of vitamin D_3 supplementation may be attributed to the possible anti-oxidative capacity that has been linked to this vitamin (Hansen et al., 2011; Lahucky et al., 2007; Wiegand et al., 2002), which may be related to increased concentrations of antioxidant enzymes (Hamden et al., 2009). However, the lower degree of yellowness found in samples from animals protected from sunlight could be the result of pro-oxidant activity stimulated by the higher total muscle calcium concentrations, which were observed in the muscles of those animals. An increasing muscle calcium concentration could increase the number of free radical electrons, providing more catalysts for myoglobin oxidation (Lawrence et al., 2003).

Although the levels of vitamin D_3 supplementation affected the chroma parameters, we did not test whether such effects would be perceptible by beef consumers, considering that the differences were small. Within the muscle pH range typical of dark-cutting carcasses (5.6 to 6.8), as occurred with 87.5% of the carcasses in our study (pH at 24 h pm: MEAN = 5.72, SD = 0.16, MIN = 5.50, and MAX = 6.22), the colorimeter values may vary only slightly, whereas muscle pH can vary considerably (Wulf and Wise, 1999).

5. Conclusion

Myofibrillar fragmentation may be influenced by the relationship between levels of vitamin D_3 supplementation and sunlight exposure without negatively affecting animal performance or carcass traits of beef cattle. Although vitamin D_3 supplementation may not be effective for increasing the tenderness of beef from mature *B. indicus* cattle, it may modify beef colour.

Conflict of interest statement

No conflict declared.

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