Effect of dietary beta-agonist treatment, Vitamin D_3 supplementation and electrical stimulation of carcasses on colour and drip loss of steaks from feedlot steers

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Abstract

In this study, 20 young steers received no beta-agonist (C) and 100 animals all received zilpaterol hydrochloride (Z), with 1 group receiving Z while the other 4 groups receiving Z and vitamin D_3 at the following levels (IU/animal/day) and durations before slaughter: 7 million for 3 days (3D7M) or 6 days (6D7M), 7 million for 6 days with 7 days no supplementation (6D7M7N) and 1 million for 9 days (9D1M). Left carcass sides were electrically stimulated (ES) and right sides not (NES). Samples were analysed fresh or vacuum-aged for 14 days post mortem. Parameters included drip loss and instrumental colour measurements. In general, zilpaterol showed increased drip loss, lighter meat, and reduced redness. Vitamin D_3 supplementation could not consistently overcome these negative effects. All vitamin D_3 treatments reduced drip loss of stimulated aged steaks.

Keywords: Vitamin D_3, zilpaterol, electrical stimulation, calpains, meat tenderness

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1. Introduction

Physical appearance of retail meat cuts during display is a critical link in the first step of the consumer satisfaction process. The bright red colour of red meat is the most important quality attribute to a consumer at the point of purchase (Jeremiah et al., 1972, Killinger et al., 2004 and Kropf, 1980). In addition, unacceptable water holding capacity, resulting in increased driploss in packaging that is unattractive to consumers, more susceptible to bacterial growth and that leads deterioration of eating quality (Lagerstedt et al., 2011 and Vázquez et al., 2004). Various pre-harvest, harvest and post-harvest processes could affect the colour and water holding qualities of meat.

Beta-agonists are supplemented to feedlot cattle to improve weight gain and feed efficiency and increase carcass yield (Dikeman, 2007, Dunshea et al., 2005, Kellermeier et al., 2009 and Shook et al., 2009). However, beta-agonists also induce muscle toughness in supplemented animals (Brooks et al., 2009 and Holmer et al., 2009) especially in ruminants, mainly due to an increase in activity of the inhibitor calpastatin and a reduction in calpain activity (Koohmaraie and Shakelford, 1991, Strydom et al., 2009 and Wheeler and Koohmaraie, 1997). In addition, various studies have reported effects on other quality attributes like colour and water holding capacity (Avendaño-Reyes et al., 2006, Geesink et al., 1993, Gonzalez et al., 2009, Hilton et al., 2009, Rogers et al., 2010 and Vestergaard, Sejrsen and Klastrup, 1994), which could probably be associated with the shift in fibre type composition (more glycolytic or white) (Geesink et al., 1993, Gonzalez et al., 2009, Strydom et al., 2009 and Vestergaard, Henckel, Oksbjerg and Sejrsen, 1994) and muscle hypertrophy (Kellermeier et al., 2009) caused by beta-agonists. It is also well-known that processes like electricalstimulation and *post mortem* ageing may affect colour (Devine et al., 2002, Ledward, 1985, Ledward et al., 1968, [MacDougall, 197] and Renerre, 1990) and water holding capacity (Den Hertog-

Meischke et al., 1997, Devine, 2009 and Strydom et al., 2005) and that this procedure combined with beta-agonists may have additive effects on these parameters (Geesink et al., 1993).

Supplementation with ultra-high levels of vitaminD_3 over the final days before slaughter has been used to improve meat tenderness in steers (Montgomery et al., 2002). This method is motivated by the suggestion that an increased calcium ion level, stimulated by high vitaminD_3 levels (Swanek et al., 1999), contributes to meat tenderization directly by weakening of myofibrillar structures (Takahashi, 1992) as well as indirectly through activation of μ-calpain (Koohmaraie, 1992). Lahucky et al. (2007) showed that supplementation with vitaminD_3 could improve the antioxidative capacity of pork loin muscle, thereby maintaining the cell structure, which could affect colour and water binding qualities of muscle.

The beta-agonist zilpaterol is approved for use in Mexico, South Africa and USA and, together with ractopamine, is probably the most commonly utilised beta-agonist in commercial beef production. In a recent study Strydom, Hope-Jones, Frylinck, and Webb (2011) compared the effect of supplementation of ultra-high levels of vitaminD_3 on the tenderness (Warner–Bratzler shear force) of shorter (3 days) and longer vacuum-aged (14 days) beef loins from carcasses that were either not stimulated or subjected to electricalstimulation within 30 min of killing. As subpart of this study, the effect of various combinations of vitaminD_3 supplement on the instrumental colour and driploss of fresh and vacuum-aged beef loin steaks was investigated. Our hypothesis is that the anti-oxidative protection of vitaminD_3 observed in pork could play a role in alleviating the negative effects of zilpaterol combined with other processes on moisture and colour quality parameters of beef loin.

2. Materials and methods

2.1. Experimental design and feedlot phase

One hundred and twenty Bonsmara steers of approximately 9 months of age were raised on a commercial feedlot diet for 120 days. The animals were divided into six groups (n = 20) so that the average weight and variation was the same for all groups. The groups represented 6 treatments, namely a control (C), which received the feedlot diet only, while the five remaining groups were supplemented with the beta-agonist, zilpaterol hydrochloride, (Intervet/Schering-Plough Animal Health, South Africa) at 0.15 mg/kg live weight for 30 days during the final weeks of finishing. One of the five groups only received zilpaterol (Z), while the other four groups received zilpaterol and vitamin D_3 (vitamin D_3 500, Advit Animal Nutrition S.A. (Pty) Ltd, Sebenza, South Africa) at the following levels and durations before slaughter (D = days supplemented; M = million IU; N = days withdrawn): 7 x 10^6 IU/animal /day for 3 days prior to slaughter (3D7M); 7 x 10^6 IU/animal /day for 6 days prior to slaughter (6D7M); 7 x 10^6 IU/animal /day for 6 days followed by 7 days of no supplementation prior to slaughter (6D7M7N) and 1 x 10^6 IU/animal /day for 9 days prior to slaughter (9D1M). In abbreviations of treatments D = days, M = million, N = no supplement. Zilpaterol was withdrawn from feed 4 days prior to slaughter. The vitamin D_3 supplement was mixed with maize to increase the volume and supplied as (1 kg) top dressing on the daily supply of the feedlot diet (10 animals per pen). To ensure equal and complete consumption of the supplement, a clean bunk approach was followed, i.e. all feed was consumed before new feed was given each morning. All animals in each pen therefore approached the bunk at the same time and sufficient bunk space was provided so that all animals per pen could have access to feed at the same time.

2.2. Slaughter and sampling procedures

The animals were slaughtered (at an average age of 12 months and average weight of 420 kg) at the abattoir of the Animal Production Institute (Agricultural Research Council, Irene, Gauteng Province). Carcasses were split and the left sides were electrically stimulated for 30 s (400 V peak, 5 ms pulses at 15 pulses per second) within 30 min of slaughter (ES) and the right sides were not stimulated (NES). Carcass sides were then chilled at 2 ± 2 °C (chiller temperature at loading). pH and temperature measurements of the *M. longissimus lumborum* (LL) were recorded every hour for 4 h and a final measurement was recorded at 18 h *post mortem*. All samples were collected from the LL on the day after slaughter. Measurements were performed on fresh (24 h *post mortem*) or vacuum-aged (14 days *post mortem* at 2 ± 1 °C) samples. For both fresh and aged samples, a steak of 30 mm was sampled from the LL at the position of the last rib.

2.3. Colour and drip loss of fresh meat

Instrumental meat colour was measured with a Minolta meter (Model CR200, Osaka, Japan) on fresh (24 h *post mortem*) and vacuum-aged samples (14 days *post mortem*). The sampled 30 mm LL steaks were divided into two steaks of 15 mm thickness and 1 steak was allowed to bloom for 60 min at 2 ± 1 °C, with its freshly cut surface facing upwards before colour recording. Recordings were done in triplicate at 3 positions on the steak surface. Colour measurements followed the CIE colour convention, where the three fundamental outputs are L*, a* and b*. L* is lightness on a scale of 0 (all light absorbed) to 100 (all light reflected); a* spans from + 60 (red) to − 60 (green) and b* from + 60 (yellow) to − 60 (blue). Saturation index, also known as chroma, was calculated as the square root of $a^{*2} + b^{*2}$ and hue angle, defined as $\tan^{-1}$

(b/a) that describes the fundamental colour of a substance (MacDougall, 1977). Mean values per sample were used for statistical analysis.

Two cubes of 10 mm × 10 mm × 20 mm were cut from the remaining fresh steak used for colour measurement to determine driploss of fresh loin muscle. The cubes were suspended on a pin inside a sample bottle (200 ml) taking care that the meat did not touch the sides of the bottle and stored for 72 h at 2 ± 1 °C. The amount of drip measured between 24 h and 96 h *post mortem*, as the difference between the sample mass before and after, was expressed as a percentage of the starting mass. Driploss or purge for aged samples was determined by measuring the amount of purge remaining in the vacuum bag after removing the 30 mm LL steak for colour measurement. The steak was removed and lightly dried with tissue paper. Drip was expressed as a percentage of the combined mass of the aged steak and the drip.

2.4. Statistical analyses

Data of colour and driploss were subjected to analysis of variance for a split-plot design (GenStat® VSN International, Hemel Hempstead, UK; Payne, Murray, Harding, Baird, & Soutar, 2007) with the 6 treatment groups (C, Z, 3D1M, 9D1M, 6D7M7N, 6D7M) as whole plots and the 2 electricalstimulation sub-treatments (ES and NES) as sub-plots and the 2 ageing periods (24 h and 14 days). Means for the interactions between the whole plot and sub-plots were separated using Fisher's protected t-test least significant difference (LSD) at the 5% level (Snedecor & Cochran, 1980).

3. Results

3.1 Effect of treatment, electrical stimulation and vacuum-aging on drip loss

Treatment ($P = 0.024$), electricalstimulation ($P < 0.001$) and ageing ($P < 0.001$) had significant effects on driploss (Table 1). Furthermore, significant interactions between treatment and electricalstimulation ($P < 0.001$), treatment and ageing ($P = 0.053$) and between electricalstimulation and ageing ($P = 0.014$) were recorded. According to Table 4 and Table 5 driploss was generally higher for stimulated samples than for non-stimulated samples and vacuum-ageing increased driploss compared to fresh samples.

Fresh control samples recorded lower driploss than zilpaterol samples (Table 4; $P < 0.05$). Drip values of vitaminD_3 samples were closer to those of zilpaterol samples, except for non-stimulated 6D7M7N that was the same as the non-stimulated control. Zilpaterol samples showed the largest increase in drip when carcass sides were stimulated and 9D1M showed no difference between the two applications (ES and NES). Aged non-stimulated samples of all treatments recorded similar driploss values irrespective of treatment (Table 5). However for aged stimulated samples, zilpaterol showed a significant increase ($P < 0.05$) in driploss, while the control and vitaminD_3 samples recorded smaller increases ($P < 0.05$ for 6D7M7N only).

3.2 Effect of treatment, electrical stimulation and vacuum-aging on instrumental colour

Treatment, electricalstimulation and *post mortem* ageing had significant effects on all aspects of instrumental colour measurements (Table 2 and Table 3). Furthermore, various interactions between the main effects were recorded.

The three way interaction between treatment, electricalstimulation and ageing for lightness (L*) approached significance ($P = 0.088$; Table 2). In general, L* increased with

ageing for both stimulated and non-stimulated samples ($P < 0.001$) (Table 4 and Table 5). In fresh and aged samples electricalstimulation had no significant effect on L* except for 3D7M ($P < 0.05$) and C ($P < 0.10$) where electricalstimulation reduced the L* for aged samples compared to fresh samples. In fresh samples the control had lower ($P < 0.05$) L* values than all zilpaterol treatments (vitaminD_3 included) and 6D7M7N had lower values ($P < 0.05$) than Z, 9D1M and 6D7M (Table 4). Similar patterns among treatments were found for aged samples, except that the control, 3D7M and 6D7M7N were grouped together and non-stimulated 6D7M7N samples recorded uncharacteristically low L* values for non-stimulated samples ($P < 0.05$) (Table 5).

Vacuum-aged samples recorded higher ($P < 0.001$) values for redness, yellowness, chroma and hue angle than fresh samples. Ageing interacted significantly with electricalstimulation and treatment for redness, yellowness, chroma and hue angle ($P < 0.001$; Table 2 and Table 3).

In fresh samples electricalstimulation had no effect on redness, yellowness or chroma. Zilpaterol recorded the lowest and the C, 9D1M and 3D7M the highest ($P < 0.05$) values for redness (Table 4). Redness values for 6D7M and 6D7M7N were closer to zilpaterol, except for electrically stimulated 6D7M7N samples that showed slightly higher values than non-stimulated samples.

In aged samples electricalstimulation tended to decrease redness values ($P < 0.05$ for zilpaterol and 3D7M), while 6D7M was not affected by electricalstimulation (Table 5). The control samples recorded higher redness values ($P < 0.05$) than all other treatments. The range in yellowness values was less than 1 unit in aged and fresh samples (Table 4 and Table 5). In aged samples only 3D7M showed lower ($P < 0.05$) values for stimulated samples. Chroma values mirrored redness values for the different treatment combinations. Hue values for zilpaterol samples indicated a greater shift (higher hue angle) from red to yellow than control samples in fresh and aged samples

(Table 5). In fresh samples electricalstimulation tended to reduce hue angle (closer to red) ($P < 0.05$; for control and zilpaterol) but this effect was not evident in aged samples (Table 4).

4. Discussion

Strydom et al. (2011) showed that ultra-high dosages of vitaminD_3 could not overcome beta-agonist induced toughness (according to Warner–Bratzler shear force; WBSF), while electricalstimulation (ES) and/or *post mortem* ageing reduced the difference in WBSF between zilpaterol and control samples significantly. In agreement with Avendaño-Reyes et al., 2006, Kellermeier et al., 2009 and Strydom et al., 2009, this study also showed that zilpaterol increased driploss compared to control samples which was exacerbated by electricalstimulation especially in aged samples (Table 4). This combination of factors could be regarded as a worst case scenario for drip due to proliferation and/or hypertrophy of predominantly white fibres (fast glycolytic; FG) (Gonzalez et al., 2009, Strydom et al., 2009 and Vestergaard, Henckel, Oksbjerg and Sejrsen, 1994; Wheeler & Koohmaraie, 1997) in beta-agonist treated animals that are more susceptible to protein denaturation (Maltin et al., 1997) especially when electricalstimulation is applied and glycolysis is accelerated. This study also showed that the effect of electricalstimulation was enhanced with longer ageing which was supported by the study of Simmons et al. (2008). This effect was also more pronounced in steaks from zilpaterol-treated steers.

According to Table 4 vitaminD_3 (in particular 3D7M) reduced driploss in aged stimulated steaks from zilpaterol-supplemented steers, while no other study on beef supported these results. Montgomery et al. (2002) found no effect with various supplementation levels of vitaminD_3 on free water content of beef loin. In addition, Montgomery et al. (2004) eported higher driploss at 5 million IU vitaminD_3 supplemented

for 8 days, which they attributed to higher proteolyses and instability of the muscle structure. Several studies on pork reported lower driploss when high supplement levels of vitaminD_3 were used (Enright et al., 1998, Lahucky et al., 2007 and Wilborn et al., 2004). The study of Lahucky et al. (2007) associated the reduced driploss with an increased anti-oxidative capacity in steaks from vitaminD_3-supplemented steers after incubation of muscle homogenates with Fe^{2+}/ascorbate and suggested that a higher level of Ca^{2+} (bivalent ion) due to vitaminD_3 in muscles was causing the positive influence on lipid oxidation. Under similar increased anti-oxidative conditions obtained by vitamin E supplementation, Pettigrew and Esnaola (2001) suggested that the reduction in the oxidation of membrane lipids may improve the integrity of those membranes, and thus reduce fluid leakage. That would translate into reduced driploss (increased water-binding capacity) and collectively these effects could explain the positive effects of vitaminD_3 seen in the present study.

Paler meat, i.e. higher L* values, in steaks from zilpaterol-treated steers was expected. Firstly higher driploss could increase surface moisture resulting in higher reflectance by the aqueous layer as reported by Geesink et al. (1993). In addition, the shift towards whiter glycolytic fibre types and hypertrophy of these fibres caused by beta-agonisttreatment (Gonzalez et al., 2009, Strydom et al., 2009 and Vestergaard, Henckel, Oksbjerg and Sejrsen, 1994; Wheeler & Koohmaraie, 1997) would have reduced the heme iron pigment concentration and hence result in paler meat (Geesink et al., 1993). Certain vitaminD_3treatments reduced the effect of the beta-agonist on lightness for both fresh and aged meat. In fresh samples, 6D7M7N, and to some extent 3D7M were closer to those of the control but still significantly higher ($P < 0.05$), but in aged meat the effects of both treatments were more pronounced. No effect on lightness was reported for vitaminD_3 supplemented beef in the literature (Montgomery et al., 2002, Montgomery et al., 2004 and Reiling and Johnson, 2003), while Enright et al. (1998); Lahucky et al.

(2007) (tendency; $P < 0.05$), Wiegand et al. (2002) and Wilborn et al. (2004) reported lower L* values for vitaminD_3 supplemented pig. Wiegand et al. (2002) found no effect on day 1 *post mortem* but a significant effect on day 14 which agrees with the present results for beef, in particular for 3D7m and 6D7M7N. None of the studies cited suggested a mechanism for the effect of vitaminD_3 but considering the relationship between driploss, surface moisture and light reflection, it is reasonable to believe that lower L* values in certain vitaminD_3treatments were the result of lower driploss which was more evident in aged samples. However, the reason for the lack of effect in other treatment combinations (9D1M and 6D7M) cannot be explained.

Existing evidence for the effect of zilpaterol and other beta-agonists on the typical colour of meat (bright redness) is contradictive probably due to specific trial conditions in different studies. For zilpaterol in particular, Avendaño-Reyes et al. (2006) recorded lower values for redness (a*) and chroma and higher hue angle values for zilpaterol samples, in agreement with the present results, although their colour recordings were done on thawed steaks previously frozen at − 20 °C. In addition, Hilton et al. (2009) recorded poorer visual colour scores and lower redness, yellowness and chroma values on freshly cuts loins (probably not bloomed properly) during chiller assessment. However, better colour scores (brighter red) were recorded for steaks from zilpaterol-treated steers displayed under simulated retail conditions after vacuum-ageing for 14 days. Rogers et al. (2010) also recorded more desirable visual colour scores and instrumental colour values for loin steaks from zilpaterol treated steers in a colour shelf life study, while VanOverbeke et al. (2009) found no effect of zilpaterol on visual colour and discolouration of displayed top sirloin steaks. Variable results were also recorded for other beta-agonists. Despite reduced heme iron concentrations in meat from clenbuterol-treated veal calves, Geesink et al. (1993) found no clear differences in a*- and b*-values between treated and non-treated animals. In contrast, Garssen, Geesink, Hoving-Bolink,

and Verplanke (1995) reported lower values for redness for steaks from clenbuterol and salbutamol-treated steers. The lower redness correlated with lower heme pigment and myoglobin which they attributed to a general shift in muscle fibre composition towards white muscle type accompanied by muscle fibre hypertrophy in steaks from beta-agonist-treated steers. In a colour shelf-life study, Gonzalez et al. (2009) reported poorer subjective scores for several muscles from ractopamine-treated steers. However, no differences in objective colour measurements, such as ratios of oxy- and metmyoglobin, percentage reduced nitric oxide metmyoglobin and Hunterlab a*- and b*-values were reported despite an expected decrease in the metmyoglobin reducing ability (MRA) due to increased glycolytic fibre types with fewer mitochondria and less intracellular NADH in most of the studied muscle types of treated animals. NADH is key to colour development and stability in meat (Howlett & Willis, 1998). Since previous studies on the effect recorded a shift towards whiter glycolytic muscle fibre types (Strydom et al., 2009) in zilpaterol-treated steers, it is reasonable to believe that poorer instrumental colour values were due to lower MRA in the loin muscles of treated steers as discussed by Gonzalez et al. (2009).

The significant effect of ageing on overall colour values (a*, b* and therefore chroma) was unexpected as Renerre (1990) speculated that low oxygen partial pressure may exist in vacuum packs that will cause brown metmyoglobin (MetMb) due to higher oxidation rates. However, Hood, 1980 and Ledward, 1985 suggested that in fresh meat a high oxygen consumption rate (OCR) will favour the reduced form of myoglobin causing the formation of MetMb (Atkinson & Follett, 1973). Then, as the OCR decreases, so does MetMb formation during storage (Bendall & Taylor, 1972) and will become more dependent on the relative effectiveness of the enzymatic reducing system that is still active after several weeks *post mortem* and could therefore maintain colour. It further explains the increased variation between the control and zilpaterol in aged samples

since muscle with different fibre compositions would respond differently to these effects (Ledward, 1985). In the same line of reasoning, results from Ledward (1985) explained why aged ES meat in the present study tended to have lower values for redness (and chroma). High temperatures and low pH values found with electricalstimulation advance the depletion of OCR and MRA and hence the buffering effect of the latter will be less at the same stage of ageing as non-stimulated meat.

Under these combined conditions of zilpaterol treatment, ES and ageing, variable effects of vitaminD_3supplementation on colour were recorded. In aged samples, only 9D1M and 6D7M in ES samples showed increased redness of 1 unit ($P < 0.05$), while in fresh ES and non-electrically stimulated (NES) samples, 3D7M and 9D1M (and 9D7M7N in ES) redness increased to similar levels as those of the control. Lahucky et al. (2007) (5 days PVC overwrapped display) and Wiegand et al. (2002) (vacuum-aged for 14 days) reported improved redness (CIE a*) values for vitaminD_3 supplemented pork loins (500,000 IU/day) and Wilborn et al. (2004) reported no effect on instrumental colour readings (a*), but recorded better visible colour scores in vitaminD_3 supplemented pigs. No effect on any colour attribute was recorded in the literature for beef (Montgomery et al., 2002 and Montgomery et al., 2004; Reiling & Johnson, 2003). The anti-oxidative effect of vitaminD_3 discussed earlier for driploss and lightness, was probably also involved here (Lahucky et al., 2007) with the small but positive response for selected treatment combinations. The specific conditions (ageing and electricalstimulation) and variation in muscle composition as discussed by Ledward (1985) between the control and other treatments (zilpaterol with and without vitaminD_3) probably reduced the effect of certain vitaminD_3treatments relative to the control. Since yellowness varied within a small range, chroma followed more or less the pattern of redness under the different scenarios. For hue angle, indicating the shift from redness to yellowness, 6D7M7N and

6D7M treatments showed some success in buffering the colour shift of zilpaterol treatments in aged and fresh meat.

5. Conclusion

This study confirmed the negative effects of the beta-agonist, zilpaterol, on driploss and instrumental colour of loin steaks and that electricalstimulation and ageing enhanced these negative effects on important meat quality traits. We hypothesized that very high levels of vitaminD_3 would exhibit anti-oxidative behaviour and alleviate negative effects of zilpaterol combined with other processes on moisture and colour quality of beef. This hypothesis was found to be true for driploss under certain conditions but in particular when samples were stimulated and aged. For instrumental colour quality certain vitaminD_3treatments gave significant positive results but the effects were often small in magnitude and were also affected by ageing and electricalstimulation. Considering the lack of positive effects on meat tenderness, ultra high levels of vitaminD_3 are probably not a viable option to improve the quality of meat that was compromised by feeding beta-agonists.

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Table 1. General statistics for the effects of treatment, electricalstimulation and ageing and their first order interactions for driploss of the *M. longissimus lumborum* (data pooled for treatment, electricalstimulation and *post mortem* ageing; degrees of freedom for main effects and error in parenthesis).

Effect	Driploss		
	Significance	***F* ratio**	**SEM[a]**
Main effect			
Treatment	$P = 0.024$	2.70 (5, 114)	0.1386
Stimulation	$P < 0.001$	69.08 (1, 114)	0.0516
Ageing	$P < 0.001$	76.19 (1, 218)	0.0467
Interactions			
T × S	$P < 0.001$	4.69 (5, 114)	0.1650
T × A	$P = 0.053$	2.23 (5, 218)	0.1605
S × A	$P = 0.014$	6.13 (1, 218)	0.0696
T × S × A	$P = 0.442$	0.96 (5, 218)	0.2007

T: Treatment (control, zilpaterol and vitaminD_3 supplemented groups).S: Stimulation (electrically stimulated and not electrically stimulated).A: Ageing (1 and 14 days *post mortem*; “1 day” represents the amount of drip measured between 24 h and 96 h *post mortem*).

a Standard error of means.

Table 2. General statistics for the effects of treatment, electricalstimulation and ageing and their first order interactions on instrumental colour attributes of *M. longissimus lumborum* (data pooled for treatment, electricalstimulation and *post mortem* ageing; degrees of freedom for main effects and error in parenthesis).

Effect	Lightness (L*)			Redness (a*)			Yellowness (b*)		
	Significance	***F* ratio**	**SEM[a]**	**Significance**	***F* ratio**	**SEM[a]**	**Significance**	***F* ratio**	**SEM[a]**
Main effect									
Treatment	$P < 0.001$	6.51 (5, 114)	0.3543	$P < 0.001$	4.99 (5, 114)	0.2505	$P = 0.002$	4.03 (5, 114)	0.1127
Stimulation	$P = 0.128$	2.35 (1, 113)	0.0709	$P = 0.026$	5.07 (1, 114)	0.0800	$P = 0.002$	10.16 (1, 114)	0.0505
Ageing	$P < 0.001$	90.44 (1, 224)	0.0676	$P < 0.001$	526.69 (1, 228)	0.0782	$P < 0.001$	3711.2 (1, 228)	0.0422
Interactions									
T × S	$P = 0.147$	1.67 (5, 113)	0.3749	$P = 0.398$	1.04 (5, 114)	0.2862	$P = 0.459$	0.94 (5, 114)	0.1426
T × A	$P < 0.001$	9.23 (5, 224)	0.3731	$P < 0.001$	6.12 (5, 228)	0.2848	$P < 0.001$	6.31 (5, 228)	0.1343
S × A	$P = 0.501$	0.45 (1, 224)	0.0980	$P < 0.001$	16.45 (1, 228)	0.1118	$P = 0.044$	4.11 (1, 228)	0.0658
T × S × A	$P = 0.088$	1.94 (5, 224)	0.4099	$P = 0.497$	0.88 (5, 228)	0.3444	$P = 0.204$	1.46 (5, 228)	0.1762

T: Treatment (control, zilpaterol and vitaminD_3 supplemented groups).S: Stimulation (electrically stimulated and not electrically stimulated).A: Ageing (1 and 14 days *post mortem*).

a Standard error of means.

Table 3. General statistics for the effects of treatment, electricalstimulation and ageing and their first order interactions on chroma and hue angle of *M. longissimus lumborum* (Data pooled for treatment, electricalstimulation and *post mortem* ageing; degrees of freedom for main effects and error in parenthesis).

Effect	Chroma			Hue angle		
	Significance	***F* ratio**	**SEM[a]**	**Significance**	***F* ratio**	**SEM[a]**
Main effect						
Treatment	$P < 0.001$	4.72 (5, 114)	0.2578	$P < 0.001$	6.52 (5, 114)	0.2945
Stimulation	$P = 0.013$	6.34 (1, 114)	0.0902	$P = 0.011$	6.76 (1, 111)	0.0837
Ageing	$P < 0.001$	1093.2 (1, 228)	0.0844	$P < 0.001$	3570.9 (1, 225)	0.0771
Interactions						
T × S	$P = 0.378$	1.07 (5, 114)	0.3014	$P = 0.605$	0.73 (5, 111)	0.3283
T × A	$P < 0.001$	6.05 (5, 228)	0.2964	$P < 0.001$	4.79 (5, 225)	0.3234
S × A	$P < 0.001$	14.43 (1, 228)	0.1235	$P < 0.001$	11.73 (1, 225)	0.1138
T × S × A	$P = 0.454$	0.94 (5, 228)	0.3655	$P = 0.217$	1.42 (5, 225)	0.3787

T: Treatment (control, zilpaterol and vitaminD_3 supplemented groups).S: Stimulation (electrically stimulated and not electrically stimulated).A: Ageing (1 and 14 days *post mortem*).

a Standard error of means.

Table 4. Mean values for the effects of treatment and electricalstimulation on driploss and colour properties of fresh *M. longissimus lumborum* samples (24 h *post mortem*).

	Treatment					
	Control	**9D1M**	**3D7M**	**6D7M**	**6D7M7N**	**Zilpaterol**
Driploss[e]						
NES	1.30[a]	1.98[b]	1.78[ab]	1.86[b]	1.34[a]	1.84[b]
ES	1.45[a]	2.07[bc]	2.18[bc]	2.35[c]	2.03[bc]	2.68[d]
Lightness						
NES	39.2[a]	41.2[c]	40.6[bc]	41.3[c]	40.2[b]	41.3[c]
ES	38.8[a]	41.3[c]	40.7[bc]	41.0[c]	40.1[b]	41.5[c]
Redness						
NES	14.8[b]	14.9[b]	14.7[b]	13.9[ab]	14.0[ab]	13.6[a]
ES	14.9[b]	15.0[b]	14.8[b]	14.1[ab]	14.6[b]	13.8[a]
Yellowness						
NES	6.46[ab]	6.88[b]	6.80[b]	6.56[ab]	6.30[a]	6.66[ab]
ES	6.22[a]	6.90[b]	6.78[b]	6.39[ab]	6.27[a]	6.45[ab]
Chroma						
NES	16.2[b]	16.4[b]	16.2[b]	15.4[ab]	15.4[ab]	15.2[a]
ES	16.1[b]	16.5[b]	16.3[b]	15.5[ab]	15.9[ab]	15.2[a]
Hue angle						
NES	23.5[b]	24.8[c]	24.8[c]	25.3[c]	24.2[b]	26.1[d]
ES	22.7[a]	24.7[c]	24.6[c]	24.3[b]	23.2[ab]	25.1[c]

ES and NES = electrically stimulated and not electrically stimulated.Control: no zilpaterol and no vitaminD_3 supplement.Zilpaterol: 0.15 mg zilpaterol hydrochloride per kg live weight for 30 days during the final weeks of finishing.VitaminD_3treatments: 3D7M, 9D1M, 6D7M, 6D7M7N: D = number of days supplemented; N = number of days vitaminD_3 was withdrawn; M = millions IU vitaminD_3/animal/day, all vitaminD_3 received 0.15 mg zilpaterol hydrochloride per kg live weight for 30 days during the final weeks of finishing.[a,b,c,d] Means in the 2 rows of the same property and with different superscripts, differ significantly ($P < 0.05$).[e]Measured as the amount of drip between 24 h and 96 h *post mortem*.

Table 5. Mean values for the effects of treatment and electricalstimulation on driploss and colour properties of vacuum-aged (14 days *post mortem*) *M. longissimus lumborum* samples.

	Treatment					
	Control	**9D1M**	**3D7M**	**6D7M**	**6D7M7N**	**Zilpaterol**
Driploss						
NES	2.20^{ab}	2.14^{ab}	2.12^{ab}	2.17^{ab}	2.01^{ab}	1.94^{a}
ES	2.49^{b}	2.74^{b}	2.52^{b}	2.92^{b}	2.88^{b}	3.66^{c}
Lightness						
NES	41.2^{bc}	42.0^{d}	41.3^{c}	42.5^{de}	39.8^{a}	42.9^{e}
ES	40.5^{b}	42.1^{d}	40.4^{ab}	42.4^{de}	40.5^{b}	42.4^{de}
Redness						
NES	18.7^{c}	17.5^{bc}	17.1^{b}	16.8^{b}	16.8^{b}	17.1^{b}
ES	18.2^{c}	16.8^{b}	15.8^{a}	16.9^{b}	16.1^{ab}	16.0^{a}
Yellowness						
NES	10.80^{c}	10.58^{bc}	10.55^{bc}	9.83^{ab}	9.84^{ab}	10.59^{bc}
ES	10.51^{bc}	10.27^{b}	9.70^{a}	9.79^{ab}	9.85^{ab}	9.99^{ab}
Chroma						
NES	21.6^{d}	20.4^{c}	20.1^{bc}	19.4^{b}	19.5^{b}	20.1^{bc}
ES	21.0^{cd}	19.7^{bc}	18.5^{a}	19.5^{b}	18.9^{ab}	18.9^{ab}
Hue angle						
NES	30.0^{a}	31.2^{b}	31.7^{b}	30.4^{ab}	30.4^{ab}	31.9^{b}
ES	30.0^{a}	31.4^{b}	31.4^{b}	30.2^{a}	31.1^{b}	31.8^{b}

ES and NES = electrically stimulated and not electrically stimulated.Control: no zilpaterol and no vitaminD_3 supplement.Zilpaterol: 0.15 mg zilpaterol hydrochloride per kg live weight for 30 days during the final weeks of finishing.VitaminD_3treatments: 3D7M, 9D1M, 6D7M, 6D7M7N: D = number of days supplemented; N = number of days vitaminD_3 was withdrawn; M = millions IU vitaminD_3/animal/day, all vitaminD_3 received 0.15 mg zilpaterol hydrochloride per kg live weight for 30 days during the final weeks of finishing.^{a,b,c,d,e} Means in the 2 rows of the same property and with different superscripts, differ significantly ($P < 0.05$).