The effect of high supplement levels of Vitamin D_3 on instrumental colour and drip loss of beta agonist treated beef

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Abstract— **Twenty young steers received no beta agonist (C), while 5 groups of 20 animals each received the beta agonist, zilpaterol hydrochloride (Z), with 4 of these groups also 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). *M. longissimus lumborum* (LD) were 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. Certain treatment combinations improved the colour measurements, probably due to reduced oxidation and the consequent improved cell membrane integrity.**

Keywords— **beta agonist, vitamin D_3, colour**

I. INTRODUCTION

It is widely known, that beta agonists supplemented animals produce tougher meat and that this increase in toughness, in part, is caused by a lower ageing potential due to a reduction in calpain activity and an increase in calpastatin activity [1, 2]. In addition, various studies have reported effects on other quality attributes like colour and water holding capacity [3, 4], which is mainly associated with the shift in fibre type composition (more glycolytic or white) and muscle hypertrophy caused by beta-agonists [2, 4].

Electrical stimulation and extended aging are often used to improve the tenderness of beta agonist treated beef. It is also well-known that these processes may affect colour [5, 6, 7, 8] and that these procedures combined with beta-agonists may have additive effects on quality parameters [4].

Supplementation with ultra high levels of vitamin D_3 over the final days before slaughter has been used to improve meat tenderness [9]. This method is motivated by the suggestion that an increased calcium ion level, stimulated by high vitamin D_3 levels [10], contributes to meat tenderization directly by weakening of myofibrillar structures [11] as well as indirectly through activation of μ-calpain [12]. [13] investigated the effect of high vitamin D_3 supplement levels when meat tenderness was compromised by beta agonists. As sub-part of the study they also investigated the effect of vitamin D_3 on drip loss and colour. [14] showed that supplementation of vitamin D_3 could improve antioxidative capacity of pork loin muscle, thereby maintaining the cell structure, which could affect colour and water binding qualities of muscle. Therefore, it is possible that high vitamin D supplement levels could improve colour and drip loss, especially under conditions where carcasses are electrically stimulated and meat is first vacuum aged to overcome the negative effect of beta agonists on tenderness.

II. MATERIALS AND METHODS

One hundred and twenty young Bonsmara steers (~9 months) were purchased, processed and raised in the research feedlot facilities of the Agricultural Research Council (Irene, Gauteng Province) on a commercial feedlot diet (120 days). The animals were identified and allocated to 6 treatment groups of 20 animals each so that the average weight and variation for each group was the same. One group (C) receive no beta agonist or vitamin D_3, while 5 groups of 20 animals each received the beta agonist, zilpaterol hydrochloride (Z) (Intervet/ Schering-Plough Animal Health, South Africa) at 0.15mg/kg live weight/day, for thirty days during the final weeks of finishing. Four of these groups also received a supplement of vitamin D_3 (Vitamin D_3 500, Advit Animal Nutrition S.A. (Pty) Ltd, Sebenza, South Africa) 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). At slaughter, the left carcass sides were electrically stimulated (ES) for 30 seconds (400V peak, 5 ms pulses at 15 pulses per second) and right sides not (NES). Sub-samples (30 mm thickness) of the LD were removed the day following slaughter and vacuum-aged for 14 days *post mortem* for measurement of drip loss and colour.

Following the 14 day aging, vacuum packed samples were opened and drip loss or purge were determined by measuring the amount of purge remaining in the bag after removing expressed as a % of the cut and drip combined

The sample were then divided in two and 1 steak was allowed to bloom for 60 minutes at chiller temperatures at 2 ± 1 ºC, with its freshly cut surface facing upwards before colour recording in triplicate with a Minolta meter (Model CR200, Osaka, Japan). Colour measurements followed the CIE colour convention, where the 3 fundamental outputs are L*, a* and b*. L* is lightness, a* is redness and b* is yellowness. Chroma, was calculated as square root of $a^{*2} + b^{*2}$ [15].

Data were subjected to analysis of variance for a split-plot design [16] with the 6 treatment groups (C, Z, 3D1M, 9D1M, 6D7M7N, 6D7M) as whole plots and the 2 stimulation sub-treatments (ES and NES) as a sub-plots. 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 [17]

III. RESULTS

Treatment ($P = 0.024$), electrical stimulation ($P < 0.001$) had significant effects on drip loss (Table 1). Furthermore, significant interactions between treatment and stimulation ($P < 0.001$), were recorded. According to Fig 1a drip loss was generally higher for stimulated samples than for non-stimulated samples. Non-stimulated samples of all treatments recorded similar drip loss values irrespective of treatment. However for stimulated samples, zilpaterol showed a significant increase ($P < 0.05$) in drip loss, while the control and vitamin D_3 samples recorded smaller increases ($P < 0.05$ for 6D7M7N only)..

Table 1 General statistics for the effects of treatment and stimulation and their first order interactions for drip loss, lightness (L*) and chroma of the *M. longissimus lumborum* (Data pooled for treatment and stimulation).

Effect	Drip loss		Lightness		Chroma	
	Significance	SEM	Significance	SEM	Significance	SEM
Main effect						
Treatment	P < 0.024	0.1386	P < 0.001	0.3543	P < 0.001	0.2578
Stimulation	P < 0.001	0.0516	P < 0.026	0.0800	P < 0.013	0.0902
Interactions						
T x S	P < 0.001	0.1650	P = 0.398	0.2862	P = 0.378	0.3014

T: Treatment (control, zilpaterol and vitamin D_3 supplemented groups)
S: Stimulation (stimulated and non-stimulated)

Treatment and electrical stimulation recorded significant effects on all aspects of instrumental colour measurements (Table 1). Electrical stimulation had no significant effect on L* except for 3D7M ($P < 0.05$) and C ($P < 0.10$) where ES reduced the L*. For stimulated samples, the control, 3D7M and 6D7M7N; and Z, 9D1M and6D7M, were grouped together. Non-stimulated 6D7M7N samples recorded uncharacteristically low L* values (Fig 1b, $P < 0.05$).

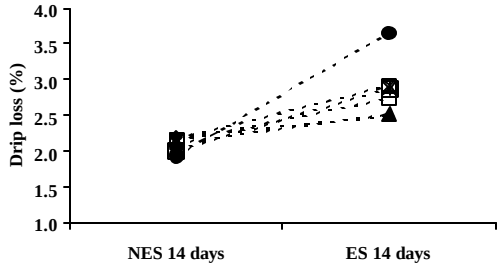
Redness and yellowness were combined in a chroma value for the purpose of results and discussion. Stimulation tended to decrease chroma values ($P < 0.05$ 3D7M), while 6D7M was not affected by ES (Fig. 1c). The control samples recorded higher chroma values ($P < 0.05$) than all other treatments.

IV. DISCUSSION

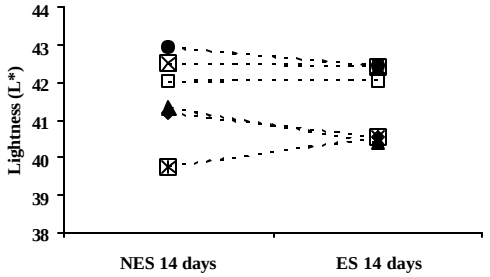
The condition of this experiment can be regarded as a worst case scenario for drip loss due to proliferation of predominantly white fibres in zilpaterol treated animals [2, 18] that are more susceptible to protein denaturation [19] especially when stimulation is applied and glycolyses is accelerated. Our study also showed that the effect of stimulation was enhanced with longer aging which was supported by the study of [20]. Vitamin D_3 (in particular 3D7M) reduced drip loss in aged stimulated zilpaterol treated steaks (Fig 1a), while no other study on beef supported these results. Several studies on pork reported lower drip loss when high supplement levels of vitamin D_3 were used. [14] associated reduced drip loss in pork with an increased anti-oxidative capacity in vitamin D_3 supplemented samples after incubation of muscle homogenates with Fe^{2+}/ascorbate and suggested that a higher level of Ca^{2+} (bivalent ion) due to vitamin D_3 in muscles were causing the positive influence on the lipid oxidation.

Paler meat, i.e. higher L* values, in zilpaterol treated samples was expected and could be associated with the increased surface moisture due to higher drip loss reported earlier [4]. The 6D7M7N, and to some extent 3D7M, treatments reduced this effect which agrees with other results on aged and unaged pork [14, 21] but none on beef. Considering the relationship between drip loss, surface moisture and light reflection, it is reasonable to believe that lower L* values in certain vitamin D_3 treatments was the result of lower drip loss and will probably be more evident in aged samples.

a)



b)



c)

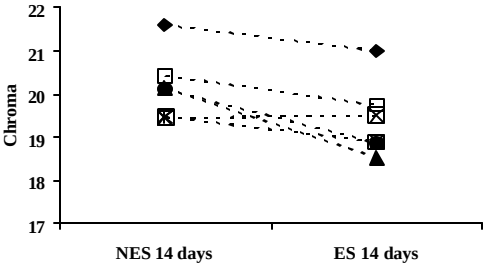


Fig. 1 Interaction between treatment (Control, 3D7M, 9D1M, 6D7M7N, 6D7M, Zilpaterol) and electrical stimulation (a) Drip loss, (b) Lightness (L*), (c) Chroma, Legend**:**
□ - 9D1M, ▲- 3D7M , ● - Zilpaterol, ◆- Control, **X** – 6D7M ✳ – 6D7M7N

ES and NES = stimulated and non-stimulated

In support of our results, [3, 22] reported lower values for redness for beta agonist treated meat. The lower redness in the latter study correlated with lower heme pigment and myoglobin which they related to hypertrophy of especially white muscles fibres and a consequent dilution effect and also to a general shift in muscle fibre composition towards white muscle type in beta-agonist treated samples. The lower values for chroma of stimulated meat in our study (Fig 1c) could be the result of high temperatures and low pH values associated with electrical stimulation that advance the depletion of oxygen consumption rate and metmyoglobin reducing ability [5]. This in turn could reduce the buffering effect of the latter in stimulated samples at the same stage of aging as non-stimulated samples.

No effect on any colour attribute was recorded in the literature for beef [9, 23]. [14] and [21]reported positive results in pork which could be ascribe to anti-oxidative effect of vitamin D_3 discussed earlier for drip loss and lightness [14]. The effect in our study was small and only 9D1M (and to some extent) 6D7M recorded chroma values closer to the control. The specific conditions (aging and stimulation) and variation in muscle composition as discussed by [5] between the control and other treatments (zilpaterol with and without vitamin D_3) could have reduced the effect of certain vitamin D_3 treatments relative to the control.

V. CONCLUSION

In this study we confirmed the negative effects of the beta agonist, zilpaterol, on drip loss and instrumental colour of beef loin steaks and that electrical stimulation enhanced these effects for aged meat. High supplement levels of vitamin D_3 probably exhibited anti-oxidative behaviour and alleviated the negative effects of zilpaterol combined with electrical stimulation and aging on drip loss but not on colour. Considering the lack of positive effects on meat tenderness reported in a previous study, ultra high levels of vitamin D_3 is probably not a viable option to improve the quality of meat that was compromised by feeding beta agonists.

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REFERENCES

1. Koohmaraie M, Shackelford SD, Mugglicockett NE, et al. (1991) Effect of the beta-adrenergic agonist l644,969 on muscle growth, endogenous proteinase activities, and postmortem proteolysis in wether lambs. J Anim Sci 69:, 4823-4835
2. Strydom PE, Frylinck L, Montgomery JL, et al. (2009) The comparison of three B-agonists for growth

performance, carcass characteristics and meat quality of feedlot cattle. Meat Sci 81: 557-564.
3. Avendaño-Reyes L, Torres-Rodriques V, Meraz-Murillo F, et al. (2006) Effects of two Badrenergic agonists on finishing performance, carcass characteristic and meat quality of feedlot steers. J Anim Sci 84: 3259-3265.
4. Geesink GH, Smulders FJM, Van Laack HLJM, et al. (1993) Effects on meat quality of the use of clenbuterol in veal calves. J Anim Sci 71: 1161-1170.
5. Ledward DA (1985) Post-slaughter influences on the formation of metmyoglobin in beef muscle. Meat Sci 15: 149-171.
6. Ledward D A, Dickinson, RF, Powell VH, et al. (1968) The colour and colour stability of beef Longissimus Dorsi and Semimembranosus Muscles after effective electrical stimulation. Meat Sci 16: 245-265.
7. Renerre M (1990) Review: Factors involved in the discoloration of beef meat. Int Jl of Fd Sci Tech 25: 613-630.
8. Devine C (2009) Advantages of electrical stimulation. . Meat Sci, 83, 584-585.
9. Montgomery JL, Carr MA., Kerth CR et al. (2002) Effect of vitamin D_3 supplementation level on the postmortem tenderization of beef from steers. J Anim Sci 80: 971-981.
10. Swanek SS, Morgan JB, Owens FN et al. (1999) Vitamin D_3 supplementation of beef steers increases longissimus tenderness. J Anim Sci 77: 874-881.
11. Takahashi K (1992) Nonenzymatic Weakening of Myofibrillar Structures During conditioning of Meat - Calcium-Ions at 0.1-Mm and Their Effect on Meat Tenderization. Bioch 74: 247-250.
12. Koohmaraie M (1992) The Role of Ca2+-dependent Proteases (Calpains) in post mortem Proteolysis and Meat Tenderness. Bioch 74: 239-245.
13. Strydom PE, Hope-Jones M, Frylinck L et al. (2011) Effect of dietary B-agonist treatment, vitamin D_3 supplementation and electrical stimulation of carcasses on meat quality of feedlot steers. Meat Science, DOI: 10.1016/j.meatsci.2011.05.01.
14. Lahucky R, Bahelka I, Kuechenmeister U et al. (2007) Effects of dietary supplementation of vitamin D_3 and E on quality characteristics of pigs and longissimus muscle antioxidative capacity. Meat Sci 77: 264-268.
15. MacDougall DB (1977) Sensory properties of foods. Applied Science Publishers, London.
16. Payne RW, Murray DA, Harding SA et al. (2007) GenStat for Windows® (10th Edition) Introduction. VSN International, Hemel Hempstead, UK.
17. Snedecor GW, Cochran WG (1980) Statistical methods 7th Edition, Iowa State University Press.
18. Wheeler TL, Koohmaraie M (1997) Effects of the B-adrenergic agonist L644,969 on muscle protein turnover, endogenous proteinases activities, and meat tenderness in steers. J Anim Sci 70: 3035-3043.
19. Maltin CA, Warkup CC, Matthews KR, Grant CM et al. (1997). Pig muscle fibre characteristics as a source of variation in eating quality. Meat Sci, 47, 237-248.
20. Simmons NJ, Daly CC, Cummings TL et al. (2008) Reassessing the principles of electrical stimulation. . Meat Sci 80: 110-122.
21. Wiegand BR, Sparks JC, Beitz DC et al. (2002) Short-term feeding of vitamin D_3 improves colour but does not change tenderness of pork-loin chops. J Anim Sci80: 2116-2121.
22. Garssen GJ, Geesink GH, Hoving-Bolink AH et al. (1995). Effects of dietary clenbuterol and salbutamol on meat quality in veal calves. Meat Sci 40: 337-350.
23. Montgomery JL, King MB, Gentry JG et al. (2004) Supplemental vitamin D_3 concentration and biological type of steers. II. Tenderness, quality, and residues of beef. J Anim Sci 82: 2092-2104.