THE EFFICIENCY OF ELECTRICAL STIMULATION TO COUNTERACT THE NEGATIVE EFFECTS OF β-AGONISTS ON MEAT TENDERNESS OF FEEDLOT CATTLE.

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***Abstract*—Parameters included Warner Bratzler shear force (WBSF) and calpastatin and calpain enzyme activity measured at 24 h *post mortem*. Treatment groups were a control group (C) and a zilpaterol hydrochloride supplemented group (Z). After slaughter carcasses were split, the left side electrically stimulated (ES) and the right side not stimulated (NES). Samples were aged for 3 days. Zilpaterol resulted in increased ($P < 0.001$) WBSF mainly due to an increased ($P < 0.001$) calpastatin activity. ES improved tenderness ($P < 0.001$) in general by early onset of rigor triggering the activity of calpains. ES also reduced the calpastatin activity ($P < 0.001$), which partially countered the effect of high calpastatin activity on the aging potential of Z loins. ES can therefore be implemented to improve meat tenderness in zilpaterol supplemented steers, although having no zilpaterol will still have an advantage in final tenderness.**

***Index Terms*—calpains, electrical stimulation, meat tenderness, Zilpaterol.**

I. INTRODUCTION

It is the aim of any livestock industry to improve efficiency and economic return. β-agonists are compounds fed to animals to improve rate of gain and feed efficiency and to increase carcass meat yield efficiency (Dikeman, 2007).

It is however common knowledge that β-agonist supplemented animals produce tougher meat due to an increase in the activity of calpastatin and a reduction in calpain activity (Wheeler & Koohmaraie, 1997). The β-agonist zilpaterol was recently registered in Mexico, South Africa and the USA and is probably the most commonly utilised β-agonist in commercial beef production. Recent studies have found that tenderness problems generally related to β-agonists also occur with this product (Strydom, Frylinck, Montgomery & Smith, 2009).

A common way of improving tenderness is to age meat for longer although this leads to extended storage costs. Electrical stimulation has been shown to hasten rigor and cause the tenderisation process to start earlier at a higher temperature (Dransfield, Etherington & Taylor, 1992) thereby reducing aging time. In this study we look at the efficiency of electrical stimulation to improve meat tenderness of β-agonist treated meat and the mechanisms behind the process.

II. MATERIALS AND METHODS

Forty Bonsmara steers, approximately 9 months old, were raised on a commercial feedlot diet for 120 days. The animals were divided in two groups (n=20) so that the average weight and variation was the same for both groups. The groups represented two treatments, a control (C), which received the feedlot diet only, and a zilpaterol group (Z), which received zilpaterol hydrochloride (Intervet/ Schering-Plough Animal Health, South Africa) at 0.15mg/kg live weight/day, for thirty days during the final weeks of finishing and withdrawn four days prior to slaughter.The animals were slaughtered at the abattoir of the Animal Production Institute (Agricultural Research Council, Irene, Gauteng Province). Carcasses were split and the left sides electrically stimulated (400V peak, 5ms pulses at 15 pulses per second) within 30 minutes of killing. Carcasses were then chilled at 0-5ºC. Samples for Warner Bratzler shear force (WBSF) and

proteolytic enzyme studies were collected from the *M. longissimus* lumborum (LL) on the day following slaughter.

Samples collected for WBSF measurement were vacuum-packed and aged for 14 days, processed into 30 mm steaks and frozen at -20°C. Frozen loin steaks, thawed at 4°C for 24 hours and prepared according to an oven-broiling method using direct radiant heat (AMSA, 1995) to 70°C internal temperature. Six round cores (12.7 mm diameter) were removed from cooled down steaks (18°C) parallel to the long axis of the muscle fibers and sheared perpendicular to the fiber direction, by a Warner Bratzler shear device mounted on an Instron Universal Testing apparatus (Model 4301, Intsron Ltd, Buckinghamshire, England;.cross head speed = 200 mm/min).

Samples collected for enzyme studies (24 hours *post-mortem*) were snap-frozen in liquid nitrogen and preserved at –70ºC. Calpastatin and μ-calpain were extracted from 5 g of the frozen samples as described by Dransfield (1996) and separated by means of the two-step gradient ion-exchange chromatography-method according to Geesink and Koohmaraie (1999). Calpain assays were determined by using azo-casein as substrate according to Dransfield (1996). One unit of calpain activity was defined as an increase in absorbance at 366 nm of 1.0 per hour, at 25°C. One unit of calpastatin activity was defined as the amount that inhibited one unit of m-calpain activity. Data were expressed as units per gram of muscle.

Data of WBSF and two enzyme activities 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 two treatment groups (C and Z) as whole plots and the two stimulation sub-treatments (ES and NES) as 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 (Snedecor & Cochran, 1980).

III RESULTS AND DISCUSSION

WBSF was affected negatively (Table 1) by Z which agrees with various other studies regarding the effect of β-agonists on beef tenderness (for a review: Dunshea, D'Souza, Pethick, Harper & Warner, 2005; Dikeman, 2007). Higher WBSF of Z loins coincided with higher calpastatin and μ-calpain activity which explain the delay in *post mortem* aging (Table1). Lower calpain activity for C means less inhibition by calpastatin and consequently higher rate of proteolysis and autolysis of this enzyme took place over the first 24 *post mortem*. In support, Geesink, Smulders, Van Laack, Van der Kolk, Wensing and Breukink (1993) and Simmons, Young, Dobbie, Singh, Thompson and Speck (1997) also recorded higher shear values and higher calpastatin levels with β-agonist treatment on calpain activity were less consistent in these reports and dependant on the time of analysis *post mortem* and the product in question.

The main objective of this experiment was to investigate the ability of ES to overcome the negative effect of beta agonists on meat tenderness. The interaction between stimulation and treatment in Figure 1 ($P = 0.003$) shows that ES reduced the difference in WBSF for aged meat between C and Z significantly, although ES loins of C still had an advantage over Z. Strydom, Osler, Leeuw and Nel (1999) reported similar results but gave no supportive evidence. According to Table 1, ES reduced the activity of 24 hour calpastatin and μ-calpain for both treatments. Reports of Ducasting, Valin, Schollmeyer and Cross (1985), Dransfield et al. (1992) and Hwang and Thompson (2001) suggest that ES advances the onset of rigor (pH = 6.1; Dransfield et al., 1992) whereby activation of μ-calpain is initiated due to the release of Ca+ ions and which causes and advance in proteolyses and tenderisation. Ducasting et al. (1985) also recorded an overall decline in calpastatin activity for ES samples similar to our study (Table 1). If relative changes occurring in the calcium dependent proteinase (CDP) system in the first 24 hours *post mortem* are accurate predictions for tenderness development later on as suggested by Koohmaraie and Geesink (2006); Dransfield et al. (1992), the effect of ES on the CDP system in the present study could explain the advantage gained by Z. The proportionally greater reduction in calpastatin activity in Z due to ES (Figure 2; $P = 0.015$) coupled with the advanced rigor (and higher rigor temperatures) and subsequent action of calpain could have benefitted Z to the extent that the final WBSF was closer to C than without ES. In support, Ferguson, Jiang, Hearnshaw, Rymill and Thompson (2000) also recorded beneficial effects of ES when tenderness potential was compromised by the CDP system of *Bos indicus* vs. *Bos Taurus* breeds.

IV CONCLUSION

The study confirmed that the negative effect on meat tenderness by the β-agonist, zilpaterol, was mainly caused by increased calpastatin activity. Electrical stimulation improved loin tenderness of both β-agonist supplemented and non-supplemented animals, mainly mediated through early onset of rigor, triggering the CDP system and advancing tenderisation in general. However, Z probably gained additional advantage through the significant reduction in calpastatin activity (relative to C) with ES, although ES could not completely cancel out the effect of Z on the aging process.

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Table 1
Effect of the β-agonist, zilpaterol, and electrical stimulation on Warner Bratzler shear force (WBSF) and 24 hour calcium dependent proteinase activity of *M. longissimus lumborum*

Treatment	β-agonist treatment		SEM[a]	*P* value
	Control	Zilpaterol		
WBSF (kg)(aged 14 days)	3.53	4.75	0.1922	<0.001
Calpastatin activity[b]	2.15	2.61	0.0525	<0.001
μ-calpain activity[c]	0.64	0.80	0.0344	0.021
	Stimulation			
	ES	NES	SEM[a]	*P* value
WBSF (kg) (aged 14 days)	3.81	4.47	0.1205	<0.001
Calpastatin activity[b]	2.21	2.55	0.0377	<0.001
μ-calpain activity[c]	0.53	0.91	0.0306	<0.001

[a] Standard error of means
[b] Calpastatin activity: One unit of calpastatin activity is defined as the amount that inhibited one unit of m-calpain activity.
[c] One unit of calpain activity is defined as an increase in absorbance at 366 nm of 1.0 absorbance unit per g of muscle per hour, at 25 °C.

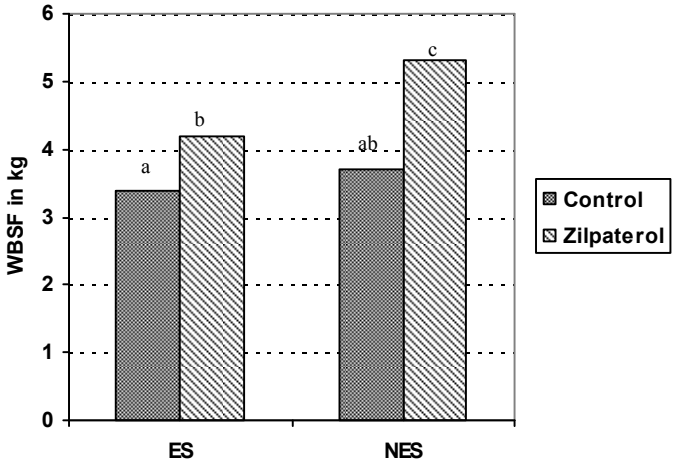

Fig. 1. Interaction between treatment (control and zilpaterol) and electrical stimulation in relation to Warner Bratzler shear force (WBSF; $P = 0.003$). (Bars with different superscripts differ significantly, $P < 0.05$; ES and NES = stimulated and non-stimulated

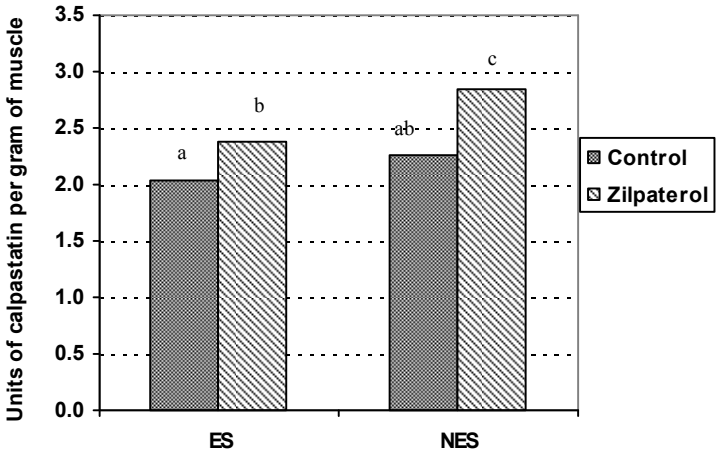

Fig. 2. Interaction between treatment (control and zilpaterol) and electrical stimulation in relation to calpastatin activity ($P = 0.015$). (Bars with different superscripts differ significantly, $P < 0.05$; ES and NES = stimulated and non-stimulated

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