GENETIC MECHANISMS THAT INFLUENCE SHEAR FORCE OF BEEF FROM CARCASSES SUBJECTED TO ELECTRICAL STIMULATION

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I. INTRODUCTION

Beef tenderness is important for consumer satisfaction. Post-mortem application of an electrical current to a carcass is commonly used to improve end-product tenderization [1]. Electrical stimulation (ES) likely improves tenderness, in part, by increasing proteolysis, but much variation can remain [2], particularly in beef from *Bos indicus*-influenced cattle. In this study, we investigated how genetic differences in steers may affect post-ES beef tenderness variation. Our objectives were to utilize a group of genetically defined animals to (1) identify differentially expressed genes in skeletal muscle from animals that produced the highest or lowest Warner-Bratzler shear force (WBSF) measures; and (2) utilize gene expression phenotypes with SNP genotypes to identify genetic variants associated with variability in WBSF that remains in post-ES treated meat.

II. MATERIALS AND METHODS

This experiment utilized F_2 Nellore (*Bos indicus*)-Angus (*Bos taurus*) steers from a genetic mapping herd (N = 321). Steers fed as yearlings were harvested at approximately 18 months of age. At harvest, carcasses were split and the right half was subjected to ES (Koch Britton Stimulator 350, Kansas City, MO). Carcasses were chilled for 48 h. Loins were cut into 2.54 cm thick steaks and aged at 2°C until 14d *postmortem*, then stored frozen. WBSF was measured as previously described [3] and ranged from 1.47 kg to 6.81 kg across all individuals and treatments. Approximately 1 g *Longissimus lumborum* tissue, obtained prior to ES, was flash frozen in liquid nitrogen and stored at -80°C until RNA extraction. Gene expression was evaluated by bovine oligo microarray analysis (Agilent 4X44K GPL11649). Expression was evaluated for both ES-treated group and non-ES groups. A mixed-model statistical approach was used for sample selection in which fixed-class effects were contemporary group (year and season) and harvest date [4]. Analysis was performed separately for WBSF measurements of both ES and non-ES beef. Samples were assigned to tenderness groups based on residual differences in WBSF. For differential expression, 12 extremes of tenderness (12 highest or 12 lowest WBSF residuals) from each group (ES vs non) were evaluated. Expression pathway analysis [4] was validated by quantitative real-time reverse transcription PCR (qRT-PCR). For DNA haplotype analysis, individuals (n=776) from three generations of the experimental population were genotyped (Illumina, Inc., San Diego, CA) [4].

III. RESULTS AND DISCUSSION

Bovine microarray and genetic data revealed genes within the extracellular matrix (ECM) and focal adhesion (FA) gene networks associated with reduced WBSF of beef subjected to ES. The effects of several gene sequence variants and mode of inheritance associated with differences in WBSF are summarized in Table 1. In beef not exposed to ES (NON-ES), lower expression of

calpastatin (*CAST*) was associated with more favourable WBSF, as expected [5]. Genetic haplotype analysis showed that *CAPN1, CAPN2*, and *CAST* alleles did not affect post-ES tenderness.

Table 1 Impact of genotype on WBSF variation in beef subjected to ES

GENE	Favourable allele breed type, direction of inheritance	Change in WBSF	P value
Actinin 3 (*ACTN3*)	Angus, paternal	5.5% lower (-0.16 kg)	0.03
Fibronectin 1 (*FN1*)	Angus, paternal	7.0% lower (-0.23 kg	0.03
Integrin alpha 6 (*ITGA6*)	Nellore, paternal	5.5% lower (-0.15 kg)	0.03
Integrin alpha 6 (*ITGA6*)	Nellore, maternal	6.2% lower (-0.18 kg)	0.01
Integrin beta 5 (*ITGB5*)	Nellore, paternal	5.9% lower (-0.17 kg)	0.02
Integrin beta 5 (*ITGB5)*	Nellore, maternal	5.2% lower (-0.17 kg)	0.05

Because commercial genetic tests for tenderness are based on *CAPN1, CAPN2*, and *CAST* genes [6], our results suggest these genes are poor predictors of aged tenderness in beef subjected to ES. In contrast, genes in the ECM and FA pathways (*ITGA6, ITGB5, ACTN3* and *FN1)* are likely to be better predictors of tenderness in production systems that utilize ES.

IV. CONCLUSION

We identified novel gene variants in *ITGA6*, *ACTN3*, *FN1*, and *ITGB6* genes that are associated with improved beef tenderness. We conclude that breed and expression differences in the closely related ECM and FA pathways partially explain genetic influences on variation in tenderization that remains in beef despite post-mortem ES treatment. These gene network pathways may provide novel targets for selection or intervention to improve beef quality and are of particular importance in sub-tropical environments where *Bos indicus*-influenced cattle are prevalent.

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