GENE EXPRESSION OF SKELETAL MUSCLE OF RED FACED HEREFORD STEERS

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Impact Statement

This research provides insight into the growth and developmental differences in meat quality grade. This may enhance our ability to control variation in meat quality, as well as understand that underlying genetic mechanisms controlling muscle growth and fat deposition.

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SUMMARY

The objective of this study was to evaluate the relationship between quality grade and genetic growth patterns on meat tenderness. The research aimed to evaluate the impacts of gene expression on quality grade. Muscle samples from 16 different Hereford cross steers were taken following harvest and RNA was extracted from these samples to evaluate which genes were being actively transcribed in the muscle at time of harvest. Loin samples were collected and allowed to age for 1, 3, 7, 14, and 21 d postmortem and frozen. After harvest, carcasses were quality graded by an experienced grader. RNA samples were pooled and sequenced based upon carcass quality grade. Gene set enrichment analysis, transcription factor analysis and network and pathway analysis was used to identify genes and gene networks that relate to growth rate and carcass quality.

Grading of carcasses resulted in six carcasses grading Choice and five carcasses each for Select and Standard. Standard carcasses were significantly lighter, less fat with smaller loin muscle area than the Select and Choice carcasses. Suggesting the steers yielding Standard carcasses were not at the same growth phase as the steers yielding Choice or Select carcasses despite belonging to the same cohort. Shear force measurements unexpectedly indicated that there was no difference in the tenderness of steaks from Choice and Standard carcasses, while steaks from Select carcasses were significantly less tender. Upon analysis of differentially expressed genes, a significant number of differences were observed between Choice and Standard carcass pools (1258 genes, $P < 0.01$). A functional analysis was run using DAVID bioinformatics software, which revealed differences in the underlying pathways regulating muscle cell growth and proliferation. Suggesting that the previously observed differences in the growth patterns between Choice and Standard cattle were due to identifiable differences in the regulation of cellular processes and growth.

Meat quality and tenderness are two of the most important traits for beef production, and this research helps to shed light on the genetic and molecular basis of these traits, and how selection and growth may interact in these economically significant characteristics.

INTRODUCTION

Meat quality and tenderness are two of the most consumer-valued characteristics of a steak. Tenderness is one of the most important palatability attributes of meat (Shackelford et al., 2001). Consequently, providing this highly demanded product becomes a major concern for beef cattle producers. However, despite that this issue is on the forefront of improvement initiatives, the industry still experiences difficulties producing a tender product. The findings of the National Beef Tenderness Survey

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(Morgan et al., 1991) suggest that approximately 20% of rib and loin steaks are not acceptably tender. Ongoing research is required in order to gain a better understanding of the genetic expression of these traits, and how selection plays a role in the transmission and expression of these economically significant characteristics.

PROCEDURES

Sixteen Red Faced Herford steers will be fed at Fort Keogh Agricultural Research Station. Birth weight, weaning weight, feedlot growth data, and fatness measurements have been collected. Animals within the same cohort that produced carcasses that graded standard, select and choice were sampled.

The animals were harvested after all steers had been fed a minimum of 270 days in the feedlot. Animals were harvested at a federally inspected facility. Carcass data was collected, as was a muscle sample from the loin, which was snap frozen in liquid nitrogen and stored for subsequent RNA extraction. Loin samples were collected and aged for 1, 3, 7, 14, and 21 days. These samples were used to determine tenderness as measured with Warner Bratzler shear. The extracted RNA was depleted of ribosomal RNA using an Invitrogen Ribominus kit and then will be used to create individual cDNA libraries that were then randomly allocated to one of two pools for each quality grade of standard, select and choice. These pools were then sequenced on an Ion Proton next-generation sequencing platform according to manufacturer instructions. The sequencing reads generated were aligned to the known bovine consensus sequence and a normalized count of reads were generated to determine expression of each known gene and gene isoforms using CLC Bio Genome Workbench software. Differentially expressed genes and transcripts will be calculated using Golden Helix RNA seq. Module and gene set enrichment analysis, transcription factor analysis and network and pathway analysis will be used to identify genes and gene networks that relate to growth rate and carcass quality. This will also help elucidate the mechanisms underlying muscle development, which may provide valuable information for beef meat quality improvement. The raw data will be made publically available to the Gene Expression Omnibus. A functional analysis will be run using DAVID bioinformatics software.

Shear force was determined using previously published methods (Boles et al. 2008). Steaks were thawed at 4°C for 24 hours. Each steak was weighed before and after cooking to determine cook loss. Eight to ten samples (1.27 x 1.27 x 2.54 cm) for shear force evaluation were removed parallel to the fiber direction from each steak that was cooked and cooled. Samples were sheared once perpendicular to the fiber direction with a TMS 30 Food Texturometer fitted with a Warner-Bratzler shear attachment. The average of the samples sheared were used for statistical analysis in relation to the differentially expressed genes and transcripts.

RESULTS AND DISCUSSION

The carcasses were placed into categories by quality grades- Standard, Select and Choice- and were evaluated for statistical differences between carcass traits and shear force. The carcass traits evaluated included HCW, fat thickness, REA, KPH, YG and marbling. There was a significant difference in REA, KPH and marbling between all the categories. Choice carcasses had the largest REA, KPH and marbling scores where as Standard measured the lowest in each catagory. Boles et al (2009) also reported larger rib-eye areas with larger carcass weights. When comparing steaks from Select and Choice carcasses, Obuz et al (2004) reported increased marbling scores decreased

the shear force value, as also reported by and Tatum et al (1982).

Steaks from Standard carcasses had the lowest shear force values, but weren't significantly different from Choice ($P > 0.05$). Select, having the highest shear force value was significantly different from the other two categories ($P < 0.0001$). A report from Obuz et al (2004) confirmed our findings of Select being tougher than Choice. Standard carcasses were significantly heavier with less fat than Select and Choice carcasses ($P < 0.0001$). As fat thickness increased, shear force values decreased, as also found by Tatum et al (1982). Select and Choice carcasses had significantly different yield grades.

Every carcass had a steak that was aged for 1, 3, 7, 14 and 21 days. The longer a steak is aged, the more tender it will become (Marino et al, 2013). Steaks aged 1 d were toughest. With longer aging, the shear force decreased, so those that aged 21 d were most tender. These values correlate directly to the study done by Marino et al (2013), having the shear force values decrease with aging, with 1 d measuring the highest and 21 d measuring the lowest values.

As expected, we found Choice carcasses had a lower shear value then Select carcasses. However, Standard carcasses had a lower shear force value than Select. This is potentially due to the physiological age of the animals that were Standard. This is supported by the lighter carcass weights, lower marbling values, and less fat on the carcasses. To get the most tender product possible, Standards need to be utilized or the animals need to be fed long enough to reach Choice.

Upon analysis of differentially expressed genes, a significant number of differences were observed between Choice and Standard carcass pools (1258 genes, $P < 0.01$). A functional analysis was run using DAVID bioinformatics software, which revealed differences in the underlying pathways regulating muscle cell growth and proliferation. Biological processes such as growth, muscle hypertrophy, protein kinase activity, and lipid biosynthetic pathway were found to enriched in the differentially expressed gene set. This will provide new insight into the molecular and genetic basis of meat quality grade.

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