Molecular Pathways for Muscle and Adipose Tissue Are Altered Between Animals Classed as Choice or Standard

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IMPACT STATEMENT

Beef tenderness is the driving force behind consumers decisions to consume beef. When the eating experience is poor or inconsistent, genetic improvement can be one strategy to improve meat eating experience. Identification of patterns of gene expression in muscle from carcasses of different quality grades allows a better understanding of the metabolic changes that occur as fat deposition increases both intramuscularly and in adipose depots and will provide targets for genetic improvement and increased tenderness and consistency.

SUMMARY

Genetic improvement of beef product quality benefits both producers and consumers. Beef tenderness is a leading influence in consumer decisions to consume beef. Insufficient marbling and subsequent lower quality grades, lack of a uniform product, reduced or insufficient tenderness, high carcass weight or high yield grade can negatively impact beef acceptability. Improvements can be achieved in many of these characteristics by making selection decisions based on the results of genetic evaluations in the form of EPDs, real-time ultrasound imaging, and physical evaluation of candidate breeding animals. The gene expression profiles of muscle and adipose tissues in Standard and Choice carcasses was evaluated. Gene expression differences were profiled to determine the altered metabolic pathways to increase our understanding of the changes that occur in these tissues with increasing fat deposition. This can be used to identify genetic improvement targets to increase product quality and consistency.

INTRODUCTION

Intramuscular fat has been used for quality assurance systems within the US and Australia. Marbling at high levels have been related to tenderness. Tenderness has been identified as the most important factor affecting a consumer perception of palatability of meat. However, Platter et al. (2003) found that when a trained tasting panel evaluated strip loin steaks from different USDA Quality grade categories, they reported significant variation in tenderness was within a category of quality grades. This suggests that quality grade alone will not guarantee tenderness. As an animal approaches maturity it exhibits significant changes in its physiology. These changes encourage the deposition of fat, including marbling. Thus, there is a need to identify what changes occur at the molecular level that initiates fat deposition that are related to tenderness.

While advances have been made with regards to genetics, and the influence they have on meat tenderness, the question of what specific genes and how these genes function to determine meat quality remains. Previous work at MSU (Engle, 2015) utilized a cohort of Hereford steers that produced carcasses grading Standard, Select and Choice. *Longissimus lumborum* muscle was sampled at slaughter and a significant number of differences were observed between Choice and Standard carcass pools (1,258 genes < 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 the lipid biosynthetic pathway were found to be enriched

in the differentially expressed gene set. The objective of this work was to provide new insight into the molecular and genetic basis of meat quality grade.

PROCEDURES

Data collections from these animals were in compliance with the Montana State University Agriculture Animal Care and Use Committee (Protocol No. 2015-AA17).

Fifteen steers, born at the Red Bluff Research Ranch, Norris, MT, were selected at weaning based on weight and date of birth, the selection parameters were: ± 22.7 kg and birthdates within a 14-day window. Steers were relocated to the Montana State University Bozeman Area Research and Teaching Farm and placed in a single pen in the feedlot. At the start of the study, steers weighed an average of 315 kg. Each steer received a Synovex One Feedlot implant per standard feedlot protocol. Steers were fed an ad libitum standard feedlot diet and had free access to water. Steers were randomly allocated to one of three endpoints based on body weight, with average endpoint weights of 431 kg, 522 kg, and 612 kg for Standard, Select, and Choice quality grades, respectively. These weight-based endpoints achieved the desired marbling endpoints reported in Table 1.

Steers were harvested following normal harvest procedures utilizing a mechanical stun. Intermuscular and subcutaneous adipose tissue samples were taken at time of harvest and homogenized immediately. *Longissimus thoracis* muscle samples were snap-frozen in liquid N_2 for later gene expression analysis. Twenty-four hours after slaughter, carcass data were collected by trained personnel. The decline in pH of each carcass was monitored in the *longissimus lumborum* muscle approximately 6 cm from the hip bone using an Accumet Portable AP110 meter, (Fisher Scientific) equipped with an Orion 8163BNWP electrode. Measurements were taken hourly up to 8 h, then at 24 h postmortem to measure 'ultimate' pH.

RNA Extraction and quantification

Frozen muscle samples and homogenized intramuscular and intermuscular adipose tissue samples RNA was extracted using a Qiagen RNAeasy Plus Universal Midi kit according to manufacturer recommendations. Extracted RNA was stored in a -80° C freezer. Due to RNA degradation in some samples we were unable to utilize samples from each adipose depot from each animal. The highest quality RNA sample from each animal was sent for further analysis, as well as sending random samples from each depot for 3 samples to verify that the adipose samples, regardless of depot, had a sample correlation of greater than 0.80.

RNA sequencing and quantification

Three µg of RNA per sample was used for library preparation. Sequencing libraries were generated using NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) following the manufacturer's recommendations and index codes were added to attribute sequences to each sample. RNA libraries were sequenced and then mapped to the bovine reference genome UMD 3.1. An index of the reference genome was built using Bowtie v2.2.3 and paired-end clean reads were aligned to the reference genome using TopHat v2.0.12. The use of HTSeq v0.6.1 was employed to count the read numbers mapped to each gene. The FPKM, expected number of fragments per kilobase of transcript sequence per millions base pairs sequenced of each gene was then calculated based on the length of the gene and the reads count mapped to said gene.

Differential expression analysis

Prior to differential gene expression analysis, data was normalized. Differential expression was analyzed for two conditions and the p-values were adjusted using the Benjamini-Hochberg method. This was done to minimize false positives and to ensure the observed differences were real.

GO and KEGG enrichment analysis

Gene Ontology (GO) enrichment analysis of differentially expressed genes was implemented by the GOseq R package, in which gene length bias was corrected. GO terms with FDR corrected p-values of less than 0.05 were

considered significantly enriched by differentially expressed genes. KEGG, a database resource for understanding high-level functions and utilities of the biological system was used, along with KOBAS software, to test the statistical enrichment of differentially expressed genes in KEGG pathways.

RESULTS AND DISCUSSION

Carcass weight from animals classified in the Choice quality grade were greater (P = 0.002) than carcass weights from animals classified as Select or Standard (Table 1). Furthermore, fat thickness was greater (P = 0.007) for carcasses from animals classified as Choice and Select than from animals classified as Standard. The use of the weights as endpoints did place most of the carcasses in the correct grade categories as indicated by the significant differences in marbling for each category. In contrast, there was no difference in ribeye area between the different categories, indicating that muscle growth had slowed even at the lower fat categories.

Table 1. Carcass characteristics of steaks from carcasses classified as choice, select and standard

	Choice	Select	Standard
Hot carcass wt (lb)	748[a]	608[b]	536[b]
Fat thickness (in)	0.55[a]	0.39[a]	0.20[b]
Ribeye area (in^2)	11.0	9.9	10.4
Marbling score[y]	510[a]	382[b]	285[c]

[a-c]Means within a row with differing superscripts are significantly different ($P \leq 0.05$)
[y]Marbling scores: 200 = traces, 300 = slight, 400 = small, 500 = modest, 600= moderate.
[Z]N= 9.81 kg

KEGG pathway enrichment

KEGG (Kyoto Encyclopedia of Genes and Genomes) is a collection of manually curated databases dealing with genomes, biological pathways, diseases, drugs and chemical substances. Pathway enrichment analysis identifies significantly enriched metabolic pathways of signal transduction pathways associated with DEGs compared with the whole genome background. The greatest differences in pathway enrichment was seen in adipose and muscle tissues between the carcasses classified as Choice and Standard. Thus, these differences are the focus in this report.

In the comparison between muscle from Standard and Choice carcasses, 15 genes were down regulated and 20 were up regulated. The insulin receptor substrate 1 (IRS 1) gene was the only known functionally important gene to be differentially expressed. Interestingly, there was an increased expression of Major Histocompatability Complex genes in muscle, generally indicating a sign of inflammation or increased macrophage migration to the tissue in fattening animals. Alternatively, this could mean an altered stress response.

There were 49 down-regulated genes and 113 up-regulated genes in the comparison between adipose tissue from Standard and Choice carcasses. Upregulated genes included: CAB39L, FGF-1, GRIN1, LEP, HK2, YWHAG, ACC1, SCD1 and ELOVL3. These genes are mostly related to the metabolism of fat and energy. For example, up-regulation of ACC1, HK2, and the down-regulation of EIF43BP1 would inhibit protein synthesis and promote lipogenesis and glycolysis. This suggests a change from actively growing muscle to depositing fat in Steers that graded Choice. Furthermore, up regulation of leptin which can cause the inhibition of insulin resistance, and the down regulation of gluconeogenesis, which can contribute to altered fat metabolism.

In conclusion, many changes were observed in gene expression in adipose and muscle tissues between steers that were classified as Choice or Standard. KEGG pathway enrichment indicated some overlap and interaction between the different genes that would tend to have contradictory responses in the tissue. However, organisms are complex systems, with metabolic processes under multiple levels of regulation, so considerably more research is necessary to clarify what these changes mean to determining and predicting quality grade in cattle.

REFERENCES

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