Molecular pathways for adipose tissue are altered between animals classed by marbling as Choice or Standard

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I. INTRODUCTION

Intramuscular fat has been used for quality assurance systems within the US and Australia. Marbling at high levels has been related to tenderness. However, Platter et al. [1] reported differences in tenderness within USDA quality grade categories. This suggests that quality grade alone will not guarantee tenderness. While genetic advances have been made in meat tenderness and quality grade, the question of the number of genes and how they are expressed to determine meat quality remains. Previous work at Montana State University [2] showed a significant number of differentially expressed genes between Choice and Standard carcass pools (1258 genes <0.01). A functional analysis using DAVID bioinformatics software suggested that biological processes such as growth, muscle hypertrophy and lipid biosynthetic pathways were enriched. The objective of this work was to provide new insight into the molecular and genetic basis of meat quality grade.

II. MATERIALS AND METHODS

Fifteen steers were selected at weaning based on weight and date of calving. They were then placed in a feedlot. 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 the average end point weights being 431kg, 522kg, and 612kg. Steers were harvested following normal harvest procedures. Intermuscular and subcutaneous adipose tissue samples were taken at harvest and homogenized immediately while *longissimus thoracis* muscle samples were snap frozen for later gene expression analysis. Twenty-four hours after slaughter, carcass data was collected.

RNA Extraction and quantification

Frozen muscle samples and homogenized intramuscular and intermuscular adipose tissue samples underwent RNA extraction using a Qiagen RNeasy Plus Universal Midi kit according to manufacturer recommendations. Extracted RNA was stored in a -80°C freezer. 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 great than 0.8.

RNA sequencing and quantification

A total of 3 µg of RNA per sample was taken from total sample as input material for RNA sample 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. Library preparations were sequenced on an Illumina Hiseq platform and 125 bp/150 bp paired-end reads were generated. Reads were mapped to the 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, for each sequenced library the read counts were adjusted by edgeR program package through one scaling normalized factor. Differential expression analysis of two conditions was performed using the DEGSeq R package (1.20.0). The p-values were adjusted using the Benjamini-Hochberg method. A corrected p-value of 0.005 and $\log_2$ (fold change) of 1 were set as the threshold for significantly differential expression.

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-value 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.

III. RESULTS AND DISCUSSION

The use of the weights as endpoints did place most of the carcasses in the correct grade categories as indicated by the significant difference in marbling for each category (Table 1). Carcass weight from animals classified in the Choice grade were significantly (P=0.002) higher than other grades and fat thickness was significantly (P=0.007) higher in Choice and Select than Standard. 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 animals categorized as quality grade Choice, Selec and Standard.

	Choice	Select	Standard
Carcass Wt (kg)	339.3^{a}	275.7^{b}	243.1^{b}
Fat Thickness (cm)	1.4^{a}	1.0^{a}	0.5^{b}
Ribeye area (cm^2)	70.9	64.0	66.9
Marbling score^{y}	510^{a}	382^{b}	285^{c}

a,b,c Means within a row with differing superscripts are significantly different (P≤ 0.05)

^{y} Marbling scores: 200 = traces, 300 = slight, 400 = small, 500 = modest, 600= moderate.

^{Z} N= 9.81 kg

The greatest differences in KEGG pathway enrichment was seen in adipose tissue between the carcasses classified as Choice and Standard so this is the data that is focused on here. Many genes were observed to be different between adipose tissue from Standard and Choice carcasses. Upregulated genes, CAB39L, FGF-1, GRIN1, LEP, HK2, YWHAG, ACC1, SCD1 and ELOVL3, were mostly related to the metabolism of fat and energy. This suggests the change from actively growing muscle to fat deposition. Furthermore, down regulation of NGF, RELN, and EIF4EBP1 within the PI3K-Akt signaling pathway and up regulation of EIF4E, YWHAG and FGF1 could affect protein synthesis, cell cycle progression and survival.

CONCLUSION

Many changes were seen in gene expression in adipose tissue between animals that were classified as Choice or Standard. KEGG pathway enrichment indicates some overlap and interaction between the different genes that would suggest opposite effects. Organisms are complex systems, with metabolic processes under multiple levels of regulation so more research is necessary to clarify what these changes mean to determining quality grade.

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