RESEARCH ARTICLE

Ruminal Transcriptomic Analysis of Grass-Fed and Grain-Fed Angus Beef Cattle

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Abstract

Beef represents a major diet component and one of the major sources of protein in human. The beef industry in the United States is currently undergoing changes and is facing increased demands especially for natural grass-fed beef. The grass-fed beef obtained their nutrients directly from pastures, which contained limited assimilable energy but abundant amount of fiber. On the contrary, the grain-fed steers received a grain-based regime that served as an efficient source of high-digestible energy. Lately, ruminant animals have been accused to be a substantial contributor for the green house effect. Therefore, the concerns from environmentalism, animal welfare and public health have driven consumers to choose grass-fed beef. Rumen is one of the key workshops to digest forage constituting a critical step to supply enough nutrients for animals’ growth and production. We hypothesize that rumen may function differently in grass- and grain-fed regimes. The objective of this study was to find the differentially expressed genes in the ruminal wall of grass-fed and grain-fed steers, and then explore the potential biopathways. In this study, the RNA Sequencing (RNA-Seq) method was used to measure the gene expression level in the ruminal wall. The total number of reads per sample ranged from 24,697,373 to 36,714,704. The analysis detected 342 differentially expressed genes between ruminal wall samples of animals raised under different regimens. The Fisher’s exact test performed in the Ingenuity Pathway Analysis (IPA) software found 16 significant molecular networks. Additionally, 13 significantly enriched pathways were identified, most of which were related to cell development and biosynthesis. Our analysis demonstrated that most of the pathways enriched with the differentially expressed genes were related to cell development and biosynthesis. Our results provided valuable insights into the molecular mechanisms resulting in the phenotype difference between grass-fed and grain-fed cattle.

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Background

As the integral part of food production system, cattle not only make valuable contributions to the diversity of human food supply, but also play a main role in nutrient recycling and still constitute a significant work force in some countries. In the next decades, large demand for beef could be foreseeable for most developing countries and particularly for those with large populations and rapid demographic growth rate. Therefore, it is necessary for researchers to enhance the animal productivity through the application of appropriate technologies, particularly in production system and nutrient digestion [1]. Although the increment of meat production is critical for the years to come, the improvement in composition and quality of the beef is also essential. Among the beef characteristics, flavor, as the combination of taste and aroma, is one of the most important factors affecting consumer preference [2]. Nowadays, the market is more demanding for the flavor, quality and composition of beef. Additionally, with regard to the perceived divergence in nutritional quality between grass-fed and grain-fed cattle, growing consumers were interested in grass-fed beef products due to the decreasing fatty acid content [3]. It was reported that grass finished beef had higher concentration of diterpenoids and derivatives of chlorophyll, which changed the aroma and flavor of the cooked beef [4]. In addition, studies on lamb suggested that the meat from concentrate feeding was more tender and juicer than the grass-fed lambs; meanwhile, the carcass was heavier, with more fatty and less liver flavor in animals from concentrate diets [5]. The beef composition also diverged between grass-fed and grain-fed cattle. For instance, beta-carotene was the precursor of retinol (Vitamin A), a fat-soluble vitamin critical for bone growth, cell differentiation and division, and reproduction [6]. As compared to grain-fed animals, pasture-fed cattle had significantly larger amounts of beta-carotene in their muscles. Vitamin E, another fat-soluble vitamin with eight different isoforms, had powerful antioxidant activity [7]. Numerous studies have demonstrated higher concentration of vitamin E in the meat of grass-fed cattle compared with products from concentrate diets [8–10].

In the course of evolution, ruminants developed a forestomach where bacteria, fungi and protozoa disintegrated the forage under anaerobic conditions. Functionally, the reticulum and rumen constituted a unit called reticulorumen, where the ingesta mixed constantly to facilitate microbial digestion. Ruminal pillars projected the aliment into the rumen and the contraction of the walls promoted the circulation of contents in the reticulorumen. Additionally, rumination drove the decrease of size and the increase of density in the particles [11]. This series of processes were necessary to supply adequate nutrition for animals’ maintenance, growth and production. Rumen formed the larger part of the reticulorumen and served as the main site for plant material digestion and microbial fermentation.

Angus, as one of the most famous cattle breeds of the world, contributed to large proportion of beef yield, especially in America. Surrounding it, numerous valuable researches were carried on. For instance, most studies were carried on focusing on rumen microbes and its fermentation effects [12–15]. Whereas, little information about ruminal transcriptome was reported; the molecular mechanism of feed digestion and nutritional absorption remained largely unknown.

In the project, we hypothesize that rumen may function differently under grass-fed and grain-fed regimes, which result in different compositions and flavor of beef. To test it, we choose the ruminal wall tissue as our primary experiment material. The RNA sequencing (RNA-Seq) method was used to identify differentially expressed genes (DEGs) in the ruminal wall of grass-fed and grain-fed bovines. Then, based on the DEGs list, we performed a computational function analysis and found potential mechanisms contributing to the difference between the two groups.
**Materials and Methods**

**Sample collection**

Ruminal wall samples from two randomly chosen animals per group were obtained, totaling four samples. The animals were born, raised and maintained at the Wye Angus farm. This herd, which has been closed for almost 75 years and yielded genetically similar progenies, constitutes an excellent resource to perform transcriptomic analysis. The genetic resemblance among individuals permits us to better control the cause of variation between experimental clusters and individuals. The randomly chosen pairs of animals were part of larger sets of steers that received a particular treatment. All animals received the same diet until weaning. The grain group received conventional diet consisting of corn silage, shelled corn, soy bean and trace minerals. The grass fed steers consumed normally grazed alfalfa; during wintertime, bai-lage was utilized. The alfalfa has been harvested from land without any fertilizers, pesticides or other chemicals. The steers ate no animal, agricultural or industrial byproducts and never receive any type of grain. Then, the calves were randomly assigned to one diet and exclusively received that regimen until termination. Grain—fed animals reached the market weight around the age of 14 month-old, however, grass—fed steers required approximately 200 additional days to achieve the same weight. Immediately after termination at the Old Line Custom Meat Company (Baltimore, MD) a small piece of ruminal wall was excised, cleaned and preserved at -80°C for posterior processing.

**RNA extraction and sequencing**

Total RNA was extracted individually (two animals per group) using Trizol (Invitrogen, Carlsbad, CA, USA) followed by DNase digestion and Qiagen RNeasy column purification (Invitrogen, Carlsbad, CA, USA), as previously described [16]. The RNA sample was dissolved in RNAse-free H2O; the integrity and quality of RNA were then checked by a NanoDrop 1000 spectrophotometer and by resolution on a 1.5% non-denaturing agarose gel. Each library was identified by adding 6-bp adaptors and sequenced at 50 bp/sequence read using an Illumina HiSeq 2000 sequencer, as described previously [17]. Approximately 30 million reads per sample were generated.

**Data analysis and bioinformatics**

After we got the raw sequenced reads data, we checked the quality through FastQC [18], which is an online tool with the capability to report the quality profile of the reads. Then, alignment to the reference genome (Bos_taurus_UMD3.1/bosTau6) obtained from the UCSC (http://genome.ucsc.edu/) was performed employing Bowtie (Ultrafast, memory-efficient short read aligner). During this step, the first 15 bases of each read (50 bp) were trimmed to avoid low Phred quality scores, resulting in 35 bp tags. The counting of reads per gene was executed by the summarizeOverlaps function implemented in R. The identification of differentially expressed genes was achieved employing the edgeR software package and the included generalized linear model (GLM) approach, which requires a design matrix to describe the treatment conditions (grass-fed and grain-fed). In edgeR, an effective library size was computed using a scaling factor based on library sizes. The normalization was model-based and the original counts were not transformed. For variance calculation, edgeR first estimated a common dispersion for all reads and then employed a Bayesian strategy to force the tagwise variation towards the common dispersion, increasing the detection sensitivity. The threshold for calling a differentially expressed gene was false discovery rate (FDR) <0.1.
Through online software David Bioinformatics Resources 6.7, we performed the GO enrichment analysis and analyzed the biological process, cellular component and molecular function associated with the DEGs [19,20]. The enrichment of the GO terms was decided by Fisher’s exact test. Ingenuity Pathways Analysis (IPA, Ingenuity Systems, and www.ingenuity.com) was further utilized to analyze the genetic networks, molecular functions and molecular pathways enriched in the DEGs [21]. IPA, a highly convenient software application, can sanction biologists to classify the pathways, molecular networks and functions most relevant to genes of interest or experimental datasets [22–26]. Fisher’s exact test was utilized to calculate a p-value across the process of IPA analysis.

Quantitative real-time PCR (qRT-PCR) analysis
qRT-PCR was conducted to validate and compare the expression of several randomly selected DEGs found in the RNA-Seq analysis on the iCycler iQ PCR system (Bio-Rad, Hercules, CA, USA). The template cDNA was obtained through the iScript First Strand Synthesis System Kit (Bio-Rad) for reverse transcriptase-PCR with 500 ng of total RNA. The RT-PCR reactions were performed with a QuantiTect SYBR Green PCR Kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions. An online primer system (http://frodo.wi.mit.edu/primer3/) was used to design the primers. Three technical replicates and two independent biological replicates were performed for each product. GAPDH was selected as the control gene [27]. All the primer sequences were listed in S1 Table.

Results
Alignment of sequencing reads
In total, 24,697,373 to 36,714,704 sequence reads were generated per sample. Table 1 summarized the alignment results. For the four samples, the alignment level exceeded 82%. The number of reads in the grain-fed group was greater than that in the grass-fed group; however, the alignment proportion comparison demonstrated the opposite direction (S1 Fig). Sequencing results showed that 24,616 genes could be considered for the analysis (S2 Table). Among these genes, 342 displayed significantly differential expression levels for the FDR less than 0.1 (S3 Table).

Gene expression analysis
The edgeR package implemented in R environment was applied during the statistical analysis to detect the genes with divergent expression profiles in the ruminal wall of grass-fed and grain-fed steers. The threshold criteria to call a significant difference was FDR<0.1. In total, 342 genes with distinct expression levels in both groups were distinguished following this methodology (Fig 1). From these genes, 267 were highly expressed in the ruminal wall of grass-fed bovines compared to the grain-fed group. The other 75 genes were down-regulated in grass-fed steers. The expression level of 82 genes in grass-fed steers ruminal wall was up-regulated with log2FC (fold-change) ≥5. The reads amount of 44 genes in grain-fed group increased

Table 1. Alignment of RNA-Seq Reads to the Bovine Genome.

| Sample | Total reads | Total aligned reads | % Aligned |
|--------|-------------|---------------------|-----------|
| Grass1 | 24697373    | 20914897            | 84.68%    |
| Grass2 | 28894211    | 24923677            | 86.26%    |
| Grain1 | 36714704    | 30181834            | 82.21%    |
| Grain2 | 35576341    | 29870573            | 83.96%    |

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significantly with the log2 FC \geq 5. The top 10 DEGs in the ruminal wall of the two groups were provided in Table 2. Among these genes, the expression levels of GALNT15, MFAP5, ADAMTS15 and RSPO3 were all higher in grain-fed group than that in grass-fed with the log2 FC \geq 5. For the other 6 genes, the expression abundance was lower in grain-fed steers. The whole DEGs discovered between the two groups can be found in S3 Table.

### Validation of DEGs by qPCR

Twelve DEGs were randomly selected and analyzed by qPCR as described previously [28]. The results were then compared to the same genes analyzed by edgeR (Fig 2). Among these twelve genes, all of them were in good agreement for consistency of response. For gene THBS4, the expression level in grain-fed ruminal wall was significantly higher than in grass-fed ruminal wall, and the results of qPCR and RNA-Seq suggested the same direction with almost the same log2 FC value. For the other eleven genes, the abundance level of grass-fed steers was higher

| Ensemble Gene ID     | Symbol   | Log2 FC (grass/grain) | CPM (Grass/Grain) | FDR        |
|----------------------|----------|-----------------------|------------------|------------|
| ENSBTAG00000008240   | GALNT15  | -10.2324              | 87.60/60.11      | 2.22 x 10^{-5} |
| ENSBTAG00000012584   | GJB3     | 10.03519              | 45.47/78.42      | 2.22 x 10^{-5} |
| ENSBTAG0000003484    | F2RL1    | 9.832995              | 29.64/76.81      | 1.42 x 10^{-4} |
| ENSBTAG00000013831   | DSG1     | 10.24195              | 10.94/126.94     | 3.73 x 10^{-4} |
| ENSBTAG00000003010   | MFAP5    | -9.58044              | 51.10/42.88      | 5.73 x 10^{-4} |
| ENSBTAG00000014329   | LOC512548| 10.38487              | 5.89/145.61      | 7.81 x 10^{-4} |
| ENSBTAG00000016857   | ADAMTS15 | -9.47078              | 45.41/41.68      | 8.67 x 10^{-4} |
| ENSBTAG00000008121   | RSPO3    | -9.40866              | 40.54/42.89      | 1.14 x 10^{-3} |
| ENSBTAG00000033510   | MPZL2    | 5.518227              | 124.84/797.15    | 1.17 x 10^{-3} |
| ENSBTAG00000018647   | SLC2A11  | 5.49098               | 67.23/82.15      | 1.34 x 10^{-3} |

Note: CPM means counts per million.

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than grain-fed group. Overall, the validation of the twelve selected genes by qPCR confirmed the accuracy of RNA-Seq analysis results.

**Gene Ontology enrichment analysis**

To explore the specific functional features shared by the DEGs, online software David Bioinformatics Resources 6.7 was used to perform the GO enrichment analysis. Results showed that some biological processes, cellular components and molecular functions were significantly enriched in the DEGs (Table 3). The most significant GO terms in the above three categories were: homophilic cell adhesion in biological process \((P = 9.31 \times 10^{-6})\), plasma membrane in cellular component \((P = 2.00 \times 10^{-8})\) and calcium ion binding in molecular function \((P = 1.54 \times 10^{-2})\). Other enriched GO terms included oxidation reduction, regulation of cell proliferation, negative regulation of cell proliferation, positive regulation of cell differentiation, cell junction, plasma membrane part, enzyme inhibitor activity and carbohydrate binding.

**Pathway enrichment by Ingenuity Pathway Analysis**

Through Fisher’s exact test in the Ingenuity Pathway analysis (IPA) system, we then detected the genes that were involved in the canonical pathways. The 13 most significantly enriched pathways were shown in Table 4. Importantly, majority pathways were related to cell development and biosynthesis. Among them, we emphasized PXR/RXR activation, glutathione redox reactions II, LPS/IL-1 mediated inhibition of RXR function, vitamin-C transport, agranulocyte adhesion and diapedesis, estrogen biosynthesis and triacylglycerol biosynthesis. This result would provide prior knowledge to explain the difference between grass-fed and grain-feed Angus cattle.

**Molecular subnetwork**

With additional criteria that each pathway should have at least 10 DEGs and the pathway’s score should be above 10, a total of 16 significant molecular networks were found by Fisher’s
The pathway’s score was calculated by the transformation from —logP, where P is generated by Fisher’s exact test [29]. Fig 3 showed the four most significant networks. In the first network (Fig 3A), 28 DEGs were observed, and the most important functions of this network consisted of molecular transport and organ morphology. The second network, including 27 DEGs, was enriched with the function of cell-to-cell signaling and interaction, immunological disease and connective tissue disorders (Fig 3B). The third network involved 26 DEGs; the top function of this network was embryonic development, organ development and organismal development (Fig 3C). The fourth network, in which we observed 24 DEGs, was mainly related to the function of drug metabolism and gastrointestinal disease (Fig 3D).

The exact test in the IPA system (S4 Table). The pathway’s score was calculated by the transformation from —logP, where P is generated by Fisher’s exact test [29]. Fig 3 showed the four most significant networks. In the first network (Fig 3A), 28 DEGs were observed, and the most important functions of this network consisted of molecular transport and organ morphology. The second network, including 27 DEGs, was enriched with the function of cell-to-cell signaling and interaction, immunological disease and connective tissue disorders (Fig 3B). The third network involved 26 DEGs; the top function of this network was embryonic development, organ development and organismal development (Fig 3C). The fourth network, in which we observed 24 DEGs, was mainly related to the function of drug metabolism and gastrointestinal disease (Fig 3D).

Table 3. Gene Ontology (GO) terms enriched with differentially expressed genes (GO level > 6).

| GO terms                                      | Observed* | P         | FDR       |
|----------------------------------------------|-----------|-----------|-----------|
| **Biological process**                       |           |           |           |
| GO:0007156: homophilic cell adhesion         | 10        | 9.31x10^-6| 9.40x10^-3|
| GO:0016337: cell-cell adhesion               | 12        | 1.70x10^-5| 8.60x10^-3|
| GO:0055114: oxidation reduction              | 22        | 1.43x10^-4| 4.73x10^-2|
| GO:0007155: cell adhesion                    | 16        | 2.06x10^-4| 5.10x10^-2|
| GO:0022610: biological adhesion              | 16        | 2.06x10^-4| 5.10x10^-2|
| GO:0042127: regulation of cell proliferation | 13        | 2.44x10^-3| 3.90x10^-1|
| GO:0008285: negative regulation of cell proliferation | 8 | 2.60x10^-3| 3.56x10^-1|
| GO:0045597: positive regulation of cell differentiation | 7 | 3.94x10^-3| 4.35x10^-1|
| GO:0006955: immune response                  | 12        | 1.10x10^-2| 7.13x10^-1|
| GO:0051094: positive regulation of developmental process | 7 | 1.23x10^-2| 6.81x10^-1|
| GO:0006611: response to wounding             | 8         | 2.92x10^-2| 8.12x10^-1|
| GO:0006952: defense response                 | 9         | 3.58x10^-2| 8.14x10^-1|
| GO:0008284: positive regulation of cell proliferation | 7 | 4.56x10^-2| 8.38x10^-1|
| GO:0006811: ion transport                    | 14        | 9.31x10^-2| 9.16x10^-1|
| **Cellular component**                       |           |           |           |
| GO:0005886: plasma membrane                  | 51        | 2.00x10^-8| 2.95x10^-6|
| GO:0030054: cell junction                    | 16        | 8.04x10^-6| 5.95x10^-4|
| GO:0005911: cell-cell junction               | 10        | 7.58x10^-5| 3.73x10^-3|
| GO:0044459: plasma membrane part             | 30        | 9.27x10^-5| 3.42x10^-3|
| GO:0005576: extracellular region             | 33        | 3.77x10^-4| 1.11x10^-2|
| GO:0070161: anchoring junction               | 7         | 1.35x10^-3| 3.28x10^-2|
| GO:0031012: extracellular matrix             | 10        | 7.87x10^-3| 1.22x10^-1|
| GO:004421: extracellular region part         | 17        | 8.73x10^-3| 1.22x10^-1|
| GO:0005578: proteinaceous extracellular matrix| 9         | 1.42x10^-2| 1.61x10^-1|
| GO:0009986: cell surface                     | 7         | 3.40x10^-2| 3.25x10^-1|
| **Molecular function**                       |           |           |           |
| GO:0005509: calcium ion binding              | 17        | 1.54x10^-2| 7.40x10^-1|
| GO:0030414: peptidase inhibitor activity     | 7         | 1.72x10^-2| 6.99x10^-1|
| GO:0004857: enzyme inhibitor activity        | 8         | 2.03x10^-2| 6.94x10^-1|
| GO:0030246: carbohydrate binding             | 7         | 3.80x10^-2| 8.54x10^-1|

*Number of the differentially expressed genes in the category.

“GO level > 6” means that each GO term in this table contains more than 6 differentially expressed genes of the 342 genes of interest discovered in our study.

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Discussion

Indeed, development of animal genetic improvement and breeding methodology can bring about leaner beef products [30]. Regardless of the genetic makeup, species, age, gender and geographic location, grass and grain rations of the diet can also contribute to remarkable discrepancy in the general fatty acid profile and antioxidant content in the body tissues and lipid depots [8,31,32]. And the potential changes of rumen metabolism function may have effects on the quality and quantity of protein reaching other digestive organs, such as reticulum, small intestine and large intestine; the ratio of protein from the digestion in the rumen may alter as the rumen started to digest the dry feed [33]. The disparate proportion between undegraded feed protein and bacterial protein reaching the lower gut would also lead to the shift of the quality and quantity of the protein available for absorption [33]. Therefore, our research mainly focused on the ruminal wall based on the grass-fed and grain-fed regimen to explore the underlying molecular mechanisms and biopathways.

In response to dietary changes, 60 differentially expressed proteins in sheep ruminal epithelium were detected after two days of hay-fed and concentrate-fed; 6 weeks later, only 14 proteins displayed disparate expression level [34]. By altering the dietary plane of nutrition, Aisha et al. detected 208 genes with distinct expression level in the ruminal epithelium tissue of young Holstein calves, which lead to a strong transcriptomic response [35]. In our present study, 342 DEGs were found between grass-fed and grain-fed ruminal wall of Angus cattle. Seventy-eight percent of these genes displayed significantly higher expression level in grass-fed steers. This might be explained by that the distinct feed style caused the transcriptome difference. Studies also suggested that, compared to grain-finished beef, grass-finished beef had higher concentration of beta-carotene [36], glutathione [37] and less total fat [3]. Our analysis indicated that, among the top 10 DEGs, DSG1 is related to embryonic, organ and organismal development; RSPO3 is related to abnormal morphology and organismal death. According to previous studies, the structure characterization of DSG1 encoding the pemphigus antigen has been analyzed [38]; in the absence of DSG1, the phosphorylation of the RNA polymerase II carboxy-terminal domain may be transformed, which would affect the recruitment of RNA processing machinery [39]. RSPO3 is a novel protein in the Wnt signaling pathway, which was one of the key pathways controlling cell differentiation, cell proliferation and morphogenesis.

| Ingenuity Canonical Pathways                              | Observed* | P value | FDR  
|----------------------------------------------------------|-----------|---------|------|
| Granulocyte adhesion and diapedesis                       | 8         | 0.0024  | 0.4332|
| PXR/RXR activation                                       | 5         | 0.0025  | 0.2229|
| Glutathione redox reactions II                           | 2         | 0.0035  | 0.2088|
| Ascorbate recycling (cytosolico)                         | 2         | 0.0089  | 0.3960|
| LPS/IL-1 mediated inhibition of RXR function            | 8         | 0.0098  | 0.3519|
| Retinoate biosynthesis I                                 | 3         | 0.0118  | 0.3520|
| Glutathione-mediated detoxification                      | 3         | 0.0147  | 0.3759|
| Triacylglycerol biosynthesis                             | 3         | 0.0215  | 0.4811|
| Estrogen biosynthesis                                    | 3         | 0.0242  | 0.4813|
| Vitamin-C transport                                      | 2         | 0.0310  | 0.5549|
| Glutathione redox reactions I                            | 2         | 0.0367  | 0.5972|
| Agranulocyte adhesion and diapedesis                     | 6         | 0.0375  | 0.5594|
| CDP-diaclyglycerol biosynthesis I                        | 2         | 0.0459  | 0.6320|

*Number of the differentially expressed genes in the category.
Till now, there is little information about the function of these two genes, which may be the potential genetic factors that gave rise to the different carcass and growth rate between grass-fed and grain-fed cattle.

After identification of DEGs, pathway analysis was performed to better understand the biological function of the DEGs in the context of the regulatory system. Providing the information about the molecular networks and the pathways enriched in the DEGs, it becomes possible for us to explore the gene action and regulation, searching for the explanation of the underlying molecular mechanism in the discrepancy between the two groups. IPA analysis showed that the DEGs GLRX, GSTO1 were involved in the canonical pathway vitamin-C transport, which may alter beef color, lipid stability and fatty acid composition between grass-fed and grain-fed cattle [41]. In our study, molecular network analysis elucidated that there were 26 DEGs involved in the third significant network; the prior function of this network consisted of embryonic, organ and organismal development. Between grass-fed and grain-fed cattle, studies have demonstrated that the carcass composition was also different [42].

Fig 3. The top four molecule networks identified by Ingenuity Pathway Analysis (IPA). A: The most significant molecular network by IPA pathway analysis. B: The second most significant molecular network. C: The third most significant molecular network. D: The fourth most significant molecular network.

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to grain-fed beef, glutathione was particularly high in grass-fed beef [3]. Our studies indicated that, between grass-fed and grain-fed cattle, the differentially expressed gene GSR was involved in the pathways glutathione redox reactions II and glutathione redox reactions I; another two DEGs GLRX and GPX7 respectively functioned in glutathione redox reactions II and glutathione redox reactions I. Accordingly, it might be intriguing to perform functional experiment of these genes on cattle to better comprehend the mechanisms causing the varied production performance.

Our results might be informative to explain the molecular mechanisms leading to the differences between grass-fed and grain-fed cattle, including the beef color, fatty acid content, vitamin concentration and carcass. However, there are still some limitations in our current work. The DEGs and the follow-up pathway/network analysis were conducted merely relying on the computational strategies; extensive experimental validation work is still needed. Thus, overexpressing and inhibiting the important differential genes in the pathways or networks could be considered for the functional validation, which can provide more supportive and valuable evidence for our findings.

Conclusions

Through genome-wide RNA-Seq of the genes expressed in the ruminal wall of grass-fed and grain-fed Angus cattle, we were able to identify the genes and pathways that may affect the growth and meat quality traits of cattle. Totally, 342 DEGs were discovered between grass-fed and grain-fed cattle. Combining network and differential gene expression analysis, we detected the genes related to embryonic and organ development, organismal development and death, including DSG1 and RSPO3. According to those DEGs, a total of 16 significant molecular networks, involved in organ morphology, immunological disease, embryonic development, organ and organismal development, were found in the IPA system. Most of the pathways enriched in the DEGs were associated with cell development and biosynthesis. While expanding the scope of future studies with putative genes relevant to bovine growth and meat quality traits, our findings provided more insights into the mechanisms to enhance the productivity of animals.

Supporting Information

S1 Fig. Alignment of RNA-Seq reads to the Bovine Genome.
(TIF)

S1 Table. Primers used for quantitative real-time PCR validation.
(XLS)

S2 Table. Common genes shared by all samples.
(XLS)

S3 Table. Differentially expressed genes in the ruminal wall of grass-fed and grain-fed cattle at a strict false discovery rate (FDR) < 0.1.
(XLS)

S4 Table. The 16 most significant molecular networks found by Fisher’s exact test in the IPA system.
(XLS)
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Author Contributions

Conceived and designed the experiments: JS. Performed the experiments: CZ YL. Analyzed the data: JAC YD YH. Contributed reagents/materials/analysis tools: LZ. Wrote the paper: YL JS.

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