ISSN 2616-5910 Vol. 5, Issue 1: 63-76, DOI: 10.25236/AJALS.2024.050110

Key Differentially Expressed Genes in Qinghai Yak Meat: A Comparative Transcriptome Study

Yunna Liu[1,a], Rong Hu[2,b], Zhongxin Yan[2,3,4,c,*], Haiyue Wu[2,d], Qing Lin[1,e]

[1]*College of Agriculture and Animal Husbandry, Qinghai University, Xining, China*
[2]*Qinghai Academy of Animal Science and Veterinary Medicine, Qinghai University, Xining, China*
[3]*Qinghai Engineering Research Center of Yak, Xining, China*
[4]*Key Laboratory of Plateau Grazing Animal Nutrition and Feed Science of Qinghai Province, Xining, China*
[a]*912534809@qq.com*, [b]*451189962@qq.com*, [c]*421312454@qq.com*, [d]*35490084@qq.com*, [e]*2416334119@qq.com*
[*]*Corresponding author*

Abstract: *Yak meat is extremely rich in protein, amino acids, and trace elements such as calcium and phosphorus. Due to its low-fat content, high calories, and good nutritional value, yak meat is a preferred choice for consumers. However, different cuts of yak meat vary in taste and nutritional properties. This study examined the gene expression profiles and related major signaling pathways that differ across three cuts of Qinghai yak meat: the lateral spine cut (12/13th rib to the last lumbar vertebra region), shoulder cut (front shoulder blade and upper part of front legs) and cucumber strip cut (posterior femur and lateral ischium). The yak meat samples from the three cut sites were collected from the same breed of adult yaks to extract total RNA and subsequently prepare the corresponding cDNA libraries. These libraries were used for transcriptome analysis using Illumina MiSeq high-throughput sequencing technology. In total, 28.84 Gb of sequencing data was obtained including 152,227,776 pairs of two-end reads. The Gene Ontology functional annotation of differentially expressed genes (DEGs) among the meat samples from three different cuts of yak meat revealed significant differences mainly related to the functions of cytokines, protein transport, and metabolic enzymes. Furthermore, the Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis indicated that DEGs were enriched in ribosomes, oxidative phosphorylation, MAPK, and nitrogen metabolism pathways. Particularly, the role of genes including Ndufa3, Ndufa12, Cox513, COX2, COX5B, CA14, CA3, and CA7 that participate in regulating yak meat quality and flavor material metabolism were highlighted. These screened genes may be responsible for the variation in flavor and quality of 3 different cuts of yak meat. Our results help annotate the yak meat genome and can be used to improve yak meat quality.*

Keywords: *yak meat, transcriptome, high-throughput sequencing, differential genes, biological analysis*

1. Introduction

Yak, famously called the "boat of the plateau", is a unique and important cattle species in China [1]. China is rich in yak resources, and yak is a large native mammal living in the plateau area[2]. Worldwide, there are more than 20 million yaks; 95% of these are found in the alpine region of China, mainly in the Qinghai-Tibet plateau region [3]. Yak distribution areas are characterized by extreme environmental conditions, such as an annual low temperature of about 0 ℃, a huge day and night temperature difference of about 15 ℃, a short forage growth season of 110-135 days, strong radiation of over 140~195 KJ/cm2, low oxygen partial pressure below 110 mm Hg, etc. Due to the special ecological environment and strong natural selection, yaks have tenacious vitality with unique physical morphological structures and physiological mechanisms that make them resistant to coarse material, cold, and hypoxic environments [4]. Yak meat is protein-rich but has low-fat content exhibiting good meat color and unique flavor [5], making it a popular choice among consumers. Yak not only differs from other cattle breeds in body performance and physical form but also has unique meat quality [6-7], allowing the production of nutritional meat products. Most past research on meat quality often focused on the appearance, nutrition, flavor, hygiene, and other physical and chemical properties after animal slaughter and processing. With the advancement of omics technologies, recent studies have begun to

ISSN 2616-5910 Vol. 5, Issue 1: 63-76, DOI: 10.25236/AJALS.2024.050110

understand the molecular factors determining meat quality such as in pig and sheep breeds[8][9][10].

Nevertheless, the molecular level quality of Qinghai yak meat has rarely been examined. With the development of high-throughput sequencing technology, scientific research has entered the post-genomic era utilizing omics technologies such as transcriptomics and proteomics [11]. Transcriptomics studies focus on factors that can modify gene expression profiles [12-13], involving transcriptional regulators such as protein-coding mRNAs and non-coding mRNAs. Wang et al [14] used transcriptomics to study regulatory differential genes determining intramuscular fat (IMF) content through regulating lipid metabolism between Jinnan cattle and Simmental cattle such as *CYP21A2*, *PC*, *ACACB*, *APOA1*, and *FADS2*). Sun et al [15] used transcriptomics to reveal key transcriptional regulators related to muscle growth and development between merino sheep and small-tail cold sheep. They identified 960 differentially expressed genes (DEGs) between the two breeds including the *MRF*, *GXP1*, and *STAC3* genes that play an important role in muscle growth and development. Xiaoyun et al. [16] studied differential metabolites between oxidized skeletal muscle and glycolytic skeletal muscle of yaks. They found numerous lncRNA-regulated demRNA and circRNAs related to the PPAR signaling, citric acid cycle (TCA cycle), and muscle fiber type transformation pathways, resulting in characteristic metabolic differences between oxidized muscle and glycolytic muscle. Gui et al [17] found that CEBP-β transcriptionally activates CEBP-α and *FABP4* in yak adipose tissue, regulating the cellular transport of fatty acids. Gui et al. [18] examined genetic polymorphisms in growth hormone 1 (GH1) in Chinese yaks, discovering its link to yak growth traits. The SH2B2 gene influences energy balance and weight by enhancing cytokine, leptin, and GH signaling. Four SH2B2 gene polymorphisms were studied in Qinchuan cattle, showing associations with body measurements [19].

Notably, similar to beef, different cuts of Yak meat, such as sirloin, chuck, brisket, neck, etc., are used for various meat preparations. Yak cuts in different regions have different meat quality due to their distinct physiological function and unique muscle fiber distribution. These portions vary in nutritional composition (such as fatty acid profile), cooking method, taste, and flavor, influencing consumer choice. Uncovering molecular patterns responsible for these changes can help improve the yak meat quality. Therefore, in this study, three different cuts of Qinghai yak were subjected to transcriptomic analysis to reveal key genes determining meat quality. Our results provide a basis for further metabolic analysis to understand the quality traits of yak meat.

2. Materials and Methods

2.1 Test materials and equipment

Nine healthy grazing bulls (aged 36±2 months, living weight 248.6±16.7 kg) were selected. After slaughter, meat samples from three distinct regions, shoulder meat (front shoulder blade, upper front leg; named JR), outer spinal pork (12/13th rib to the last lumbar spine; named WJR), and cucumber strip (back femur, lateral sciatic; named HGT), were transported to the laboratory for analysis. The meat samples for transcriptomics were obtained within 60 min of slaughter, cleaned with buffer, and transported back to the laboratory in liquid nitrogen, where they were stored at -80 ℃.

Equipment: Frozen centrifuge (Thermo Fisher, China); UV1900 UV spectrometer (Beijing Ruili Analytical Instruments Co., Ltd., China); Agilent2100 bioanalyzer (Experimental Instrument Technology Co., Ltd., China); Sonics ultrasonic crusher (Pengqi Scientific Instrument Co., Ltd., China); Thermomixer (Thermo Fisher, China); PCR instrument (Bio-rad, China); 3100ABI gene sequencer (Applied Biosystems, USA); Illumina Hi Seq2000 sequencing system; gel imager (Bio-rad, China), etc

Reagents: RNA extraction kit, RNA purification kit, DNA Ladder Mark (100 bp), RNA-seq kit, and RNAset-free were purchased from Thermo Fisher, China. A PCR kit was from Annotech Biotechnology, China. All reagents were of analytical grade.

2.2 Experimental Methods

2.2.1 Preparation of cDNA library and transcriptome sequencing

The cDNA library was established by reverse transcription of high-quality muscle tissue RNA and analyzed against the yak reference genome. Sequencing was outsourced to Beijing Bermics. In data processing, the low-quality joints Q10 reads accounted for more than 3% of half the number of whole reads, and "N" bases were evaluated by FastQC software for subsequent analysis.

ISSN 2616-5910 Vol. 5, Issue 1: 63-76, DOI: 10.25236/AJALS.2024.050110

2.2.2 DEGs screening

The DEGs in the three yak meat samples (WJR, JR, and HGT) were screened using the criteria of FPKM (Fragment Per Kilobase of exon model per Million mapped read; [20-21]), with a threshold of > 0.1, fold change 2 or 0.5, p-value 0.01 and FDR (False Discovery Rate) of <0.01. The upregulated and downregulated genes were identified and the gene Venn diagram was created according to the method of Bardou et al [22].

2.2.3 Functional enrichment analysis of DEGs

The Gene Ontology (GO) functional annotations are categorized into three groups: cellular components (CC), molecular functions (MF), and biological processes (BP) [23]. The GO annotation was performed at http://wego.genomics.org. Functional clustering of differential genes was done at cn/cgi/bin/wego/index.pl. the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed using the KEGG platform (http://www.genome.jp/kegg/pathway.htML). The key signal transduction pathways related to DEGs were identified.

2.2.4 Quantitative real-time PCR validation of DEGs

Six differentially expressed genes (3 upregulated and 3 downregulated) were randomly selected for quantitative real-time PCR (qRT-PCR) validation of transcriptome sequencing data using the ABI7300 real-time reaction system. The original sample RNA was reverse transcribed to obtain cDNA using the FastKing RT Kit with gDNase. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was the internal reference gene. The 20 μL qRT-PCR system included 10 μL TB Green Premix Ex Taq II, 0.4 μL ROX Reference Dye II, and 0.8 μL (m 0 l / L), 2 μL cDNA, and 6 μL ddH2O. The reaction conditions were as follows: 95 ℃ for 30 s; followed by 40 cycles of melting at 95 ℃ for 10 s and annealing for 30 s at 65-95 ℃, adding 0.5 ℃ every 5 s to improve the melting curve. Each sample was repeated three times and the relative expression of each gene was calculated using the 2- ΔΔ Ct method.

2.3 Data analysis

2.3.1 Differential expression analysis

The DEGs among yak samples were analyzed by DESeq2 software, and Benjamini and Hochberg method was used to adjust the P-value and control accuracy [24]. DEG analysis between the comparison combinations was performed using the edgeR software package. Corrected P <0.05 and log2Fold Change > 1.0 were used as thresholds for significant differential expression.

3. Results and analysis

3.1 Sequencing data analysis

Table 1: Sequencing data quality.

Sample	Raw_reads	Clean_reads	Clean_bases	Error_rate	Q20	Q30	GC%
WJR_1	45720072	45337658	6.8G	0.03	97.16	92.42	54.97
WJR_2	49317164	48976452	7.35G	0.03	97.2	92.44	54.59
WJR_3	58633632	58048990	8.71G	0.03	97.15	92.33	53.05
JR_1	49701862	48919934	7.34G	0.03	97.23	92.56	55.46
JR_2	52119806	51271628	7.69G	0.03	97.35	92.71	53.64
JR_3	51126132	50524470	7.58G	0.03	97.17	92.38	53.09
HGT_1	53127890	52130912	7.82G	0.03	97.49	92.97	52.15
HGT_2	48383828	47612860	7.14G	0.03	97.22	92.49	52.75
HGT_3	54441498	53860424	8.08G	0.03	97.18	92.37	53.18

Note: Sample, sample name; Raw_reads, number of reads in the raw data; Clean_reads, the number of reads filtered by the raw data; Clean_bases, the number of bases filtered from the raw data (clean bases=clean reads *150bp); Error_rate, the overall sequencing error rate; Q20: percentage of total bases with a Phred value greater than 20; Q30, percentage of total bases with a Phred value greater than 30; GC%, percentage of G and C bases in clean reads.

cDNA libraries (3 replicates per sample) were constructed from three different cut sites of Yak meat to explore their expression profiles (Table 1). In total, 28.84 Gb sequencing data was generated,

ISSN 2616-5910 Vol. 5, Issue 1: 63-76, DOI: 10.25236/AJALS.2024.050110

involving 152,227,776 double reads. The GC content of each sample library ranged from 52.15% to 54.97%, with Q20 and Q30 values of 97.16% and 92.33%, respectively. The overall sequencing error rate of the data was 0.03. These results indicate that the sequencing data were of reliable quality and can be used for subsequent analysis.

3.2 DEGs screening

A Venn diagram showing the numbers of DEGs between pairwise samples from the three Yak cut sites is shown in Figure 1. There were 607 DEGs in the JRvsHGT group (419 upregulated and 188 downregulated genes), 713 DEGs in WJRvsHGT (445 upregulated and 268 downregulated genes), and 295 DEGs in WJRvsJR (132 upregulated and 163 downregulated genes). There were 16 DEGs among the three samples, i.e., JRvsHGTvsWJR.

The DEGs from HGT, WJR, and JR samples were merged and normalized. In the clustering analysis of gene expression, the abscissa is the sample name, and the ordinate is the FPKM normalized value (Figure 2). Red and green colors indicate the upregulation and downregulation of genes. A cluster map of 1261 genes in the three sites indicates significantly different expression profiles in the three samples, with the largest difference between WJR and HGT (WJRvsHGT).

Figure 1: A Venn diagram of DEGs in different cuts of yak meat. WJR, 12/13th rib to the last lumbar spine; JR, front shoulder meat, upper front leg; HGT, back femur, lateral sciatic shoulder.

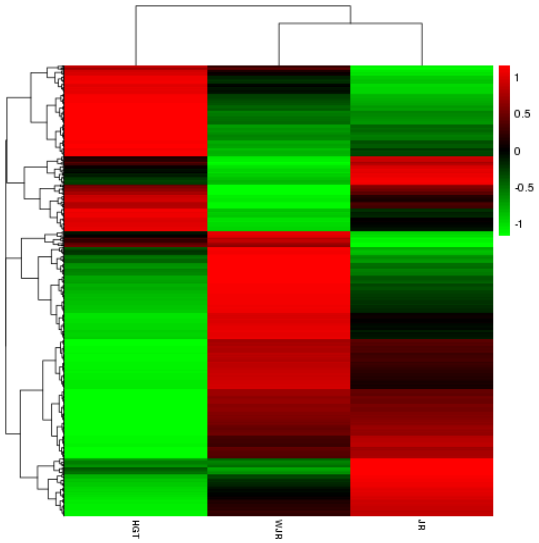

Figure 2: The cluster analysis of differentially expressed genes. The abscissa is the sample name, and the ordinate is the normalized value of FPKM of the differentially expressed gene; the red and green colors indicate higher and lower expressions, respectively.

ISSN 2616-5910 Vol. 5, Issue 1: 63-76, DOI: 10.25236/AJALS.2024.050110

3.3 Functional enrichment analysis of DEGs

3.3.1 GO functional enrichment analysis of DEGs

DEGs were subjected to GO functional classification and annotation in MF, CC, and BP categories. Three control libraries were subjected to the GO enrichment test ($p < 0.05$).

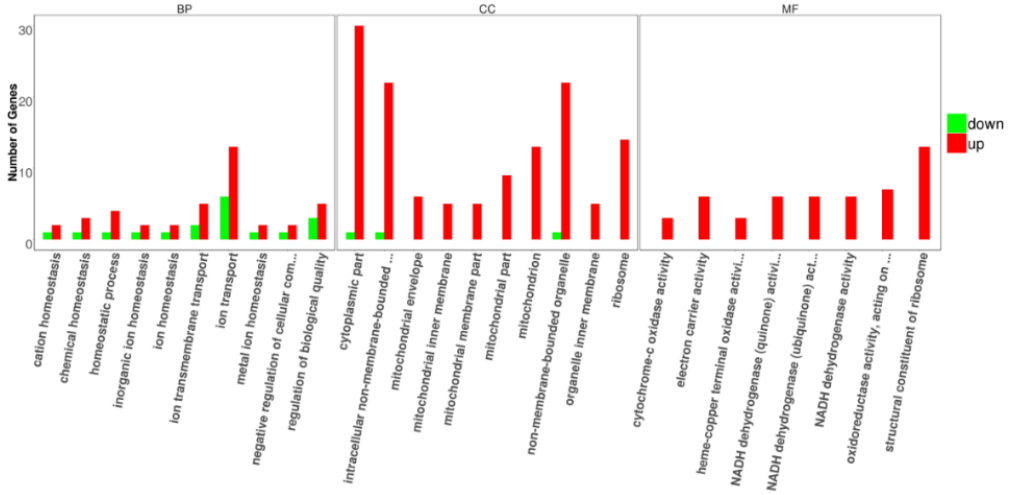

Figure 3 (A): Gene ontology classification of differentially expressed genes in the JRvsHGT comparison group.

In Figure 3 (A), the top BP of DEGs in the JRvsHGT comparison group included biological activity adjustment, biological cell component negative regulation, metal ion transport, ion transport, ion transmembrane transport, inorganic ion homeostasis, homeostasis process, in vivo chemical balance, cationic homeostasis. The top CC were ribosomes, inner membrane organelles, non-bound membrane organelles, mitochondria, mitochondrial components, mitochondrial membrane components, mitochondrial outer membrane, intracellular non-bound membrane organelles, and cytosolic components. The top MF included oxidoreductase activity, acting on NAD(P)H, NADH dehydrogenase activity, NADH dehydrogenase (ubiquinone) activity, oxidoreductase activity, quinone or similar compound as acceptor, NADH dehydrogenase (quinone) activity, electron carrier activity, structural constituent of ribosome, cytochrome-c oxidase activity, heme-copper terminal oxidase activity, oxidoreductase activity, acting on a heme group of donors. Particularly, more ion transport and ion transmembrane transport accumulated in BP, more non-bound membrane organelles and cytosolic components accumulated in CC, and more ribosome structure, component oxidoreductase activity, and nicotinamide coenzyme aggregated in MF categories.

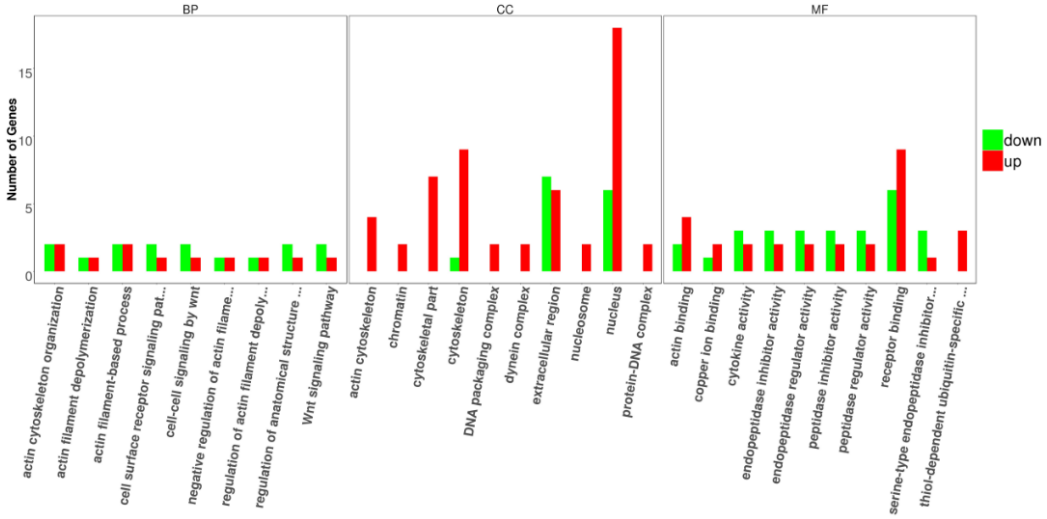

Figure 3 (B): Gene ontology classification of differentially expressed genes in the WJRVvsHGT comparison group.

ISSN 2616-5910 Vol. 5, Issue 1: 63-76, DOI: 10.25236/AJALS.2024.050110

In Figure 3 (B), the top BP of DEGs in the WJRvsHGT comparison group were Wnt signaling pathway anatomy regulation, actin fiber depolymerization regulation, actin fiber depolymerization negative regulation, cell surface receptor signaling pathway, actin basal fiber protrusion, actin fiber depolymerization, and actin cytoskeleton configuration. The top CC included protein-DNA complex, cell nucleus, nucleosomes, extracellular regions, dynein polymer, DNA group, cytoskeleton, cytoskeletal components, chromatin, and actin cytoskeleton. The top MF were sulfate-dependent ubiquitin-specific protease activity, serine-type endopeptidase inhibitor activity, receptor binding, peptide chain endonuclease regulation activity, peptidase inhibitor activity, peptide chain endonuclease regulation activity, peptide chain endonuclease inhibitor activity, cytokine activity, copper ion binding, and dynamic binding. Particularly, there were more clusters of actin basal fiber processes and actin cytoskeleton functions in BP, more nucleus and cytoskeleton functions in CC, and more receptor binding and actin binding functions in MF categories.

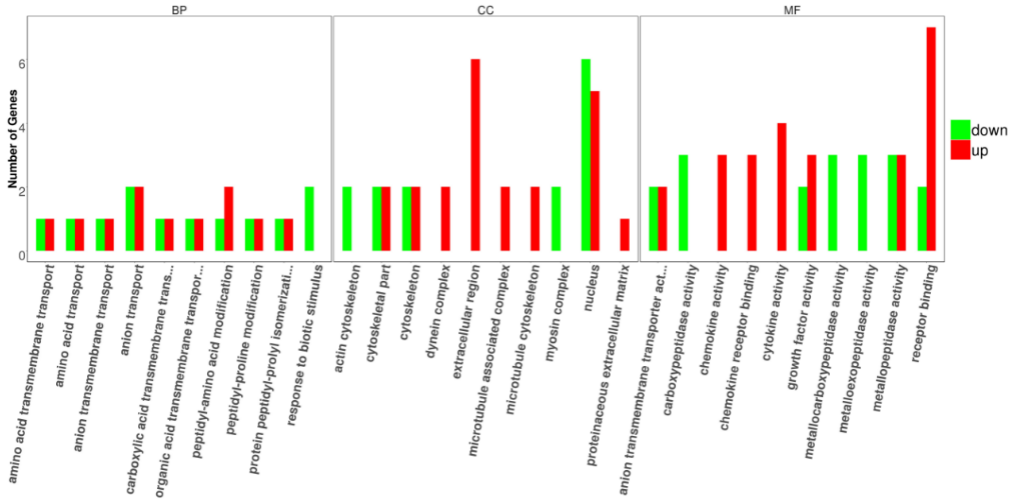

Figure 3 (C): Gene ontology classification of differentially expressed genes in the WJRvsJR comparison group.

As shown in Figure 3 (C), the top BP of DEGs in the WJRvsJR comparison group were biological stress response, peptidyl-prolyl isomerase, proline peptidyl modification, amino acid peptidyl modification, organic acid transmembrane transport, carboxyl transmembrane transport, anion transport, anion transmembrane transfer, amino acid transport, and amino acid transmembrane transport. The top CC included extracellular matrix proteins, nucleus, sarcomososin polymer, tubulin skeleton, microtubule-associated protein polymers, extracellular regions, dynein polymer, cytoskeleton, cytoskeletal components, and actin skeleton. The top MF were receptor binding, metallopeptidase activity, metalloexopeptidase activity, metallocarboxypeptidase activity, growth factor activity, cytokine activity, chemokine receptor binding, chemokine, carboxypeptidase activity, and anion transmembrane transporter activity. Particularly, anion transport and amino acid peptidyl modifications were highlighted in BP, nucleus and extracellular regions in CC, and receptor binding and cytokine activity in MF categories.

Concisely, the biological functions of DEGs varied in different yak cuts potentially influencing their quality, flavor, and tenderness.

3.3.2 KEGG pathway enrichment analysis of DEGs

The KEGG pathway enrichment analysis revealed the 20 most significant pathways related to DEGs in three types of yak meat (p-value < 0.05, Figure 4).

ISSN 2616-5910 Vol. 5, Issue 1: 63-76, DOI: 10.25236/AJALS.2024.050110

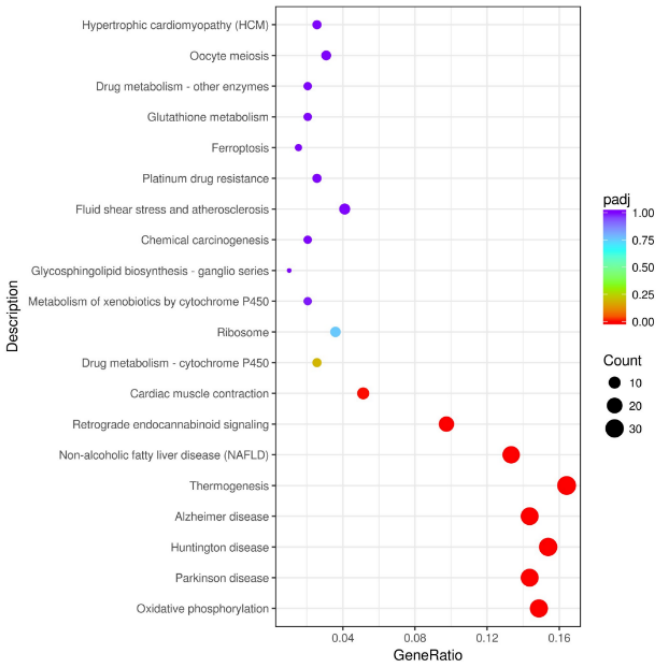

Figure 4 (A): The top 20 KEGG pathways are shown in a bubble diagram in the JRvsHGT comparison group.

Figure 4 (A) shows the top 20 KEGG-enriched metabolic pathways in the JRvsHGT comparison group, including oxidative phosphorylation, necrotizing apoptosis, retrograde nerve signaling, and cardiac muscle contraction. Particularly, more DEGs were related to ribosomes and the oxidative phosphorylation metabolic pathways in the JRvsHGT group.

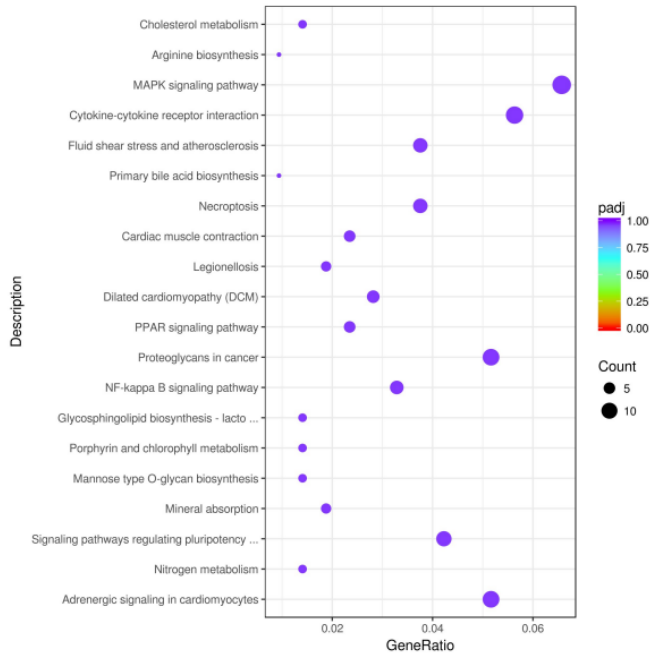

Figure 4 (B): The top 20 KEGG pathways are shown in a bubble diagram in the WJRvsHGT comparison group.

In the WJRvsHGT comparison group (Figure 4 (B)), none of the KEGG pathways of DEGs showed significant enrichment. Nevertheless, some of the important enriched pathways were adrenergic signaling in cardiomyocytes, the signaling pathway regulating the pluripotency of stem cells, the NF-kappa B signaling pathway, and particularly the MAPK signaling pathway.

ISSN 2616-5910 Vol. 5, Issue 1: 63-76, DOI: 10.25236/AJALS.2024.050110

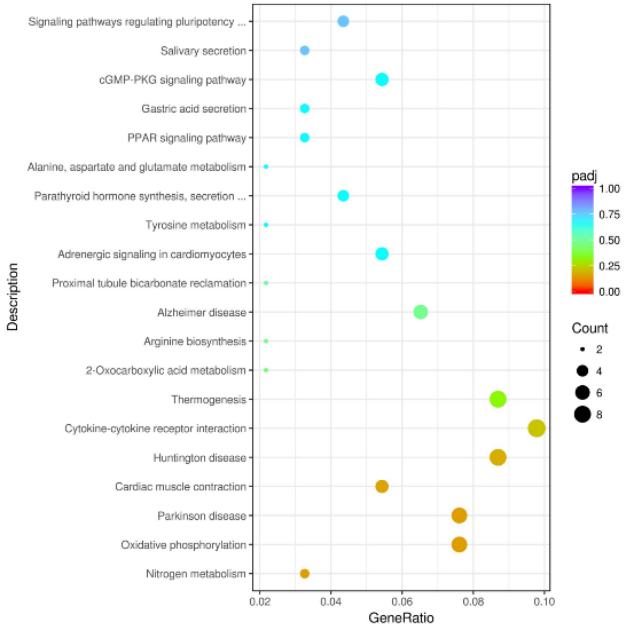

Figure 4 (C): The top 20 KEGG pathways are shown in a bubble diagram in the WJRvsJR comparison group.

As shown in Figure 4 (C), there were no significant KEGG-enriched metabolic pathways in the WJRvsJR comparison group. Nevertheless, some of the important enriched pathways were oxidative phosphorylation, cytokine receptor interactions, and nitrogen metabolism pathways.

3.3.3 Differential gene pathway

In the JRvsHGT group, ribosomes and oxidative phosphorylation were the most enriched metabolic pathways. The ribosomal metabolic pathways are shown in Figure 5. Among them, L3e, L36, L11, S18, L27, L15e, L24e, and other ribosome-related genes were slightly upregulated. The key oxidative phosphorylation metabolic pathways are shown in Figure 6, where Ndufa3, Ndufa12, and Cox51B were significantly upregulated, influencing the quality of yak meat. The other oxidative phosphorylation related genes such as Ndufs3, Ndufs4, Ndufs6, Ndufs8, Ndufa1, Ndufa2, Ndufa13, Ndufb1, Ndufb2, Ndufb4, Ndufb6, Ndufb7, Ndufb8, Ndufb9, Ndufb10, QCR6, QCR9, COX4, COX6A, COX6B, COX7A, c, e, and f6/h were slightly upregulated.

Similarly, the most affected genes in the WJRvsHGT group were related to MAPK metabolic pathway (Figure 7), where NIK, NFKB, Tau, RasGRP, MNK1/2, MKP, FLNA, MKP, and HSP27 were upregulated, and GF, IL1, and MAPK 2 were downregulated.

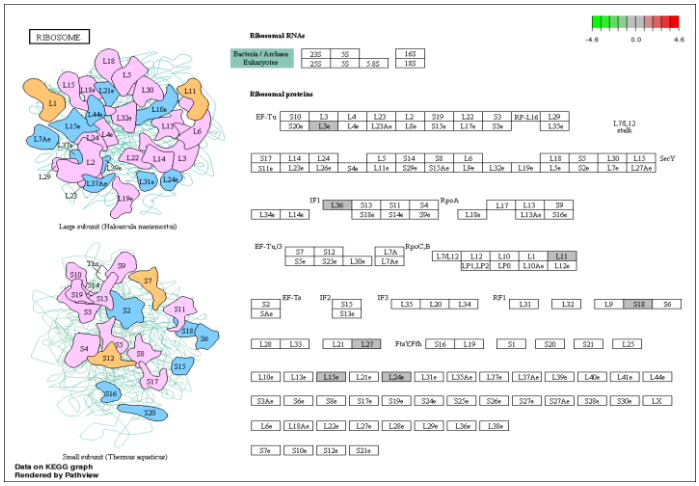

Figure 5: The map of the ribosome pathway in KEGG analysis.

ISSN 2616-5910 Vol. 5, Issue 1: 63-76, DOI: 10.25236/AJALS.2024.050110

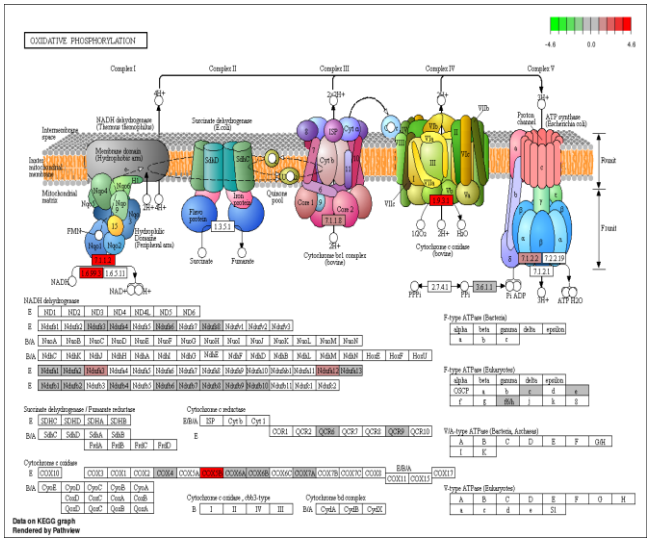

Figure 6: The map of the oxidative phosphorylation pathway in KEGG analysis.

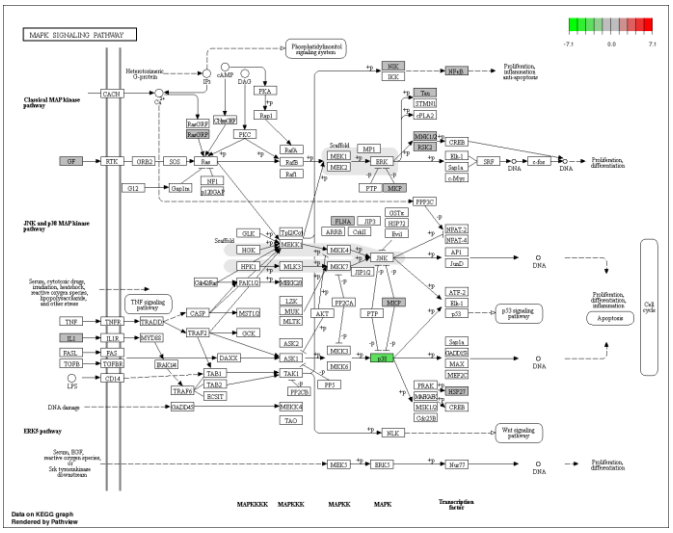

Figure 7: The map of the MAPK signaling pathway in KEGG analysis.

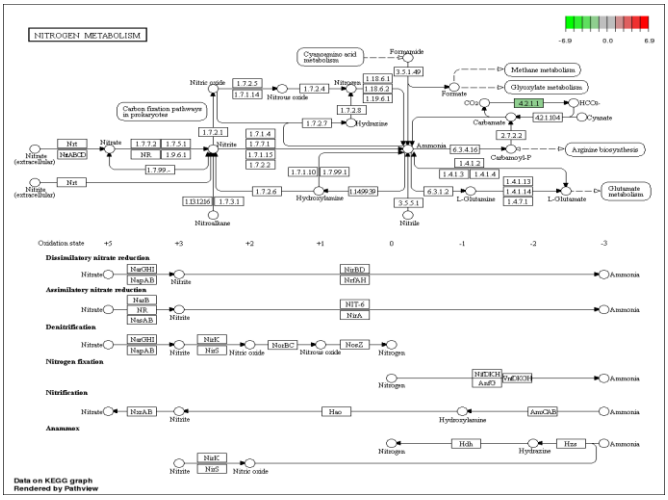

Figure 8: The map of the nitrogen metabolism pathway in KEGG analysis.

ISSN 2616-5910 Vol. 5, Issue 1: 63-76, DOI: 10.25236/AJALS.2024.050110

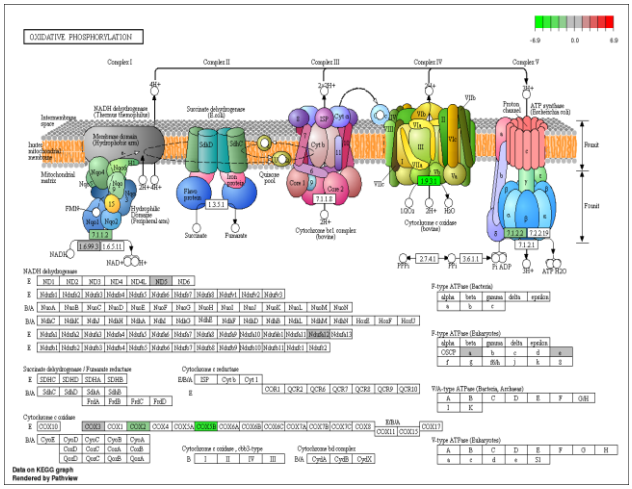

Figure 9: The map of the oxidative phosphorylation pathway in KEGG analysis

Table 2: Results of partial enrichment of KEGG pathway with corresponding DEGs in the three comparison groups.

N0.	Description	Gene Ratio	Differentially expressed genes	P-value	P.adj
JRvsHGT					
1	Oxidative phosphorylation	29/195	*NDUFS6*, *NDUFB8*, *NDUFA2*, *COX7A1*, *LOC101906178*, *UQCR10*, *LOC101906363*, *NDUFA13*, *COX4I1*, *LOC100296311*, *NDUFA1*, *NDUFB9*, *COX6B1*, *NDUFB2*, *NDUFB10*, *NDUFS8*, *COX6A2*, *UQCRH*, *NDUFB4*, *NDUFB1*, *NDUFS4*, *NDUFA3*, *NDUFB6*, *NDUFS3*, *ATP5PF*, *ATP5MC1*, *ATP5ME*, *NDUFB7*, and *LHPP*	1.842	4.144
2	Ribosome	7/195	*MRPS18A*, *MRPL11*, *MRPL27*, *RPL24*, *RPL3L*, *MRPL36*	0.034	0.757
WJRvsHGT					
1	MAPK signaling pathway	14/213	*FGF7*, *FLNC*, *MAPT*, *ANGPT1*, *DUSP10*, *NFKB2*, *HSPB1*, *IL1B*, *BDNF*, *MAPK13*, *MAP3K14*, *RASGRP4*, *RPS6KA3*, and *MKNK2*	0.128	0.970
WJRvsJR					
1	Nitrogen metabolism	3/92	*CA14*, *CA3*, and *CA7*	0.001	0.150
2	Oxidative phosphorylation	7/92	*ATP6*, *COX3*, *ATP5ME*, *COX2*, *ND5*, *LOC100296311*, and *LOC101906178*	0.002	0.150

The most affected metabolic pathways in the WJRvsJR group were nitrogen metabolism (Figure 8 and Table 2) and oxidative phosphorylation (Figure 9). The key DEGs in the nitrogen metabolism pathway were CA14, CA3, and CA7. In the oxidative phosphorylation metabolism pathway, COX2 and COX5B were significantly downregulated, while ND5, Ndufa12, A, e, and COX3 were slightly downregulated.

Using KOBAS (KEGG Orthology-Based Annotation System), KEGG analysis revealed DEGs enrichment with 500 pathways, including 195 in the JRvsHGT group, 213 in the WJRvsHGT group, and 92 in the WJRvsJR group. The top 20 metabolic pathways selected based on the small to large P values are shown in a scatter plot in Figure 4 and the details are listed in Table 2.

3.4 Validation of sequencing data by RT-qPCR

To verify the reliability of the RNA-Seq data, six DEGs (three upregulated and three downregulated genes) were randomly selected for RT-qPCR validation (Figure 10). The selected genes were HOXC8 and LOC112446667 from the WJRvsJR group; BARX2, HOXA13, and HOXA11 from the JRvsWJR group; and LOC112441511 from the WJRvsJR group. The RT-qPCR results were consistent with the

ISSN 2616-5910 Vol. 5, Issue 1: 63-76, DOI: 10.25236/AJALS.2024.050110

RNA-Seq data, indicating the reliability and reproducibility of transcriptome results.

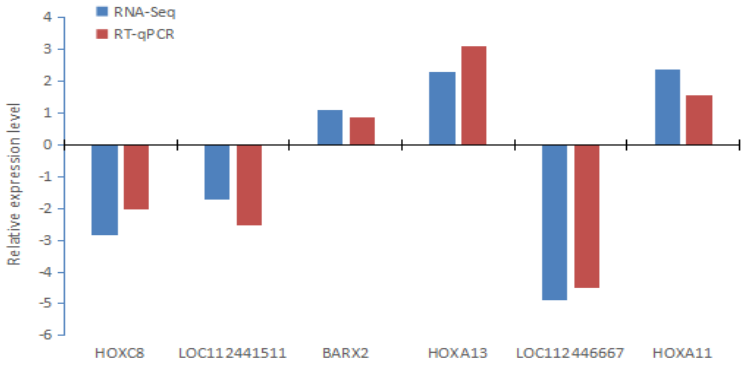

Figure 10: RT-qPCR validation of selected DEGs from RNA-seq data.

4. Discussion

The quality and flavor of meat are important determinants of consumer choice, affecting the economies of the poultry and meat food industries. Meat flavor is closely related to precursor substances such as fat, amino acids, nucleotides, and organic acids. Differences in the content of these flavor substances provide differential flavors to various meat cuts, such as in cattle meat [25][26]. Transcriptome technology, utilizing high-throughput sequencing, can help us understand the genetic basis of these differences among different meat portions. Here, we conducted transcriptome analysis on three cuts of yak meat to screen out key DEGs and enriched pathways affecting their flavor and quality. Our results aim to help improve the quality of yak meat. Through pairwise comparison of transcriptome DEGs in three samples, we found 419 upregulated and 188 downregulated genes in the JRvsHGT group, 445 upregulated and 268 downregulated genes in WJRvsHGT, and 132 upregulated and 163 downregulated genes in WJRvsJR. Notably, 16 DEGs were common in all three comparison groups, i.e., JRvsHGTvsWJR (Figure 1). This indicated a relatively smaller difference in JRvsHGT (607 DEGs) and WJRvsJR (295 DEGs) groups, while the difference was more significant in the WJRvsHGT group (713 DEGs).

The GO and KEGG pathway functional enrichment analysis of DEGs can provide insight into the biological functions of the genes and related metabolic pathways. GO functional enrichment analysis revealed that many DEGs were involved in BP, such as cell binding, metabolism, cellular processes, etc. These results are consistent with Damon et al [27] showing that interactions between DEGs lead to divergent meat quality phenotypes affecting muscle metabolism and structure in pigs. The KEGG pathway annotation analysis revealed that key DEGs were involved in important signaling pathways related to ribosomes, oxidative phosphorylation, MAPK, and nitrogen metabolism, influencing meat flavor and quality.

The MAPK pathway is not only important for adipocyte proliferation and differentiation [28] but also participates in myofiber development, affecting meat quality traits by regulating muscle fiber types [29-30]. In mammals, MAPK kinases can be divided into three families: ERKs, JNKs, and p38/SAPKs [31]. Different extracellular signals inducing the MAPK pathway can elicit different cellular responses. Moreover, the MAPK pathway can interact with other ligand-induced signaling pathways to coordinate a complex set of cellular events that determine the cellular response [32]. In this study, we identified FGF7, FLNC, MAPT, and ANGPT1 genes related to the MAPK pathway that might influence yak meat quality.

There are two ways to generate required ATPs for skeletal muscle movement: glycolysis and oxidative phosphorylation [33]. Oxidative phosphorylation occurs in the mitochondria and generates a proton electrochemical gradient during the respiratory electron transport chain. This gradient is then utilized to drive ATP synthesis by ATP synthase. The activity of glycolysis and oxidative phosphorylation varies in different tissue regions. Chen et al [34] found that the LDH activity in the longest dorsal length muscle was significantly lower, while the activities of succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) were significantly higher in Bama pigs than in large white pigs. In this study, NDUFS6, NDUFB8, and NDUFA2 and other genes of the oxidative phosphorylation metabolic pathway in JR and HGT samples may have influenced yak meat quality. Additionally, ATP6, COX3, and ATP5ME related to oxidative phosphorylation metabolic pathways in

ISSN 2616-5910 Vol. 5, Issue 1: 63-76, DOI: 10.25236/AJALS.2024.050110

WJR and JR cuts may have influenced the yak quality.

Ribosomes are responsible for cellular protein synthesis and are composed of many proteins with different functions. RplB, located near the peptidyltransferase activity center (PTC) in the 50S subunit, participates in the binding of tRNA to the A and P positions. RplD plays an important role in the assembly and functioning of the 50S subunit and regulates the genes of its operon. In this study, some genes encoding ribosomal constituent proteins were differentially expressed likely due to variation in ribosome content at different muscle sites. Notably, the gene expression of MRPS18A, MRPL11, MRPL27, RPL24, RPL3L, and MRPL36 was increased. We reasoned that the changes in the ribosomal protein expression can affect ribosome structure and function, subsequently impacting protein expression, and thus the yak meat quality.

Several in vitro and in vivo studies showed that amino acids and insulin-like growth factor-1 (IGF 1) can promote the synthesis and utilization of animal skeletal myin and mammary protein through the mammalian target of rapamycin (mTOR) signaling pathway. In this study, the main DEGs affecting nitrogen metabolism were CA14, CA3, and CA7, suggesting they may influence yak meat quality.

5. Conclusion

In this study, using high-throughput sequencing, we identified key DEGs from three yak cuts that influence meat characteristics. Specifically, DEGs such as *Ndufa3*, *Ndufa12*, *Cox513*, *COX2*, *COX5B*, *CA14*, *CA3*, and *CA7*, as well as key enriched pathways such as ribosomal, oxidative phosphorylation, MAPK, and nitrogen metabolism, determine the site-specific meat features through regulating muscle growth and development. Our work lays the foundation for further metabolic studies aimed at understanding the molecular details of yak meat quality.

Acknowledgements

This study was supported by the National Key R&D Program of China（2022YFD1602304）, Qinghai Provincial Science and Technology Programmes (2023-NK-135) and Qinghai Province "Kunlun Talents-Science and Technology Leading Talents" Program (2023).

Ethics Approval

All animal treatments have been approved by the Laboratory Animal Management Committee and the Institutional Animal Care and Use Committee of Qinghai University. Nine healthy grazing male yaks with a live weight of (248.6 ± 16.7) kg, aged 36 ± 2 months, were selected from the Qinghai Lake area.. Free feeding and humane slaughter at the National Beef Cattle Improvement Center. According to the National Regulations on the Administration of Experimental Animals (Order No. 2 of the National Science and Technology Commission of the People's Republic of China, 1988) and the Measures for the Administration of Laboratory Animal Licenses (Trial) (Guo Ke Fa Cai Zi (2001) No. 545), all institutional and government regulations shall be followed.

References

[1] Wang Yongjie,Wang Zhisheng,Hu Rui,Peng Quanhui,Xue Bai,Wang Lizhi. Comparison of carcass characteristics and meat quality between Simmental crossbred cattle, Cattle-yaks, and Xuanhan yellow cattle.[J]. Journal of the science of food and agriculture,2020,101(9).
[2] Ruan Chong-Mei,Wang Jing,Yang Yong-Xin,Hu Jun-Jie,Ma You-Ji,Zhang Yong,Zhao Xing-Xu. Proteomic analysis of Tianzhu White Yak (Bos grunniens) testis at different sexual developmental stages.[J]. Animal science journal = Nihon chikusan Gakkaiho,2019,90(3).
[3] Bu Xinrong,Wang Huihui,Wang Yujiao,Ojangba Theodora,Nan Huaili,Zhang Li,Yu Qunli. Effects of iron-catalyzed oxidation and methemoglobin oxidation systems on endogenous enzyme activity and myofibrillar protein degradation in yak meat[J]. Food Chemistry,2023,404(PB).
[4] Sapkota S.,Acharya K. P.,Laven R.,Acharya N.. Possible Consequences of Climate Change on Survival, Productivity and Reproductive Performance, and Welfare of Himalayan Yak (Bos grunniens)[J]. Veterinary Sciences,2022,9(8).
[5] Jia-Chun Tian,Ling Han,Qun-Li Yu,Xi-Xiong Shi,Wen-Ting Wang. Changes in tenderness and cathepsins activity during post mortem ageing of yak meat[J]. Canadian Journal of Animal Science,

ISSN 2616-5910 Vol. 5, Issue 1: 63-76, DOI: 10.25236/AJALS.2024.050110

2013,93(3).
[6] Van Donkersgoed J,Jewison G,Bygrove S,Gillis K,Malchow D,McLeod G. Canadian beef quality audit 1998-99.[J]. The Canadian veterinary journal = La revue veterinaire canadienne,2001,42(2).
[7] Zhang Xiaowei,Zhu Linxian,Song Lushan,Song Li,Shi Shuyuan,Liu Hai,Wu Jiameng,Si Kai,Gong Tingting,Liu Huiping. Combined treatment of lactic acid-ultrasound-papain on yak meat and its tenderization mechanism[J]. Meat Science,2023,196.
[8] Wang Xiaoyu,Xu Rong,Tong Xian,Zeng Jianhua,Chen Meilin,Lin Zhuhu,Cai Shufang,Chen Yaosheng,Mo Delin. Characterization of different meat flavor compounds in Guangdong small-ear spotted and Yorkshire pork using two-dimensional gas chromatography–time-of-flight mass spectrometry and multi-omics[J]. LWT,2022,169.
[9] Xi Bin,Luo Jin,Gao YaQin,Yang XiaoLing,Guo TianFen,Li WeiHong,Du TianQing. Transcriptome-metabolome analysis of fatty acid of Bamei pork and Gansu Black pork in China.[J]. Bioprocess and biosystems engineering,2020(prepublish).
[10] An Xuejiao,Zhang Shengwei,Li Taotao,Chen Nana,Wang Xia,Zhang Baojun,Ma Youji. Transcriptomics analysis reveals the effect of Broussonetia papyrifera L. fermented feed on meat quality traits in fattening lamb.[J]. PeerJ,2021,9.
[11] Pan Qun,Shai Ofer,Lee Leo J,Frey Brendan J,Blencowe Benjamin J. Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing.[J]. Nature genetics, 2008,40(12).
[12] Li Wei Vivian,Li Jingyi Jessica. Modeling and analysis of RNA-seq data: a review from a statistical perspective.[J]. Quantitative biology (Beijing, China),2018,6(3).
[13] Hrdlickova Radmila,Toloue Masoud,Tian Bin. RNA-Seq methods for transcriptome analysis.[J]. Wiley interdisciplinary reviews. RNA, 2017,8(1).
[14] Xi Wang,Yuanqing Zhang,Xizhong Zhang,Dongcai Wang,Guang Jin,Bo Li,Fang Xu,Jing Cheng, Feng Zhang, Sujun Wu,Su Rui,Jiang He,Ronghua Zhang,Wenzhong Liu. The comprehensive liver transcriptome of two cattle breeds with different intramuscular fat content[J]. Biochemical and Biophysical Research Communications,2017,490(3).
[15] Sun Limin,Bai Man,Xiang Lujie,Zhang Guishan,Ma Wei,Jiang Huaizhi. Comparative transcriptome profiling of longissimus muscle tissues from Qianhua Mutton Merino and Small Tail Han sheep.[J]. Scientific reports,2016,6(1).
[16] Wu Xiaoyun,Zhou Xuelan,Chu Min,Guo Xian,Pei Jie,Xiong Lin,Ma Xiaoming,Bao Pengjia,Liang Chunnian,Yan Ping. Whole transcriptome analyses and comparison reveal the metabolic differences between oxidative and glycolytic skeletal muscles of yak[J]. Meat Science,2022,194.
[17] Gui L, Raza SHA, Ma B, Easa AA, Althobaiti F, Shukry M, Alotaibi MA, Al Hazani TMI, Dawood MAO, Khan R, Jahejo AR, Shater AF, Abd El-Aziz AH, Quan G, Alanbari KH, Wang L. CEBPβ binding directly to the promoter region drives CEBPa transcription and improves FABP4 transcriptional activity in adipose tissue of yak (Bos grunniens). Res Vet Sci. 2021 Dec;141:174-179. doi: 10.1016/j.rvsc.2021.10.018. Epub 2021 Oct 30. PMID: 34749102.
[18] Gui L, Raza SHA, Sun Y, Sabek A, Abbas SQ, Shah MA, Khan R, Abdelnour SA. Molecular characterization and analysis of the association of growth hormone 1 gene with growth traits in Chinese indigenous yak (Bos grunniens). Trop Anim Health Prod. 2021 Mar 22;53(2):221. doi: 10.1007/s11250-021-02671-w. PMID: 33754201.
[19] Raza SHA, Khan R, Gui L, Schreurs NM, Wang X, Mei C, Yang X, Gong C, Zan L. Bioinformatics analysis and genetic polymorphisms in genomic region of the bovine SH2B2 gene and their associations with molecular breeding for body size traits in qinchuan beef cattle. Biosci Rep. 2020 Mar 27;40(3):BSR20192113. doi: 10.1042/BSR20192113. PMID: 32110807; PMCID: PMC7069895.
[20] Yoav Benjamini,Daniel Yekutieli. The Control of the False Discovery Rate in Multiple Testing under Dependency[J]. The Annals of Statistics,2001,29(4).
[21] Wickramasinghe Saumya,Rincon Gonzalo,Islas-Trejo Alma,Medrano Juan F. Transcriptional profiling of bovine milk using RNA sequencing.[J]. BMC genomics,2012,13(1).
[22] Bardou Philippe,Mariette Jérôme,Escudié Frédéric,Djemiel Christophe,Klopp Christophe. jvenn: an interactive Venn diagram viewer.[J]. BMC bioinformatics,2014,15(1).
[23] Young MD, Wakefield MJ, Smyth GK, et al. Gene ontology analysis for RNA-seq: accounting for selection bias [J]. Genome Biol, 2010, 11: R14.
[24] Anders S, Huber W. Differential expression analysis for sequence count data [J]. Genome Biol, 2010, 11: R106.
[25] Arshad Muhammad Sajid,Sohaib Muhammad,Ahmad Rabia Shabir,Nadeem Muhamad Tahir, Imran Ali,Arshad Muhammad Umair,Kwon Joong-Ho,Amjad Zaid. Ruminant meat flavor influenced by different factors with special reference to fatty acids.[J]. Lipids in health and disease,2018,17(1).
[26] Vaikundamoorthy Ramalingam,Zhen Song,Inho Hwang. The potential role of secondary

ISSN 2616-5910 Vol. 5, Issue 1: 63-76, DOI: 10.25236/AJALS.2024.050110

metabolites in modulating the flavor and taste of the meat[J]. Food Research International,2019,122.
[27] Damon Marie,Wyszynska-Koko Joanna,Vincent Annie,Hérault Frédéric,Lebret Bénédicte. Comparison of muscle transcriptome between pigs with divergent meat quality phenotypes identifies genes related to muscle metabolism and structure.[J]. PloS one,2012,7(3).
[28] Sun W X,Wang H H,Jiang B C,Zhao Y Y,Xie Z R,Xiong K,Chen J. Global comparison of gene expression between subcutaneous and intramuscular adipose tissue of mature Erhualian pig.[J]. Genetics and molecular research : GMR,2013,12(4).
[29] Wu H,Olson EN. Activation of the MEF2 transcription factor in skeletal muscles from myotonic mice.[J]. The Journal of Clinical Investigation: The Official Journal of the American Society for Clinical Investigation,2002,109(10).
[30] Liao Simeng,Liu Guang,Tan Bie,Qi Ming,Wu Xin,Li Jianjun,Li Xiaoqing,Zhu Changfeng,Huang Jiamei,Zhang Shuo,Tang Yulong,Yin Yulong. Dietary zero-dimensional fullerene supplementation improves the meat quality, lipid metabolism, muscle fiber characteristics, and antioxidative status in finishing pigs[J]. Animal Nutrition,2022,11.
[31] Matsunaga Yuki,Nakagawa Shinsuke,Morofuji Yoichi,Dohgu Shinya,Watanabe Daisuke,Horie Nobutaka,Izumo Tsuyoshi,Niwa Masami,Walter Fruzsina R.,SantaMaria Ana Raquel,Deli Maria A.,Matsuo Takayuki. MAP Kinase Pathways in Brain Endothelial Cells and Crosstalk with Pericytes and Astrocytes Mediate Contrast-Induced Blood–Brain Barrier Disruption[J]. Pharmaceutics, 2021,13(8).
[32] Jordan J D,Landau E M,Iyengar R. Signaling networks: the origins of cellular multitasking.[J]. Cell,2000,103(2).
[33] Dewar Caroline E,CasasSanchez Aitor, Dieme Constentin,Crouzols Aline,Haines Lee R,AcostaSerrano Álvaro,Rotureau Brice,Schnaufer Achim. Oxidative Phosphorylation Is Required for Powering Motility and Development of the Sleeping Sickness Parasite Trypanosoma brucei in the Tsetse Fly Vector.[J]. mBio,2022.
[34] Baojian Chen,Qiuwei Ao,Shaomei Chen,Yingming Wei,Yafen Guo,Ganqiu Lan,Qinyang Jiang, Hesheng Jiang. Assessment of M. longissimus fibre types and metabolic enzymes in Bama miniature pigs and Landrace swine[J]. Italian Journal of Animal Science,2018,17(1)