

Article

Fat Deposition in the Muscle of Female and Male Yak and the Correlation of Yak Meat Quality with Fat

Lin Xiong^{1,2}, Jie Pei^{1,2} , Min Chu^{1,2}, Xiaoyun Wu^{1,2}, Quadratullah Kalwar³, Ping Yan^{1,2,*} and Xian Guo^{1,2,*} 

¹ Animal Science Department, Lanzhou Institute of Husbandry and Pharmaceutical Sciences, Chinese Academy of Agricultural Sciences, Lanzhou 730050, China; xionglin@caas.cn (L.X.); peijie@caas.cn (J.P.); chumin@caas.cn (M.C.); wuxiaoyun@caas.cn (X.W.)

² Key Laboratory for Yak Genetics, Breeding, and Reproduction Engineering of Gansu Province, Lanzhou 730050, China

³ Department of Animal Reproduction, Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand 67210, Pakistan; qudratullahkalwar@gmail.com

* Correspondence: pingyanlz@163.com (P.Y.); guoxian@caas.cn (X.G.); Tel.: +86-0931-2115288 (P.Y.); +86-0931-2115271 (X.G.)

Simple Summary: The type and quantity of fat are among the key factors influencing the carcass and meat quality in yak. The effect of gender to fat in yak and the correlation of meat quality with fat were studied in this paper. The meat of male yaks (MYs) is healthier from the point of nutritional value, whereas the meat of female yaks (FYs) possesses better qualities in relation to the visual impact and mouthfeel. The below results can provide a theoretical basis for the deep processing of yak meat. Yak meat quality (especially tenderness) can be improved by increasing the content of fat, C18:0, *cis*-C18:2, and *cis*-C18:1 in muscle during yak production. These genes, including *SCD*, *PLIN5*, *LPL*, *ME1* and *DBI*, play a crucial role in the regulation of fat deposition in yak. Above candidate genes for fat deposition in yak can supply the theoretical basis in molecular breeding of yak with a high fat content in muscle.



Citation: Xiong, L.; Pei, J.; Chu, M.; Wu, X.; Kalwar, Q.; Yan, P.; Guo, X.

Fat Deposition in the Muscle of Female and Male Yak and the Correlation of Yak Meat Quality with Fat. *Animals* **2021**, *11*, 2142. <https://doi.org/10.3390/ani11072142>

Academic Editor: Riccardo Bozzi

Received: 15 June 2021

Accepted: 18 July 2021

Published: 20 July 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: This study aimed to explore the differences in fat deposition between female (FYs) and male yaks (MYs). Compared with MYs, the tenderness, L*, marbling, absolute content of fat, and most fatty acids (FAs) of *longissimus dorsi* (LD) in FYs were higher or better ($p < 0.05$), whereas the relative content of polyunsaturated fatty acids (PUFAs) and n-3 PUFAs were lower ($p < 0.01$). The absolute content of fat, C18:0, *cis*-C18:2, *cis*-C18:1, and C24:0 were positively correlated with L*_{45 min}, b*_{24 h}, tenderness, and marbling score of LD in FYs and MYs ($p < 0.05$), respectively. *LPL*, *FATP2*, *ELOVL6*, *HADH*, *HACD*, and *PLINS* genes play a crucial role in improving the marbling score and tenderness of yak meat. The results of gene expression and protein synthesis showed the effect of gender to FA biosynthesis, FA transport, lipolysis, and FA oxidation in the adipose tissue of yak was realized by the expressions of *ME1*, *SCD*, *ACSL5*, *LPL*, *FABP1*, *PLIN4*, and *PLIN2* in peroxisome proliferators-activated receptor (PPAR) signaling. This study established a theoretical basis for the improvement of the meat quality of yak and molecular breeding.

Keywords: yak; fat; PPAR signaling; meat quality; gender



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Fat and fatty acids (FAs) composition are closely related to meat quality, including the appearance, texture, flavor, and hardness [1–4], and have an essential part in avoiding cold shortening, drip loss, and dark cutting [5]. Optimal intramuscular fat is crucial in animal husbandry [6,7]. Along with the rapid development of globalization and the change of consumer philosophy, the demand, supply, and acceptance of unconventional and exotic meats are increasing around the world [8–11]. Yak (*Bos grunniens*) is a unique livestock in Tibetan Plateau and its adjacent areas at heights of 2500–6000 m above sea level [12] possess

unusual physiological adaptations to low temperature (as low as $-40\text{ }^{\circ}\text{C}$) and full-grazing style with grasses, sedges, and fobs as their sole nutritional source [13]. At present, there are approximately 14 million yaks in the world. Yak meat is a unconventional meat, and the major source of animal protein in the local human diet [14,15]. Because of its rich protein and low fat, devoid of anabolic steroids and pollutants, yak meat is increasingly popular with consumers [16]. The annual yak meat yield is more than 500 thousand tons. The carcass yield and dressing percentage of yak are 46.67–51.0% and 37.10–43.02%, respectively. Because of special natural environment and traditional view, the yak breeding model is mainly natural grazing, and the slaughtering industry of yak is scattered. For a long time, the yak industry is an extensive management and low production performance. There are very few modern yak meat processing facilities in the place of origin. Yak meat classification and grade are not put to wide use in the industry, and the deep processing of yak meat is still in the initial stage.

Genetic factors [17], diet [18], gender [19], breeding environment [20,21], and so on can influence the FA profiles in animal-derived food. The metabolic responses are different in female and male animals [22,23], and the effect of gender on fat is realized by hormones [24]. It is very difficult to explore the regulation of fat deposition by a single technique, and the introduction of omics technologies has made these analyses possible. The high-throughput profiling of transcripts has been applied in the study on fat in cattle [25,26] and pig [27,28]. The newest RNA-sequencing (RNA-Seq) analysis can be used to examine the mRNA level in the adipose tissue of yaks with different genders to estimate its gene expression profile. The protein is the ultimate bio-function executor, and the proteomic analysis may provide more direct information on key biological processes. The emerging technology of quantitative proteomics has also been applied in the systematic study of changes in proteome-wide expression profiling in fat of cattle [29] and pig [30].

The goals of the research work was to investigate the regulative mechanism of gender to fat deposition in yak and the correlation of yak meat quality with fat in the muscle of yak. The fat content, FA profiles, and meat quality in *longissimus dorsi* (LD) of female yaks (FYs) and male yaks (MYs) were determined. In addition, the correlations of fat and FAs with meat quality were analyzed. Finally, the effect of gender on fat deposition in yaks was explored by transcriptome and proteome data in the adipose tissue. Differentially expressed genes (DEGs) and proteins (DEPs) in the adipose tissue of FYs and MYs were identified and annotated by Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genome (KEGG). This research can establish a theoretical basis for the improvement of the yak meat quality and molecular breeding, and promote the development of yak breeding and deep processing of yak meat.

2. Materials and Methods

2.1. Animals and Samples Collection

An animal experiment was carried out in the pasture in Haiyan County, Qinghai province, China. Six FYs (four-years-old) and six MYs (four-years-old) were kept in grazing conditions, and were given free-choice access to diet and water. The contents of ash, crud fat, crud protein, neutral detergent fiber, acid detergent fiber, calcium, and phosphorus in grass in September were $7.66 \pm 0.06\%$, $2.63 \pm 0.20\%$, $11.93 \pm 0.40\%$, $76.14 \pm 0.79\%$, $10.09 \pm 0.62\%$, $5.22 \pm 0.32\%$, and $0.07 \pm 0.002\%$, respectively. The intramuscular fat content in yak is so low that it is impossible to obtain an intramuscular fat sample for the transcriptome and proteome analysis; moreover, when the LD samples were directly used in metabolome and proteome analysis, the pre-tests showed that only minimal information on fat in yaks was obtained. Both physiological characteristics and form of the adipose tissue on the surface of the LD are similar to intramuscular fat, and are also easily collected. Thus, the adipose tissue on the surface of LD (12th–13th rib level) of the yak was chosen and used in this study. By late September, all yaks were humanely slaughtered at a commercial abattoir, and the samples of LD (12–13th rib level, 1000 g) and adipose tissue (10 g) were collected. The adipose tissue samples were placed in enzyme-free cryopreservation tubes

and stored in liquid nitrogen; a part of LD samples were frozen at $-20\text{ }^{\circ}\text{C}$ for fat and FAs analysis, and the other part of LD samples were used for meat quality analysis.

2.2. Meat Quality Measurements

After evisceration and cleaning, carcasses were kept in cold storage ($4\text{ }^{\circ}\text{C}$) until rigor mortis completion. The LD sample was used for an ageing period of one day (24 h post-mortem) and for the analysis of color, pH, cooking holding percentage, marbling score, and shear force. Meat color was measured with a CR-400 chroma meter (KONICA MINOLTA Inc., Tokyo, Japan) at 45 min and 24 h postmortem at $4\text{ }^{\circ}\text{C}$ after slaughter, respectively, and the results were showed using International Commission on illumination (CIE) standards for lightness (L^*), redness (a^*), and yellowness (b^*). The pH was measured using the TESTO 205 pH meter (TESTO AG Inc., Lenzkirch, Germany) at 45 min and 24 h at $4\text{ }^{\circ}\text{C}$ after slaughter, respectively. The pH meter was calibrated using pH = 4 and 7 standard solutions from Mettler Toledo (Zurich, Switzerland). Shear force was measured using the C-LM4 tenderness meter (Northeast Agricultural University, Shenyang, China). The LD sample was boiled in a water bath at $80\text{ }^{\circ}\text{C}$ until the core temperature of meat was $70\text{ }^{\circ}\text{C}$, and then cooled to $4\text{ }^{\circ}\text{C}$. Three replicate blocks were cut parallel to the side of the muscle fibers from each sample and each block sheared in the center in the vertical direction to the longitudinal orientation of the fibers. Shear force was the average peak positive force value for all individual sample blocks. The marbling score was visually determined with the aid of standard United States Department of Agriculture (USDA) cards. The LD sample was cut open 24 h post-mortem, and the marbling score was evaluated by the figures of grading standards. Cooking holding percentage was expressed as the weight change percentage of the sample before and after cooking according to the method in the China national standard NY/T821–2019 [31], and was measured by weighing after boiling the sample in the water bath at $80\text{ }^{\circ}\text{C}$.

2.3. Determination of Fat Content and Fatty Acid (FA) Profiles

The fat content in LD was determined according to the Soxhlet extraction principle using a Soxtec 2050 soxhlet apparatus (FOSS Inc., Hillerød, Denmark). FA profiles in LD were analyzed according to the method described in Song et al. [32], and a total of 37 FAs were determined in this study. First, the fat in LD was extracted with the solution of chloroform and methanol (*v:v*, 2:1) for three times, and the combined extract solution was dried under nitrogen blow. Next, the extracted fat sample was decomposed into non-esterified FAs by the basic hydrolysis. Finally, the non-esterified FAs were derived with boron fluoride-methanol solution. The 7890A gas chromatography system (Agilent Corp., Santa Clara, CA, USA) coupled with a flame ionization detector and an Agilent J&WCP-Sil88 FAME capillary column ($100\text{ m} \times 0.25\text{ mm} \times 0.20\text{ }\mu\text{m}$) were used to separate and detect the content of fatty acid methyl esters (FAMES). The nitrogen constant linear flow rate was at 0.5 mL/min , and the split ratio was 1:100. The initial column temperature was held at $100\text{ }^{\circ}\text{C}$ for 5 min, increased to $180\text{ }^{\circ}\text{C}$ at $8\text{ }^{\circ}\text{C/min}$, and held for 9 min. Then, the temperature was increased to $230\text{ }^{\circ}\text{C}$ at $1\text{ }^{\circ}\text{C/min}$ and held for 15 min. The injector and detector temperature were at 260 and $280\text{ }^{\circ}\text{C}$, respectively. The injection volume was $1\text{ }\mu\text{L}$. The analytes were determined based on their retention times, and FAs contents were calculated by FAMES.

2.4. Transcriptome Analyses

Three fat samples were randomly chosen in each group. Total RNA was extracted using the mirVanaTM miRNA Isolation Kit (Ambion Inc., Foster City, CA, USA). The purity of RNA was determined by evaluating absorbance using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Santa Clara, CA, USA). RNA integrity was evaluated using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The mRNA libraries for sequencing were prepared using TruSeq Stranded mRNA LTSample

Prep Kit (Illumina, San Diego, CA, USA). Then, these libraries were sequenced on the HiSeq™ 2500 sequencing platform (Illumina, San Diego, CA, USA).

2.5. Protein Preparation and Digestion

The fat samples for transcriptome analyses were adopted for proteome analysis too. Frozen fat samples were lysed, followed by sonication and centrifugation at 12,000 r/min twice. The sample containing 100 µg protein was diluted with reducing buffer in an ultrafiltration tube and the dithiothreitol solution was added in, following by incubation at 55 °C for 30 and cooling to room temperature. The iodoacetamide solution was added into the tube, and the solution was shaken well and mix stood for 15 min. Acetone was then added to precipitate protein and leaving for over 4 h at −20 °C. Then, the solution was centrifuged and the sediment was redissolved with TEAB2, followed with sequencing-grade trypsin in each tube. The solution was incubated for digestion at 37 °C, and centrifuged and lyophilized. Eighty-eight µL acetonitrile was added into the tandem mass tag (TMT) reagent vial at room temperature, followed by vortex and centrifugation. Then, 41 µL TMT label reagent was added into the sample, and the tube was incubated for 1 h at room temperature. Finally, the reaction was terminated with hydroxylamine.

2.6. Reversed-Phase Liquid Chromatography (RPLC) Separation and Mass Spectrum (MS) Analysis

Reversed-phase liquid chromatography (RPLC) separation was performed on a 1100 HPLC System (Agilent Technologies, Santa Clara, CA, USA) using an Agilent Zorbax Extend RP column (5 µm, 150 mm × 2.1 mm). The mobile phases were A (2% acetonitrile in HPLC water) and B (98% acetonitrile in HPLC water), and the flow rate was 300 µL/min. The detection wavelengths were at 210 and 280 nm, respectively. The sample was collected over 8–60 min, and the separated peptides were lyophilized for mass spectrometry (MS). MS analyses were performed by a Q-Exactive mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA), equipped with a Nanospray Flex source (Thermo Fisher Scientific, Waltham, MA, USA). The sample was separated by a C₁₈ column (15 cm × 75 µm). The mobile phases were A (0.1% formic acid in water) and B (80% acetonitrile, in water containing 0.1% formic acid), and the flow rate was 300 nL/min and linear gradient was 0–40 min, 5–30% B; 40–54 min, 30–50% B; 54–55 min, 50–100% B; 55–60 min, 100% B. Full MS scans were acquired in the mass range of 300–1600 m/z with a mass resolution of 70,000.

2.7. Statistical Analysis

Fat content, FAs content, a*, b*, L*, pH, shear force, marbling score, and cooking holding percentage were calculated separately using independent-sample T test in SPSS 16.0, and the correlations of meat quality with fat and FAs were calculated separately using Pearson correlation analysis in SPSS 16.0 too. The numbers of reads in the RNA-seq analysis were normalized against reads per kilobase of transcripts per million to compute the gene expression levels. Fragments per kilobase of transcript per million mapped reads (FPKM) of each gene were calculated using cufflinks, and the read counts of each gene were obtained by htseq-count. DEGs were identified using the DESeq (2012) R package functions estimate SizeFactors and nbinomTest. Proteome Discoverer (v. 2.3) was used to search all of the Q Exactive raw data thoroughly against the sample protein database. A database search was performed with Trypsin digestion specificity. Alkylation on cysteine was considered as a fixed modification in the database searching. A global false discovery rate (FDR) was set to 0.01, and protein groups considered for quantification were required at least two peptides. In this study, $p < 0.05$ and Fold Change (FC) > 2 or FC < 0.5 were set as the threshold for DEGs and DEPs. The transcriptome and proteome data can reveal the molecular regulation of gender to fat deposition in yak, and the Pearson correlation analysis can explore the main factors on fat which can improve the meat quality in yak.

3. Results

3.1. Meat Quality

The results of meat quality of LD in FYs and MYs are shown in Table 1. Both $L^*_{45 \text{ min}}$ and $L^*_{24 \text{ h}}$ were higher in FYs vs. MYs ($p < 0.05$), and $b^*_{24 \text{ h}}$ was higher in FYs vs. MYs ($p < 0.05$). The shear force was lower in FYs vs. MYs ($p < 0.01$). Moreover, the marbling score was higher in FYs vs. MYs ($p < 0.05$). Meanwhile, no significant differences in other indicators of meat quality were found between FYs and MYs.

Table 1. The meat quality of *longissimus dorsi* (LD) in female (FYs) and male yaks (MYs).

Variable	FYs (Mean ± SE)	MYs (Mean ± SE)
$a^*_{45 \text{ min}}$	55.03 ± 0.72	53.27 ± 0.47
$b^*_{45 \text{ min}}$	23.34 ± 0.84	21.31 ± 1.09
$L^*_{45 \text{ min}}$	22.77 ± 0.65 ^a	19.83 ± 0.74 ^b
$a^*_{24 \text{ h}}$	55.78 ± 0.55	53.88 ± 0.59
$b^*_{24 \text{ h}}$	24.49 ± 0.87 ^a	21.41 ± 0.55 ^b
$L^*_{24 \text{ h}}$	23.98 ± 1.04 ^b	20.82 ± 0.29 ^a
pH _{45 min}	5.86 ± 0.03	5.82 ± 0.03
pH _{24 h}	5.40 ± 0.05	5.55 ± 0.06
Shear force (kg)	7.93 ± 0.16 ^A	9.29 ± 0.27 ^B
Marbling score	3.25 ± 0.16 ^a	2.75 ± 0.10 ^b
Cooking holding percentage (%)	70.60 ± 0.86	71.37 ± 0.36

SE: Standard Error. Values in the same row with different lowercase superscripts show $p < 0.05$, different capital superscripts show $p < 0.01$.

3.2. Fat Content and FA Profiles

Fat contents in LD of FYs and MYs were $2.51 \pm 0.10\%$ and $1.83 \pm 0.09\%$ ($p < 0.01$), respectively. The absolute and relative contents of FAs in LD of FYs and MYs are shown in Table 2. A total of 34 FAs were simultaneously detected in LD of FYs and MYs, including 17 saturated fatty acids (SFAs), 8 monounsaturated fatty acids (MUFAs), and 9 polyunsaturated fatty acids (PUFAs). The absolute contents of 16 FAs in LD were higher in FYs vs. MY ($p < 0.05$), whereas 10 FAs in LD were lower in FYs vs. MY ($p < 0.01$). The absolute contents of Σ SFAs, total unsaturated fatty acids (Σ UFAs), Σ MUFAs, and Σ PUFAs in LD were all higher in FYs vs. MYs ($p < 0.01$). The FAs in LD of both FYs and MYs largely consisted of C18:0, *cis*-C18:1, *cis*-C18:2, C16:0, C20:4n6, C24:0, C24:1, and *cis*-C20:5n3. On the other hand, the relative content of Σ SFAs in LD was higher in FYs vs. MYs ($p < 0.01$), whereas the relative content of Σ UFAs was lower ($p < 0.01$). Further, the relative content of Σ PUFAs in LD was lower in FYs vs. MY ($p < 0.01$), whereas the relative content of Σ MUFAs was higher in FYs vs. MY ($p < 0.01$).

The relative contents of Σ n-3 and Σ n-6 PUFAs, and the ratio of different types of FAs in LD of FYs and MYs are shown in Table 3. The Σ PUFAs/ Σ SFAs (relative content) in LD of FYs and MYs were 0.38 and 0.50 ($p < 0.01$), respectively, and the relative content of Σ n-3 and Σ n-6 PUFAs in LD of FYs and MYs were 2.15 and 16.22%, and 6.65 and 17.46% ($p < 0.05$), respectively. Meanwhile, the Σ n-6/ Σ n-3 PUFAs in LD of FYs and MYs were 8.53 and 2.44 ($p < 0.01$), respectively.

3.3. Function Enrichment Analysis for Differentially Expressed Genes (DEGs) and Differentially Expressed Proteins (DEPs)

There were 1027 DEGs in adipose tissue in FYs vs. MYs and 611 genes were up-regulated, whereas 416 genes were downregulated. Moreover, there were 82 DEPs in the adipose tissue in FYs vs. MYs, and 53 DEPs were upregulated, whereas 29 DEPs were downregulated. The biological processes of GO enrichment of DEGs were mostly involved in long-chain FA transport (GO:0015909), FA β -oxidation (GO:0006635), fructose 2,6-bisphosphate metabolic process (GO:0006003), extracellular polysaccharide biosynthetic process (GO:0045226), bicarbonate transport (GO:0015701), cell adhesion (GO:0007155), and renal water absorption (GO:0070295). The biological processes of GO enrichment of DEPs

were mostly involved in collagen fibril organization (GO:0030199), collagen biosynthetic process (GO:0032964), acute-phase response (GO:0006953), positive regulation of cell division (GO:0051781), UFA biosynthetic process (GO:0006636), and very long-chain FA biosynthetic process (GO:0042761). Total 455 DEGs were enriched in 65 KEGG pathways ($p < 0.05$) which were mainly involved in various substance metabolisms and signaling pathways (Figure 1A). Moreover, a total of 82 DEPs were enriched in 52 KEGG pathways ($p < 0.05$) (Figure 1B). The mutual pathways on KEGG enrichment of DEGs and DEPs included the biosynthesis of unsaturated fatty acids (bom01040), fatty acid elongation (bom00062), terpenoid backbone biosynthesis (bom00900), butanoate metabolism (bom00650), fatty acid degradation (bom00071), tryptophan metabolism (bom00380), synthesis and degradation of ketone bodies (bom00072), and PPAR signaling pathway (bom03320). The important DEGs and DEPs in the above crucial KEGG pathways are shown in Table 4.

Table 2. The absolute and relative content of fatty acids (FAs) in LD of FYs and MYs.

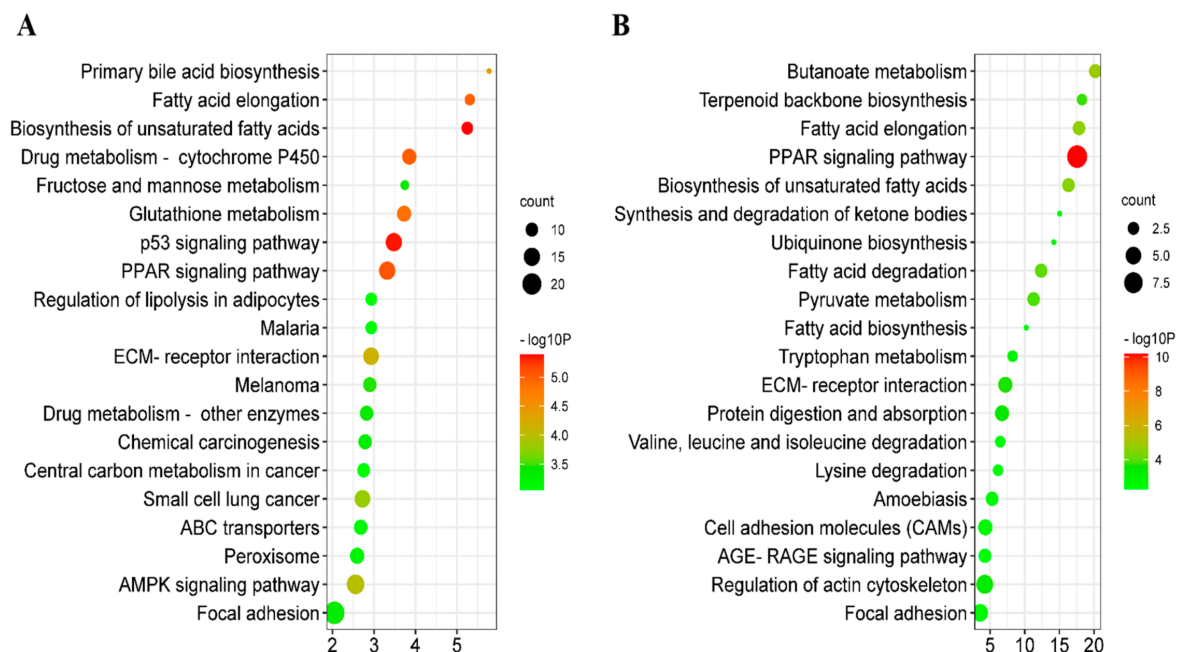
FAs	Absolute Content (mg/100 g)		Relative Content (%)	
	FYs (Mean ± SE)	MYs (Mean ± SE)	FYs (Mean ± SE)	MYs (Mean ± SE)
ΣSFAs	1002.82 ± 14.94 ^A	658.71 ± 15.74 ^B	52.28 ± 0.33 ^A	46.68 ± 0.43 ^B
C4:0	3.46 ± 0.22 ^A	2.22 ± 0.09 ^B	0.18 ± 0.01	0.16 ± 0.01
C6:0	0.55 ± 0.04 ^A	0.38 ± 0.01 ^B	0.02 ± 0.001	0.03 ± 0.002
C8:0	0.12 ± 0.02 ^A	0.55 ± 0.02 ^B	0.01 ± 0.001 ^A	0.04 ± 0.00 ^B
C10:0	0.57 ± 0.03 ^a	0.46 ± 0.02 ^b	0.03 ± 0.001	0.03 ± 0.001
C11:0	0.09 ± 0.01	0.07 ± 0.003	0.004 ± 0.0003	0.005 ± 0.0002
C12:0	0.50 ± 0.04	0.39 ± 0.01	0.03 ± 0.002	0.03 ± 0.001
C13:0	1.25 ± 0.13 ^a	0.83 ± 0.03 ^b	0.06 ± 0.01	0.06 ± 0.002
C14:0	9.86 ± 0.33 ^A	8.03 ± 0.27 ^B	0.515 ± 0.02	0.57 ± 0.02
C15:0	6.92 ± 0.56 ^A	9.42 ± 0.30 ^B	0.36 ± 0.03 ^A	0.67 ± 0.02 ^B
C16:0	271.66 ± 4.70 ^A	181.69 ± 3.47 ^B	14.18 ± 0.35 ^a	12.88 ± 0.14 ^b
C17:0	15.52 ± 1.79 ^a	23.04 ± 1.44 ^b	0.81 ± 0.08 ^A	1.63 ± 0.08 ^B
C18:0	581.96 ± 12.53 ^A	341.49 ± 9.14 ^B	30.33 ± 0.40 ^A	24.20 ± 0.32 ^B
C20:0	2.01 ± 0.25	1.60 ± 0.09	0.10 ± 0.01	0.11 ± 0.01
C21:0	6.48 ± 0.46 ^a	4.95 ± 0.26 ^b	0.34 ± 0.02	0.35 ± 0.01
C22:0	2.81 ± 0.19	2.34 ± 0.21	0.15 ± 0.01	0.17 ± 0.01
C23:0	11.66 ± 1.69	9.86 ± 0.28	0.61 ± 0.09	0.70 ± 0.02
C24:0	87.41 ± 4.92 ^a	71.41 ± 3.33 ^b	4.55 ± 0.21	5.06 ± 0.19
ΣMUFAs	536.21 ± 10.36 ^A	423.15 ± 5.61 ^B	27.95 ± 0.36 ^A	30.02 ± 0.24 ^B
C14:1	1.08 ± 0.11 ^A	18.29 ± 1.22 ^B	0.06 ± 0.01 ^A	1.30 ± 0.08 ^B
<i>cis</i> -C15:1	2.91 ± 0.28 ^A	5.10 ± 0.26 ^B	0.15 ± 0.01 ^A	0.36 ± 0.02 ^B
C16:1	36.20 ± 3.59	37.01 ± 1.50	1.88 ± 0.17 ^A	2.62 ± 0.10 ^B
<i>cis</i> -C17:1	5.62 ± 0.70 ^A	15.23 ± 0.89 ^B	0.29 ± 0.04 ^A	1.08 ± 0.05 ^B
<i>cis</i> -C18:1	424.98 ± 7.31 ^A	300.62 ± 4.82 ^B	22.16 ± 0.32	21.33 ± 0.26
<i>trans</i> -C18:1	11.24 ± 1.14 ^A	6.08 ± 0.55 ^B	0.59 ± 0.06	0.43 ± 0.04
<i>cis</i> -C20:1	4.97 ± 0.44 ^A	1.79 ± 0.05 ^B	0.26 ± 0.02 ^A	0.13 ± 0.004 ^B
C24:1	49.23 ± 3.07 ^a	39.04 ± 1.04 ^b	2.56 ± 0.14	2.77 ± 0.09
ΣPUFAs	379.29 ± 6.90 ^A	328.71 ± 7.27 ^B	19.77 ± 0.13 ^A	23.30 ± 0.32 ^B
<i>cis</i> -C18:2n6	274.54 ± 7.37 ^A	173.92 ± 4.58 ^B	14.31 ± 0.30	12.33 ± 0.23
<i>trans</i> -C18:2n6	0.53 ± 0.06	0.47 ± 0.04	0.03 ± 0.01	0.03 ± 0.01
<i>cis</i> -C18:3n6	5.33 ± 0.52 ^A	10.71 ± 0.27 ^B	0.28 ± 0.03 ^A	0.76 ± 0.02 ^B
C18:3n3	16.83 ± 2.48 ^A	47.95 ± 2.28 ^B	0.87 ± 0.12 ^A	3.39 ± 0.11 ^B
<i>cis</i> -C20:2	3.17 ± 0.50 ^A	6.21 ± 0.32 ^B	0.16 ± 0.02 ^A	0.44 ± 0.02 ^B
C20:4n6	54.56 ± 2.05 ^A	43.61 ± 1.62 ^B	2.84 ± 0.10	3.10 ± 0.13
<i>cis</i> -C20:3n3	0.25 ± 0.04	0.25 ± 0.01	0.01 ± 0.002	0.02 ± 0.001
<i>cis</i> -C20:5n3	21.57 ± 2.20 ^A	43.28 ± 1.09 ^B	1.13 ± 0.12 ^A	3.07 ± 0.10 ^B
<i>cis</i> -C22:6n3	2.51 ± 0.23	2.31 ± 0.07	0.13 ± 0.01 ^a	0.16 ± 0.01 ^b
ΣUFAs	915.50 ± 15.66 ^A	751.86 ± 12.14 ^B	47.72 ± 0.33 ^A	53.32 ± 0.43 ^B

ΣMUFAs: sum of monounsaturated fatty acids; ΣSFAs: sum of saturated fatty acids; ΣPUFAs: sum of polyunsaturated fatty acids; ΣUFAs: sum of unsaturated fatty acids. Values in the same row with different lowercase superscripts show $p < 0.05$, different capital superscripts show $p < 0.01$.

Table 3. The relative contents of $\Sigma n-3$ and $\Sigma n-6$ polyunsaturated fatty acids (PUFAs) and the ratio of different types of FAs in LD of FYs and MYs.

FAs	FYs (Mean \pm SE)	MYs (Mean \pm SE)
$\Sigma n-3$ PUFAs (%)	2.15 \pm 0.18 ^A	6.65 \pm 0.07 ^B
$\Sigma n-6$ PUFAs (%)	16.22 \pm 0.21 ^a	17.46 \pm 0.34 ^b
$\Sigma n-6/\Sigma n-3$ PUFAs	8.53 \pm 0.86 ^A	2.44 \pm 0.06 ^B
$\Sigma SFAs/\Sigma UFAs$	1.10 \pm 0.01 ^A	0.88 \pm 0.02 ^B
$\Sigma MUUFAs/\Sigma PUFAs$	1.41 \pm 0.02 ^A	1.29 \pm 0.02 ^B
$\Sigma PUFAs/\Sigma SFAs$	0.38 \pm 0.01 ^A	0.50 \pm 0.01 ^B

$\Sigma n-3$ PUFAs: sum of n-3 polyunsaturated fatty acids; $\Sigma n-6$ PUFAs: sum of n-6 polyunsaturated fatty acids. Values in the same row with different lowercase superscripts show $p < 0.05$, different capital superscripts show $p < 0.01$.

**Figure 1.** (A) The bubble diagram of the top 20 Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment pathways of differentially expressed genes (DEGs). The horizontal axis represents the enrichment score; (B) The bubble diagram of the top 20 KEGG enrichment pathways of differentially expressed proteins (DEPs).**Table 4.** The differentially expressed genes (DEGs) and differentially expressed proteins (DEPs) in the crucial Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways related to fat metabolism in the adipose tissue of FYs and MYs.

KEGG Pathway	DEGs	DEPs
Biosynthesis of unsaturated fatty acids (bom01040)	<i>SCD, ACOT7, HACD2, SCP2, ELOVL6, HSD17B12, ACAA1, HSD17B12</i>	<i>SCD, ELOVL6, HACD3</i>
Fatty acid elongation (bom00062)	<i>ACOT7, HACD2, THEM4, HADHB, ELOVL6, HADH, HSD17B12</i>	<i>ELOVL6, HADH, HACD3</i>
Terpenoid backbone biosynthesis (bom00900)	<i>PCYOX1, ACAT2, FNTB</i>	<i>PCYOX1, ACAT2</i>
Butanoate metabolism (bom00650)	<i>ACSM1, HADH, BDH1, ACAT2</i>	<i>ACSM1, HADH, ACAT2</i>
Fatty acid degradation (bom00071)	<i>ACADSB, HADHB, HADH, ACAT2, ACAA1, CPT1C</i>	<i>ACSL5, HADH, ACAT2</i>
Tryptophan metabolism (bom00380)	<i>MAOB, AADAT, HADH, ACMSD, ACAT2</i>	<i>HADH, ACAT2</i>
Synthesis and degradation of ketone bodies (Bom00072)	<i>BDH1, ACAT2</i>	<i>ACAT2</i>
PPAR signaling pathway (bom03320)	<i>SCD, SCP2, PLIN5, ACOX2, ACAA1, LPL, ME1, CPT1C, SLC27A4, SLC27A6, DBI, SCP2</i>	<i>ACSL5, SCD, PLIN4, FABP1, PLIN2, LPL, ME1, DBI</i>
Pyruvate metabolism (bom00620)	<i>ACAT2, ACSS2, LDHA, ME1, SCP2, ACYP2</i>	<i>ACAT2, ME1</i>

Further, DEGs distribution in the metabolism classification at KEGG level2 showed (Figure 2A) that a total of 41 DEGs were involved in lipid metabolism and their percentage in all DEGs was 24%, which was the largest. Meanwhile, the percentage of DEGs in carbohydrate metabolism (16%) and amino acid metabolism (16%) were larger too. On the other hand, DEPs were largely concentrated in lipid metabolism (32%), carbohydrate metabolism (20%), and amino acid metabolism (8%) in the metabolism classification at KEGG level2 (Figure 2B) too. A total of eight DEPs were involved in lipid metabolism, including ACAT2, HACD3, LPL, SRD5A3, ACSL5, HADH, SCD, and ELOVL6, and they play a crucial role in fatty acid elongation, fatty transport, and fatty acids synthesis, and their expressions were all upregulated in the adipose tissue of FYs ($p < 0.05$).

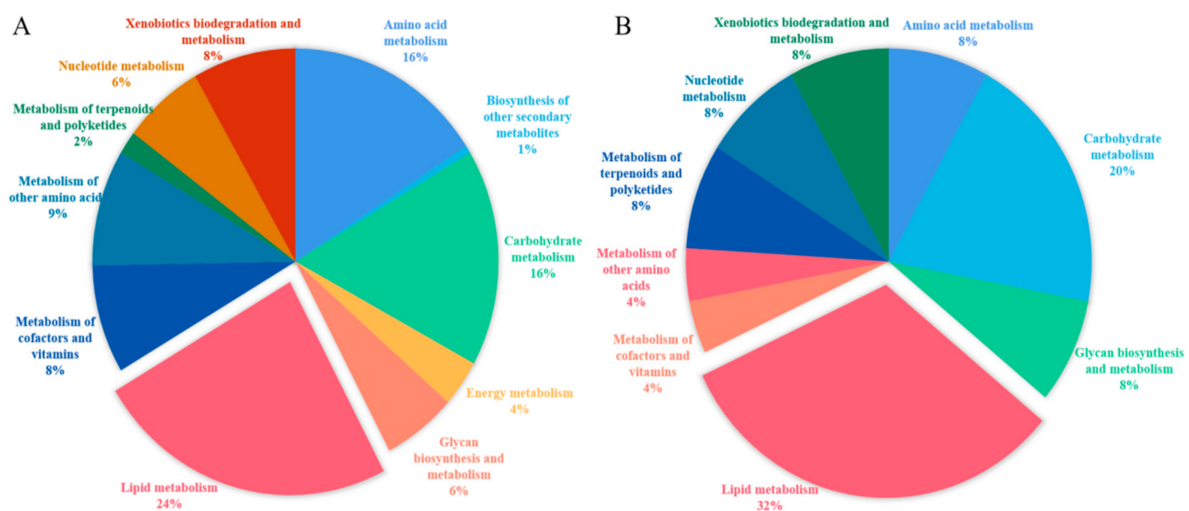


Figure 2. (A) The distribution of DEGs in the metabolism classification at KEGG level2; (B) The distribution of DEPs in the metabolism classification at KEGG level2.

4. Discussion

4.1. Correlation of Fat with Meat Quality

Consumers much prefer the beef which is low L^* and b^* and high a^* with abundant marbling and better tenderness, and when the beef possessing above quality is higher price. In this study, the L^* , tenderness, and marbling score of LD in FYs were higher than that in MYs, and the FYs meat quality is better from the view of visual impact and mouthfeel. Meat color may also be influenced by factors such as diet, meat pH, animal age, and intramuscular fat content [33]. In this study, most of these factors, except intramuscular fat content, is kept the same, and the intramuscular fat content in yaks is closely related to meat color. An increase in Σ SFAs (absolute content) in LD of FYs mainly resulted from C16:0, C18:0, and C24:0, whereas cis-C18:1, C24:1, cis-C18:2n6, and C20:4n6 were responsible for the decrease in Σ PUFAs (absolute content). These results showed that the degradation of long-chain PUFA may contribute to the increase in short-chain SFAs [34]. C18:0 has a neutral effect on blood cholesterol, and exerts a neutral effect like some MUFAs [35]. The FAs that appears in greater proportion is C18:0, which is a nutritionally positive aspect of yak intramuscular fat. Generally speaking, the C18:0 is higher in bulls compared to cows [36], and the above result is also suitable for yak. The relative content of FAs is crucial when evaluating the meat quality [37,38]. The Food and Agriculture Organization of United Nations (FAO) and the World Health Organization (WHO) suggest a reduction in the intake of SFAs and trans fatty acids (TFAs), and an increase in the intake of n-3 PUFAs [39]. The recommendation of Σ PUFAs/ Σ SFAs is above 0.4, while Σ n-6/ Σ n-3 PUFAs is below 4. Two TFAs, including *trans*-C18:2n6 and *trans*-C18:1, were determined in LD of both FYs and MYs, and the absolute content of *trans*-C18:1 was higher in LD of FYs ($p < 0.01$), but no differences in their relative contents were found; the Σ PUFA/ Σ SFA in beef

is typically 0.1 [40]. The low fat content in LD of yaks explains the Σ PUFA/ Σ SFA values, which are higher than the typical values for beef. In addition, under grazing conditions, Σ n-3 PUFAs concentrations of yak meat were higher than that of beef cattle meat [41], and the yak meat showed more favorable Σ n-6/ Σ n-3 PUFAs than reported for beef. The Σ PUFAs/ Σ SFAs (relative contents) in LD of MYs was above the recommended levels, while the Σ n-6/ Σ n-3 PUFA (relative contents) was below the recommended levels. Therefore, from the view of fat nutritional value, yak meat is healthier than usual beef, and MYs meat is healthier than FYs meat.

The results of Pearson correlation analysis between meat quality and fat content in FAs (absolute content) are shown in Table 5. There were negative correlations between fat content and shear force in LD of FYs and MYs ($p < 0.05$). There were positive correlations between the fat content and the $L^*_{24\text{h}}$, marbling score in LD of MYs ($p < 0.05$), and the fat content and $L^*_{24\text{h}}$, $b^*_{24\text{h}}$, marbling score in LD of FYs ($p < 0.05$). The absolute contents of C18:0, *cis*-C18:1, and C24:0 in LD of the MYs were positively correlated with the $L^*_{24\text{h}}$ and marbling ($p < 0.05$), respectively, whereas they were negatively correlated with shear force ($p < 0.05$). The absolute contents of C18:0 and *cis*-C18:2 in LD of the FYs were positively correlated with $L^*_{45\text{min}}$, $b^*_{24\text{h}}$, marbling score ($p < 0.05$), respectively, whereas they were negatively correlated with shear force ($p < 0.05$) too. Therefore, a higher fat content can improve the tenderness of yak meat, and higher contents of C18:0, *cis*-C18:2, *cis*-C18:1, and C24:0 in yak meat can play a crucial role in improving b^* , L^* , marbling, and tenderness. Marbling score and tenderness are regulated by multiple intramuscular fat metabolic genes. The critical lipid uptake in genes, such as *LPL* and *FATP2*, are implicated in the process of fatty acid flux into adipocytes clustered along myofiber fasciculi in the muscle [42]. *ELOVL6*, *HADH*, *ACOT7*, and *HACD* genes are responsible for de novo synthesis of fatty acids, and act as key regulatory molecules in fat deposition. The *PLINS* gene is involved in the regulation of fat storage. The marbling score and tenderness in LD of FYs were higher, likely because of the overexpression of the above lipogenic-related genes. Further, the overexpression of *LPL*, *FATP2*, *ELOVL6*, *HADH*, *HACD*, and *PLINS* proteins verified the regulation of above genes to the marbling score and tenderness in yaks with a different gender.

Table 5. The Pearson correlation analysis between meat quality and FAs, fat content (absolute content) in LD of FYs and MYs.

Gender	Variable	$L^*_{45\text{min}}$	$b^*_{24\text{h}}$	$L^*_{24\text{h}}$	Shear Force	Marbling
MYs	Fat content	0.41	0.79	0.89 ^{a,b}	−0.84 ^{a,b}	0.85 ^{a,b}
	C18:0	0.43	0.87 ^{a,b}	0.85 ^{a,b}	−0.92 ^{A,B}	0.76
	<i>cis</i> -C18:1	0.39	0.78	0.90 ^{a,b}	−0.81 ^{a,b}	0.91 ^{a,b}
	<i>cis</i> -C18:2	0.15	0.31	0.44	−0.32	0.39
	C16:0	0.42	0.58	0.52	−0.61	0.35
	C20:4n6	0.23	−0.25	−0.21	−0.30	−0.28
	C24:0	0.29	0.77	0.85 ^{a,b}	−0.81 ^{a,b}	0.88 ^{a,b}
	C24:1	−0.27	−0.55	−0.28	−0.56	−0.17
	<i>cis</i> -C20:5n3	0.56	−0.06	0.01	−0.12	−0.07
	Fat content	0.94 ^{A,B}	0.88 ^{a,b}	0.93 ^{A,B}	−0.84 ^{a,b}	0.66
FYs	C18:0	0.87 ^{a,b}	0.92 ^{A,B}	0.70	−0.93 ^{A,B}	0.72
	<i>cis</i> -C18:1	0.54	0.34	0.72	−0.51	0.48
	<i>cis</i> -C18:2	0.79	0.73	0.68	−0.94 ^{A,B}	0.81 ^{a,b}
	C16:0	−0.14	−0.14	−0.11	−0.07	−0.03
	C20:4n6	0.21	0.04	0.44	−0.40	0.01
	C24:0	0.53	0.46	0.54	−0.23	0.17
	C24:1	0.35	0.52	0.45	−0.23	−0.07
	<i>cis</i> -C20:5n3	−0.57	−0.71	−0.53	−0.76	−0.38

Values with different lowercase superscripts show $p < 0.05$, different capital superscripts show $p < 0.01$.

4.2. Effect of Gender to Fat Deposition in Yak

The absolute contents of Σ SFAs, Σ UFAs, Σ PUFAs, and Σ MUFAs in LD of FYs were higher ($p < 0.01$), and the capacity of fat deposition in FYs is more powerful than in MYs. Both DEGs and DEPs in the adipose tissue of FYs and MYs were largely concentrated in lipid metabolism, carbohydrate metabolism, and amino acid metabolism in the metabolism classification at KEGG level2, and the fat deposition in yaks with different gender was determined by the synergism of fat, amino acid, and carbohydrate metabolism. Amino acid can transfer into acetyl-Coa by butanoate metabolism (bom00650) ($p < 0.05$), and the expression of DEGs (*MAOB*, *AADAT*, *HADH*, *ACMSD*, and *ACAT2*) and DEPs (*HADH* and *ACAT2*) in tryptophan metabolism (bom00038) ($p < 0.05$) were all upregulated ($p < 0.05$) in the adipose tissue of FYs. Therefore, more amino acids, especially tryptophan in the adipose tissue of FYs, were converted into acetyl-CoA, and further acetyl-CoA was converted into triglyceride. On the other hand, the conversion from carbohydrate into triglyceride can be realized by citric acid-pyruvate cycle, and the expression of DEGs (*MAOB*, *AADAT*, *HADH*, *ACMSD* and *ACAT2*) and DEPs (*ACAT2* and *ME1*) in pyruvate metabolism (bom00620) ($p < 0.05$) were all upregulated ($p < 0.05$) in the adipose tissue of FYs, and more carbohydrates in the adipose tissue of FYs were converted into triglyceride.

The fat deposition is most directly determined by fat metabolism, and FAs synthesis is crucial for fat synthesis and fat deposition. The interaction networks of DEPs related to fat metabolism in the adipose tissue of FYs and MYs are shown in Figure 3. The FA elongation mainly synthesizes the SFAs. The expressions of DEGs (*HADHB* [43], *HADH*, *ELOVL6* [44], *HSD17B8* [45], *HACD2* [46], and *ACOT7* [47]) and DEPs (*ELOVL6*, *HADH*, and *HACD3*) in fatty acid elongation (bom00062) ($p < 0.05$) were all upregulated in the adipose tissue of FYs ($P_{FDR} < 0.05$); these genes can positively regulate the SFAs biosynthesis. The absolute contents of Σ SFAs, C4:0, C6:0, C10:0, C13:0, C14:0, C16:0, C18:0, C21:0, and C24:0 in LD of FYs were higher than that in LD of FYs. The expressions of DEGs (*SCD* [48], *ACOT7*, *HACD2*, *SCP2* [49], *ELOVL6*, *ACAA1* [50], and *HSD17B12*) and DEPs (*SCD*, *ELOVL6*, and *HACD3*) in UFAs biosynthesis (bom01040) ($p < 0.05$) were all upregulated in the adipose tissue of FYs ($p < 0.05$), and they can positively regulate the UFAs biosynthesis, especially n-6 PUFAs (C18:2n6). *SCD* is the first enzyme converting SFA to MUFA and PUFA. More SFAs were converted to MUFAs in the adipose tissue of FYs. The absolute contents of Σ MUFAs, Σ PUFAs, Σ n-6 PUFAs, cis-C18:2n6, and C20:4n6 in LD of FYs were higher. Therefore, both SFAs and UFAs synthesis in the adipose tissue of FYs increased, which resulted in the fat content in LD of FYs being higher than that in LD of MYs. The correlations of fat with L^*_{24h} , marbling score, and tenderness were positive, so higher fat content can improve the meat quality of yak.

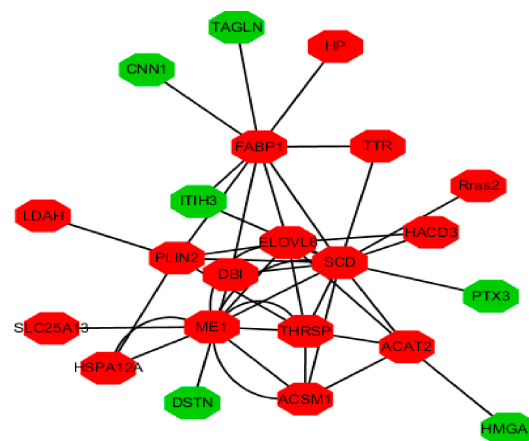


Figure 3. The interaction networks of DEPs related to fat metabolism in the adipose tissue of FYs and MYs. The red expressed the upregulated expression of protein in the adipose tissue of FY, and the green expressed the downregulated expression of protein in the adipose tissue of FY.

PPAR signal. The capacity of fat deposition in FYs was more powerful, and more fat was deposited in FYs.

Author Contributions: Conceptualization, L.X. and P.Y.; Data curation, L.X. and J.P.; Formal analysis, L.X.; Funding acquisition, P.Y. and X.G.; Investigation, L.X., X.W., X.G.; Methodology, L.X. and X.W.; Project administration, P.Y.; Resources, M.C., X.W.; Software, J.P.; Supervision, P.Y.; Writing—original draft, L.X.; Writing—review and editing, L.X., Q.K., X.W. and X.G. All authors have read and agreed to the published version of the manuscript.

Funding: This project received financial support from the innovation project of Chinese academy of agricultural sciences (grant number: CAAS-ASTIP-2014-LIHPS-01), the national beef cattle industry technology & system (grant number: CARS-37). These funding had role in the design of the study, in the collection, analysis, and interpretation of data, or in writing the manuscript.

Institutional Review Board Statement: The animal study was approved by the Ethics Committee of the Lanzhou Institute of Husbandry and Pharmaceutical Sciences, Chinese Academy of Agricultural Sciences (Permit No. SYXK-2019-0011). The methods used in this study were carried out in accordance with the Laboratory Animal Management Principles of China.

Data Availability Statement: The mRNA-Sequencing datasets generated for this study can be found in the Sequence Read Archive (<https://www.ncbi.nlm.nih.gov/sra>) at NCBI, with the BioProject ID: PRJNA686190.

Acknowledgments: The authors would like to thank all people who were involved in the experiments. The authors are grateful to the Ministry of Agriculture and Rural Affairs of the People's Republic of China for financial support. The authors also thank the coworkers of the Key Laboratory for Yak Genetics, Breeding, and Reproduction Engineering of Gansu Province, China, in performing the experiment.

Conflicts of Interest: The authors declare no conflict of interest. The funders had a role in the design of the study; in the collection, analyses, interpretation of data; in the writing of the manuscript, in the decision to publish the results.

References

1. Gagaoua, M.; Bonnet, M.; Picard, B. Protein array-based approach to evaluate biomarkers of beef tenderness and marbling in cows: Understanding of the underlying mechanisms and prediction. *Foods* **2020**, *9*, 1180. [[CrossRef](#)] [[PubMed](#)]
2. Pewan, S.B.; Otto, J.R.; Huerlimann, R.; Budd, A.M.; Mwangi, F.W.; Edmunds, R.C.; Holman, B.W.B.; Henry, M.L.E.; Kinobe, R.; Adegboye, O.A.; et al. Genetics of omega-3 long-chain polyunsaturated fatty acid metabolism and meat eating quality in Tattykeel Australian white lambs. *Genes* **2020**, *11*, 587. [[CrossRef](#)] [[PubMed](#)]
3. Ekine-Dzivenu, C.; Vinsky, M.; Basarab, J.A.; Aalhus, J.L.; Dugan, M.E.R.; Li, C. Phenotypic and genetic correlations of fatty acid composition in subcutaneous adipose tissue with carcass merit and meat tenderness traits in Canadian beef cattle1. *J. Anim. Sci.* **2017**, *95*, 5184–5196. [[CrossRef](#)] [[PubMed](#)]
4. Wen, Y.; Liu, H.; Liu, K.; Cao, H.; Mao, H.; Dong, X.; Yin, Z. Analysis of the physical meat quality in partridge (*Alectoris chukar*) and its relationship with intramuscular fat. *Poult. Sci.* **2020**, *99*, 1225–1231. [[CrossRef](#)]
5. Douny, C.; El Khoury, R.; Delmelle, J.; Brose, F.; Degand, G.; Moula, N.; Farnir, F.; Clinquant, A.; Maghuin-Rogister, G.; Scippo, M. Effect of storage and cooking on the fatty acid profile of omega-3 enriched eggs and pork meat marketed in Belgium. *Food Sci. Nutr.* **2014**, *3*, 140–152. [[CrossRef](#)]
6. Liu, S.; Huang, J.; Wang, X.; Ma, Y. Transcription factors regulate adipocyte differentiation in beef cattle. *Anim. Genet.* **2020**, *51*, 351–357. [[CrossRef](#)]
7. Guo, Q.; Kong, X.F.; Hu, C.J.; Zhou, B.; Wang, C.T.; Shen, Q.W. Fatty acid content, flavor compounds, and sensory quality of pork loin as affected by dietary supplementation with larginine and glutamic acid. *J. Food Sci.* **2019**, *84*, 3445–3453. [[CrossRef](#)]
8. Cawthorn, D.M.; Hoffman, L.C. Controversial cuisine: A global account of the demand, supply and acceptance of “unconventional” and “exotic” meats. *Meat Sci.* **2016**, *120*, 19–36. [[CrossRef](#)]
9. Maggiolino, A.; Lorenzo, J.M.; Centoducati, G.; Domínguez, R.; Dinardo, F.R.; Marino, R.; Malva, A.D.; Bragaglio, A.; Palo, P.D. How volatile compounds, oxidative profile and sensory evaluation can change with vacuum aging in donkey meat. *Animals* **2020**, *10*, 2126. [[CrossRef](#)]
10. Lorenzo, J.M.; Munekata, P.E.S.; Campagnol, P.C.B.; Zhu, Z.Z.; Alpas, H.; Barba, F.J.; Tomasevic, I. Technological aspects of horse meat products—a review. *Food Res. Int.* **2017**, *102*, 176–183. [[CrossRef](#)]
11. Popoola, I.O.; Soladoye, P.O.; Gaudette, N.J.; Wismer, W.V. A Review of Sensory and Consumer-related Factors Influencing the Acceptance of Red Meats from Alternative Animal Species. *Food Rev. Int.* **2020**, *36*, 1–20. [[CrossRef](#)]

12. Luo, J.; Huang, Z.Y.; Liu, H.N.; Zhang, Y.; Ren, F.Z. Yak milk fat globules from the Qinghai-Tibetan Plateau: Membrane lipid composition and morphological properties. *Food Chem.* **2018**, *245*, 731–737. [[CrossRef](#)] [[PubMed](#)]
13. Leslie, D.M.; Schaller, G.B. *Bos grunniens* and *Bos mutus* (Artiodactyla: Bovidae). *Mamm. Spec.* **2009**, *836*, 1–17. [[CrossRef](#)]
14. Wen, W.; Luo, X.; Xia, B.; Guan, J.; Nie, Y.; Li, L.; Duan, J.; Suman, S.; Sun, Q. Post-mortem oxidative stability of three yak (*Bos grunniens*) muscles as influenced by animal age. *Meat Sci.* **2015**, *105*, 121–125. [[CrossRef](#)] [[PubMed](#)]
15. Guo, X.; Long, R.; Kreuzer, M.; Ding, L.; Shang, Z.; Zhang, Y.; Yang, Y.; Cui, G. Importance of Functional Ingredients in Yak Milk-Derived Food on Health of Tibetan Nomads Living Under High-Altitude Stress: A Review. *Crit. Rev. Food Sci. Nutr.* **2013**, *54*, 292–302. [[CrossRef](#)]
16. Wang, L.-L.; Han, L.; Ma, X.-L.; Yu, Q.-L.; Zhao, S.-N. Effect of mitochondrial apoptotic activation through the mitochondrial membrane permeability transition pore on yak meat tenderness during postmortem aging. *Food Chem.* **2017**, *234*, 323–331. [[CrossRef](#)]
17. Brzozowska, A.M.; Lukaszewicz, M.; Oprzadek, J.M. Energy-protein supplementation and lactation affect fatty acid profile of liver and adipose tissue of dairy cows. *Molecules* **2018**, *23*, 618. [[CrossRef](#)]
18. Wang, H.; He, Y.; Li, H.; Wu, F.; Qiu, Q.; Niu, W.; Gao, Z.; Su, H.; Cao, B. Rumen fermentation, intramuscular fat fatty acid profiles and related rumen bacterial populations of Holstein bulls fed diets with different energy levels. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 4931–4942. [[CrossRef](#)]
19. Stergiadis, S.; Berlitz, C.B.; Hunt, B.; Garg, S.; Givens, D.I.; Kliem, K.E. An update to the fatty acid profiles of bovine retail milk in the United Kingdom: Implications for nutrition in different age and gender groups. *Food Chem.* **2019**, *276*, 218–230. [[CrossRef](#)]
20. Oliveira, P.P.A.; Corte, R.; Silva, S.; Rodriguez, P.; Sakamoto, L.; Pedroso, A.; Tullio, R.; Berndt, A. The effect of grazing system intensification on the growth and meat quality of beef cattle in the Brazilian Atlantic Forest biome. *Meat Sci.* **2018**, *139*, 157–161. [[CrossRef](#)]
21. Bergamaschi, M.; Cipolat-Gotet, C.; Cecchinato, A.; Schiavon, S.; Giovanni Bittante, G. Chemometric authentication of farming systems of origin of food (milk and ripened cheese) using infrared spectra, fatty acid profiles, flavor, fingerprints, and sensory descriptions. *Food Chem.* **2020**, *305*, 125480. [[CrossRef](#)]
22. Hedrington, M.S.; Davis, S.N. Sexual dimorphism in glucose and lipid metabolism during fasting, hypoglycemia, and exercise. *Front. Endocrinol.* **2015**, *6*, 61. [[CrossRef](#)] [[PubMed](#)]
23. Aaslyng, M.D.; Jensen, H.; Karlsson, A.H. The gender background of texture attributes of pork loin. *Meat Sci.* **2018**, *136*, 79–84. [[CrossRef](#)]
24. Liu, Y.N.; Albrecht, E.; Schering, L.; Kuehn, C.; Yang, R.J.; Zhao, Z.H.; Maak, S. Agouti signaling protein and its receptors as potential molecular markers for intramuscular and body fat deposition in cattle. *Front. Physiol.* **2018**, *9*, 172. [[CrossRef](#)] [[PubMed](#)]
25. Cesar, A.S.M.; Regitano, L.C.A.; Poleti, M.D.; Andrade, S.C.S.; Tizioto, P.C.; Oliveira, P.S.N.; Felício, A.M.; Nascimento, M.L.D.; Chaves, A.S.; Lanna, D.P.D.; et al. Differences in the skeletal muscle transcriptome profile associated with extreme values of fatty acids content. *BMC Genom.* **2016**, *17*, 961. [[CrossRef](#)] [[PubMed](#)]
26. Jiang, R.; Li, H.; Huang, Y.; Lan, X.; Lei, C.; Chen, H. Transcriptome profiling of lncRNA related to fat tissues of Qinchuan cattle. *Gene* **2020**, *742*, 144587. [[CrossRef](#)]
27. Xu, K.; Ji, M.; Huang, X.; Peng, Y.; Wu, W.; Zhang, J. Differential Regulatory Roles of MicroRNAs in Porcine Intramuscular and Subcutaneous Adipocytes. *J. Agric. Food Chem.* **2020**, *68*, 3954–3962. [[CrossRef](#)] [[PubMed](#)]
28. Piórkowska, K.; Żukowski, K.; Ropka-Molik, K.; Tyra, M.; Gurgul, A. A comprehensive transcriptome analysis of skeletal muscles in two Polish pig breeds differing in fat and meat quality traits. *Genet. Mol. Biol.* **2018**, *41*, 125–136. [[CrossRef](#)]
29. Bazile, J.; Picard, B.; Chambon, C.; Valais, A.; Bonnet, M. Pathways and biomarkers of marbling and carcass fat deposition in bovine revealed by a combination of gel-based and gel-free proteomic analyses. *Meat Sci.* **2019**, *156*, 146–155. [[CrossRef](#)]
30. Ma, C.; Wang, W.; Wang, Y.; Sun, Y.; Kang, L.; Zhang, Q.; Jiang, Y. TMT-labeled quantitative proteomic analyses on the longissimus dorsi to identify the proteins underlying intramuscular fat content in pigs. *J. Proteom.* **2020**, *213*, 103630. [[CrossRef](#)]
31. Ministry of National Defense of the People’s Republic of China, Beijing, China. *Technical Code of Practice for Pork Quality Assessment, China National Standard: NYT821-2019*; Ministry of National Defense of the People’s Republic of China: Beijing, China, 2019.
32. Song, S.Z.; Wu, J.P.; Zhao, S.G.; Casper, D.P.; He, B.; Liu, T.; Lang, X.; Gong, X.Y.; Liu, L.S. The effect of energy restriction on fatty acid profiles of longissimus dorsi and tissue adipose depots in sheep. *J. Anim. Sci.* **2017**, *95*, 3940–3948. [[CrossRef](#)]
33. Popova, T.; Tejada, L.; Peñarrieta, J.M.; Smith, M.A.; Bush, R.D.; Hopkins, D.L. Meat of South American camelids—Sensory quality and nutritional composition. *Meat Sci.* **2021**, *171*, 108285. [[CrossRef](#)]
34. Zhang, H.; Wang, Q.; Fan, E. Stability Profile of Fatty Acids in Yak (*Bos grunniens*) Kidney Fat During the Initial Stages of Autoxidation. *J. Am. Oil Chem. Soc.* **2009**, *86*, 1057–1063. [[CrossRef](#)]
35. Grundy, S.; Denke, M. Dietary influences on serum lipids and lipoproteins. *J. Lipid Res.* **1990**, *31*, 1149–1172. [[CrossRef](#)]
36. Reddy, B.V.; Sivakumar, A.S.; Jeong, D.W.; Woo, Y.-B.; Park, S.-J.; Lee, S.-Y.; Byun, J.-Y.; Kim, C.-H.; Cho, S.-H.; Hwang, I. Beef quality traits of heifer in comparison with steer, bull and cow at various feeding environments. *Anim. Sci. J.* **2014**, *86*, 1–16. [[CrossRef](#)]
37. Van Leeuwen, K.A.; Camin, F.; Jerónimo, E.; Vasta, V.; Prenzler, P.D.; Ryan, D.; Bessa, R.J.B. Dietary Effects on Stable Carbon Isotope Composition of Fatty Acids in Polar and Neutral Fractions of Intramuscular Fat of Lambs. *J. Agric. Food Chem.* **2017**, *65*, 9404–9411. [[CrossRef](#)]

38. Wu, K.; Zhao, T.; Hogstrand, C.; Xu, Y.-C.; Ling, S.-C.; Chen, G.-H.; Luo, Z. FXR-mediated inhibition of autophagy contributes to FA-induced TG accumulation and accordingly reduces FA-induced lipotoxicity. *Cell Commun. Signal.* **2020**, *18*, 47. [[CrossRef](#)] [[PubMed](#)]
39. Gómez, I.; Sarriés, M.V.; Ibañez, F.C.; Beriain, M.J. Quality Characteristics of a Low-Fat Beef Patty Enriched by Polyunsaturated Fatty Acids and Vitamin D3. *J. Food Sci.* **2018**, *83*, 454–463. [[CrossRef](#)] [[PubMed](#)]
40. Enser, M.; Richardson, R.; Wood, J.; Gill, B.; Sheard, P. Feeding linseed to increase the n-3 PUFA of pork: Fatty acid composition of muscle, adipose tissue, liver and sausages. *Meat Sci.* **2000**, *55*, 201–212. [[CrossRef](#)]
41. Li, P. Fatty acid composition assay and function evaluation of white yak meat. *Food Sci.* **2008**, *29*, 106–108.
42. Jeong, J.; Kwon, E.G.; Im, S.K.; Seo, K.S.; Baik, M. Expression of fat deposition and fat removal genes is associated with intramuscular fat content in longissimus dorsi muscle of Korean cattle steers. *J. Anim. Sci.* **2012**, *90*, 2044–2053. [[CrossRef](#)]
43. Dumbrepatil, A.B.; Zegalia, K.A.; Sajja, K.; Kennedy, R.T.; Marsh, E.N.G. Targeting viperin to the mitochondrion inhibits the thiolase activity of the trifunctional enzyme complex. *J. Biol. Chem.* **2020**, *295*, 2839–2849. [[CrossRef](#)]
44. Jung, Y.H.; Bu, S.Y. Suppression of long chain acyl-CoA synthetase blocks intracellular fatty acid flux and glucose uptake in skeletal myotubes. *BBA-Mol. Cell Biol.* **2020**, *1865*, 158678. [[CrossRef](#)] [[PubMed](#)]
45. Hiltunen, J.K.; Kastaniotis, A.J.; Autio, K.J.; Jiang, G.Y.; Chen, Z.J.; Glumoff, T. 17 β -hydroxysteroid dehydrogenases as acyl thioester metabolizing enzymes. *Mol. Cell. Endocrinol.* **2019**, *489*, 107–118. [[CrossRef](#)]
46. Sawai, M.; Uchida, Y.; Ohno, Y.; Miyamoto, M.; Nishioka, C.; Itohara, S.; Sassa, T.; Kihara, A. The 3-hydroxyacyl-CoA dehydratases HACD1 and HACD2 exhibit functional redundancy and are active in a wide range of fatty acid elongation pathways. *J. Biol. Chem.* **2017**, *292*, 15538–15551. [[CrossRef](#)]
47. Krause, K.; Weiner, J.; Hönes, S.; Klötting, N.; Rijntjes, E.; Heiker, J.T.; Gebhardt, C.; Köhrle, J.; Führer, D.; Steinhoff, K.; et al. The Effects of Thyroid Hormones on Gene Expression of Acyl-Coenzyme A Thioesterases in Adipose Tissue and Liver of Mice. *Eur. Thyroid. J.* **2015**, *4* (Suppl. 1), 59–66. [[CrossRef](#)] [[PubMed](#)]
48. Oliveira, D.M.; Chalfun-Junior, A.; Chizzotti, M.L.; Barreto, H.G.; Coelho, T.C.; Paiva, L.V.; Teixeira, P.D.; Schoonmaker, J.P.; Ladeira, M.M. Expression of genes involved in lipid metabolism in the muscle of beef cattle fed soybean or rumen-protected fat, with or without monensin supplementation. *J. Anim. Sci.* **2014**, *92*, 5426–5436. [[CrossRef](#)]
49. Ko, M.H.; Puglielli, L. The sterol carrier protein SCP-x/pro-SCP-2 gene has transcriptional activity and regulates the alzheimer disease gamma-secretase. *J. Biol. Chem.* **2007**, *282*, 19742–19752. [[CrossRef](#)]
50. Li, G.; Fu, S.; Chen, Y.; Jin, W.; Zhai, B.; Li, Y.; Sun, G.; Han, R.; Wang, Y.; Tian, Y.; et al. MicroRNA-15a Regulates the Differentiation of Intramuscular Preadipocytes by Targeting ACAA1, ACOX1 and SCP2 in Chickens. *Int. J. Mol. Sci.* **2019**, *20*, 4063. [[CrossRef](#)] [[PubMed](#)]
51. Anton, I.; Kovács, K.; Holló, G.; Farkas, V.; Lehel, L.; Hajda, Z.; Zsolnai, A. Effect of leptin, DGAT1 and TG gene polymorphisms on the intramuscular fat of Angus cattle in Hungary. *Livest. Sci.* **2011**, *135*, 300–303. [[CrossRef](#)]
52. Mazzucco, J.P.; Goszczynski, D.; Ripoli, M.; Melucci, L.; Pardo, A.; Colatto, E.; Rogberg-Muñoz, A.; Mezzadra, C.; Depetris, G.; Giovambattista, G.; et al. Growth, carcass and meat quality traits in beef from Angus, Hereford and cross-breed grazing steers, and their association with SNPs in genes related to fat deposition metabolism. *Meat Sci.* **2016**, *114*, 121–129. [[CrossRef](#)] [[PubMed](#)]
53. Gallego, S.F.; Sprenger, R.R.; Neess, D.; Pauling, J.K.; Færgeman, N.J.; Ejsing, C.S. Quantitative lipidomics reveals age-dependent perturbations of whole-body lipid metabolism in ACBP deficient mice. *BBA-Mol. Cell Biol. L.* **2017**, *1862*, 145–155. [[CrossRef](#)]
54. Bowman, T.A.; O’Keeffe, K.R.; D’Aquila, T.; Yan, Q.W.; Griffin, J.D.; Killion, E.A. Acyl CoA synthetase 5 (ACSL5) ablation in mice increases energy expenditure and insulin sensitivity and delays fat absorption. *Mol. Metab.* **2016**, *5*, 210–220. [[CrossRef](#)] [[PubMed](#)]
55. Itabe, H.; Yamaguchi, T.; Nimura, S.; Sasabe, N. Perilipins: A diversity of intracellular lipid droplet proteins. *Lipids Health Dis.* **2017**, *16*, 83. [[CrossRef](#)] [[PubMed](#)]