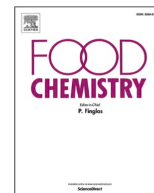
Food Chemistry 504 (2026) 147974

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Insight into the meat quality differences of Tibetan sheep from different altitudes based on metabolomics

Ruisi Liu, Jianing Fu, Shaobo Li, Minghui Gu, Liang Li, Le Xu, Jiangying Yu, Dequan Zhang, Li Chen *

Institute of Food Science and Technology, Chinese Academy of Agriculture Sciences, Key Laboratory of Agro-Products Quality & Safety Harvest, Storage, Transportation, Management and Control, Ministry of Agriculture and Rural Affairs, Beijing 100193, China

A R T I C L E I N F O

Keywords:
High-altitude adaptation
Cooking loss
Meat color
HIF-1α signaling
Lysine degradation

A B S T R A C T

Tibetan sheep from different altitudes exhibit unique meat quality attributes, whereas the molecular mechanisms remain unelucidated. Such meat quality attributes are associated with underlying metabolic processes, which are influenced by high-altitude conditions. Herein, meat quality and muscle metabolism in Tibetan sheep from Oula (2900 m), Huoerba (4000 m), and Duoma (5100 m) were compared. Oula sheep showed higher moisture and lighter color, while Duoma sheep had the lowest fat content. A total of 419 differential metabolites were identified based on metabolomics. Among them, peptide contents decreased with increasing altitude of Tibetan sheep's residence, while carnitine derivatives increased. Bioinformatics analysis indicated that high altitude regulates HIF-1α signaling through hypoxic conditions and oxidative stress mediates glutathione oxidation, thus affecting meat L^* value. This study reveals meat quality and the metabolic basis in Tibetan sheep at different altitudes, establishing a scientific foundation for resource utilization and development of Tibetan sheep at specific altitudes.

1. Introduction

Livestock such as Tibetan sheep and yaks constitute a critical source for local herders on the Qinghai-Tibet Plateau (Deng et al., 2025; Jing et al., 2022). They have developed distinct adaptations to high-altitude environments (cold and hypoxia tolerance) through long-term inhabitation of the Qinghai-Tibet Plateau, and correspondingly, genomic analyses have revealed strong signatures of natural selection (Yang et al., 2016). Notably, the nutritional profile of plateau-sourced meat is distinguished by high protein and low fat content (Li et al., 2025). However, there are some differences in meat quality of various livestock at different altitudes.

Meat quality of livestock animals exhibits significant differences with varying altitudes (Han et al., 2020). Zhu et al. (2023) examined the physicochemical properties of yak meat from various altitudes, demonstrating that high-altitude yak meat had increased protein content and decreased fat content, along with notable differences in lightness and redness values. Costa et al. (2011) investigated the fatty acids of the biceps femoris muscle in Barrosã calves from different altitudes and found significant variations in the α-tocopherol and cholesterol levels. Panjono Kang et al. (2011) analyzed the meat quality of Hanwoo cattle from different altitudes, finding that high-altitude regions produced yak meat with higher pH and methemoglobin content, as well as a lower saturated fatty acid (SFA) profile. These differences likely result from adaptive responses to high-altitude environments, particularly involving energy metabolism and blood oxygen transport (Storz & Cheviron, 2021), reflecting signatures of natural selection. These alterations in proteins and fatty acids are likely adaptive responses to high-altitude conditions (Ayalew et al., 2021), which entail critical processes such as energy metabolism and oxygen transport. However, the underlying mechanisms and key substances involved remain to be fully elucidated.

Metabolomics enables the identification of regulatory enzymes and genes within metabolic pathways through the analysis of small-molecule metabolites (<1000 Da). In muscle biology, metabolomics can comprehensively characterize muscle composition and resolve altitude-adapted molecular metabolites that cannot be detected by conventional techniques (Zhang et al., 2021). For instance, inosine monophosphate,

* Corresponding author at: Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, No.2 Yuanmingyuan West Road, Hai-Dian District, Beijing 100193, China.

E-mail address: chenliwork@126.com (L. Chen).

https://doi.org/10.1016/j.foodchem.2026.147974
Received 1 July 2025; Received in revised form 24 December 2025; Accepted 11 January 2026
Available online 13 January 2026
0308-8146/© 2026 Elsevier Ltd. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

along with creatine, carnosine, and conjugated linoleic acid, has been identified as a regulator of beef tenderness in the Longissimus lumborum of Nellore cattle (De Zawadzki et al., 2017). Through non-targeted metabolomics, Zhang et al. (2021) identified the downregulation of adipocyte lipolysis and the upregulation of protein digestion and absorption as metabolic underpinnings for the enhancement of tenderness and water-holding capacity in Tibetan mutton. NMR-based metabolomics employed by Wang et al. (2017) indicated that energy metabolites, particularly creatine, underlie the variations in pH and water-holding capacity of duck meat. Therefore, metabolomics can be used to identify the corresponding metabolic pathways by characterizing the comprehensive features of small molecule metabolites such as fatty acyls, glycerophospholipids and amino acids, and then elucidate the key metabolites and pathways that affect energy metabolism and phenotypic characteristics, which becomes a key tool to explain the molecular mechanism of meat quality differences. However, the specific metabolic basis for meat quality variations in Tibetan sheep at different altitudes has not been fully elucidated and requires systematic investigation.

This study systematically compared the meat quality of Tibetan sheep raised at three different altitudes, focusing on eating quality and nutritional composition. Meanwhile, non-targeted metabolomics combined with multivariate data was used to analyze differential metabolite profiles and identify enriched metabolic pathways. This research aimed to investigate the quality differences of Tibetan sheep at different altitudes and identify metabolic factors responsible for these differences, while providing data support for exploring the meat quality characteristics and subsequent processing of Tibetan sheep.

2. Materials and methods

2.1. Sample collection and preparation

Oula Tibetan sheep (Henan County, 2900 m), Huoerba Tibetan sheep (Zhongba County, 4000 m), and Duoma Tibetan sheep (Amdo County, 5100 m) were collected. Two years old, rams were six each. All sheep, with an average hot carcass weight of 19.6 ± 1.26 kg, were managed under a traditional free-range grazing system on natural alpine pastures. Their diet was solely derived from native vegetation, composed predominantly of Gramineae, Salicaceae and Rosaceae, with no supplemental feed provided. All Tibetan sheep were slaughtered using standard routines of the commercial slaughterhouse (Qinghai Yutai Livestock Products Co., Ltd., Qinghai, China) based on National Standards of China (GB/T 17237–2008). Our study was in accordance with the Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences Ethical and Welfare Committee (IF No.20250627c029), and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

A total of 18 *Longissimus Dorsi* from carcasses ($n = 6$, Oula, Huoerba, Duoma, six each) were collected. The whole transverse section of the *Longissimus Dorsi* muscle, with a thickness of approximately 8 cm, was excised from the 9th to 13th ribs and the sample from each carcass weighed approximately 720 g. The obtained muscle was divided into three distinct portions for analysis: the first portion (100 g of meat samples at the caudal end of the *Longissimus Dorsi*) was stored at 4 °C for 24 h for the determination of meat color and pH; the second portion (6 g, 1 g each in frozen tubes) was frozen in liquid nitrogen and stored at −80 °C for metabolomic analysis; the third portion (300 g of meat samples from the same part of the *Longissimus Dorsi*) was stored at −20 °C for the determination of cooking loss, amino acids, and fatty acids, and so on. When needed, the samples were thawed gradually in a constant-temperature room at 4 °C until their internal temperature reached 0 °C, then surface tissue and fat were removed for analysis.

2.2. Color

The color parameters (L^*, a^*, b^*) of the *Longissimus Dorsi* muscle were determined using a CM-600d spectrophotometer (Konica Minolta, Tokyo, Japan). The instrument was calibrated with a standard white calibration tile before measurement. The operating conditions were set with D65 standard illuminant, 10° standard observer angle, and specular component excluded (SCE) mode to characterize the intrinsic color properties of the meat samples.

Before analysis, all samples were equilibrated at room temperature (25 ± 1 °C) for 20 min. The measurement aperture was placed in full contact with the meat surface. Three measurements were taken on each sample at distinct locations, and the mean values were calculated for statistical analysis.

2.3. pH

The pH was measured after 24 h of storage at 4 °C using a calibrated Testo 205 pH meter (Testo, Lenzkirch, Germany). For each independent sample ($n = 6$), the probe was inserted to a depth of 3 cm at the caudal end of the *Longissimus Dorsi*, and the reading was recorded after stabilization. Three measurements were taken at distinct locations within the same muscle sample, and the average was calculated for statistical analysis.

2.4. Protein, fat and moisture

All measurements for chemical composition (moisture, ash, crude protein, and crude fat) were performed following AOAC methods (aoac, 2020): oven drying at 105 °C (AOAC 934.01) for moisture, muffle furnace ignition at 550 °C (AOAC 934.01) for ash, the Kjeldahl method (AOAC 990.03) for crude protein, and Soxhlet extraction (AOAC 920.39) for crude fat. All indicators were tested at least three times and the average value was calculated.

2.5. Cooking losses

Cooking losses of the samples were determined following the method of Li et al. (2022). 50 g lamb meat was sliced, recorded as M1, then sealed in a bag and cooked in a HWS-24 water bath (Yiheng, Shanghai, China) that had been pre-equilibrated at 71 °C. Cooking was conducted for 35 min with the temperature held constant throughout. The sealed bag containing the cooked sample was then immediately cooled under running cold water for 30 min. After reaching room temperature, the sample was removed from the bag, blotted dry, and weighed again (M2). The formula used was:

$$\text{Cooking loss}(\%) = \frac{\text{M1} - \text{M2}}{\text{M1}} \times 100\%$$

2.6. Amino acid

The amino acid content in the lamb back muscle was determined using the method of Liang et al. (2024) with high-performance liquid chromatography. The *Longissimus Dorsi* samples (0.5 g) were homogenized with 10 mL of 6 M hydrochloric acid by vortexing for 2 min in a hydrolysis tube. The tube was sealed and placed in a constant-temperature LBAO-150H drying oven (STIK, Guangzhou, China) at 110 ± 1 °C for 22 h. After cooling to room temperature, the sample was filtered 6–7 times with purified water and then adjusted to a final volume of 50 mL in a volumetric flask. For amino acid analysis, the amino acid standard solution and the sample solution were injected in equal volumes into the amino acid analyzer (L-8900; Hitachi, Tokyo, Japan). Seventeen amino acids were identified and quantified using standard curves, which were constructed using a mixture of amino acid standards (Sigma Aldrich, St Louis, MO, USA).

2.7. Fatty acid

0.5 g of the meat sample was transferred to a 2 mL glass centrifuge tube, 1 mL of chloroform-methanol solution was added, and the sample was sonicated for 30 min. The separation was carried out according to Zhang et al. (2022) on a capillary column (30 m × 0.25 mm ID × 0.25 μm, DB-WAX) in a gas chromatograph, and mass spectrometry analysis was performed using GC–MS (7890/5975C, Agilent, Palo Alto, CA, USA).

The program temperature was set as follows: the initial temperature was maintained at 40 °C for 5 min, then increased to 220 °C at a rate of 10 °C/min and held for 5 min. The carrier gas was helium with a flow rate of 1.0 mL/min. The temperature of the inlet, ion source, and transfer line was 280 °C, 230 °C, and 250 °C, respectively. The MS was operated in the Electron Impact Ionization (EI) source with electron energy set at 70 eV.

2.8. Metabolomics

2.8.1. Metabolite extraction

Metabolite extraction was conducted based on Guo et al. (2024) with modifications. Briefly, meat samples were ground in liquid nitrogen and homogenized with a pre-cooled mixture of methanol, acetonitrile, and water (2:2:1, *v*/*v*/v). The homogenate was vortexed for 60 s, sonicated for 30 min, and incubated at 20 °C for 10 min. After centrifugation at 14,000 rpm (radius = 83 mm, 18,200 xg) in the 5424 R Centrifuge (Eppendorf, Leipzig, Germany) for 20 min, the supernatant was collected and dried in a vacuum centrifuge. The residue was reconstituted in 100 μL of acetonitrile-water (1:1, v/v), vortexed, and centrifuged again under the same conditions. The final supernatant was subjected to UHPLC-QTOF-MS analysis.

2.8.2. UHPLC-QTOF-MS

The samples were separated by ultra-high performance liquid chromatography (UHPLC) (1290 Infinity, Agilent) following the method of Guo et al. (2024) with slight modifications. During the analysis, the samples were kept at 4 °C, with a column temperature of 25 °C, a flow rate of 0.5 mL/min and an injection volume of 2 μL. The mobile phase system consisted of A (25 mM ammonium acetate and ammonium solution) and B (acetonitrile).

AB Triple TOF 6600 Mass Spectrometry (SCIEX, Framingham, MA, USA) was used to collect primary and secondary spectra of the sample. Data were acquired in both positive and negative electrospray ionization (ESI) modes with the following source parameters: ion source gas 1 (GS1): 60, ion source gas 2 (GS2): 60, curtain gas (CUR): 30, source temperature: 600 °C, and ion spray voltage: ± 5500 V. Full scans were recorded over *m/z* 60–1000 with an accumulation time of 0.20 s/spectrum. Product ion scans were collected over m/z 25–1000 using information-dependent acquisition (IDA) with a collision energy of 35 ± 15 eV.

2.8.3. Quality control assessment

Quality control (QC) samples were prepared by mixing equal aliquots from all 18 individual sample extracts. These QC samples were analyzed throughout the analytical sequence to assess instrument stability, with the retention time deviation, coefficient of determination (R^2), relative standard deviation (RSD), and mass accuracy monitored as key criteria for data reliability.

2.9. Data analysis

2.9.1. Statistical analysis

To ensure statistical reliability, each experimental group consisted of six independent samples ($n = 6$). Each sample was measured in at least three technical replicates and the mean values were calculated for statistical analysis. Prior to one-way ANOVA, the assumptions of normality and homogeneity of variances were verified using the Shapiro-Wilk and Levene's tests, respectively. Having verified that the data met these parametric assumptions, significant inter-group differences ($P < 0.05$) were identified using Tukey's HSD post-hoc test following the ANOVA. The data are expressed as mean ± standard deviation. Statistical significance ($P < 0.05$) was determined by one-way ANOVA using SPSS 22.0 software package (SPSS Inc., Chicago, IL, USA).

2.9.2. Metabolite statistics and analysis

For the analysis of metabolomics data, the raw data were first normalized, including sample normalization, normalization by sum, data scaling, auto scaling, data transformation, none. Principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA) were conducted using the R package (4.1.2) MetaboAnalystR. Prior to OPLS-DA, the data were $\log_2$-transformed and scaled using unit variance scaling (UV). The OPLS-DA models were validated through permutation testing. Differential metabolites were screened using thresholds of VIP > 1.5 and $P < 0.05$. A two-tiered screening strategy was employed: initial screening (VIP ≥ 1, |FC| ≥ 2, $P < 0.05$) for a broad overview; refined screening (VIP > 1.5, $P < 0.05$) for downstream analysis. These were used for Venn diagrams, hierarchical clustering (top 50 shown), KEGG pathway enrichment, and Mantel test correlation with meat quality traits. Visualization analyses, including volcano plots, heatmaps, and Sankey diagrams, were generated using the R language (4.1.2) and the online platform MetWare Cloud (https://cloud.metware.cn. Accessed September 25, 2024).

Table 1
Edible quality and basic nutritional indicators of Oula, Huoerba and Duoma.

Index	Oula	Huoerba	Duoma
pH	6.42 ± 0.05[a]	5.66 ± 0.07[b]	5.72 ± 0.12[b]
*L**	41.99 ± 1.23[a]	36.87 ± 2.75[b]	37.09 ± 2.63[b]
*a**	16.98 ± 4.05[a]	16.98 ± 2.05[a]	16.18 ± 2.44[a]
*b**	12.04 ± 0.07[a]	10.63 ± 0.84[b]	10.99 ± 0.92[ab]
Cooking Loss (%)	35 ± 3.5[a]	27 ± 3.9[b]	26 ± 2.9[b]
Moisture (%)	75 ± 0.7[a]	73 ± 3.1[a]	68 ± 0.6[b]
Protein (%)	21 ± 0.2[b]	21 ± 0.6[b]	23 ± 1.2[a]
Fat (%)	6 ± 1.5[a]	5 ± 0.8[a]	2 ± 0.7[b]

Lower case letters represent significant differences in indicators among Tibetan sheep at different altitudes ($P < 0.05$).

Table 2
Amino acid composition and content of Oula, Huoerba and Duoma.

Amino acids (g/100 g)	Oula	Huoerba	Duoma
Asp	1.84 ± 0.08	2.02 ± 0.13	1.86 ± 0.01
Thr	0.93 ± 0.04	1.01 ± 0.07	0.93 ± 0.01
Ser	0.79 ± 0.03	0.87 ± 0.06	0.8 ± 0.01
Glu	3.04 ± 0.17	3.42 ± 0.21	3.04 ± 0.01
Pro	0.68 ± 0.03[b]	0.78 ± 0.04[a]	0.7 ± 0.02[b]
Gly	0.84 ± 0.04	0.9 ± 0.05	0.82 ± 0.03
Ala	1.13 ± 0.05	1.24 ± 0.07	1.12 ± 0.01
Val	0.95 ± 0.03	0.98 ± 0.06	0.9 ± 0.01
Met	0.54 ± 0.03	0.58 ± 0.04	0.52 ± 0.01
Ile	0.91 ± 0.04	0.97 ± 0.05	0.88 ± 0.01
Leu	1.61 ± 0.07	1.77 ± 0.11	1.61 ± 0.01
Tyr	0.69 ± 0.04	0.76 ± 0.05	0.69 ± 0.01
Phe	0.79 ± 0.03	0.88 ± 0.06	0.8 ± 0.01
Lys	1.79 ± 0.08	1.98 ± 0.12	1.81 ± 0.01
His	0.72 ± 0.03	0.71 ± 0.07	0.74 ± 0.01
Arg	1.24 ± 0.06	1.39 ± 0.09	1.25 ± 0.01
EAA	7.51 ± 0.32	8.16 ± 0.5	7.46 ± 0.02
NEAA	10.97 ± 0.3	12.08 ± 0.43	11.03 ± 0.03
TAA	18.48 ± 0.83	20.25 ± 1.24	18.5 ± 0.05

EAA: Threonine + Valine + Methionine + Lysine + Phenylalanine + Isoleucine + Leucine;
NEAA: Glycine + Glutamic + Aspartic + Alanine + Argine + Histidine + Serine + Proline + Tyrosine + Ornithine + Glutamine + Asparagine;
Lower case letters represent differences in indicators among Tibetan sheep at different altitudes.

Table 3
Fatty acids composition and content of Oula, Huoerba, and Duoma.

Fatty Acids(mg/100 g)	Oula	Huoerba	Duoma
C6:0	/	0.51 ± 0.00[a]	0.58 ± 0.15[a]
C10:0	/	0.67 ± 0.00[a]	1.07 ± 0.30[a]
C12:0	/	/	1.02 ± 0.36[a]
C14:0	3.16 ± 1.92[b]	5.61 ± 1.20[b]	21.37 ± 1.18[a]
C14:1n5	/	/	0.50 ± 0.11[a]
C15:0	0.43 ± 0.00[b]	0.36 ± 0.01[b]	1.45 ± 0.57[a]
C16:0	33.67 ± 1.00[b]	55.40 ± 12.04[b]	187.00 ± 4.78[a]
C16:1n7	2.83 ± 0.87[b]	4.06 ± 0.10[b]	14.19 ± 5.63[a]
C17:0	1.51 ± 0.17[b]	1.52 ± 0.10[b]	5.88 ± 2.06[a]
C17:1n7	1.33 ± 0.42[ab]	1.07 ± 0.17[b]	2.80 ± 1.01[a]
C18:0	23.70 ± 5.58[b]	35.27 ± 4.47[b]	112.83 ± 8.25[a]
C18:1n9t	2.88 ± 2.02[a]	2.00 ± 0.70[a]	3.13 ± 0.64[a]
C18:1n9c	44.00 ± 3.46[b]	88.03 ± 11.21[b]	292.33 ± 1.02[a]
C18:2n6c	4.17 ± 1.09[b]	10.12 ± 1.04[ab]	16.17 ± 4.66[a]
C18:3n3	1.37 ± 0.41[a]	0.32 ± 0.02[b]	0.81 ± 0.19[ab]
C20:4n6	1.51 ± 0.32[b]	3.31 ± 1.08[ab]	4.01 ± 0.72[a]
C24:0	1.13 ± 0.29[a]	/	/
SFA	64.01 ± 3.91[b]	99.01 ± 0.17[b]	331.00 ± 11.30[a]
MUFA	51.07 ± 3.40[b]	95.08 ± 1.10[b]	313.06 ± 10.90[a]
PUFA	7.00 ± 2.01[b]	14.03 ± 0.42[b]	21.09 ± 0.52[a]

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA (C6:0 + C8:0 + C162:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C24:0).
MUFA (C14:1 N5 + C16:1 N7 + C17:1 N7 + C18:1N9t + C18:1N9c).
PUFA (C18:2N6c + C18:3 N3 + C20:4 N6).
Lower case letters represent differences in indicators among Tibetan sheep at different altitudes.

Functional annotation and pathway enrichment analysis were performed utilizing the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.genome.jp/kegg/. Accessed October 16, 2024).

3. Results and discussion

3.1. Nutritional quality

3.1.1. Protein, fat and moisture

The conventional nutritional composition of meat (protein, fat and moisture) plays a decisive role in its nutritional value and eating quality. As shown in Table 1, Duoma Tibetan sheep exhibited a significantly higher protein content compared to other Tibetan sheep, whereas the fat content was significantly lower. This observation was consistent with the results of Zi et al. (2004), whose research showed higher protein content in high-altitude animals. This might be related to intense ultraviolet radiation at high altitudes, which promoted the synthesis of antioxidant proteins and consequently increased protein content (Geihs et al., 2020). Additionally, Rellinger et al. (2015) has demonstrated that hypoxic conditions at high altitudes upregulate relevant metabolic enzymes, thereby facilitating protein synthesis.

3.1.2. Amino acid

The amino acid composition of Tibetan sheep at three altitudes was analyzed in Table 2. Proline exhibited higher levels in Huoerba and Duoma sheep, while there were no significant differences in the other amino acid content among the Tibetan sheep at three altitudes. Furthermore, no significant differences were observed in the concentrations of total amino acids (TAA), non-essential amino acids (NEAA), and essential amino acids (EAA) among the three groups ($P > 0.05$). These findings demonstrated that altitude had relatively limited effects on the amino acid profile, whereas potential variations in undetected rare amino acids cannot be excluded.

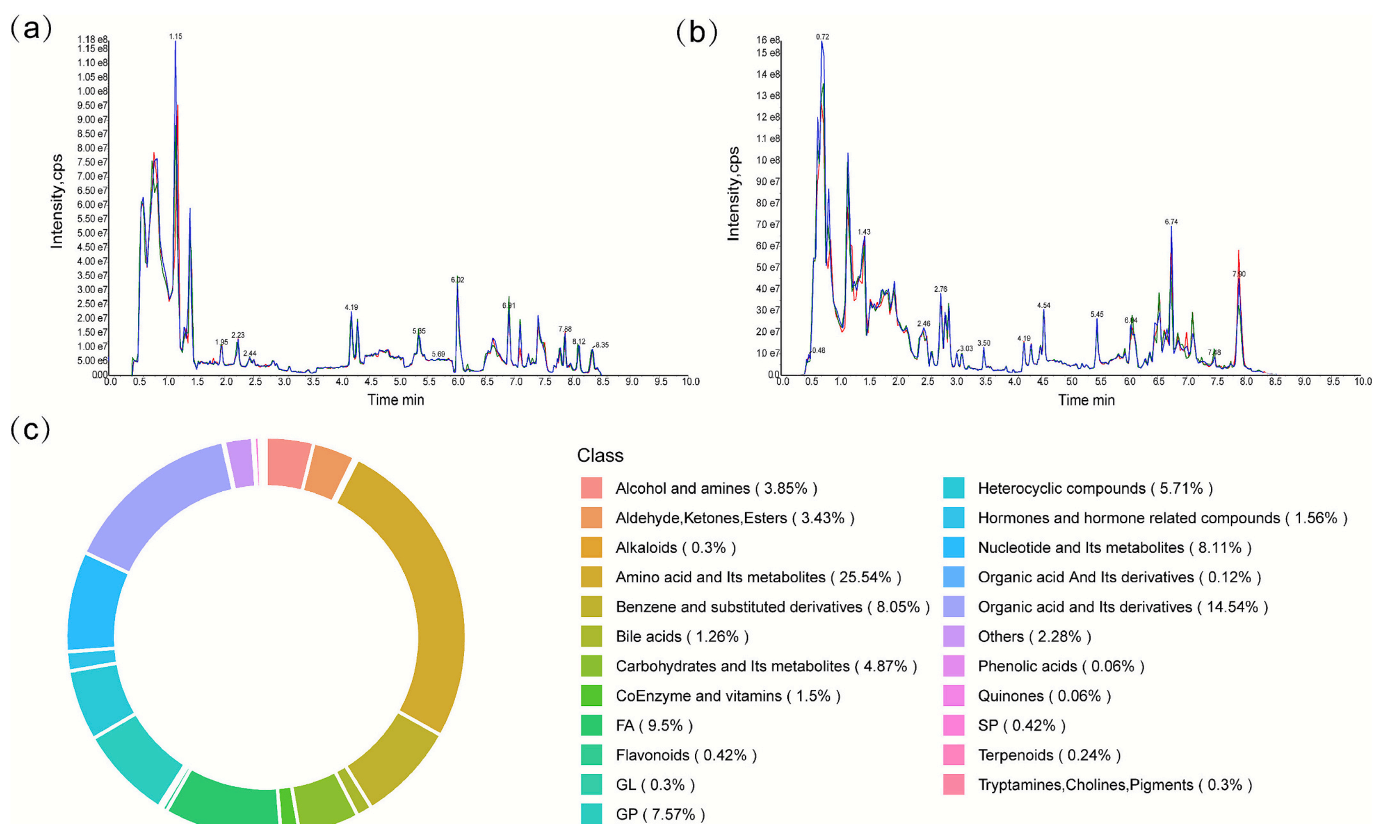


Fig. 1. QC sample mass spectrometry detection plots and results. (a) Total Ion Current (TIC) overlay of T3 negative ion mode, (b) T3 positive ion mode, (c) Metabolite classes and percentages. (a, b) The x-axis represents the retention time (RT) of metabolite detection, and the y-axis represents the ion signal intensity (measured in cps, counts per second).

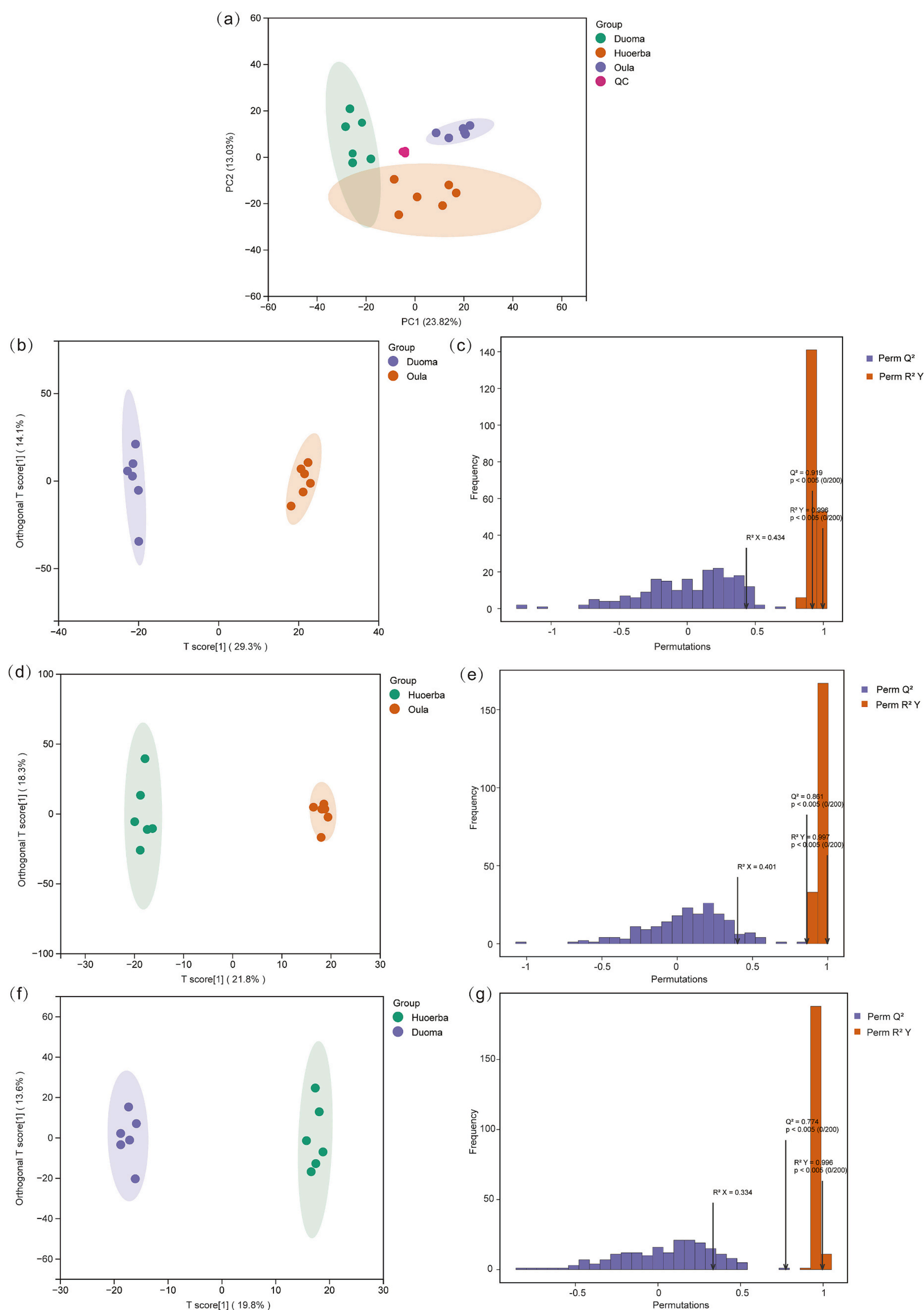


Fig. 2. Multivariate statistical analysis of differential metabolites plots. (a) Principal component analysis plots for the three sheep, (b, d, f) Partial least squares discriminant analysis (OPLS-DA) scores plot for the Duoma vs Oula, Huoerba vs Oula, and Huoerba vs Duoma groups, respectively, (c, e, g) Plots of permutation tests for the OPLS-DA model. Green = Duoma, Orange = Huoerba, Purple = Oula, Pink = Quality Control. In the plots of permutation tests, the horizontal axis represents the model's R^2Y and Q^2 values, while the vertical axis shows the frequency of model classification results in 200 random permutation experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

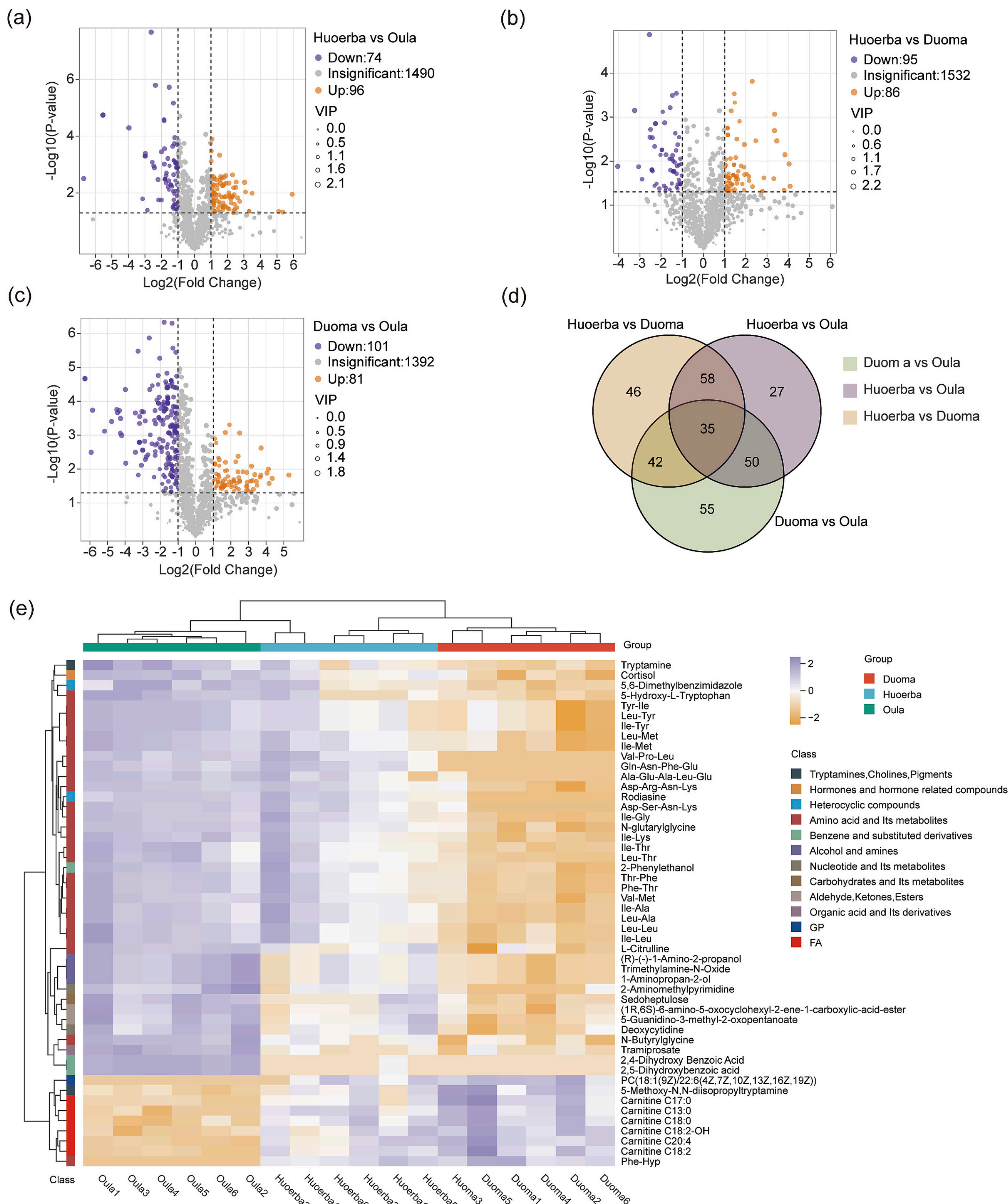


Fig. 3. Comparison of the metabolomics data of the *Longissimus Dorsi* among Oula, Huoerba and Duoma groups. (a, b, c) Volcano plots of comparisons between Huoerba vs Oula, Huoerba vs Duoma and Duoma vs Oula groups, respectively. The horizontal axis represents the logarithm of the fold change in the relative content of a metabolite between two groups of samples; the larger the value, the greater the difference. (d) Venn diagrams of differential metabolites in the three comparisons (Duoma vs Oula, Huoerba vs Duoma and Huoerba vs Oula); (e) Oula, Huoerba and Duoma Tibetan sheep clustered heat maps of differential metabolites (plotted at $P < 0.05$, VIP value >1.5, top 50 selected for presentation). The horizontal axis represents sample names, and the vertical axis represents differential metabolites. Different colors indicate content values obtained after standardization (Purple = high content, Orange = low content). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.1.3. Fatty acid

The fatty acid profiles of Tibetan sheep at three altitudes are shown in Table 3. A total of 13, 14, and 16 distinct fatty acids were detected in Oula, Huoerba, and Duoma Tibetan sheep, respectively. C12:0 (lauric acid) and C14:1n5 (myristoleic acid) were exclusively present in Duoma Tibetan sheep, while C24:0 was uniquely identified in Oula Tibetan sheep. Similar results were obtained by Richter et al. (2012), who demonstrated significant altitude-dependent variations in fatty acid profiles of lambs.

Meanwhile, Duoma Tibetan sheep displayed higher contents of saturated (SFA), polyunsaturated (PUFA), and monounsaturated fatty acids (MUFA) compared to the others ($P < 0.05$). Generally, SFA and MUFA (Krishnan & Cooper, 2014) serve as efficient energy sources and contribute to thermoregulation, while PUFA enhance cellular membrane stability and provide antioxidant protection against oxidative stress induced by UV radiation and hypoxia (Crescenzo et al., 2017). Hence, the compositional difference in meat samples might represent an adaptation of the Tibetan sheep to high-altitude environments.

3.2. Eating quality

The eating quality of meat includes color, pH, and cooking loss (Li et al., 2022). The results of the eating quality of Oula, Huoerba, and Duoma Tibetan sheep are shown in Table 1. The L^* values and b^* values of Oula are significantly higher ($P < 0.05$), which might be due to the changes in myoglobin levels and differences in lipid composition. Xin et al. (2022) and Zhu et al. (2023) found significant reductions in L^* values, b^* values, and water-holding capacity of yak meat with ascending elevations (3000–4500 m). Generally, increasing altitude leads to reduced oxygen concentration, and the hypoxic environment's impact on animal myoglobin levels results in meat browning and decreased L^* values (Xu et al., 2023).

The pH values at postmortem 24 h were within the normal range (5.8–6.5) (Tao et al., 2021). Duoma and Huoerba Tibetan sheep exhibited significantly lower cooking loss than Oula Tibetan sheep ($P < 0.05$), indicating superior water-holding capacity in the high-altitude groups. It was demonstrated that hypoxic plateau conditions enhance aerobic metabolic efficiency in animals, significantly increasing the proportion of slow-twitch fibers (Type I) rich in mitochondria and myoglobin (Zhang et al., 2014). The elevated slow-twitch fiber content helps stabilize muscle pH, thereby improving water-holding capacity. Consequently, Duoma and Huoerba Tibetan sheep adapted to higher altitudes exhibit superior water retention properties.

3.3. Metabolomics

3.3.1. Metabolite composition

The close clustering of the QC (Quality Control) samples confirmed the reproducibility and reliability of the metabolomic data. The metabolite profile of *Longissimus Dorsi* samples from Oula, Huoerba, and Duoma Tibetan sheep was analyzed using UPLC-QTRAP-MS/MS as shown in Fig. 1. A total of 1664 subclasses of metabolites from 16 major groups were detected, including 425 (25.54%) amino acids and their derivatives, 242 (14.54%) organic acids and their derivatives, 158 (9.50%) fatty acids, 135 (8.11%) nucleotides and their metabolites, 134 (8.05%) benzene and substituted derivatives and others. It can be observed that amino acids and their derivatives, organic acids and other substances are the main components in muscle metabolites.

3.3.2. Multivariate statistical analysis

To better understand the metabolic differences between Tibetan sheep at 3 altitudes, PCA and OPLS-DA were used for pairwise comparisons of three breeds in Fig. 2. The PCA results after removing QC samples explained 35.58% of the variance. To optimize inter-group separation and better display differences, OPLS-DA was used for further analysis of the two groups. It was found that the Q^2 (predictive ability) of the three models: Duoma vs Oula, Duoma vs Huoerba, and Oula vs Huoerba, were all greater than 0.7, with the robustness of the predictions indicated by R^2Y values of 0.996, 0.997, and 0.996, all greater than 0.9, indicating that the analysis model has high consistency and reliability, and the analysis in this study is effective. Since the Oula sheep showed a clear trend of intra-group aggregation and inter-group separation compared to Duoma and Huoerba sheep, the Tibetan sheep at 3 altitudes can be well distinguished.

3.3.3. Differential metabolite analysis

A total of 419 differential metabolites were screened according to the criteria of FC ≥ 2 or FC ≤ 0.5 and VIP value ≥ 1 at $P < 0.05$. Volcano (Fig. 3a,b,c) showed that a total of 81 metabolites were observed to be up-regulated in the Duoma vs Oula group, with a clear predominance of fatty acyls (23), glycerophospholipids (15), amino acids and their metabolites (13), and organic acids and their derivatives (9). Herein, in the Huoerba vs Oula group, Up-regulation of 96 metabolites was observed, of which the prominent ones were fatty acyls (43), glycerophospholipids (15), amino acids and their metabolites (10), and alcohols and amines (4). The results indicated that organic acids, alcohols, and amines were the primary differential metabolites in Duoma and Huoerba Tibetan sheep, while fatty acyls, organic acids, and benzene derivatives were predominant in Duoma and Oula Tibetan sheep. Fatty acyls directly influence the fat content and distribution in muscle (Bjorntorp, 1987), which aligns with the present study's findings of differences in crude fat among Tibetan sheep at three altitudes. In addition, the accumulation of organic acids in the muscle affects the final pH of the meat, and lower pH may lead to a darker color and tougher texture (Sun et al., 2018), which explains the lower pH and L^* values of Huoerba Tibetan sheep. Then, shared and endemic metabolites (VIP > 1.5 and $P < 0.05$) were shown through the overlapping regions of Venn diagrams (Fig. 3d). Among them, 58 metabolites were shared differential metabolites in the Huoerba group, 42 metabolites were shared differential metabolites in the Duoma group, and 50 metabolites were shared differential metabolites in the Oula group.

All differential metabolites in the three groups were visualized in a heat map (Fig. 3e). Hierarchical cluster analysis was conducted on Tibetan sheep meat from different altitudes to assess differences in accumulation patterns with the conditions of $P < 0.05$ and VIP > 1.5, and the top 50 major metabolites were selected for presentation. The results showed that there was a significant difference in the metabolites of *Longissimus Dorsi* between Oula and Duoma sheep, with the former having a higher content of amino acids and their metabolites, heterocyclic compounds, alcohols and amines, while the opposite was true for fatty acids and glycerophospholipids. Further analysis revealed that the concentrations of dipeptides and tripeptides, such as Tyr-IIE, Leu-Tyr, and Val-Pro-Leu, progressively decreased with increasing altitude, as these compounds are primarily involved in protein degradation and metabolism. In contrast, the levels of carnitine analogues, including Carnitine C17:0, Carnitine C13:0, and Carnitine C18:0, exhibited a significant increase in Tibetan sheep across all three altitudes. A clear upward trend in the content of carnitine analogues was observed among the Tibetan sheep at three altitudes. Carnitine analogues and their metabolites mainly play an important role in fatty acid metabolism, antioxidative stress and energy production (Li & Zhao, 2021). Specifically, carnitine is a key molecule in β-oxidation, which helps fatty acids to enter the mitochondria for oxidative catabolism and energy production. Additionally, its antioxidant properties and role in energy supply assist the organism in adapting to stresses such as hypoxia and cold environmental conditions (Chicco et al., 2018). Carnitine derivatives are compounds created when carnitine bonds with specific fatty acids, aiding in their metabolism. For example, Carnitine C17:0 is involved in the metabolic processes of C17:0.

Generally, the elevated levels of carnitines and their derivatives in Tibetan sheep and goats residing at high altitudes reflect their active lipid metabolism and adaptation to hypoxic conditions. Elevated

Fig. 4. Sankey diagram of pathway enrichment for differential metabolites in Tibetan sheep at three altitudes (The nodes on the left are the substances enriched in the pathway, and Rich Factor is the ratio of the differential metabolite in the corresponding pathway to the total number of metabolites annotated to that pathway. The closer the *P*-value is to 0, the more significant the enrichment).

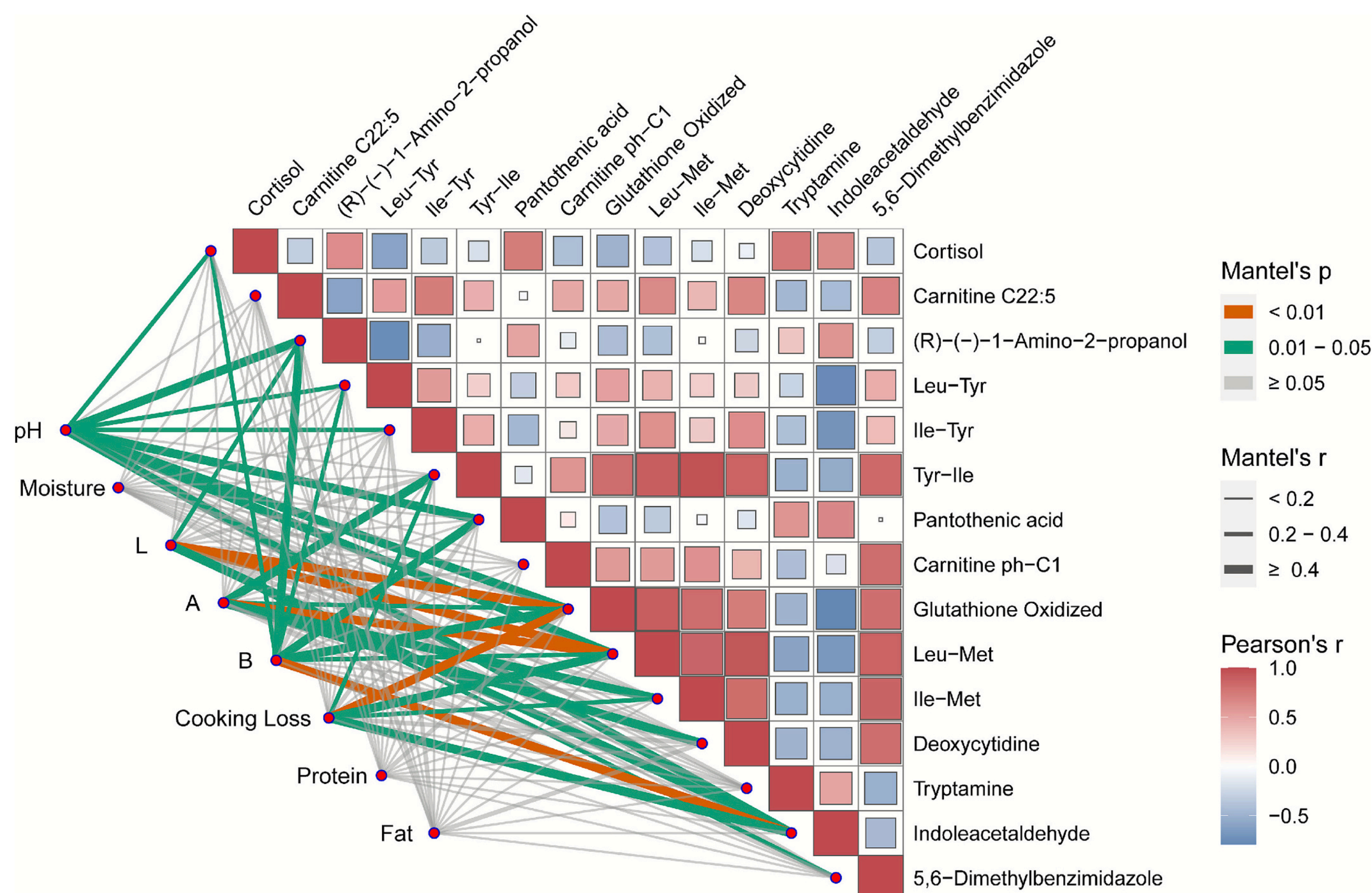


Fig. 5. Mantel correlation analysis plot of phenotypic baseline metrics with major differential metabolites. The color gradient represents the Pearson correlation coefficient. (Red = 1, Blue = −1) Line width represents the significant correlation coefficient from the Mantel test ($P < 0.05$ is significant). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

carnitine content was also shown to contribute to counteracting hypoxia in a study by Xu et al. (2023) in high-altitude hypoxic mice, which is consistent with the findings of this paper. Meanwhile, the content data of carnitine derivatives in the metabolomic data are in good agreement with the fatty acid content data (Table 3) in the present study.

3.3.4. KEGG enrichment of core difference products

A total of 185 differential metabolites were identified in this study, with screening criteria set at $P < 0.05$ and VIP value >1.5. KEGG pathway analysis was conducted using MetaboAnalyst 5.0 to elucidate the altered metabolic pathways among the three groups. The pathways predominantly associated with the differential metabolites in the *Longissimus Dorsi* of Oula, Huoerba, and Duoma Tibetan sheep are illustrated in Fig. 4, including thermogenesis, ABC transporters, lysine degradation, and coenzyme A biosynthesis. While thermogenesis and ABC transporters represent major common pathways across organisms, the minor pathways, such as lysine degradation, warrant further attention due to their potential functional significance. For instance, lysine degradation is intrinsically linked to the synthesis of acetyl-CoA (Davies et al., 2016). This suggests that in post-mortem muscle, changes in acetyl-CoA availability and protein acetylation may regulate the glycolytic rate and lactate accumulation, ultimately modulating key meat quality attributes such as tenderness and color (Liu et al., 2025). Additionally, the enrichment of bile acid biosynthesis, though minor, suggests a potential modulation of lipid digestion and metabolism. Specifically, alterations in this pathway could affect lipid absorption and utilization, thereby influencing the muscle fatty acid profile and subsequent sensory quality of the meat (Yi et al., 2023). A comparative analysis between the two groups is presented in Fig. S1. In the *Longissimus Dorsi* of Duoma and Oula, the metabolic pathways identified include ABC transporters, lysine degradation, and porphyrin metabolism, among others. In the comparison between Huoerba and Duoma, several pathways were significantly enriched, notably ABC transporters, nicotinate and nicotinamide metabolism, cysteine and methionine metabolism, and HIF-1α signaling pathways. Additionally, the differential metabolites in the dorsal muscles of Huoerba and Oula were primarily associated with thermogenesis, primary bile acid biosynthesis, bile secretion, and cortisol synthesis and secretion.

Of interest, the HIF-1α signaling pathway (Chiu et al., 2019; Xin et al., 2023) serves as a major regulator of numerous hypoxia-inducible genes under hypoxic conditions and has a variety of important functions, including angiogenesis, gluconeogenesis, erythropoiesis and metabolism, as well as immune response and inflammation. Differential metabolites detected in sheep at different altitudes were significantly enriched in the HIF-1α signaling pathway, suggesting that altitudinal changes may persistently activate this pathway to drive the unique hypoxic metabolic remodeling in plateau animals by enhancing the stability of HIF-1α proteins. Similarly, Xin et al. (2022) reported that HIF-1α protein increased with elevation, and its protein expression was associated with pH, glycolysis, and hardness (Zhu et al., 2025). Therefore, HIF-1α protein could be considered as a marker protein affecting the meat quality of yaks at different altitudes.

3.4. Correlation analysis between meat quality and major differential metabolites

The Mantel heat map was constructed to correlate the meat quality parameters and 15 major differential metabolites to explore their

relationship. As shown in Fig. 5, there was a significant relationship between major differential metabolites (such as Cortisol, Carnitine C22:5, Leu-Met, Oxidized glutathione) and phenotypic characteristics (moisture content, pH, color, cooking loss, crude protein, and crude fat content) in the breed. It is worth mentioning that Mantel's $P < 0.01$ for L^* value and cooking loss with Oxidized glutathione, Mantel's $P < 0.01$ for L^* value and a^* value with Leu-Met, suggesting that correlations between these variables are highly statistically significant. It has been shown that Oxidized glutathione (GSSG), the oxidized form of glutathione (GSH), reflects the level of oxidative stress in cells (Herzog et al., 2019). High levels of GSSG usually indicate enhanced oxidative stress, leading to protein oxidation. On one hand, this may alter the structure of muscle fibers, affecting light reflection and influencing the L^* values. On the other hand, increased oxidative stress may cause protein cross-linking and denaturation, thereby reducing the water-holding capacity of muscle fibers (Bao et al., 2018; Bertram et al., 2007; Jiang et al., 2021), thus increasing cooking loss. Similarly, Leu-Met is a dipeptide that can scavenge free radicals (Zheng et al., 2016), reducing the oxidation of myoglobin and thereby maintaining the stability of oxymyoglobin to keep a higher a^* value, which is consistent with this article.

Our correlation analysis revealed significant associations between cortisol levels and both pH and b^* values (Mantel's $P < 0.05$), suggesting this stress hormone may directly modulate key meat quality. As a stress hormone, prolonged cortisol elevation triggers muscle catabolism and lipid metabolic dysregulation, ultimately compromising core meat quality, including hardness, L^* value, and water-holding capacity (Xia et al., 2012). Acute pre-slaughter stress induces cortisol spikes, accelerating the conversion of glycogen to lactate and leading to a rapid decline in postmortem pH. This process results in DFD (Dark, Firm, Dry) meat, which is characterized by dark purple coloration, a tough texture, and a reduced shelf life (O'Neill et al., 2003).

4. Conclusion

Comprehensive comparative analyses were conducted on the meat quality and metabolites among Oula, Huoerba, and Duoma Tibetan sheep. The results indicated that low-altitude Oula Tibetan sheep contained higher moisture and brighter color, Huoerba Tibetan sheep had lower pH, and high-altitude Duoma Tibetan sheep had higher protein content, lower fat content and richer fatty acids. Metabolomic analysis identified 419 differential metabolites, and carnitine levels increase with altitude in Tibetan sheep. Correlation analysis showed that 15 key differential metabolites are strongly associated with phenotypic characteristics. These findings provide novel perspectives on the altitudinal adaptation of meat quality in Tibetan sheep.

CRediT authorship contribution statement

Ruisi Liu: Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jianing Fu:** Visualization, Project administration, Data curation, Conceptualization. **Shaobo Li:** Writing – review & editing, Methodology, Formal analysis. **Minghui Gu:** Software, Project administration, Conceptualization. **Liang Li:** Visualization, Validation, Methodology, Investigation, Conceptualization. **Le Xu:** Visualization, Investigation, Conceptualization. **Jiangying Yu:** Validation, Methodology, Conceptualization. **Dequan Zhang:** Resources, Project administration, Funding acquisition. **Li Chen:** Writing – review & editing, Supervision, Funding acquisition, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by National Key Research and Development Program of China (2023YFD1600100) and Agriculture Research System of China of MOF and MARA (CARS-38). We are deeply grateful to Chongxin Liu for his invaluable guidance and insightful advice throughout the revision of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2026.147974.

Data availability

Data will be made available on request.

References

AOAC (Ed.). (2020). *Official methods of analysis* (17th ed.). AOAC International https://www.aoac.org/scientific-solutions/calls-for-methods/.

Ayalew, W., Chu, M., Liang, C., Wu, X., & Yan, P. (2021). Adaptation mechanisms of yak (Bos grunniens) to high-altitude environmental stress. *Animals, 11*(8). https://doi.org/10.3390/ani11082344

Bao, Y., Boeren, S., & Ertbjerg, P. (2018). Myofibrillar protein oxidation affects filament charges, aggregation and water-holding. *Meat Science, 135*, 102–108. https://doi.org/10.1016/j.meatsci.2017.09.011

Bertram, H. C., Kristensen, M., Ostdal, H., Baron, C. P., Young, J. F., & Andersen, H. J. (2007). Does oxidation affect the water functionality of myofibrillar proteins? *Journal of Agricultural and Food Chemistry, 55*(6), 2342–2348. https://doi.org/10.1021/jf0625353

Bjorntorp, P. (1987). Fat cell distribution and metabolism. *Annals of the New York Academy of Sciences, 499*, 66–72. https://doi.org/10.1111/j.1749-6632.1987.tb36198.x

Chicco, A. J., Le, C. H., Gnaiger, E., Dreyer, H. C., Muyskens, J. B., D'Alessandro, A., … Roach, R. C. (2018). Adaptive remodeling of skeletal muscle energy metabolism in high-altitude hypoxia: Lessons from altitude omics. *Journal of Biological Chemistry, 293*(18), 6659–6671. https://doi.org/10.1074/jbc.RA117.000470

Chiu, D. K.-C., Tse, A. P.-W., Law, C.-T., Xu, I. M.-J., Lee, D., Chen, M., … Wong, C. C.-L. (2019). Hypoxia regulates the mitochondrial activity of hepatocellular carcinoma cells through HIF/HEY1/PINK1 pathway. *Cell Death & Disease, 10*, 934. https://doi.org/10.1038/s41419-019-2155-3

Costa, P., Costa, A. F., Lopes, P. A., Alfaia, C. M., Bessa, R. J. B., Roseiro, L. C., & Prates, J. A. M. (2011). Fatty acid composition, cholesterol and α-tocopherol of Barrosa-PDO veal produced in farms located in lowlands, ridges and mountains. *Journal of Food Composition and Analysis, 24*(7), 987–994. https://doi.org/10.1016/j.jfca.2010.11.008

Crescenzo, R., Mazzoli, A., Cancelliere, R., Bianco, F., Giacco, A., Liverini, G., … Iossa, S. (2017). Polyunsaturated fatty acids stimulate de novo lipogenesisand improve glucose homeostasis during refeeding with high fat diet. *Frontiers in Physiology, 8*, 178. https://doi.org/10.3389/fphys.2017.00178

Davies, M. N., Kjalarsdottir, L., Thompson, J. W., Dubois, L. G., Stevens, R. D., Ilkayeva, O. R., … Muoio, D. M. (2016). The acetyl group buffering action of carnitine acetyltransferase offsets macronutrient-induced lysine acetylation of mitochondrial proteins. *Cell Reports, 14*(2), 243–254. https://doi.org/10.1016/j.celrep.2015.12.030

De Zawadzki, A., Arrivetti, L. O. R., Vidal, M. P., Catai, J. R., Nassu, R. T., Tullio, R. R., … Cardoso, D. R. (2017). Mate extract as feed additive for improvement of beef quality. *Food Research International, 99*, 336–347. https://doi.org/10.1016/j.foodres.2017.05.033

Deng, J., Bao, P., Li, N., Kong, S., Wang, T., Zhang, M., Yu, Q., Cao, X., Jia, J., & Yan, P. (2025). Integrated multi-omics of the longissimus dorsal muscle transcriptomics and metabolomics reveals intramuscular fat accumulation mechanism with diet energy differences in yaks. *Biomolecules, 15*(7), 1025. https://www.mdpi.com/2218-273X/15/7/1025.

Geihs, M. A., Moreira, D. C., Lopez-Martinez, G., Minari, M., Ferreira-Cravo, M., Carvajalino-Fernandez, J. M., & Hermes-Lima, M. (2020). Commentary: Ultraviolet radiation triggers "preparation for oxidative stress" antioxidant response in animals: Similarities and interplay with other stressors. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology, 239*, Article 110585. https://doi.org/10.1016/j.cbpa.2019.110585

Guo, T., Zhang, Q., Wang, X., Xu, X., Wang, Y., Wei, L., Li, N., Liu, H., Hu, L., Zhao, N., & Xu, S. (2024). Targeted and untargeted metabolomics reveals meat quality in grazing yak during different phenology periods on the Qinghai-Tibetan plateau. *Food Chemistry, 447*, Article 138855. https://doi.org/10.1016/j.foodchem.2024.138855

Han, G., Zhang, L., Li, Q. X., Wang, Y., Chen, Q., & Kong, B. H. (2020). Impacts of different altitudes and natural drying times on lipolysis, lipid oxidation and flavour profile of traditional Tibetan yak jerky. *Meat Science, 162*. https://doi.org/10.1016/j.meatsci.2019.108030

Herzog, K., Ijlst, L., van Cruchten, A. G., van Roermund, C. W. T., Kulik, W., Wanders, R. J. A., & Waterham, H. R. (2019). An uplc-ms/ms assay to measure glutathione as marker for oxidative stress in cultured cells. *Metabolites, 9*(3), 45. https://doi.org/10.3390/metabo9030045

Jiang, Y., Li, D., Tu, J., Zhong, Y., Zhang, D., Wang, Z., & Tao, X. (2021). Mechanisms of change in gel water-holding capacity of myofibrillar proteins affected by lipid oxidation: The role of protein unfolding and cross-linking. *Food Chemistry, 344*, Article 128587. https://doi.org/10.1016/j.foodchem.2020.128587

Jing, X., Wang, W., Degen, A. A., Guo, Y., Kang, J., Liu, P., … Long, R. (2022). Small intestinal morphology and sugar transporters expression when consuming diets of different energy levels: Comparison between Tibetan and small-tailed Han sheep. *Animal, 16*(3). https://doi.org/10.1016/j.animal.2022.100463

Krishnan, S., & Cooper, J. A. (2014). Effect of dietary fatty acid composition on substrate utilization and body weight maintenance in humans. *European Journal of Nutrition, 53*(3), 691–710. https://doi.org/10.1007/s00394-013-0638-z

Li, N., & Zhao, H. (2021). Role of carnitine in non-alcoholic fatty liver disease and other related diseases: An update. *Frontiers in Medicine, 8*, Article 689042. https://doi.org/10.3389/fmed.2021.689042

Li, S. B., Xiang, C., Ge, Y., Liu, H., Zhang, D. Q., & Wang, Z. Y. (2022). Differences in eating quality and electronic sense of meat samples as a function of goat breed and postmortem rigor state. *Food Research International, 152*. https://doi.org/10.1016/j.foodres.2021.110923

Li, X., Han, B., Liu, D., Wang, S., Zhao, J., Wang, L., Li, R., Bao, G., Pei, Q., Sun, D., Zhang, Z., Huang, B., Zhang, F., Zhao, K., & Tian, D. (2025). Transcriptomic and flavor metabolomic exploration of the genetic basis of meat quality and flavor in Tibetan sheep. *BMC Genomics, 26*(1), 867. https://doi.org/10.1186/s12864-025-12098-4

Liang, P., Liang, P., Chen, K. M., Chen, Z. X., Liu, Y. M., Lin, Y. J., … Wang, D. Y. (2024). Important nutrient sources and carbohydrate metabolism patterns in the growth and development of spargana. *Parasites & Vectors, 17*(1). https://doi.org/10.1186/s13071-024-06148-1

Liu, X., Xu, Y., Zhao, X., Bai, Y., Ren, C., Li, X., Hou, C., & Zhang, D. (2025). The role of lactate in meat beyond pH regulation: A study on lactylation and its effects on meat metabolism. *Food Chemistry, 489*, Article 144975. https://doi.org/10.1016/j.foodchem.2025.144975

O'Neill, D. J., Lynch, P. B., Troy, D. J., Buckley, D. J., & Kerry, J. P. (2003). Influence of the time of year on the incidence of PSE and DFD in Irish pigmeat. *Meat Science, 64*(2), 105–111. https://doi.org/10.1016/S0309-1740(02)00116-X

Panjono Kang, S. M., Lee, I. S., & Lee, S. K. (2011). The quality characteristics from Hanwoo (Korean cattle) steer with different raising altitudes and slaughter seasons. *Livestock Science, 136*(2–3), 240–246. https://doi.org/10.1016/j.livsci.2010.09.015

Rellinger, E. J., Romain, C., Choi, S., Qiao, J., & Chung, D. H. (2015). Silencing gastrin-releasing peptide receptor suppresses key regulators of aerobic glycolysis in neuroblastoma cells. *Pediatric Blood & Cancer, 62*(4), 581–586. https://doi.org/10.1002/pbc.25348

Richter, E. K., Spangenberg, J. E., Willems, H., Kreuzer, M., & Leiber, F. (2012). Stable carbon isotope composition of perirenal adipose tissue fatty acids from Engadine sheep grazing either mountain or lowland pasture. *Journal of Animal Science, 90*(3), 905–913. https://doi.org/10.2527/jas.2011-4284

Storz, J. F., & Cheviron, Z. A. (2021). Physiological genomics of adaptation to high-altitude hypoxia. *Annual Review of Animal Biosciences, 9*, 149–171. https://doi.org/10.1146/annurev-animal-072820-102736

Sun, B., Zhang, Y., Ding, M., Xi, Q., Liu, G., Li, Y., Liu, D., & Chen, X. (2018). Effects of Moringa oleifera leaves as a substitute for alfalfa meal on nutrient digestibility, growth performance, carcass trait, meat quality, antioxidant capacity and biochemical parameters of rabbits. *Journal of Animal Physiology and Animal Nutrition, 102*(1), 194–203. https://doi.org/10.1111/jpn.12678

Tao, Y. M., Ma, L., Li, D. D., Tian, Y. T., Liu, J., & Liu, D. H. (2021). Proteomics analysis to investigate the effect of oxidized protein on meat color and water holding capacity in tan mutton under low temperature storage. *Lwt-Food Science and Technology, 146*. https://doi.org/10.1016/j.lwt.2021.111429

Wang, X. R., Fang, C. K., He, J. H., Dai, Q. Z., & Fang, R. J. (2017). Comparison of the meat metabolite composition of Linwu and Pekin ducks using 600 MHz H nuclear magnetic resonance spectroscopy. *Poultry Science, 96*(1), 192–199. https://doi.org/10.3382/ps/pew279

Xia, X., Kong, B., Liu, J., Diao, X., & Liu, Q. (2012). Influence of different thawing methods on physicochemical changes and protein oxidation of porcine longissimus muscle. *LWT - Food Science and Technology, 46*(1), 280–286. https://doi.org/10.1016/j.lwt.2011.09.018

Xin, K., Tian, K., Yu, Q., Han, L., & Zang, Z. (2022). Effects of altitude on meat quality difference and its relationship with HIF-1α during postmortem maturation of beef. *Journal of Food Biochemistry, 46*(12). https://doi.org/10.1111/jfbc.14470

Xin, K.-Q., Tian, K., Yu, Q.-L., & Han, L. (2023). High oxygen-modified packaging (HiOx-MAP) mediates HIF-1α regulation of tenderness changes during postmortem aging of yak meat. *Food Chemistry-X, 17*, Article 100573. https://doi.org/10.1016/j.fochx.2023.100573

Xu, X., Liu, H., Wang, X., Zhang, Q., Guo, T., Hu, L., & Xu, S. (2023). Evaluation of the longissimus thoracis et lumborum muscle quality of Chaka and Tibetan sheep and the analysis of possible mechanisms regulating meat quality. *Animals, 13*(15). https://doi.org/10.3390/ani13152494

Yang, J., Li, W. R., Lv, F. H., He, S. G., Tian, S. L., Peng, W. F., … Liu, M. J. (2016). Whole-genome sequencing of native sheep provides insights into rapid adaptations to extreme environments. *Molecular Biology and Evolution, 33*(10), 2576–2592. https://doi.org/10.1093/molbev/msw129

Yi, W., Huang, Q., Wang, Y., & Shan, T. (2023). Lipo-nutritional quality of pork: The lipid composition, regulation, and molecular mechanisms of fatty acid deposition. *Animal Nutrition, 13*, 373–385. https://doi.org/10.1016/j.aninu.2023.03.001

Zhang, M., Liu, Y. L., Fu, C. Y., Wang, J., Chen, S. Y., Yao, J., & Lai, S. J. (2014). Expression of genes, composition of muscle fiber type and their association with intramuscular fat, tenderness in skeletal muscle of hybrids. *Molecular Biology Reports, 41*(2), 833–840. https://doi.org/10.1007/s11033-013-2923-6

Zhang, T., Chen, C., Xie, K. Z., Wang, J. Y., & Pan, Z. M. (2021). Current state of metabolomics research in meat quality analysis and authentication. *Foods, 10*(10), 2388. https://doi.org/10.3390/foods10102388

Zhang, X., Han, L., Hou, S., Raza, S. H. A., Wang, Z., Yang, B., … Al Hazani, T. M. I. (2022). Effects of different feeding regimes on muscle metabolism and its association with meat quality of Tibetan sheep. *Food Chemistry, 374*, Article 131611. https://doi.org/10.1016/j.foodchem.2021.131611

Zheng, L., Zhao, Y., Dong, H., Su, G., & Zhao, M. (2016). Structure–activity relationship of antioxidant dipeptides: Dominant role of Tyr, Trp, Cys and met residues. *Journal of Functional Foods, 21*, 485–496. https://doi.org/10.1016/J.JFF.2015.12.003

Zhu, X., Yang, C., Song, Y., Qiang, Y., Han, D., & Zhang, C. (2023). Changes provoked by altitudes and cooking methods in physicochemical properties, volatile profile, and sensory characteristics of yak meat. *Food Chemistry-X, 20*, Article 101019. https://doi.org/10.1016/j.fochx.2023.101019

Zhu, X., Yang, C., Yu, Q., & Han, L. (2025). Hypoxia-inducible factor-1α promotes ferroptosis by inducing ferritinophagy and promoting lactate production in yak longissimus thoracis et lumborum postmortem. *Meat Science, 220*, Article 109692. https://doi.org/10.1016/j.meatsci.2024.109692

Zi, X. D., Zhong, G. H., Wen, Y. L., Zhong, J. C., Liu, C. L., Ni, Y. A., … Ashi, M. C. (2004). Growth performance, carcass composition and meat quality of Jiulong-yak (Bos grunniens). *Asian-Australasian Journal of Animal Sciences, 17*(3), 410–414. https://doi.org/10.5713/ajas.2004.410