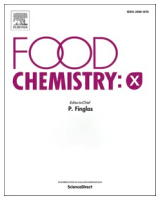
Food Chemistry: X 22 (2024) 101411

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



# Insights into the effects of saline forage on the meat quality of Tibetan sheep by metabolome and multivariate analysis

Nana Ma, Lijuan Han *, Shengzhen Hou, Linsheng Gui, Zhenzhen Yuan, Shengnan Sun, Zhiyou Wang, Baochun Yang, Chao Yang

*College of Agriculture and Animal Husbandry, Qinghai University, Xining, China*

ARTICLE INFO

*Keywords:*
Tibetan sheep
Saline and alkaline
Forage quality
Meat quality
Metabolomics

ABSTRACT

This work aimed to investigate how two different types of forage (saline and alkaline) impact the meat quality and muscle metabolism of Tibetan sheep. An integrative multi-omics analysis of meat quality and different metabolites was performed using untargeted and targeted metabolomics approaches. The research results indicated that GG grass (saline and alkaline forage) possessed superior characteristics in terms of apparent quality and secondary metabolite content compared with HG grass (Non saline alkali forage), regardless of the targeted metabolites or non-targeted ones. Simultaneously, under stress conditions, the carbohydrates-rich salt-alkali grass play a significant role in slowing down the decline in pH, increasing the unsaturated fatty acid content and reducing the thawing loss in Tibetan sheep. This study provides an understanding of the impact of different salt-alkali grass on the quality of Tibetan sheep meat, while providing a scientific basis for the future development of salt-alkali livestock industry.

## 1. Introduction

Pasture-based livestock farming, often considered as an ideal and natural method (Majdoub-Mathlouthi, Hamdi, & Kraiem, 2021), faces challenges due to overgrazing (Li et al., 2015; Zhang et al., 2017) and environmental changes (Wang et al., 2020) that gradually deteriorate and reduce the availability of Earth's grasslands. In contrast, saline-alkali grasslands have been gaining prominence globally and are now of significant importance (Wang et al., 2011). In fact, saline-alkali lands were initially considered to possess unsuitable environments for agriculture and livestock (Tavakkoli et al., 2011) as the alkaline soil stress could negatively affect plant growth and development through ion permeability, high pH stress and the generation of reactive oxygen species. However, subsequent research has shown that such unique conditions actually help plants to adapt by adjusting their root structure and function, regulating physiological metabolism and activating specific gene expressions. Altogether, these adaptations result in the production of higher-quality feed.

Numerous studies suggest that saline forages are of better quality. For instance, Jia et al.(Jia et al., 2020). observed a significantly higher abundance of sucrose, amino acids, alkaloids, flavonoids and carotenoids in *Malus halliana* seedlings under alkaline stress, with this feature helping to enhance salt-alkali resistance by maintaining a balanced redox state. According to Hayley C. Norman et al. (Norman, Duncan, & Masters, 2019)., it has been discovered that saline pasture grasses contain high concentrations of minerals and vitamins that are associated with antioxidant defences in response to saline stress. This finding suggests that these grasses may be a valuable source of antioxidant mineral and vitamin supplements for ruminants. In addition, Wang (Wang et al., 2021) found that leaves of cordgrass and European cordgrass contained flavonoids, alkaloids and coumarins which are of significant medical value, while Zhang (Zhang et al., 2016) reported increased levels of proteins involved in glycolysis, the tricarboxylic acid (TCA) cycle, the photosystem I (PSI), reactive oxygen species (ROS) scavenging and signal transduction under alkaline stress, hence suggesting the potential to resist such stress by regulating carbohydrate metabolism and redox homeostasis.

Tibetan sheep, a unique breed on the Qinghai-Tibetan Plateau (Wu et al., 2023) and primarily produced in the Qinghai Province, graze on natural pastures all year round. Since the animals are bred in a clean ecological environment where they mainly consume high-quality natural pasture that are free from antibiotics, their meat is nutritionally rich and is characterized by high protein, low fat and high mineral content as well as elevated amounts of essential amino acids and fatty acids (Su

* Corresponding author.
*E-mail address:* 2016990034@qhu.edu.cn (L. Han).

https://doi.org/10.1016/j.fochx.2024.101411
Received 20 February 2024; Received in revised form 21 April 2024; Accepted 22 April 2024
Available online 26 April 2024

et al., 2022). Currently, the Qinghai Province possesses mostly saline-alkali types of grassland, and therefore, in areas such as the Hainan Tibetan Autonomous Prefecture, the Haixi Mongolian-Tibetan-Kazakhstan Autonomous Prefecture or other areas along the Qinghai Lak (Yang et al., 2023), the Tibetan sheep have traditionally been recognized for their superior food and nutritional values. For instance, Guo (Guo et al., 2022) revealed that, compared to traditional grazing, the high-salt environments experienced by Chaka Tibetan sheep increased the fatty acids and metabolic products in the longest muscle on their backs, and this was translated into improved meat quality, with Xu (Xu et al., 2023) further reporting the enhanced texture and flavor of such meat. In addition, K.L. Pearce (Pearce, Norman, & Hopkins, 2010) discovered that salt-based pastures had the potential to produce carcasses with a higher percentage of lean meat (lower fat), while according to Hassan (El Shaer, 2010), the salt-tolerant plants used as feed for ruminants vary widely in palatability, chemical composition, nutritional value and animal response. Building upon previous research, this study hypothesized that feeding Tibetan sheep with salt-tolerant alkaline grass can enhance muscle quality, with the process being dependent on muscle metabolism. This hypothesis serves as a basis for exploring the specific factors influencing meat quality in Tibetan sheep.

The focus on salt-affected pastureland not only allows the practice of sustainable agriculture and livestock production, but also provides a means for the rational use of land resources, while ensuring environmental conservation and food production. However, the specific mechanisms through which halophytic plants affect meat quality are yet to be investigated. To clarify the mechanism by which pasture cultivation in saline soil affects the quality of Tibetan sheep meat, and to identify the key components in pasture that influence this change, we conducted the following experiments. This study applied non-targeted metabolomics techniques, including ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS), to identify the differential metabolites in the *longestissimus lumborum* (LL) muscle and forage of Tibetan sheep. The functions and metabolic pathways of these differential metabolites were then determined using Kyoto Encyclopedia of Genes and Genomes (KEGG) and Human Metabolome Database (HMDB). Furthermore, targeted metabolomics approaches, based on ultrahigh-performance liquid chromatography-mass spectrometry (UHPLC–MS) and gas chromatography–mass spectrometry (GC–MS), were applied to identify amino acid (AA) and fatty acid (FA) metabolites, hence providing a comprehensive understanding of variations in the amino acid and fatty acid composition of muscles induced by different forages and feeding methods across diverse regions. Finally, correlation analyses between metabolism and meat quality were performed to investigate how halophytic plants adapt to saline-alkali environments and elucidate their potential value in livestock and agriculture. The findings are expected to offer valuable guidance to farmers, enabling them to implement scientifically sound feeding practices in saline-alkaline areas, thereby enhancing the quality and yield of meat. Additionally, it will provide effective methods to promote the sustainable development of animal husbandry in high-altitude regions while achieving a harmonious balance between production and ecology.

## 2. Materials and methods

The Committee of Experimental Animal Care approved all experimental procedures involving animals, while the Qinghai University of Animal Care approved handling techniques (QUA- 2020- 0709).

### 2.1. Tibetan sheep, meat samples and forage samples

Forage samples, collected according to the National Forage Testing Association (NFTA), were simultaneously sampled from the same batch in Haiyan (HG: Latitude: 36°59′36”N; Longitude: 100°55′53″E; Altitude: 3111 m) and Gonghe (GG: Latitude: 36°28′2”N; Longitude: 99°16′26″E; Altitude: 3168.1 m). They were then placed in a refrigerator at −80 °C for subsequent testing of grass quality in different regions.

In this experiment, 90 healthy Tibetan ewe sheep, aged 2–3 months and having similar body conditions, were randomly assigned to 3 groups of 30 sheep each. The experiment involved rearing Tibetan sheep in different groups and feeding them different types of pasture and concentrate feeds. Group M sheep were reared at Jinzang farm in Haiyan county and fed on non-saline pasture grown in the same county. Groups H0 and H1 were reared at Xiangka Meiduo farm in Gonghe county. Group H1 was fed on saline pasture grown in Gonghe county, while group H0 was fed on non-saline pasture grown in Haiyan county. The standards for concentrated feed and feed intake were consistent across groups as shown in Table 1. The three groups were housed in separate pens with exercise yards that were sheltered from wind, while being sunny, dry and well ventilated. The animals were fed twice daily at 8:30 a.m. and 16:30 p.m. through free-feeding and free-watering, with the residual feed weighed before each feeding. In addition, the sheep house was cleaned with daily sweeping and cleaning of the water troughs. Weekly disinfection and sterilization of the sheep house and exercise yard were also performed, while keeping the enclosure clean and hygienic. All sheep were immunized against sheep tetralogy of fallot, small ruminant, foot-and-mouth disease and sheep pox, with ivermectin and abamectin also used on a regular basis to prevent and control the spread of internal and external parasites.

At the end of a feeding trial of 120 days, all animals were fasted solid and liquid for 12 h in accordance with animal welfare procedures, transported to a local commercial abattoir, and humanely slaughtered according to the standard, i.e. (FAO—Food and Agriculture Organization, 2001), the lambs were bled unconscious. Then the *longissimus lumborum* between the 12th and 13th ribs was harvested, and after removing the fat and fascia, the samples were placed in dry ice for transport to the laboratory where they were stored at −80 °C for backup.

### 2.2. Forage quality analysis

#### 2.2.1. Analysis of forage nutritive values

To determine the nutritional value of the feeds, the latter were dried in an oven, pulverized and passed through a 40-mesh sieve for subsequent chemical analysis. Forage moisture, ash, crude protein and crude fat were analyzed according to the standard procedures of Al-Mentafji (2016), while NIR was used for analyzing neutral detergent fiber (NDF) and acid detergent fiber (ADF). The polyphenol content was then determined using the Folin-Ciocalteu as described by Huang (Huang et al., 2023) before applying Jing's (Jing, Dong, & Tong, 2015) method for ultrasound-assisted extraction of feed flavonoids. The polysaccharide content in the crude extract was eventually determined by the phenol-sulphuric acid method as reported by Tepsong kroh (Tepsongkroh et al., 2023).

**Table 1**
Diet composition and nutritional level (dry matter basis, %).

| Dietary composition | Groups | | |
|---|---|---|---|
| | M(%) | H1(%) | H0(%) |
| Corn | 46 | 46 | 46 |
| Wheat | 13 | 13 | 13 |
| Soybean meal | 6 | 6 | 6 |
| Canola meal | 11 | 11 | 11 |
| Cottonseed meal | 16 | 16 | 16 |
| Salts | 1 | 1 | 1 |
| Stone power | 1 | 1 | 1 |
| Baking soda | 1 | 1 | 1 |
| Premix | 5 | 5 | 5 |
| Total | 100 | 100 | 100 |
| Roughage | 4(Nonsaline-alkali pasture) | 4(Saline-alkali pasture) | 4(Nonsaline-alkali pasture) |
| Farming area | Nonsaline-alkali Farming | Saline-alkali Farming | Saline-alkali Farming |

#### 2.2.2. Calculation of forage quality index

The relative feeding value (RFV) was calculated as described by Gao (Gao et al., 2023) using the formula:

$$RFV = DMI\ (\%DW) \times DDM\ (\%DW)/1.29)$$

Where DMI represents the dry matter intake and DDM is the digestible dry matter. RFV integrates both the intake and digestibility as predicted from NDF and ADF concentrations. This single index allows the nutrient quality among forages to be compared based on their ability to support digestible DM intake (Rohweder, Barnes, & Jorgensen, 1978).

### 2.3. Meat analysis

#### 2.3.1. Analysis of carcass quality

Carcass quality was measured during the splitting process. In this case, the eye muscle area (EMA), rib fat thickness (GR), abdominal fat thickness (AFT) and backfat thickness (BFT) were measured according to previous methods (Zhang, Han, Gui, et al., 2022).

#### 2.3.2. Sensory analysis of Tibetan sheep

The reference standard (ISO 13299:2016(en), 2024, *Sensory analysis — Methodology — General guidance for establishing a sensory profile*, no date) and Ma Ying's (Ma, Han, Zhang, et al., 2023) method were used to conduct sensory evaluations on three groups of Tibetan sheep samples.

#### 2.3.3. Analysis of the edible quality of meat

Three pH measurements were taken on a carcass before recording the average as its pH value. An automated calibrated color-meter (TCP2-AE/BE/CE, Kett Japan Kite) was then used to measure the color of the meat on the cut surface of the *longissimus thoracis* muscle. In addition, the water-holding capacity was determined by the cooking loss, thawing loss and cooked meat percentage.

Thawed meat was cut into pieces of 2 cm × 1 cm × 1 cm in the direction of muscle fibers, with the shearing force of the samples subsequently measured using a tenderizer. Cut meat samples (1 cm × 1 cm × 1 cm) were also pressed to 50% of their original heights at a speed of 1 mm/s to determine their hardness, viscosity, elasticity, cohesion and adhesion properties.

#### 2.3.4. Analysis of the nutritional component of meat

AOAC standard methods were used to assess water, crude protein, crude fat and other nutritional components of muscles ((PDF) A.O.A.C, *2005*, no date).

### 2.4. Untargeted metabolomics profiling of meat and forage

#### 2.4.1. Metabolites extraction

Metabolites were extracted from samples that had been thawed at 4 °C by mixing them with cold methanol/acetonitrile (1:1, *v*/v). QC samples were regularly included and analyzed alongside six samples.

#### 2.4.2. UHPLC-q-TOF MS

An UHPLC (1290 Infinity LC, Agilent Technologies), coupled to a quadrupole TOF (AB Sciex Triple TOF 6600), was used for the analysis. The conditions for HILIC separation and the ESI source were set as previously described (Ma, Han, Raza, et al., 2023).

### 2.5. Targeted metabolomic analysis of forage and meat

#### 2.5.1. Analysis of the amino acid composition

Samples stored at −80 °C were weighed, and 60 mg was then added to 150 ul of water for MP homogenization. After vortex mixing for 60 s, 800 ul of a methanol-acetonitrile solution (1:1, v/v) was added, followed by 50 ul of an internal standard mixture (50 um 16 isotopes). The final mixture was vortex mixed for 60 s, and after low-temperature sonication, performed twice for 30 min each, it was left at −20 °C for 1 h to precipitate proteins. This was followed by a 20-min centrifugation at 14,000 rcf and 4 °C, with the resulting supernatant freeze-dried prior to storage at −80 °C. An Agilent 1290 Infinity LC Ultra performance liquid chromatography system was finally used to separate the samples, while an AB SCIEX 5500 QTRAP mass spectrometer in positive ion mode was used for the mass spectrometry analysis.

#### 2.5.2. Analysis of the fatty acid composition

Methyl n-nonadecanoate, used as an internal standard, was mixed well and added to the injection bottle for GC–MS analysis on Agilent DB-WAX capillary columns (30 m × 0.25 mm ID × 0.25 μm). An Agilent 7890/5975C gas-mass spectrometer was then used for the mass spectrometry analysis.

### 2.6. Targeted metabolomics for analyzing reducing sugar content in forage

To begin, transfer 20 μL of polysaccharide extract to each well of a 96-well plate. Then, add 120 μL of pre-chilled methanol solution with internal standards to each well. Vigorously vortex the plate for 5 min. Next, add 20 μL of freshly prepared derivatization reagent to each well. Seal the plate and incubate it at 30 °C for 60 min. Following this, dilute the samples by adding 330 μL of 50% methanol solution on ice. After centrifugation, collect 135 μL of the supernatant and carefully transfer it to a new 96-well plate. On the left side of the plate, add a gradient dilution of the derivatized standard solution to the respective wells. Finally, seal the plate for LC-MS analysis. To evaluate the stability and reproducibility of the system, include quality control (QC) samples. For specific UPLC-MS/MS analysis conditions, refer to Supplementary Table 1.

### 2.7. Data processing and analysis

Results for the meat quality (carcass quality, edible quality, nutritional quality, AA, FA) and forage quality (nutritional quality, FA, AA) of Tibetan sheep from different regions were presented as means and standard error of the means (SEM) before being analyzed using one-way analysis of variance (ANOVA) in IBM SPSS Statistic 26 software. In this case, values were considered to be of statistical significance at $P < 0.05$. Pearson's correlation coefficient was also used to analyze the relationship between meat quality, meat metabolism and forage metabolism in Tibetan sheep, with $P$ values $<0.05$ and $|r| > 0.50$ considered as significant.

## 3. Results

### 3.1. Forage quality and metabolites

#### 3.1.1. Analysis of forage nutritive values

Forage samples were significantly different in terms of their nutritive profiles. Specifically, as shown in Table 2. the GG group had a slightly higher moisture content (8.31%) but a significantly greater crude protein content (7.85%) compared with the HG group ($p < 0.01$). The HG group also had a lower crude fat content of 6.2 g/kg, compared with that of the GG group (12.1 g/kg). Significant differences were further noted in the amount of crude fiber, NDF and ADF, with the HG group showing significantly higher values compared with the GG group ($p < 0.01$). Additionally, the GG group exhibited a high amount of crude polysaccharides, quantified at 16.32 mg/100 g, alongside higher polyphenol and flavonoid content.

#### 3.1.2. Calculation of forage quality index

The quality of forage has been linked to ingestion rates and the efficiency of nutrient utilization. Its value, which represents the nutritional quality of the feed, was negatively correlated with the ADF and NDF content. As shown in Table 2, GG groups also showed higher DMI,

**Table 2**
Differences in forage quality between regions.

| Items | Groups | | *P* |
|---|---|---|---|
| | HG | GG | |
| Analysis of forage nutritive values | | | |
| Moisture (%) | 7.06 ± 0.43 | 8.31 ± 0.6* | 0.013 |
| Crude protein (%) | 2.45 ± 0.3 | 7.85 ± 0.35** | <0.01 |
| Crude fat (g/kg) | 6.2 ± 0.1 | 12.1 ± 0.1** | <0.01 |
| Ash (%) | 8 ± 0.00 | 7.97 ± 0.057 | 0.374 |
| Crude fiber (%) | 47.2 ± 0.26 | 27.1 ± 0.1* | <0.01 |
| NDF (%) | 92.93 ± 0.68 | 54.67 ± 0.51* | <0.01 |
| ADF (%) | 65.27 ± 1.13 | 35.93 ± 1.03* | <0.01 |
| Crude polysaccharide(mg/100 g) | 1.766 ± 0.89 | 16.32 ± 0.84** | <0.01 |
| Polyphenol(mg/100 g) | 0.005 ± 0.00 | 0.006 ± 0.00** | <0.01 |
| Flavonoid(mg/100 g) | 0.301 ± 0.00 | 0.342 ± 0.01** | <0.01 |
| Calculation of forage quality index | | | |
| DM% | 92.84 ± 0.36** | 91.69 ± 0.06 | <0.01 |
| OM% | 84.84 ± 0.36** | 83.72 ± 0.01 | <0.01 |
| NEF% | 28.99 ± 0.33 | 36.67 ± 0.09** | <0.01 |
| DMI% | 1.29 ± 0.01 | 2.20 ± 0.02** | <0.01 |
| DDM% | 38.06 ± 0.89 | 60.91 ± 0.80** | <0.01 |
| RFV% | 20.24 ± 1.41 | 60.13 ± 0.72** | <0.01 |
| IVDMD% | 1.26 ± 0.19 | 6.17 ± 0.16** | <0.01 |
| ME(MJ/kg) | 92.84 ± 0.36** | 91.69 ± 0.06 | <0.01 |
| Amino acid composition(mg/100 g) | | | |
| serine | 0.09 ± 0.06 | 6.93 ± 0.39** | 0.0000 |
| threonine | 0.04 ± 0.02 | 4.19 ± 0.37** | 0.0000 |
| valine | 0.09 ± 0.08 | 2.98 ± 0.27** | 0.0001 |
| Isoleucine | 0.03 ± 0.02 | 0.83 ± 0.08** | 0.0001 |
| proline | 0.17 ± 0.18 | 5.74 ± 0.63** | 0.0001 |
| alanine | 0.29 ± 0.29 | 7.99 ± 0.95** | 0.0002 |
| leucine | 0.03 ± 0.02 | 0.89 ± 0.12** | 0.0003 |
| lysine | 0.09 ± 0.02 | 2.01 ± 0.29** | 0.0003 |
| Glutamine | 0.193 ± 0.24 | 8.995 ± 1.44** | 0.0005 |
| Tyrosine | 0.025 ± 0.01 | 0.46 ± 0.09** | 0.0013 |
| Tryptophan | 0.01 ± 0.00 | 0.87 ± 0.18** | 0.0013 |
| glycine | 0.12 ± 0.05 | 0.71 ± 0.12** | 0.0013 |
| aspartate | 0.03 ± 0.01 | 0.66 ± 0.14** | 0.0016 |
| asparagine | 0.10 ± 0.11 | 2.14 ± 0.47** | 0.0019 |
| histidine | 0.01 ± 0.00 | 0.13 ± 0.03** | 0.0019 |
| hydroxyproline | 0.01 ± 0.00 | 0.09 ± 0.02** | 0.0024 |
| phenylalanine | 0.03 ± 0.02 | 0.79 ± 0.19** | 0.0027 |
| aminoadipic acid | 0.04 ± 0.01 | 0.29 ± 0.08** | 0.0050 |
| citrulline | 0.0002 ± 0.00 | 0.07 ± 0.03* | 0.0120 |
| arginine | 0.05 ± 0.01 | 0.73 ± 0.29* | 0.0164 |
| ornithine | 0.01 ± 0.00 | 0.1 ± 0.04* | 0.0318 |
| glutamate | 0.26 ± 0.11 | 0.69 ± 0.23* | 0.0462 |
| EAAs | 0.27 ± 0.91 | 8.36 ± 0.03** | <0.01 |
| NEAAs | 1.43 ± 0.09 | 39.89 ± 3.16** | <0.01 |
| Fatty acid composition(mg/100 g) | | | |
| C21:0 | 0.07 ± 0.01 | 0.30 ± 0.02** | 0.000 |
| C18:2 N6 | 4.97 ± 2.25 | 47.12 ± 5.74** | 0.000 |
| C22:6 N3 | 0.06 ± 0.02 | 0.46 ± *0.09* | 0.001 |
| C24:0 | 0.57 ± 0.09 | 1.09 ± 0.12** | 0.003 |
| C24:1 N9 | 0.12 ± 0.06 | 0.37 ± 0.05** | 0.004 |
| C18:3 N3 | 1.60 ± 1.72 | 54.71 ± 20.25* | 0.011 |
| C22:5 N6 | 0.01 ± 0.001 | 0.04 ± 0.01* | 0.011 |
| C22:0 | 0.83 ± 0.12 | 1.52 ± 0.28* | 0.017 |
| C16:0 | 16.03 ± 0.69 | 42.46 ± 11.66* | 0.017 |
| C6:0 | 0.001 ± 0.00 | 0.003 ± 0.00* | 0.017 |
| C22:2 N6 | 0.20 ± 0.18 | 3.11 ± 1.36* | 0.022 |
| C18:2TTN6 | 0.01 ± 0.01 | 0.03 ± 0.01* | 0.036 |
| C18:1 N9 | 0.29 ± 0.13 | 3.43 ± 1.78* | 0.039 |
| SFA | 23.83 ± 0.69 | 52.52 ± 13.42* | 0.021 |
| MUFA | 9.94 ± 6.07 | 30.79 ± 10.69* | 0.043 |
| PUFA | 18.88 ± 10.94 | 130.36 ± 35.30** | 0.006 |
| N3 | 1.74 ± 1.67 | 55.35 ± 20.33* | 0.010 |
| N6 | 17.14 ± 9.43 | 75.01 ± 15.01** | 0.005 |
| Reducing sugar content (umol/L) | | | |
| D-Maltose | 0.45 ± 0.04 | 3.88 ± 0.05** | <0.01 |
| Melibiose | 0.39 ± 0.01 | 1.23 ± 0.05** | <0.01 |
| D-Glucose | 192.71 ± 3.71 | 2172.93 ± 20.16** | <0.01 |
| D-Galactose | 3.06 ± 0.23 | 14.02 ± 0.46** | <0.01 |
| D-Mannose | 4.03 ± 0.18 | 7.39 ± 0.22** | <0.01 |
| D-Fructose | 224.22 ± 2.36 | 1295.08 ± 35.01** | <0.01 |
| D-Ribose\DXylose\LArabinose | 4.08 ± 0.23** | 2.74 ± 0.20 | <0.01 |
| D-Ribulose | 1.48 ± 0.12 | 1.35 ± 0.05 | 0.157 |
| D-Xylulose | 0.11 ± 0.01 | 0.47 ± 0.04** | <0.01 |
| D-Fucose | 0.15 ± 0.02 ** | 0.09 ± 0.01 | 0.01 |
| Rhamnose | 0.19 ± 0.03 | 0.36 ± 0.06** | 0.01 |
| Ribonolactone | 5.22 ± 0.45 | 9.40 ± 0.23** | <0.01 |
| Glyceraldehyde | 0.28 ± 0.04 | 0.59 ± 0.06 ** | <0.01 |
| Erythrose | 0.06 ± 0.02 | 0.31 ± 0.14 * | 0.04 |

Note: *$P < 0.05$ and **P < 0.01. EAAs, essential amino acids; NEAAs: non-essential amino acids; TAAs, total amino acids. MUFA, sum of monounsaturated fatty acids; PUFA, sum of polyunsaturated fatty acids; SFA, sum of saturated fatty acid, n-3 PUFAs: omega-3 polyunsaturated fatty acids, n-6 PUFAs: omega-6 polyunsaturated fatty acids.

DDM and RFV ($P < 0.01$) compared with the HG group, and hence, for ruminants, group GG feeds were of higher feeding value.

### 3.1.3. Untargeted metabolomics profiling of forage

*3.1.3.1. Identification and differential analysis of metabolites.* Principal Component Analysis (PCA), performed on all experimental and QC samples' extracted peaks as depicted in Fig. 1A and B, further showed close clustering of the QC samples in both positive and negative ion modes, thereby indicating excellent experimental reproducibility.

To enhance the differentiation between groups, PLS-DA and OPLS-DA were performed for further analysis of the two sample sets after excluding QC samples. As depicted in Fig. 1C and D, the GG and HG groups exhibited intragroup clustering and intergroup separation in both analysis modes, with OPLS-DA demonstrating a more pronounced effect. To prevent the risk of overfitting in the supervised modeling process, a permutation test was also applied to validate the models, with Fig. 1E and F illustrating the permutation test results for PLS-DA (R2X = 0.626, R2Y = 0.998, Q2 = 0.978) and OPLS-DA (R2X = 0.626, R2Y = 0.998, Q2 = 0.976) conducted on the GG and HG groups. As the permutation iterations progressed, both the R2 and Q2 values of the random model steadily decreased, confirming the absence of overfitting in the original model. The results suggest that the observed separation of metabolites between the groups during PLS-DA and OPLS-DA analyses was statistically significant.

*3.1.3.2. Bioinformatics analysis of differential metabolites.* Predictor variable importance (VIP), derived from OPLS-DA, was used to identify differential metabolites of biological significance. In the positive ion mode, 216 such metabolites were identified in both the GG and HG groups (VIP > 1.0, $P < 0.05$), with 96 of these associated with KEGG metabolic pathways (Supplementary Table 2).

The KEGG pathway enrichment analysis, shown in Fig. 2A, presents the top 20 pathways, most of which were associated with AA metabolism, lipid metabolism and nucleotide metabolism. In order to further compare the differential metabolic pathways responsible for variations in forage metabolites between regions, a differential enrichment score map, shown in Fig. 2B, was obtained. In this case, 2 metabolic pathways were found to be down-regulated and 10 were up-regulated in group GG compared with group HG (DA score > 0.5 and $P < 0.05$).

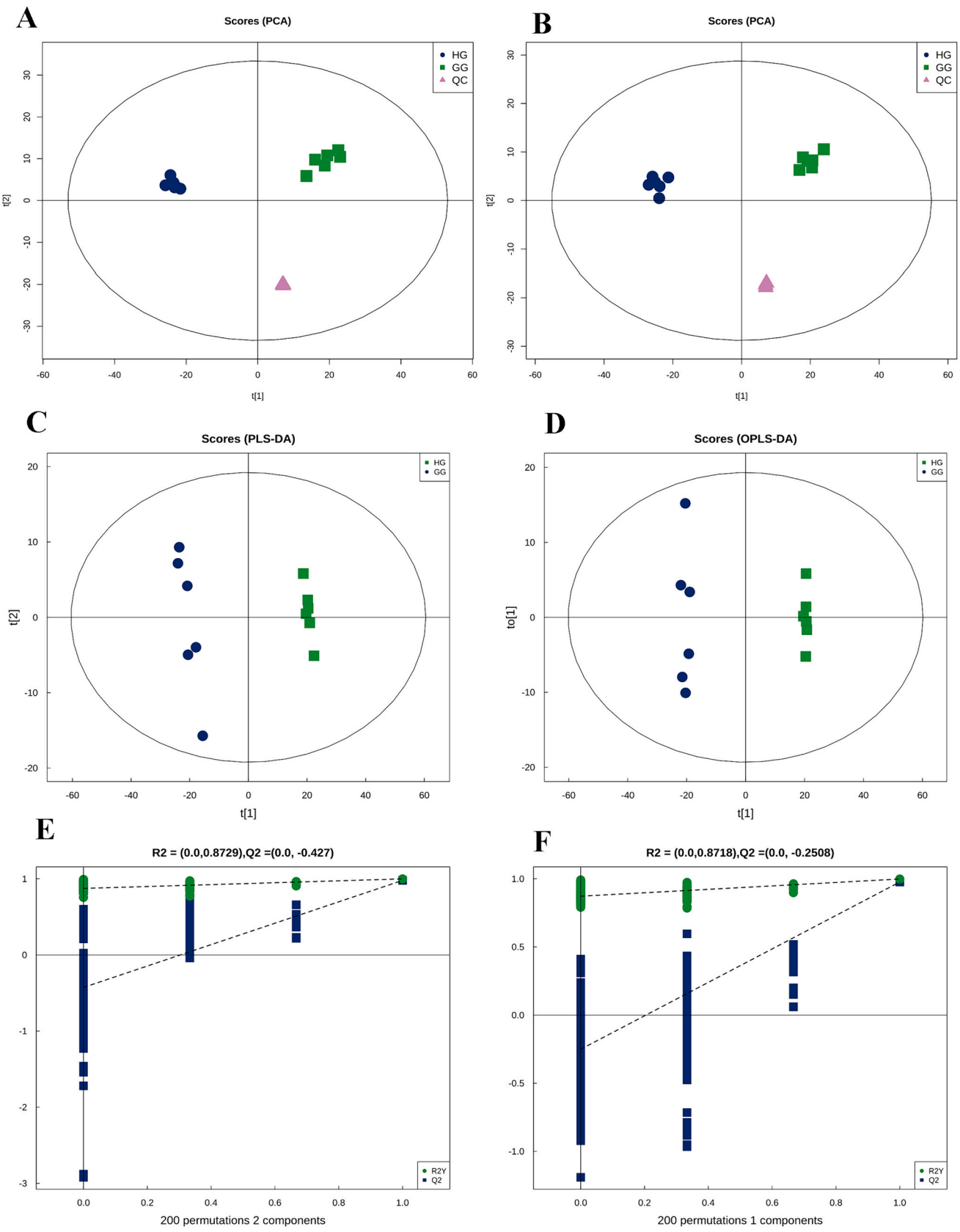



**Fig. 1.** Quality control of forage samples. A: PCA analysis of all the samples based on peaks detected in positive ion modes. B: PCA analysis of all the samples based on peaks detected in negative ion modes. Multivariate statistical analysis of forage in different regions: PLS-DA (C) OPLS-DA (D) scores of the overall sample in the positive ion mode and permutations test of PLS-DA (E), OPLS-DA (F) in the positive ion detection mode.

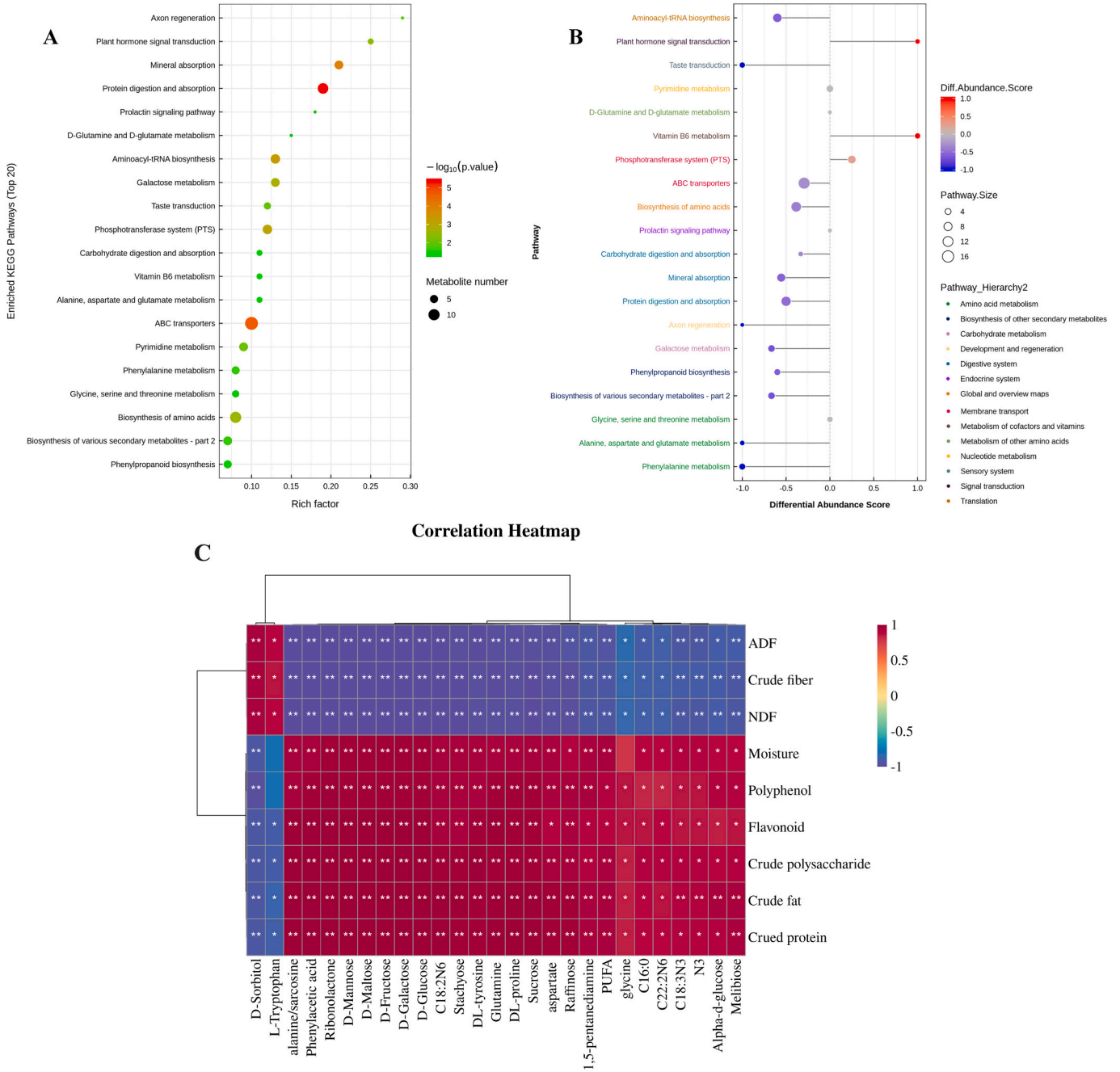



**Fig. 2.** A: Top 20 enriched KEGG pathways of the comparison between HG and GG groups. B: A differential abundance score map of differential metabolic pathways. C: The correlation heat map between forage quality parameters and forage AA, FA and forage metabolomics. The color red and blue represent positive and negative correlations, respectively. $*P < 0.05$and $**P < 0.01$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The prominently down-regulated pathway was vitamin B6 metabolism, while aminoacyl-tRNA biosynthesis, protein digestion and absorption, galactose metabolism, alanine, aspartate and glutamate metabolism, biosynthesis of various secondary metabolites - part 2 and phenylalanine were the key up-regulated pathways. As a result, the main up-regulated metabolites included DL-proline, L-tryptophan, L-alanine, D-glutamine, L-leucine, 2-phenylacetamide, benzoic acid, citrate, glutamine, propionic acid, 1,5-pentanediamine, sucrose, raffinose, alpha-D-glucose, stachyose, D-sorbitol, DL-tryptophan, alanine, phenylalanine, P-coumaric acid and melibiose. Specifically, sucrose, raffinose, alpha-D-glucose, stachyose, D-sorbitol and melibiose were involved in the galactose metabolism. The up-regulation of these metabolites could also be responsible for the observed differences in the crude polysaccharide content between the GG and HG groups. In addition, significant regional effects on the amino acid metabolism of pasture grasses can be seen in aminoacyl-tRNA biosynthesis, alanine, aspartate and glutamate metabolism, phenylalanine metabolism and protein digestion and absorption pathways. DL-proline, L-tryptophan, L-alanine, D-glutamine, *L*-phenylalanine, DL-tyrosine, L-leucine, glutamine, phenylacetic acid, and nine other amino acids have been involved in the metabolism of amino acids.

### *3.1.4. Targeted metabolomic analysis of forage*

Based on the results of the untargeted metabolism, the amino acids

and *reducing sugar content* and the fatty acids were further targeted in order to validate the above speculations.

*3.1.4.1. Amino acid.* The forage contained 22 common amino acids, of which 7 were essential ones. Specifically, alanine was the most abundant amino acid in the forage. Furthermore, as shown in Table 2, significant regional differences were noted in the amino acid content, especially with regards to the amount of essential and non-essential amino acids ($P < 0.05$). The above results reflected the up-regulation of amino acids observed during non-targeted metabolomics analysis.

*3.1.4.2. Fatty acid.* Significant differences in the fatty acid composition were observed between forage from different regions as shown in Table 2 ($P < 0.05$). In this case, a total of 13 Fatty acids were detected in forage, with the main one being C18:2 N6.

*3.1.4.3. Reducing sugar content.* The content of reducing sugars in hay feed varies across different regions, as shown in Table 2. An analysis of the table reveals that the GG group has significantly higher levels of D-Maltose, Melibiose, D-Glucose, D-Galactose, D-Mannose, D-Fructose, D-Xylulose, Rhamnose, Ribonolactone, Glyceraldehyde, and Erythrose compared to the HG group. On the other hand, the HG group exhibits higher levels of D-Ribose, D-Xylose, L-Arabinose, and D-Fucose compared to the GG group. However, there is no significant difference in the content of D-Ribulose between the two groups.

### 3.1.5. Correlation analysis

The correlation heatmap, presented in Fig. 2C, illustrated the relationships between grass quality and grass amino acids, fatty acids and reducing sugar content and non-target metabolites. Overall, a significant association was noted between forage metabolites and forage quality (Fig. 2C).

In particular, the levels of DL-tyrosine, phenylacetic acid, 1,5-pentanediamine, sucrose, raffinose, alpha-D-glucose, stachyose, melibiose, alanine/sarcosine, glutamine, glycine, aspartate, arginine, C18:2 N6, C18:3 N3, C16:0, C22:2 N6, PUFA, D-Glucose, D-Maltose, D-Galactose, D-Mannose, D-Fructose, Ribonolactone, and N3 were positively correlated with the moisture, polyphenol, flavonoid, crude polysaccharide, crude fat and crude protein content but negatively correlated with ADF, NDF and crude fiber. Besides, D-sorbitol and L-tryptophan were negatively correlated with moisture, polyphenol, flavonoid, crude polysaccharide, crude fat and crude protein, but positively correlated with ADF, NDF and crude fiber. Therefore, forage quality is significantly influenced by amino acid biosynthesis and galactose metabolism. The following features of the forage were used for further joint analyses with meat: ADF, Crude fiber, NDF, Crude polysaccharide, Crude fat, Crude protein, Flavonoid, Moisture and Polyphenol, and the metabolites of the forage, D-sorbitol, alanine, phenylacetic acid, DL-tyrosine, glutamine, DL-proline, sucrose, C18:2 N6, stachyose, aspartate, raffinose, D-Glucose, D-Maltose, D-Galactose, D-Mannose, D-Fructose, Ribonolactone, and 1,5-pentanediamine.

## 3.2. Meat quality and metabolites

### 3.2.1. Carcass quality

Regional differences in the carcass quality of Tibetan sheep are shown in Table 3. There were no significant differences in carcass weight, BFT, EMA and AFT among the H0, H1 and M. However, the GR value of the M group was different between the H1 and H0 group.

### 3.2.2. Analysis of meat quality

*3.2.2.1. Sensory analysis of meat.* Table 3 displays the results of the sensory evaluation for the three meat groups. Surprisingly, the three groups of samples showed significant differences ($p < 0.01$). The

**Table 3**
The effect of different regional feeds on the Tibetan sheep meat.

| | Groups | | | *P* |
|---|---|---|---|---|
| | H0 | H1 | M | |
| Sensory analysis | | | | |
| Tenderness | 6.53$^{b}$ | 7.93$^{a}$ | 5.07$^{c}$ | 0.00 |
| Flavor | 6.00$^{b}$ | 7.40$^{a}$ | 4.93$^{b}$ | 0.00 |
| Juiciness | 5.87$^{b}$ | 7.80$^{a}$ | 5.27$^{b}$ | 0.00 |
| Texture | 7.47$^{a}$ | 7.93a | 6.00$^{b}$ | 0.00 |
| General acceptability | 6.87$^{b}$ | 8.33a | 6.33$^{b}$ | 0.00 |
| Carcass quality | | | | |
| Carcass weight (kg) | 16.16 ± 1.19 | 15.68 ± 0.77 | 16.20 ± 0.98 | 0.664 |
| Backfat thickness (cm) | 1.09 ± 0.17 | 1.34 ± 0.27 | 1.12 ± 0.24 | 0.203 |
| Rib fat thickness (cm) | 1.18 ± 0.15$^{b}$ | 1.37 ± 0.17$^{b}$ | 1.92 ± 0.32$^{a}$ | 0.001 |
| Eye muscle area ($cm^2$) | 16.91 ± 1.9 | 17.12 ± 2.56 | 20.29 ± 3.02 | 0.121 |
| Abdominal fat thickness (cm) | 1.79 ± 0.12 | 1.70 ± 0.28 | 1.88 ± 0.41 | 0.664 |
| Edible quality | | | | |
| pH | 6.24 ± 0.15$^{a}$ | 6.42 ± 6.21$^{a}$ | 5.91 ± 0.13$^{b}$ | 0.001 |
| Color(45 min) | | | | |
| L* | 34.03 ± 1.26 | 34.65 ± 1.12 | 35.25 ± 2.21 | 0.502 |
| a* | 17.46 ± 1.19b | 19.14 ± 2.03$^{ab}$ | 20.57 ± 1.87$^{a}$ | 0.046 |
| b* | 3.53 ± 1.71$^{b}$ | 6.89 ± 1.28$^{a}$ | 6.97 ± 2.64$^{a}$ | 0.026 |
| dE | 28.89 ± 21.92 | 38.90 ± 27.86 | 63.03 ± 6.89 | 0.062 |
| Cooking loss (%) | 36.94 ± 3.48 | 38.50 ± 1.46 | 35.99 ± 2.12 | 0.312 |
| Thawing loss (%) | 5.66 ± 1.57$^{b}$ | 4.78 ± 1.43$^{b}$ | 9.23 ± 2.09$^{a}$ | 0.004 |
| Cooked meat percentage (%) | 67.16 ± 3.88 | 63.58 ± 1.88 | 62.32 ± 3.49 | 0.083 |
| water holding capacity | 35.65 ± 8.22 | 35.30 ± 5.26 | 26.97 ± 6.86 | 0.12 |
| Shear force (N) | 93.46 ± 14.46 | 129.31 ± 34.82 | 105.28 ± 30.45 | 0.168 |
| Texture | | | | |
| Viscosity (mJ) | 2.05 ± 0.79 | 1.51 ± 0.69 | 1.18 ± 0.60 | 0.273 |
| Elasticity (mm) | 7.49 ± 6.91 | 10.96 ± 13.89 | 13.57 ± 17.93 | 0.799 |
| Cohesion (g) | 0.35 ± 0.11 | 0.4 ± 0.14 | 0.4 ± 0.11 | 0.771 |
| Hardness (g) | 2754.83 ± 1412.32 | 2702.53 ± 1216.13 | 2932.44 ± 1393.11 | 0.972 |
| Adhesion (g) | 942.73 ± 572.92 | 987.20 ± 273.61 | 1240.88 ± 854.32 | 0.752 |
| Chewability (mJ) | 109.28 ± 137.97 | 80.71 ± 85.51 | 144.53 ± 161.59 | 0.788 |
| Nutritional quality | | | | |
| Moisture | 75.10 ± 0.87 | 74.58 ± 0.80 | 72.43 ± 2.62 | 0.215 |
| Protein | 21.52 ± 0.91 | 22.58 ± 0.29 | 19.7 ± 0.26 | 0.375 |
| fat | 1.74 ± 0.43$^{b}$ | 2.01 ± 0.56$^{b}$ | 5.76 ± 2.34$^{a}$ | 0.023 |
| Amino acids composition(mg/100 g) | | | | |
| Isoleucine | 0.22 ± 0.01$^{a}$ | 0.21 ± 0.00$^{a}$ | 0.17 ± 0.02$^{b}$ | 0.004 |
| Serine | 0.53 ± 0.03$^{a}$ | 0.43 ± 0.06$^{b}$ | 0.37 ± 0.03$^{b}$ | 0.007 |
| Asparagine | 0.30 ± 0.04$^{a}$ | 0.26 ± 0.02$^{ab}$ | 0.21 ± 0.01$^{b}$ | 0.01 |
| Valine | 0.45 ± 0.05$^{a}$ | 0.39 ± 0.01$^{ab}$ | 0.34 ± 0.02$^{b}$ | 0.01 |
| Leucine | 0.38 ± 0.02$^{a}$ | 0.36 ± 0.00$^{ab}$ | 0.32 ± 0.03$^{b}$ | 0.03 |
| EAAs | 2.58 ± 0.13$^{a}$ | 2.41 ± 0.04$^{ab}$ | 2.21 ± 0.14$^{b}$ | 0.022 |
| NEAAs | 43.15 ± 3.65 | 40.50 ± 3.61 | 39.28 ± 0.21 | 0.332 |
| TAAs | 45.73 ± 3.78 | 42.90 ± 3.62 | 41.49 ± 0.33 | 0.293 |
| Fatty acids composition (mg/100 g) | | | | |
| C18:2 N6 | 1821.26 ± 194.97$^{b}$ | 2831.63 ± 228.15$^{a}$ | 1352.14 ± 364.42$^{b}$ | 0.001 |
| C20:3 N3 | 9.31 ± 1.43$^{b}$ | 14.36 ± 1.19$^{a}$ | 6.69 ± 1.61$^{b}$ | 0.001 |
| C17:1 N7 | 373.84 ± 83.55$^{b}$ | 920.92 ± 93.97$^{a}$ | 310.79 ± 173.13$^{b}$ | 0.001 |
| C22:5 N3 | 226.34 ± 30.62$^{a}$ | 237.96 ± 19.73$^{a}$ | 132.23 ± 31.38$^{b}$ | 0.006 |

(*continued on next page*)

**Table 3** (*continued*)

| | Groups | | | *P* |
|---|---|---|---|---|
| | H0 | H1 | M | |
| C20:3 N6 | 85.91 ± 6.62[a] | 85.82 ± 7.93[a] | 57.55 ± 10.59[b] | 0.009 |
| C18:3 N3 | 188.39 ± 32.89[b] | 390.37 ± 96.23[a] | 139.87 ± 64.29[b] | 0.009 |
| C20:5 N3 | 134.69 ± 22.65[a] | 94.39 ± 23.30[ab] | 75.01 ± 7.42[b] | 0.023 |
| C20:1 N9 | 158.58 ± 51.42[b] | 690.40 ± 304.86[a] | 168.31 ± 141.76[b] | 0.025 |
| C18:3 N6 | 25.21 ± 2.99[b] | 38.86 ± 2.51[a] | 27.55 ± 7.52[ab] | 0.029 |
| C11:0 | 0.14 ± 0.13[b] | 1.90 ± 1.08[a] | 0.32 ± 0.38[ab] | 0.03 |
| C22:4 N6 | 52.29 ± 6.23[ab] | 73.10 ± 16.99[a] | 41.39 ± 8.65[b] | 0.039 |
| C20:2 N6 | 138.69 ± 7.97[b] | 188.82 ± 20.62[a] | 142.37 ± 28.63[b] | 0.046 |
| C12:0 | 30.19 ± 21.11[b] | 237.35 ± 128.34[a] | 54.77 ± 68.03[b] | 0.046 |
| SFA | 11,533.51 ± 4396.27 | 26,682.11 ± 11,536.56 | 12,568.63 ± 8326.38 | 0.158 |
| PUFA | 3753.61 ± 393.07[b] | 5173.23 ± 283.82[a] | 2755.60 ± 645.88[b] | 0.002 |
| MUFA | 14,566.25 ± 4823.76 | 32,504.90 ± 13,386.59 | 16,559.22 ± 9734.99 | 0.13 |
| N6 | 3158.09 ± 360.02[b] | 4398.48 ± 222.92[a] | 2371.39 ± 559.34[b] | 0.003 |
| N3 | 595.53 ± 76.32[a] | 774.76 ± 65.91[a] | 384.21 ± 93.54 [b] | 0.003 |
| N6/N3 | 5.35 ± 0.78 | 5.69 ± 0.27 | 6.21 ± 0.71 | 0.31 |
| PUFA/SFA | 0.35 ± 0.12 | 0.23 ± 0.11 | 0.27 ± 0.12 | 0.44 |

**Note:** The same lowercase letters or no letters indicate no significant difference ($P > 0.5$), and different lowercase letters indicate significant difference ($P < 0.5$). MUFA, sum of monounsaturated fatty acids; *P*UFA, sum of polyunsaturated fatty acids; SFA, sum of saturated fatty acid, n-3 PUFAs: omega-3 polyunsaturated fatty acids, n-6 PUFAs: omega-6 polyunsaturated fatty acids. EAAs, essential amino acids; NEAAs: non- essential amino acids; TAAs, total amino acids.

samples from the H1 group scored significantly higher ($P < 0.01$) in tenderness, flavor, juiciness and texture compared to the other two groups.

*3.2.2.2. Edible quality of meat.* Regional differences in the edible quality of Tibetan sheep meat are shown in Table 3. The results indicated that cooking loss, cooked meat percentage, water holding capacity, shear force (N) and texture did not differ significantly between the three groups. After 45 min post-slaughter, the slowest decline in muscle pH was observed in H1, while the quickest one occurred in group M. Furthermore, the a* value of the M group was significantly higher than that of the other two groups, with the b* value of the H0 group being the lowest ($P < 0.05$). Finally, in terms of water retention, the thawing loss of the H1 group was significantly lower compared with the M group ($P < 0.05$).

*3.2.2.3. Nutritional component of meat.* Moisture, protein and fat are the basic components of meat and meat products in terms of nutritional value. The nutritional component of Tibetan sheep meat, shown in Table 3, did not differ significantly across regions in terms of their moisture and protein. However, group M had the highest fat content.

### *3.2.3. Untargeted metabolomics profiling of meat*

*3.2.3.1. Evaluation of the quality of experimental data.* Total ion chromatograms (TIC) of QC samples were compared for spectral overlay, as shown in Supplementary Figures 1A and 1B. The experimental results indicated that the response intensities and retention times of each chromatographic peak largely overlapped, suggesting minimal variation caused by instrument errors throughout the entire experimental process. Principal Component Analysis (PCA), performed on all experimental and QC samples, as depicted in Supplementary Figures 1C and 1D, showed the absence of distinct separation of the three groups.

To enhance the differentiation between groups, PLS-DA and OPLS-DA were applied for further analysis of the three sample sets after excluding the QC samples. As depicted in Figs. 3, for both patterns under the positive ion model, the samples showed within-group clustering and between-group separation, and the effect of OPLS-DA (Figs. 3D, E, Fwas more obvious compared to PLS-DA (Figs. 3A, B, C. To prevent the risk of overfitting in the supervised modeling process, a permutation test was also applied to validate the models. OPLS-DA analysis revealed a separation and aggregation within and between the H0 and M groups (Fig. 3 G, R2X = 0.475, R2Y = 0.994, Q2 = 0.57), the H1 and H0 groups (Fig. 3 H, R2X = 0.454, R2Y = 0.995, Q2 = 0.518) and the M and H1 groups (Fig. 3 I, R2X = 0.22, R2Y = 0.992, Q2 = 0.377) in positive modes. As the permutation iterations progressed, both the R2 and Q2 values of the random model steadily decreased, confirming the absence of overfitting in the original model. These results indicate that the observed separation of metabolites between the groups during PLS-DA and OPLS-DA analyses was statistically significant.

*3.2.3.2. Bioinformatics analysis of differential metabolites.* The integration of differential metabolites in both ion modes led to the detection of 1290 metabolites, with 746 in the positive ion mode and 544 in the negative one. Using the predefined criteria (VIP > 1 and $P < 0.05$) for differential metabolites (DFMs), a total of 163 DFMs were identified among the three groups in the positive ion mode, with 66 of these associated with KEGG pathways.

Pairwise comparisons of M and H0, H1 and H0 as well as M and H1 identified 64, 47 and 33 DFMs, respectively, as illustrated in Fig. 4A. Of these, 6 metabolites were associated with the H1 group (3 up-regulated and 3 down-regulated in the H1 group compared with the other two groups), 15 metabolites were associated with the M group (3 up-regulated and 12 down-regulated in the M group compared with the other two groups), and 24 metabolites were associated with the H0 group (4 up-regulated and 20 down-regulated in the H0 group compared with the other two groups).

KEGG pathway enrichment analysis, shown in Fig. 4B, revealed the top 20 pathways that were enriched by each of the three different feed regimes, with most of these being associated with AA metabolism, lipid metabolism and nucleotide metabolism. To further compare the differential metabolic pathways in which the differential metabolites of Tibetan sheep were involved under different feeding conditions, a differential richness score plot was obtained (Fig. 4C, D, E).

Table 4. shows that 8 metabolic pathways were up-regulated and 2 were down-regulated in group M compared with group H0 (DA score > 0.5 and $P < 0.05$). The up-regulated ones included vitamin B6 metabolism, phenylalanine, tyrosine and tryptophan biosynthesis as well as amino acid and nucleotide sugar metabolism. Amino acid biosynthesis and the pentose phosphate pathway were also up-regulated, while arginine biosynthesis decreased. Key up-regulated metabolites comprised D-erythrose 4-phosphate, D-ribonose 5-phosphate, tryptophan, udp-galactose, D-glucosamine 6-phosphate, D-mannose 1-phosphate, D-mannose-6-phosphate, argininosuccinic acid, L-lysine, glucosinolate 6-phosphate, and D-glucosinolates.

In comparison with the H0 group (DA score > 0.5 and $P < 0.05$), the H1 group exhibited changes in 8 up-regulated and 10 down-regulated metabolic pathways. The up-regulated ones included phospholipid signaling, pentose phosphate pathway, amino acid and nucleotide sugar metabolism and the phosphotransferase system (PTS). Conversely, the down-regulated pathways encompassed pyruvate metabolism, the citric acid cycle (TCA cycle) and arginine biosynthesis, with the main enhanced metabolites being sphingosine, Pro-Trp, D-glucaric acid 6-phosphate, D-ribose-5-phosphate, glycerol, D-glucosamine, D-mannose 1-phosphate, Udp-galactose, D-glucosamine 6-phosphate, D-mannose 6-

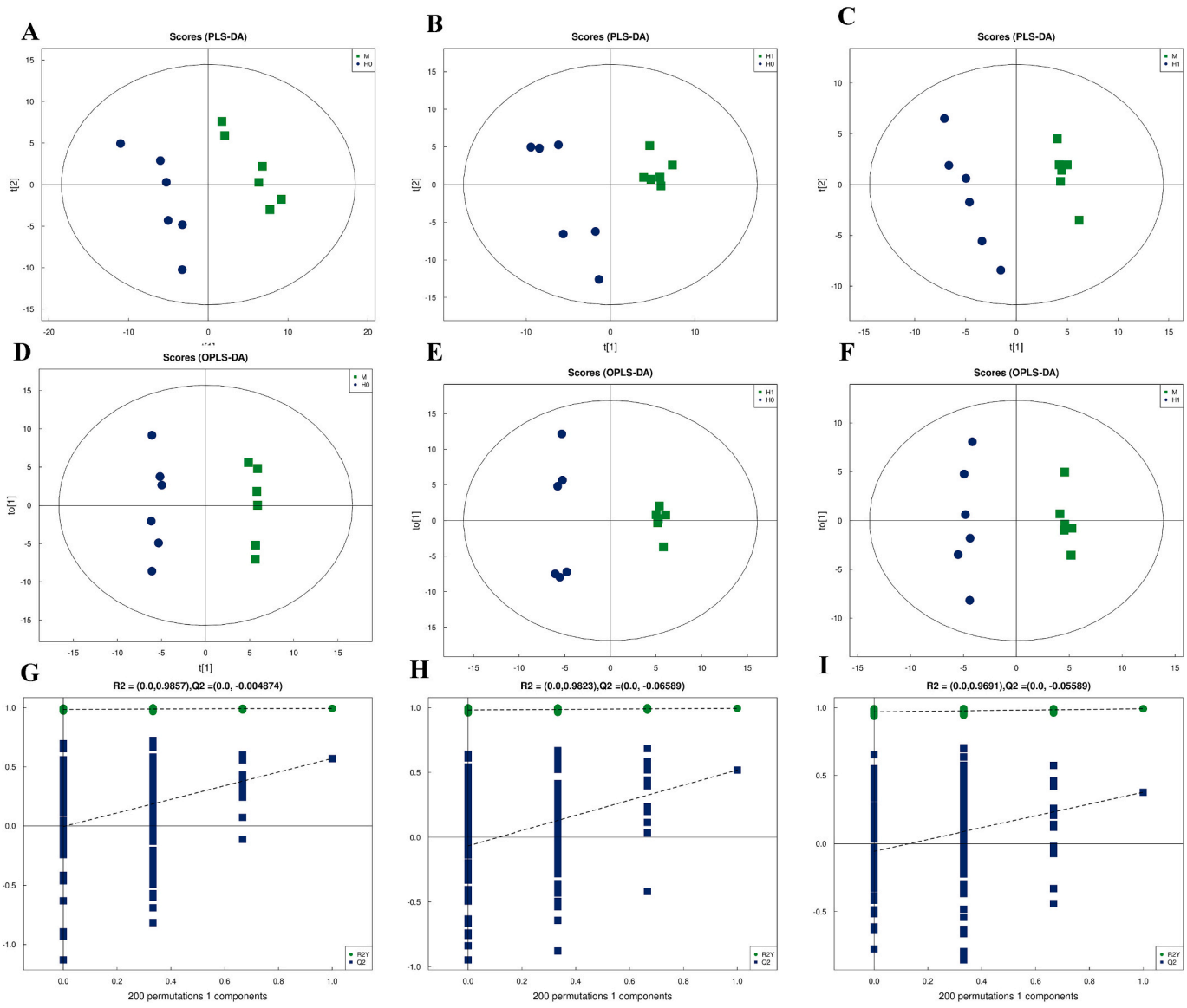



**Fig. 3.** Multivariate statistical analysis of forage in different regions: PLS-DA (A-C), OPLS-DA (D-F) scores of the overall sample in the positive ion mode and permutations test of OPLS-DA (G-I) in the positive ion detection mode.

phosphate, D-glucose 6-phosphate, phosphoenol pyruvate and L-ascorbic acid.

Compared with group M, H1 displayed changes in 10 up-regulated and 4 down-regulated metabolic pathways (DA score $> 0.5$ and $P < 0.05$). The key up-regulated ones included biosynthesis of unsaturated fatty acids, while down-regulated pathways involved D-arginine and D-ornithine metabolism. As a result, the main up-regulated metabolites consisted of arachidonic acid (peroxide free) and Cis-7,10,13,16-docosatetraenoic acid, whereas the primary down-regulated one was DL-arginine.

In general, metabolism of amino acids, sugars and unsaturated fatty acids exhibited significant upregulation in the H1 group, while amino acid metabolism was downregulated in the H0 group compared with the other groups. Interestingly, the diversity of regional diets exerted an influence on the metabolism of amino sugars and nucleotide sugars as well as on the pentose phosphate signaling pathway in the longest muscle of Tibetan sheep.

### 3.2.4. *Targeted metabolomic analysis of meat*

*3.2.4.1. Amino acid.* Regional differences in the amino acid content of Tibetan sheep meat are shown in Table 3. Overall, there were no statistically significant differences in NEAAs and TAAs, although the EAA content in H0 group was higher ($P < 0.05$). At the same time Isoleucine, Valine and Leucine were the three most abundant essential amino acids in H0 ($P < 0.05$).

*3.2.4.2. Fatty acid.* As illustrated in Table 3, monounsaturated fatty acids did not differ significantly between Tibetan sheep from different regions. However, the N3, N6 and polyunsaturated fatty acid content in H1 was significantly higher than that of the other two groups ($P < 0.05$).

### 3.2.5. *Correlation analyses*

To explore the relationship between muscle metabolism and meat quality in Tibetan sheep under different feeding conditions, correlation analyses were performed between the meat phenotype data and the non-targeted metabolomic results, as shown in Fig. 4F.

A significant association was observed between meat metabolism

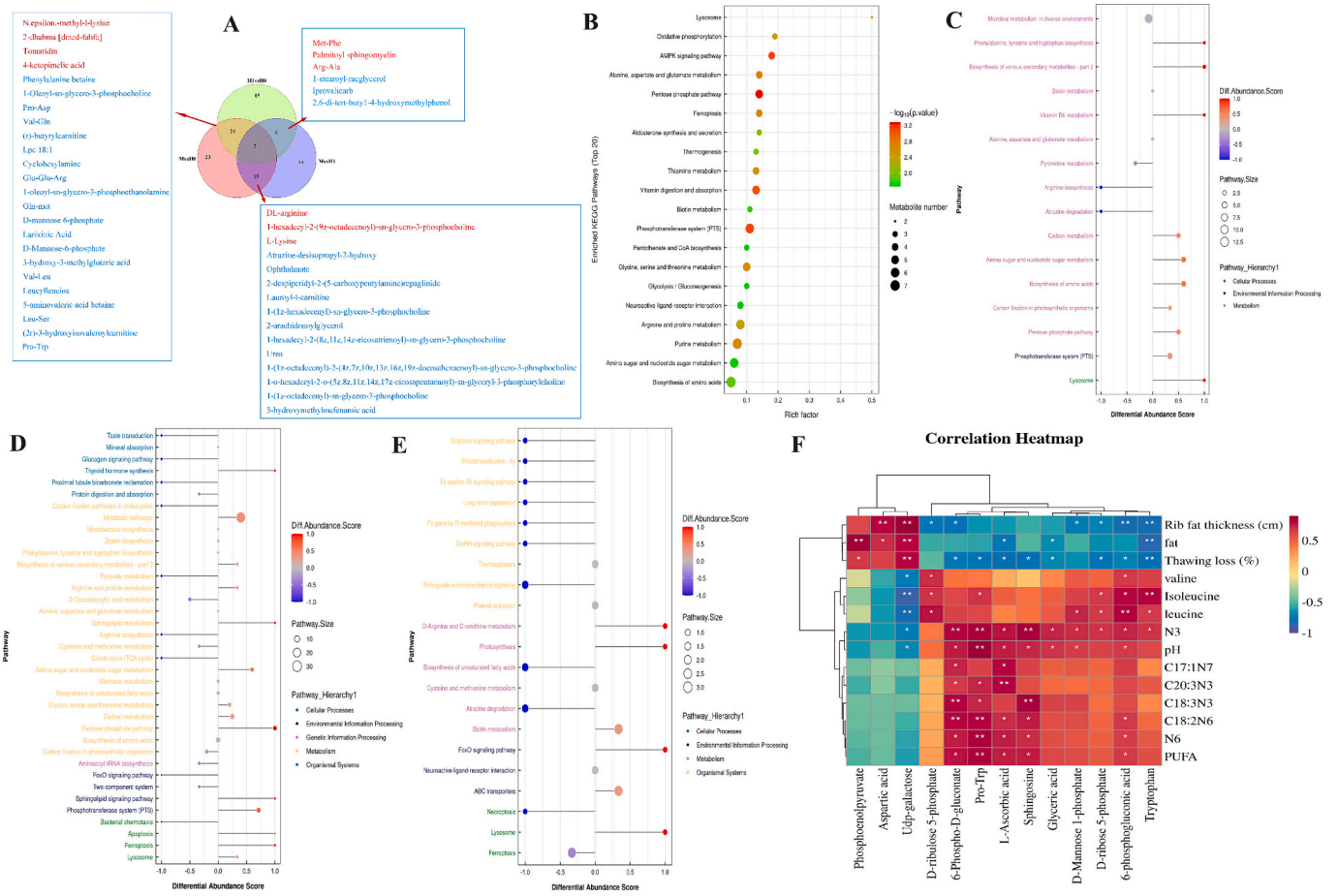



**Fig. 4.** A: Venn diagram illustrating the overlap of different group-associated DFMs between the three comparisons (M vs H0; H1 vs H0; M vs H1) in the longissimus Lumborum of Tibetan sheep. B: Top 20 enriched KEGG pathways of the comparison among three groups. A differential abundance score map of differential metabolic pathways. (C: M vs H0, D: H1 vs H0, E: M vs H1). A score of 1 indicates that the expression of all identified metabolites in the pathway is up-regulated, −1 indicates that the expression of all identified metabolites in the pathway is down-regulated. F: The correlation heat map between meat quality parameters and meat metabolomics analysis. The color red and blue represent positive and negative correlations, respectively. $*P < 0.05$ and $**P < 0.01$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and meat quality (Fig. 4F). In particular, fat, thawing loss and rib fat thickness were positively correlated with Udp-galactose, phosphoenolpyruvate and aspartic acid, but negatively correlated with tryptophan. There was also a positive correlation between isoleucine, valine and D-ribulose 5-phosphate, tryptophan, 6-phosphogluconic acid and D-ribose 5-phosphate. Moreover, there was a strong positive correlation between N3 and sphingosine, pH, Pro-Trp, C18:3 N3, sphingosine and 6-Phospho-D-gluconate. At the same time, Pro-Trp was strongly and positively correlated with C18:2 N6, N6 and PUFA. Therefore, the following meat qualities were selected: Rib fat thickness, thawing loss, isoleucine, leucine, N3 and pH and meat metabolites Udp-galactose, 6-Phospho-D-gluconate, Pro-Trp, L-Ascorbic acid, sphingosine and 6-phosphogluconic acid, to be further analyze in conjunction with forages.

### 3.3. Conjoint analysis

In Fig. 5A, the correlation clustering heatmap depicts the relationship between the feed in the HG group and the l *Longissimus lumborum* in the M group of Tibetan sheep. It should be noted that a negative correlation was observed between 6-phospho-D-gluconate in muscle and proline in feed. Similarly, 6-phosphogluconic acid showed a positive correlation with Crude fat and a negative correlation with Moisture, the pH in muscle showed a positive correlation with crude fiber and ADF in feed, while rib fat thickness was positively correlated with DL-tyrosine and stachyose in feed, as well as sphingosine displayed a negative correlation with D-Fructose, D-Mannose, and Polyphenol. Additionally, thawing loss and udp-galactose in muscle demonstrated a positive correlation with Moisture in feed. Leucine exhibited a positive correlation with D-Galactose and a negative correlation with NDF. Pro-Trp displayed a positive correlation with Ribonolactone and a negative correlation with Sorbitol. These correlations provided valuable insights into the relationship between feed composition and muscle characteristics in Tibetan sheep, specifically in the M group.

As shown in Fig. 5B, the correlation clustering heatmap demonstrated the relationship between feed in the HG group and the *Longissimus lumborum* in the H0 group of Tibetan sheep. The analysis revealed a number of interesting correlations. Specifically, L-ascorbic acid in the muscle exhibited a positive correlation with crude fat in HG group feed. Similarly, leucine in muscle showed a positive correlation with ADF and crude fiber in feed. On the other hand, isoleucine showed a positive correlation with sorbitol, but a negative correlation with ribonolactone, 6-phospho-D-gluconate in muscle was negatively correlated with D-fructose, D-mannose, and polyphenol in feed, pro-Trp and udp-galactose were positively correlated with phenylacetic acid, sphingosine in muscle was positively correlated with raffinose in feed but negatively correlated with protein, rib fat thickness was positively correlated with proline, N3 unsaturated fatty acids in muscle displayed a negative correlation with sorbitol in feed and a positive correlation with ribonolactone. Muscle pH was positively correlated with flavonoid and D-glucose in feed. These correlations provided valuable insights into the

**Table 4**
DFMs from longissimus Lumborum of Tibetan sheep in the Key Differential Enriched KEGG Pathways (False Discovery Rates <0.05).

| Metabolic pathways | Metabolites |
|---|---|
| **MvsH0** | |
| **upregulation in the M group** | |
| Vitamin B6 metabolism | D-erythrose 4-phosphate, D-ribulose 5-phosphate |
| Phenylalanine, tyrosine and tryptophan biosynthesis | Tryptophan, D-erythrose 4-phosphate |
| Amino sugar and nucleotide sugar metabolism | Udp-galactose, D-glucosamine 6-phosphate, D-Mannose 1-phosphate, D-mannose 6-phosphate, |
| Biosynthesis of amino acids | Tryptophan, D-erythrose 4-phosphate, D-ribulose 5-phosphate, Argininosuccinic acid, L-Lysine |
| Pentose phosphate pathway | D-erythrose 4-phosphate, 6-phosphogluconic acid, D-ribulose 5-phosphate, D-glucosaminic acid |
| **downregulation in the M group** | |
| Arginine biosynthesis | Argininosuccinic acid |
| **H1vsH0** | |
| **upregulation in the H1 group** | |
| Sphingolipid signaling pathway | Sphingosine, Pro-Trp |
| Pentose phosphate pathway | 6-Phospho-D-gluconate, 6-phosphogluconic acid, D-ribose 5-phosphate, D-ribulose 5-phosphate, Glyceric acid, D-glucosaminic acid |
| Amino sugar and nucleotide sugar metabolism | D-Mannose 1-phosphate, Udp-galactose, D-glucosamine 6-phosphate, D-Mannose-6-phosphate |
| Phosphotransferase system (PTS) | D-glucose 6-phosphate, Phosphoenolpyruvate, L-Ascorbic acid, D-glucosamine 6-phosphate, D-Mannose-6-phosphate, D-glucosaminic acid, D-mannose 6-phosphate |
| **downregulation in the H1 group** | |
| Pyruvate metabolism | Phosphoenolpyruvate, Malate |
| Citrate cycle (TCA cycle) | Phosphoenolpyruvate, Malate |
| Arginine biosynthesis | N-. alpha. - acetyl-l-ornithine, Aspartic acid |
| **MvsH1** | |
| **upregulation in the H1 group** | |
| Biosynthesis of unsaturated fatty acids | Arachidonic Acid (peroxide free), *cis*-7,10,13,16-Docosatetraenoic acid |
| **downregulation in the H1 group** | |
| D-Arginine and D-ornithine metabolism | DL-arginine |

relationship between feed composition and muscle characteristics in Tibetan sheep.

Fig. 5C represents the correlation clustering heatmap between feed in the GG group and the *Longissimus lumborum* in the H1 group of Tibetan sheep. From the heatmap, several important correlations could be identified. For example, thawing loss in muscle was positively correlated with C18:2 N6 and stachyose in feed, but negatively correlated with crude protein. Additionally, 6-phospho-D-gluconate in muscle showed a positive correlation with proline in feed, while being negatively correlated with sorbitol. Similarly, 6-phosphogluconic acid in muscle exhibited a positive correlation with proline and sucrose in feed, but a negative one with sorbitol. Sphingosine exhibited a positive correlation with Crude fiber and Glutamine. L-Ascorbic acid was positively correlated with D-Galactose but negatively correlated with ADF. Leucine was positively correlated with Phenylacetic acid. Muscle pH was also positively correlated with alanine, with Udp-galactose in muscle displaying a positive correlation with protein in feed, but a negative correlation with stachyose, C18:2 N6 and fat. These correlations provide valuable insights into the relationship between feed composition and muscle characteristics in Tibetan sheep, specifically in the H1 group of the GG group.

## 4. Discussion

The primary objective in formulating daily animal rations is to maximize pasture utilization, while supplementing additional feeds as needed to meet the animals' nutritional requirements (Linn & Martin, 1991). Precise analysis of pasture quality and its nutritional content is therefore crucial to ensure that animals receive the necessary nutrition for optimal growth and production. High-quality feeds with abundant protein support normal animal growth and healthy (Terler et al., 2022), while varying fat content in the feed can affect the fatty acid composition of the meat (Waszkiewicz-Robak et al., 2015). Specifically, the right fat content yields tender and juicy meat, while excessive fat may result in overly fatty or dry and lean meat (Hutchison et al., 2006). The fiber content also influences the animals' feed consumption and digestion. In particular, except for neutral detergents, more fiber has a negative impact on the digestibility of all nutrients (Paternostre, De Boever, & Millet, 2021). At the same time, the evaluation of some polysaccharides (Qiao et al., 2022), polyphenols (Pan et al., 2022), flavonoids (Zhou, Mao, & Zhou, 2019) and other secondary metabolites in plant tissues has garnered some research attention due to their strong antioxidant activity and beneficial characteristics for intestinal health, such as anti-inflammatory or antibacterial properties in animals (Myrtsi et al., 2023). In this study, aside from ash content, there were no significant differences between the values, although the GG group, as a saline feed, showed significant differences in moisture ($p < 0.05$), crude protein, crude fat, crude fiber, NDF, ADF, crude polysaccharide, polyphenol and flavonoid.

It is well established that water is the most abundant and crucial substance within plants, but under saline-alkaline conditions, the plants are often subjected to drought and pH stress (Otlewska et al., 2020). Research has revealed that a number of plants in saline-alkaline areas have evolved complex mechanisms at the physiological and molecular level to adapt to high saline-alkaline soils and drought stress (Li et al., 2022). One crucial biochemical factor related to plant water content is aquaporins, and increasing evidence from various crop plants suggests that aquaporins play a vital role in drought stress tolerance (Forrest & Bhave, 2007; Xu, Bruce, & Spivey, 2014; Zargar et al., 2017). However, the specific role of the water channel proteins in the GG group still has to be determined in more detail by means of proteomics and transcriptomics. In addition, significant accumulation of proline was noted in the GG group, with proline being a natural Osmo protectant that can enhance plant tolerance to osmotic stress (Mmf & Ef, 2017).

Referring to the quality grading of gramineous forage in China, the CP content in the GG group could be categorized as a second-grade standard (7%–9%). Furthermore, compared with the traditional alkaligrass protein content (3%) in the Chinese feed library, the GG group had a higher CP content (China forage database: http:// www.chinafeeddata.org.cn). In this context, Singer et al.(Singer et al., 2023). found that salt stress could increase the amount of protein with protective effects, and this could explain the higher CP content in GG. Furthermore, under salt stress, GG (12.1%) exhibited a significantly higher fat content compared with HG (6.2%) ($p < 0.01$). Additionally, results of the targeted metabolomics indicated that the fatty acid content in the GG group was also significantly higher than that in the HG group ($P < 0.01$). This finding was consistent with the results of a study by Petropoulos (Petropoulos et al., 2020), who found that salinity influenced the fat and fatty acid content of plants. This change is believed to be related to the remodeling of membrane lipids under salt stress

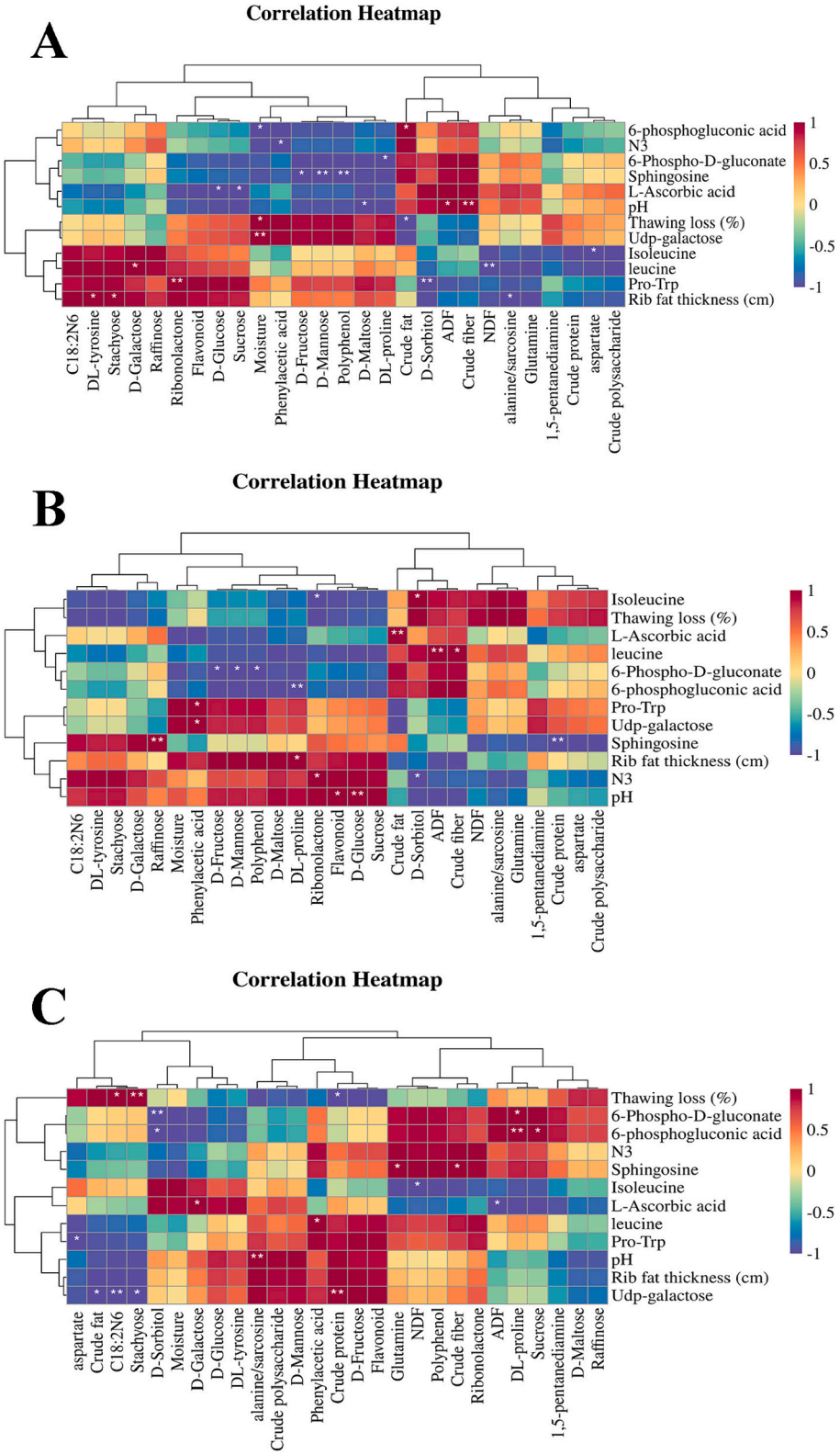



**Fig. 5.** Clustering heat map of correlation between metabolites and quality of forage and meat (A: HG vs M, B: HG vs H0, C: GG vs H1) $^{*}P < 0.05$ and $^{**}P < 0.01$.

condition (Guo, Liu, & Barkla, 2019). Interestingly, it has been confirmed that membrane lipids can alter the permeability of cell membranes during the remodeling process (Ashraf & Ali, 2008) which corresponds to previous speculations related to water content. In addition, it was found that under saline-alkali feeding conditions, sheep meat was characterized by a high protein and low-fat content, both of which were consistent with the results obtained by Friha (Friha et al., 2022). In fact, Al-Khalasi et al.(Al-Khalasi et al., 2010). also reported that for sheep raised in saline-alkali pastures, energy can be specifically allocated to protein synthesis rather than for fat production.

Although the NDF (neutral detergent fiber) in feed may stimulate the secretion of pancreatic digestive enzymes (Stock-Damgé et al., 1984),

excessive fiber content can lead to poor nutrient absorption and utilization. On the other hand, low fiber content can prevent livestock from achieving satiety and induce constipation. Therefore, maintaining an appropriate level of dietary fiber is crucial. In this study, the NDF content detected in the GG group was higher than that of corn silage (34.4%) but lower than that of purple clover silage (Uddin et al., 2020) (19.2%) and wheat straw (Li et al., 2014) (82%). However, it was similar to that of fresh oat hay (Brown et al., 2018) (58.4%–50.8%). Therefore, the dietary fiber content in the GG group could be considered to be relatively moderate. In contrast, the excessively high fiber level in the HG group could have led to lower nutrient absorption and utilization in Tibetan sheep. Feed value is a crucial economic trait of roughage, and in this case, the forage quality index of the GG group was close to that of the American Forage and Grassland Association's (AFGA) hay grade standard, hence highlighting the feed's high quality.

Numerous studies have shown that secondary metabolites such as flavonoids, polyphenols and polysaccharides in feeds exhibit good tolerance in improving meat quality and coping with abiotic stress (Cao et al., 2023; Catarino et al., 2023; Chen et al., 2023; Zhao et al., 2023). In this experiment, it was surprising to note that the GG group exhibited significant differences in terms of its crude polysaccharides, polyphenols and flavonoid content compared with the HG group ($P < 0.01$). Plants produce a large amount of reactive oxygen species (ROS) when exposed to salt stress, and this can subsequently cause oxidative damage to cells and activate stress pathways within their cells (Shabala et al., 2016). In this context, “sugars” have emerged as novel scavengers of ROS based on their role in redox balance (Nadarajah, 2020). For example, Wang et al.'s (Zhang et al., 2016) experiment on the salt-tolerant plant *Suaeda salsa* detected substantial amounts of sucrose and maltose. Numerous studies (Liu, Gai, & Qiu, 2023; Mohammadi Alagoz, Asgari La Jayer, & Ghorbanpour, 2023) have also shown that large amounts of carbohydrates accumulate in plants under salt stress, hence suggesting that they are associated with ROS accumulationInterestingly, results of the non-targeted metabolomics indicated a significant up-regulation of the KEGG pathways, namely the “Biosynthesis of various secondary metabolites - part 2” and “galactose metabolism” in the GG group. In addition, the oligosaccharides in the raffinose family, synthesized from sucrose, function as compatible solutes involved in stress tolerance defense mechanisms, with evidence further suggesting that they also act as antioxidants (ElSayed, Rafudeen, & Golldack, 2014). P-coumaric acid is a phenolic acid known for its biological properties such as high free radical scavenging, anti-inflammatory, anti-tumor and antimicrobial activities (Ferreira et al., 2019). As with the findings presented in this paper, studies have also found that the accumulation of the Melibiose increases under salt stress (Hill et al., 2013). Therefore, we suggest that saline pastures accumulate more antioxidants such as flavonoids, polysaccharides and polyphenols. Several studies have demonstrated that feed polysaccharides can impact meat quality. For example, Li et al.(Li et al., 2024). discovered that the inclusion of glycyrrhiza polysaccharides had a beneficial impact on the meat quality of broiler chickens. Similarly, Li et al. (Xiang Xiang et al., 2020). found that polysaccharides from Yingshan Yunwu tea affected the meat quality of chickens in terms of color, pH, and hardness. Additionally, the addition of *Eucommia ulmoides* leaf extract (Yan et al., 2022), which is rich in polysaccharides and polyphenols, to chicken meat improved the growth performance, meat quality, and antioxidant response of broilers.

Jorge et al.(Jorge et al., 2016) reported that the reaction between carbohydrates and amino acids is the core of metabolic product adaptation to salt stress. In fact, the results of the non-targeted metabolomics also highlighted the enrichment of amino acid pathways as well as their subsequent accumulation. Furthermore, the KEGG pathways enriched by the salt-alkaline feeds in this study were also identified in previous studies. In this context, Ashok et al.(Panda, Rangani, & Parida, 2021) found that pathways influencing drought stress included aminoacyl-tRNA biosynthesis, while Zheng et al.(Zheng et al., 2022) found that pathways significantly involved the response of *Lotus japonicus* leaves included alanine, aspartate, glutamate metabolism, as well as phenylalanine metabolism.

In conclusion, it is pleasantly surprising that through the analysis of nutritional quality and metabolomics of different regional forages, the GG group exhibits significant advantages in various aspects as feed for ruminant animals.

The rate of glycogen degradation in post-slaughter muscles as well as the accumulation of lactic acid are considered to be the primary factors affecting the decrease in pH (Zhang et al., 2020). Reactive oxygen species (ROS) are essential byproducts in post-mortem processes, but studies have shown that intraperitoneal injection of hydrogen peroxide induces ROS production (Samandari-Bahraseman et al., 2023), enhancing post-mortem muscle glycolytic metabolism, which could potentially lead to oxidative damage and a decrease in pH (Chen et al., 2017). It is worth noting that research has found that antioxidants can prevent accelerated glycolysis (Li et al., 2016).

Previous studies on the quality of feed in saline-alkali soils have revealed the presence of a significant amount of antioxidant substances. Indeed, the transfer and phosphorylation of the carbon source in PTS reduce glucose phosphorylation, resulting in decreased cAMP (cyclic adenosine monophosphate) levels. Simultaneously, this hinders the APK (cAMP-dependent protein kinase) signaling pathway as well as the phosphorylation of inactive glycogen phosphorylase, leading to a reduction in glycogen metabolism Zhang, Han, Gui, et al., 2022). For instance, Zhang et al. (Zhang, Han, Gui, et al., 2022). reported that the accumulation of D-glucosamine 6-phosphate, enriched in the up-regulated PTS pathway, can inhibit hexokinase activity, slow down glycolysis and consequently retard the decrease in pH. In this work, alkaline stress led to the inhibition of the TCA cycle associated with the glycolytic pathway. Simultaneously, the participation of malate and phosphoenolpyruvate in the TCA cycle is inhibited, thereby suppressing glycolysis. Pyruvate, as a product of glycolysis and a precursor of the TCA cycle, also undergoes inhibition in its synthesis. Guo (Guo et al., 2022) found the same characteristics in the metabolism of saline and alkaline stress in wheat seedlings.

Qinghai represents a high-altitude hypoxic region, and Mayuko et al. (Osada-Oka et al., 2010). found that, under such conditions, glucose contributed to the stabilization of hypoxia-inducible factor proteins by stimulating glucose metabolism via the pentose phosphate pathway to produce NADPH. Surprisingly, a significant up-regulation of the pentose phosphate pathway (PPP) was observed in the muscle of the H1 group, along with an increase in the level of D-ribose 5-phosphate derived from the first stage of the PPP. In this context, Zhang et al. (Zhang et al., 2016), reported that proteomic studies on sunflowers subjected to alkali stress revealed a significant up-regulation of two proteins related to the PPP, namely transketolase and transaldolase. The up-regulation of these enzymes leads to the generation of ribose 5-phosphate, which is subsequently used for nucleic acid and amino acid synthesis, as reported in the current findings. The PPP is crucial for maintaining carbon balance as it provides precursors for nucleotide and amino acid biosynthesis, while supplying reducing molecules to counteract oxidative stress (Stincone et al., 2015). Interestingly, an upregulation of the amino sugar and nucleotide sugar metabolism pathway was also observed in the H1 group. Additionally, the up-regulation of the PPP as well as the subsequent generation of NADPH, may result in an upregulation of the unsaturated fatty acid metabolism pathway (Fig. 6.)

Meat color is an important factor that affects consumer purchasing decisions (Wu et al., 2020). In general, higher a* values and lower b* and L* values are preferable for meat. In this experiment, although there were no significant differences between the L* values of the three groups, the a* value of group H1 was lower than that of group M, while its b* value was higher than that of group H0. L* (34.30) and a* (19.14) were both within the normal range for sheep meat ($L^* > 34$, $9.5 < a^* < 19$) (Khliji et al., 2010). However, the meat color in this study was not significantly different from that reported by Ma (Ma, Han, Zhang, et al., 2023) and Zhang (Zhang, Han, Hou, et al., 2022) regarding high-quality

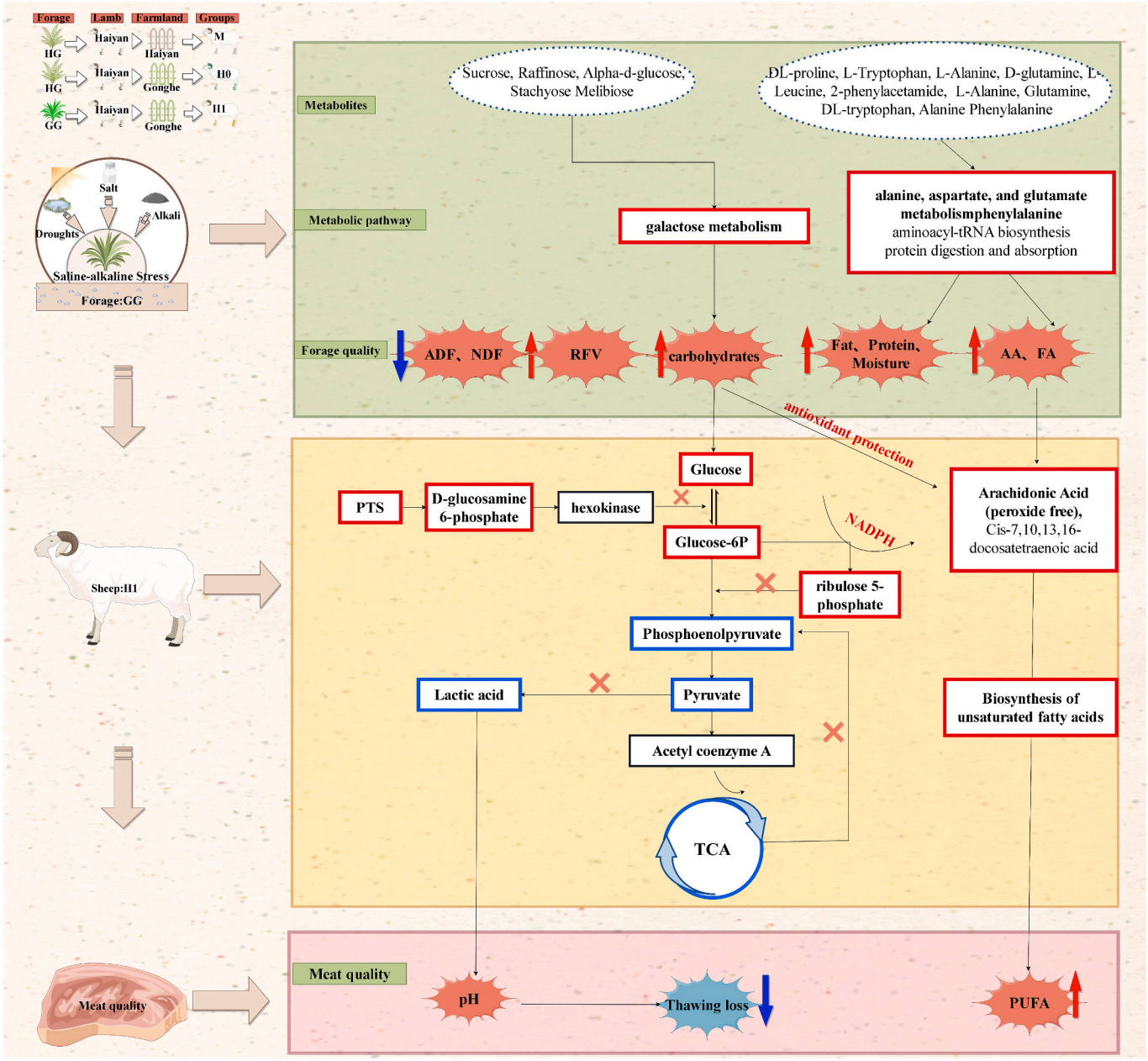



**Fig. 6.** Hypothesized scheme pathways and potential mechanisms related to the changes of forage quality, muscle metabolome and meat quality. Blue and red colors indicate significantly downregulated and upregulated metabolic pathways in each comparison, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Tibetan sheep meat. These findings suggest that the use of saline-alkali feed does not negatively impact meat color.

During the actual meat production process, freezing is an unavoidable and important step (Hasani et al., 2022). The moisture content in meat is critical as it helps maintain the meat's juiciness and tenderness. However, improper handling during the meat thawing process can lead to the loss of moisture, resulting in a dry meat with a changed texture that affects the overall eating experience (Pereira et al., 2022). Moreover, some water-soluble vitamins and minerals may be leached out during the thawing process, hence diminishing the nutritional value of the meat. Research has shown that protein denaturation during the freezing process can lead to an increase in free water, thus accelerating the formation of ice crystals. However, a higher pH may inhibit the freeze-induced protein denaturation, thereby reducing thawing losses (Nakazawa & Okazaki, 2020). Based on differences in quality listed in Table 3, it can be can concluded that the thawing losses in group H1 are significantly different compared to the other two groups ($P < 0.01$), and the pH is also higher before freezing in this group.

Fatty acids not only impact the nutritional value, flavor and culinary appeal of meat products, but also play a crucial role in their shelf life and cooking characteristics (Li et al., 2021). Therefore, it is essential to control the content and composition of fatty acids to achieve the desired quality of meat products. In particular, n-3, n-6 and PUFA in sheep meat are beneficial for human health (Calder, 2015), and interestingly, their content was significantly higher in H1 than in the other two groups ($P < 0.01$). Additionally, the n-6/n-3 ratio in the M group was >6, with this value considered as unfavorable for health (Molendi-Coste, Legry, & Leclercq, 2011). Guo et al. (Guo et al., 2022). found that up-regulated fatty acids contributed to superior meat quality in Tibetan sheep that are adapted to high-salt environments compared to traditional plateau ones. Fraser's (Fraser et al., 2004) research further suggested that the type of forage may also affect the concentration of fatty acids. For

example, Brito et al. (De Brito et al., 2017). discovered that different forages had varying capacity to promote a better fatty acid profile. Similarly, Gruffat et al.'s. (Gruffat et al., 2020) study found that sheep grazing on purple alfalfa accumulated higher levels of n-3 polyunsaturated fatty acids in their muscles, with the antioxidants present effectively preventing the oxidation of these fatty acids. Therefore, it is speculated that the upregulation of unsaturated fatty acids biosynthesis as well as the enrichment of n-3 and unsaturated fatty acids in the H1 group could be related to the GG feed. At the same time, the antioxidants in the GG group (eg: Polysaccharides, polyphenols and flavonoids) can maintain the level of unsaturated fatty acids in the meat.

Based on the above analysis (Fig. 6), it was speculated that the GG feed accumulated a large amount of carbohydrates when adapting to the salt-alkali stress. Therefore, on consuming this feed, the levels of carbohydrate compounds, such as 6-phospho-D-gluconate and D-ribose 5-phosphate in the meat were significantly increased. The up-regulation of these compounds in turn led to the up-regulation of the pentose phosphate pathway and phosphotransferase system (PTS) pathways in the *Longissimus dorsi* muscle of Tibetan sheep. In particular, the enriched 6-phospho-D-gluconate in the up-regulated PTS pathway inhibited the activity of hexokinase, thereby delaying the decrease in pH. Furthermore, the limitations of low oxygen conditions and the need to enhance cellular antioxidant capacity by increasing NADPH production enriched the metabolic pathway involving 6-phospho-glucose entering the pentose phosphate pathway. The resulting NADPH further provides reducing power for fatty acid synthesis. The down-regulation of the pyruvate metabolism pathway in Tibetan sheep fed with salt-alkali feed suggest suppressed pyruvate production which is a product of glycolysis and a precursor of the TCA cycle. As a result, the TCA cycle is down-regulated, leading to a decrease in the production of ATP and other energy-rich molecules. Normally, under anaerobic conditions or limited availability of oxygen, pyruvate is converted into lactate through the lactate fermentation pathway. However, with salt-alkali feeding, the downregulation of the pyruvate metabolism pathway inhibits lactate synthesis, further delaying the decrease in pH. This metabolic adaptation in Tibetan sheep under salt-alkali feeding conditions helps to maintain a relatively stable pH level in the meat. Such a delayed decrease in pH is also beneficial for reducing the denaturation of proteins during freezing and thawing processes, thus minimizing protein degradation and preserving the quality of the meat. In addition, the enrichment of amino acid metabolites in the feed as well as the up-regulation of amino acid metabolic pathways impact the up-regulation of unsaturated fatty acids in the muscle.

## 5. Conclusion

The results of this study indicate that differences between salt-alkali grass have a significant influence on the quality of Tibetan sheep meat. In particular, sheep in the H1 group raised under salt-alkali conditions exhibited a slower decrease in post-mortem pH through up-regulation of the PTS metabolic pathway and down-regulation of the TCA cycle thus reducing thawing loss. Additionally, the NADPH produced by the up-regulation of the pentose phosphate pathway provided precursors for nucleotide and amino acid biosynthesis, while promoting the synthesis of fatty acids, thereby increasing the unsaturated fatty acids content of muscles. In conclusion, carbohydrates enriched in salt-alkali grass play a significant role in slowing down the decline in pH, in increasing unsaturated fatty acid content and in reducing thawing loss in Tibetan sheep.

## Funding

The current work was funded by Construction of Standardized Production System for Improving quality and efficiency of Tibetan sheep industry (2022-NK-169) and Research and Integration of Production Technology for Special Tibetan Sheep in High Saline and Alkaline Habita (2022-NK-169-3).

## Ethics approval and consent to participate

This study was carried out in strict accordance with the animal protection and use guidelines established by the Ministry of Science and Technology of the People's Republic of China. All animal care and handling were approved by the Institutional Animal Care and Use Committee Guidelines of Qinghai University (protocol number 0515). Moreover, all applicable rules and regulation of the organization and government were followed regarding the ethical use of experimental animals.

## CRediT authorship contribution statement

**Nana Ma:** Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Lijuan Han:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Linsheng Gui:** Software, Formal analysis. **Zhenzhen Yuan:** Data curation. **Shengnan Sun:** Software. **Zhiyou Wang:** Investigation. **Baochun Yang:** Investigation. **Chao Yang:** Data curation.

## Declaration of competing interest

There is no conflict of interest in the submission of this manuscript, and all authors have approved the manuscript for publication. The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

## Data availability

The raw data of this study are derived from the GEO data portal (https://www.ncbi.nlm.nih.gov/geo/), which are publicly available databases.

## Acknowledgments

The authors are thankful to the support of Qinghai University for the use of laboratory facilities.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101411.

## References

(PDF) A.O.A.C. (2005) (no date). Available at: https://www.researchgate.net/publication/292783651_AOAC_2005 Accessed: 23 December 2023.

Al-Khalasi, S. S., et al. (2010). Salt tolerant fodder for Omani sheep (effects of salt tolerant sorghum on performance, carcass, meat quality and health of Omani sheep). In *A Monograph on Management of Salt-Affected Soils and Water for Sustainable Agriculture* (pp. 67–81).

Al-Mentafji, H.N. (2016) 'AOAC 1990'.

Ashraf, M., & Ali, Q. (2008). Relative membrane permeability and activities of some antioxidant enzymes as the key determinants of salt tolerance in canola (*Brassica napus* L.). *Environmental and Experimental Botany, 63*(1–3), 266–273. https://doi.org/10.1016/j.envexpbot.2007.11.008

Brown, A. N., et al. (2018). Nutritional composition and in vitro digestibility of grass and legume winter (cover) crops. *Journal of Dairy Science, 101*(3), 2037–2047. Available at: https://doi.org/10.3168/jds.2017-13260.

Calder, P. C. (2015). Functional roles of fatty acids and their effects on human health. *JPEN Journal of Parenteral and Enteral Nutrition, 39*(1 Suppl), 18S–32S. Available at: https://doi.org/10.1177/0148607115595980.

Cao, G., et al. (2023). Dietary bamboo leaf flavonoids improve quality and microstructure of broiler meat by changing untargeted metabolome. *Journal of Animal Science and Biotechnology, 14*(1), 52. Available at: https://doi.org/10.1186/s40104-023-00840-5.

Catarino, M. D., et al. (2023). Applications of antioxidant secondary metabolites of Sargassum spp. *Marine Drugs, 21*(3), 172. Available at: https://doi.org/10.3390/md21030172.

Chen, J., et al. (2023). Plant-derived polyphenols in sow nutrition: an update. *Animal Nutrition (Zhongguo Xu Mu Shou Yi Xue Hui), 12*, 96–107. Available at: https://doi.org/10.1016/j.aninu.2022.08.015.

Chen, X., et al. (2017). Hydrogen peroxide-induced change in meat quality of the breast muscle of broilers is mediated by ROS generation, apoptosis, and autophagy in the NF-κB signal pathway. *Journal of Agricultural and Food Chemistry, 65*(19), 3986–3994. Available at: https://doi.org/10.1021/acs.jafc.7b01267.

De Brito, G. F., et al. (2017). The effect of forage-types on the fatty acid profile, lipid and protein oxidation, and retail colour stability of muscles from white Dorper lambs. *Meat Science, 130*, 81–90. Available at: https://doi.org/10.1016/j.meatsci.2017.04.001.

El Shaer, H. M. (2010). Halophytes and salt-tolerant plants as potential forage for ruminants in the near east region. *Small Ruminant Research, 91*(1), 3–12. Available at: https://doi.org/10.1016/j.smallrumres.2010.01.010.

ElSayed, A. I., Rafudeen, M. S., & Golldack, D. (2014). Physiological aspects of raffinose family oligosaccharides in plants: Protection against abiotic stress. *Plant Biology (Stuttgart, Germany), 16*(1), 1–8. Available at: https://doi.org/10.1111/plb.12053.

FAO—Food and Agriculture Organization. (2001). *Guidelines for humane handling, transport and slaughter of livestock. Slaughter of livestock*. Bangkok: Food and Agriculture Organization of United Nation.

Ferreira, P. S., et al. (2019). A review of analytical methods for p-Coumaric acid in plant-based products, beverages, and biological matrices. *Critical Reviews in Analytical Chemistry, 49*(1), 21–31. Available at: https://doi.org/10.1080/10408347.2018.1459173.

Forrest, K. L., & Bhave, M. (2007). Major intrinsic proteins (MIPs) in plants: A complex gene family with major impacts on plant phenotype. *Functional & Integrative Genomics, 7*(4), 263–289. Available at: https://doi.org/10.1007/s10142-007-0049-4.

Fraser, M. D., et al. (2004). Production performance and meat quality of grazing lambs finished on red clover, lucerne or perennial ryegrass swards. *Grass and Forage Science*, 345–356. Available at: https://doi.org/10.1111/j.1365-2494.2004.00436.x.

Friha, M., et al. (2022). Potential use of natural saline pasture for grazing lambs: Effect on digestibility, growth performances, carcass and meat quality. *Small Ruminant Research, 210*, Article 106669. Available at: https://doi.org/10.1016/j.smallrumres.2022.106669.

Gao, Y., et al. (2023). Analysis of leaf forage value and screening of different populations of Pteroceltis tatarinowii, a rare and endemic species in China'. *Frontiers in Plant Science, 14*. https://doi.org/10.3389/fpls.2023.1164451. Available at:. Accessed: 29 October 2023.

Gruffat, D., et al. (2020). Comparison of muscle fatty acid composition and lipid stability in lambs stall-fed or pasture-fed alfalfa with or without sainfoin pellet supplementation. *Animal, 14*(5), 1093–1101. Available at: https://doi.org/10.1017/S1751731119002507.

Guo, Q., Liu, L., & Barkla, B. J. (2019). Membrane lipid remodeling in response to salinity. *International Journal of Molecular Sciences, 20*(17), 4264. Available at: https://doi.org/10.3390/ijms20174264.

Guo, T., et al. (2022). Comparative analysis of the composition of fatty acids and metabolites between black Tibetan and Chaka sheep on the Qinghai-Tibet plateau. *Animals: An Open Access Journal From MDPI, 12*(20), 2745. Available at: https://doi.org/10.3390/ani12202745.

Hasani, A., et al. (2022). Analyzing consumer perception on quality and safety of frozen foods in emerging economies: Evidence from Albania and Kosovo. *Foods, 11*(9), 1247. Available at: https://doi.org/10.3390/foods11091247.

Hill, C. B., et al. (2013). Characterization of ion contents and metabolic responses to salt stress of different Arabidopsis AtHKT1;1 genotypes and their parental strains. *Molecular Plant, 6*(2), 350–368. Available at: https://doi.org/10.1093/mp/sss125.

Huang, W., et al. (2023). Comparative assessment of extraction, composition, and in vitro antioxidative properties of wheat bran polyphenols. *LWT, 180*, Article 114706. Available at: https://doi.org/10.1016/j.lwt.2023.114706.

Hutchison, S., et al. (2006). Effects of adding poultry fat in the finishing diet of steers on performance, carcass characteristics, sensory traits, and fatty acid profiles. *Journal of Animal Science, 84*(9), 2426–2435. Available at: https://doi.org/10.2527/jas.2005-446.

ISO 13299:2016(en). (2024). Sensory Analysis — Methodology — General Guidance for Establishing a Sensory Profile (No Date). Available at: https://www.iso.org/obp/ui/en/#iso:std:iso:13299:ed-2:v1:en Accessed: 9 April 2024.

Jia, X., et al. (2020). Ionomic and metabolomic analyses reveal the resistance response mechanism to saline-alkali stress in *Malus halliana* seedlings. *Plant Physiology and Biochemistry, 147*, 77–90. Available at: https://doi.org/10.1016/j.plaphy.2019.12.001.

Jing, C.-L., Dong, X.-F., & Tong, J.-M. (2015). Optimization of ultrasonic-assisted extraction of flavonoid compounds and antioxidants from alfalfa using response surface method'. *Molecules (Basel, Switzerland), 20*(9), 15550–15571. Available at: https://doi.org/10.3390/molecules200915550.

Jorge, T. F., et al. (2016). Mass spectrometry-based plant metabolomics: Metabolite responses to abiotic stress. *Mass Spectrometry Reviews, 35*(5), 620–649. Available at: https://doi.org/10.1002/mas.21449.

Khliji, S., et al. (2010). Relationship between consumer ranking of lamb colour and objective measures of colour. *Meat Science, 85*(2), 224–229. Available at: https://doi.org/10.1016/j.meatsci.2010.01.002.

Li, C., et al. (2022). 'Adaptative mechanisms of halophytic *Eutrema salsugineum* encountering saline environment. *Frontiers in Plant Science, 13*, Article 909527. Available at: https://doi.org/10.3389/fpls.2022.909527.

Li, H. Y., et al. (2014). Assessment of the nutritive value of whole corn stover and its morphological fractions. *Asian-Australasian Journal of Animal Sciences, 27*(2), 194–200. Available at: https://doi.org/10.5713/ajas.2013.13446.

Li, J., et al. (2021). The age-dependent variations for fatty acid composition and sensory quality of chicken meat and associations between gene expression patterns and meat quality. *Livestock Science, 254*, Article 104736. Available at: https://doi.org/10.1016/j.livsci.2021.104736.

Li, T., et al. (2024). 'Effects of glycyrrhiza polysaccharides on growth performance, meat quality, serum parameters and growth/meat quality-related gene expression in broilers. *Frontiers in Veterinary Science, 11*. https://doi.org/10.3389/fvets.2024.1357491. Available at.

Li, X., et al. (2015). Pathways of Leymus chinensis individual aboveground biomass decline in natural semiarid grassland induced by overgrazing: A study at the plant functional trait scale. *PLoS ONE, 10*(5), Article e0124443. Available at: https://doi.org/10.1371/journal.pone.0124443.

Li, Z., et al. (2016). Antemortem stress regulates protein acetylation and glycolysis in postmortem muscle. *Food Chemistry, 202*, 94–98. Available at: https://doi.org/10.1016/j.foodchem.2016.01.085.

Linn, J. G., & Martin, N. P. (1991). Forage quality analyses and interpretation. *Veterinary Clinics of North America: Food Animal Practice, 7*(2), 509–523. Available at: https://doi.org/10.1016/S0749-0720(15)30790-8.

Liu, L, Gai, Z, Qiu, X, et al. (2023). Salt stress improves the low-temperature tolerance in sugar beet in which carbohydrate metabolism and signal transduction are involved [J/OL]. *Environmental and Experimental Botany, 208*, 105239. https://doi.org/10.1016/j.envexpbot.2023.105239

Ma, Y., Han, L., Raza, S. H. A., et al. (2023). Exploring the effects of palm kernel meal feeding on the meat quality and rumen microorganisms of Qinghai Tibetan sheep. *Food Science & Nutrition, 11*(6), 3516–3534. Available at: https://doi.org/10.1002/fsn3.3340.

Ma, Y., Han, L., Zhang, S., et al. (2023). Insight into the differences of meat quality between Qinghai white Tibetan sheep and black Tibetan sheep from the perspective of metabolomics and rumen microbiota. *Food Chemistry: X, 19*, Article 100843. Available at: https://doi.org/10.1016/j.fochx.2023.100843.

Majdoub-Mathlouthi, L., Hamdi, H., & Kraiem, K. (2021). Producing Barbarine lambs on Tunisian rangelands could be sustainable and provide healthy meat. In F. Khebour Allouche, M. Abu-hashim, & A. M. Negm (Eds.), *Agriculture Productivity in Tunisia Under Stressed Environment, Cham* (pp. 291–307). Springer International Publishing (Springer Water). Available at: https://doi.org/10.1007/978-3-030-74660-5_13.

Mmf, M., & Ef, A. (2017). Evaluation of proline functions in saline conditions'. *Phytochemistry, 140*. https://doi.org/10.1016/j.phytochem.2017.04.016. Available at.

Mohammadi Alagoz S, Asgari La Jayer B, Ghorbanpour M. Chapter 10 - Proline and soluble carbohydrates biosynthesis and their roles in plants under abiotic stresses [M/OL]//GHORBANPOUR M, ADNAN SHAHID M. Plant Stress Mitigators. Academic Press, 2023: 169-185[2023-11-02].

Molendi-Coste, O., Legry, V., & Leclercq, I. A. (2011). Why and how meet n-3 PUFA dietary recommendations? *Gastroenterology Research and Practice, 2011*, Article 364040. Available at https://doi.org/10.1155/2011/364040.

Myrtsi, E. D., et al. (2023). An interdisciplinary assessment of biochemical and antioxidant attributes of six Greek *Vicia sativa* L. varieties. *Plants, 12*(15), Article 2807. Available at: https://doi.org/10.3390/plants12152807.

Nadarajah, K. K. (2020). ROS homeostasis in abiotic stress tolerance in plants. *International Journal of Molecular Sciences, 21*(15), 5208. Available at: https://doi.org/10.3390/ijms21155208.

Nakazawa, N., & Okazaki, E. (2020). Recent research on factors influencing the quality of frozen seafood. *Fisheries Science, 86*(2), 231–244. Available at: https://doi.org/10.1007/s12562-020-01402-8.

Norman, H. C., Duncan, E. G., & Masters, D. G. (2019). Halophytic shrubs accumulate minerals associated with antioxidant pathways. *Grass and Forage Science, 74*(3), 345–355. Available at: https://doi.org/10.1111/gfs.12434.

Osada-Oka, M., et al. (2010). Glucose is necessary for stabilization of hypoxia-inducible factor-1α under hypoxia: Contribution of the pentose phosphate pathway to this stabilization. *FEBS Letters, 584*(14), 3073–3079. Available at: https://doi.org/10.1016/j.febslet.2010.05.046.

Otlewska, A., et al. (2020). When salt meddles between plant, soil, and microorganisms. *Frontiers in Plant Science, 11*, Article 553087. Available at: https://doi.org/10.3389/fpls.2020.553087.

Pan, S., et al. (2022). Current development and future application prospects of plants-derived polyphenol bioactive substance curcumin as a novel feed additive in livestock and poultry. *International Journal of Molecular Sciences, 23*(19), Article 11905. Available at: https://doi.org/10.3390/ijms231911905.

Panda, A., Rangani, J., & Parida, A. K. (2021). Physiological and metabolic adjustments in the xero-halophyte Haloxylon salicornicum conferring drought tolerance. *Physiologia Plantarum, 172*(2), 1189–1211. Available at: https://doi.org/10.1111/ppl.13351.

Paternostre, L., De Boever, J., & Millet, S. (2021). Interaction between fat and fiber level on nutrient digestibility of pig feed. *Animal Feed Science and Technology, 282*, Article 115126. Available at: https://doi.org/10.1016/j.anifeedsci.2021.115126.

Pearce, K. L., Norman, H. C., & Hopkins, D. L. (2010). The role of saltbush-based pasture systems for the production of high quality sheep and goat meat. *Small Ruminant Research, 91*(1), 29–38. Available at: https://doi.org/10.1016/j.smallrumres.2009.10.018.

Pereira, M. R., et al. (2022). Effect of freezing on the quality of breast meat from broilers affected by white striping myopathy. *Poultry Science, 101*(2), Article 101607. Available at: https://doi.org/10.1016/j.psj.2021.101607.

Petropoulos, S., et al. (2020). Chemical composition and plant growth of *Centaurea raphanina* subsp. mixta plants cultivated under saline conditions. *Molecules (Basel, Switzerland), 25*(9), 2204. Available at: https://doi.org/10.3390/molecules25092204.

Qiao, Y., et al. (2022). Polysaccharides derived from Astragalus membranaceus and Glycyrrhiza uralensis improve growth performance of broilers by enhancing intestinal health and modulating gut microbiota. *Poultry Science, 101*(7), Article 101905. Available at: https://doi.org/10.1016/j.psj.2022.101905.

Rohweder, D. A., Barnes, R. F., & Jorgensen, N. (1978). Proposed Hay grading standards based on laboratory analyses for evaluating quality. *Journal of Animal Science, 47*(3), 747–759. Available at: https://doi.org/10.2527/jas1978.473747x.

Samandari-Bahraseman, M. R., et al. (2023). Various concentrations of hesperetin induce different types of programmed cell death in human breast cancerous and normal cell lines in a ROS-dependent manner. *Chemico-Biological Interactions, 382*, Article 110642. Available at: https://doi.org/10.1016/j.cbi.2023.110642.

Shabala, S., et al. (2016). On a quest for stress tolerance genes: Membrane transporters in sensing and adapting to hostile soils. *Journal of Experimental Botany, 67*(4), 1015–1031. Available at: https://doi.org/10.1093/jxb/erv465.

Singer, S. D., et al. (2023). Elucidation of physiological, transcriptomic and metabolomic salinity response mechanisms in *Medicago sativa*. *Plants (Basel, Switzerland), 12*(10), 2059. Available at https://doi.org/10.3390/plants12102059.

Stincone, A., et al. (2015). The return of metabolism: Biochemistry and physiology of the pentose phosphate pathway. *Biological Reviews of the Cambridge Philosophical Society, 90*(3), 927–963. Available at: https://doi.org/10.1111/brv.12140.

Stock-Damgé, C., et al. (1984). Effects of wheat bran on the exocrine pancreas and the small intestinal mucosa in the dog. *The Journal of Nutrition, 114*(6), 1076–1082. Available at: https://doi.org/10.1093/jn/114.6.1076.

Su, Y., et al. (2022). Dietary alfalfa powder supplementation improves growth and development, body health, and meat quality of Tibetan sheep. *Food Chemistry, 396*, Article 133709. Available at: https://doi.org/10.1016/j.foodchem.2022.133709.

Tavakkoli, E., et al. (2011). Additive effects of Na+ and cl– Ions on barley growth under salinity stress. *Journal of Experimental Botany, 62*(6), 2189–2203. Available at: https://doi.org/10.1093/jxb/erq422.

Tepsongkroh, B., et al. (2023). Impact of high pressure pre-treatment and hot water extraction on chemical properties of crude polysaccharide extract obtained from mushroom (*Volvariella volvacea*). *Food Chemistry: X, 19*, Article 100864. Available at https://doi.org/10.1016/j.fochx.2023.100864.

Terler, G., Poier, G., Klevenhusen, F., Zebeli, Q., et al. (2022). Replacing concentrates with a high-quality hay in the starter feed in dairy calves: I. Effects on nutrient intake, growth performance, and blood metabolic profile. *Journal of Dairy Science, 105*(3). https://doi.org/10.3168/jds.2021-21078. Available at:.

Uddin, M. E., et al. (2020). Enteric methane, lactation performances, digestibility, and metabolism of nitrogen and energy of Holsteins and jerseys fed 2 levels of forage fiber from alfalfa silage or corn silage. *Journal of Dairy Science, 103*(7), 6087–6099. Available at: https://doi.org/10.3168/jds.2019-17599.

Wang, C., et al. (2011). Effects of feeding salt-tolerant forage cultivated in saline-alkaline land on rumen fermentation, feed digestibility and nitrogen balance in lamb. *Journal of the Science of Food and Agriculture, 91*(7), 1259–1264. Available at: https://doi.org/10.1002/jsfa.4308.

Wang, X., et al. (2021). A comparative metabolomics analysis of the halophyte Suaeda salsa and Salicornia europaea. *Environmental Geochemistry and Health, 43*(3), 1109–1122. Available at: https://doi.org/10.1007/s10653-020-00569-4.

Wang, Y., et al. (2020). Effects of grassland degradation on ecological stoichiometry of soil ecosystems on the Qinghai-Tibet plateau. *The Science of the Total Environment, 722*, Article 137910. Available at: https://doi.org/10.1016/j.scitotenv.2020.137910.

Waszkiewicz-Robak, B., et al. (2015). Nutritional value of raw pork depending on the fat type contents in pigs feed. *Acta Scientiarum Polonorum. Technologia Alimentaria, 14*(2), 153–163. Available at: 10.17306/J.AFS.2015.2.17.

Wu, S., et al. (2020). Understanding the development of color and color stability of dark cutting beef based on mitochondrial proteomics. *Meat Science, 163*, Article 108046. Available at https://doi.org/10.1016/j.meatsci.2020.108046.

Wu, Z., et al. (2023). Regulation mechanism and functional verification of key functional genes regulating muscle development in black Tibetan sheep. *Gene, 868*, Article 147375. Available at https://doi.org/10.1016/j.gene.2023.147375.

Xiang, L., et al. (2020). Effects of polysaccharides from *Yingshan Yunwu* tea on meat quality, immune status and intestinal microflora in chickens. *International Journal of Biological Macromolecules, 155*, 61–70. Available at: https://doi.org/10.1016/j.ijbiomac.2020.03.198.

Xu, X., et al. (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: an open access journal from MDPI, 13*(15), 2494. Available at https://doi.org/10.3390/ani13152494.

Xu, Y., Bruce, D. A., & Spivey, J. J. (2014). Preface. *Catalysis Communications, 52*, 59. Available at https://doi.org/10.1016/j.catcom.2014.04.007.

Yan, J., et al. (2022). 'Effect of *Eucommia ulmoides* leaf extract on growth performance, carcass traits, parameters of oxidative stress, and lipid metabolism in broiler chickens. *Frontiers in Veterinary Science, 9*. https://doi.org/10.3389/fvets.2022.945981. Available at.

Yang, F., et al. (2023). Prevalence and subtype distribution of blastocystis in Tibetan sheep in Qinghai Province, northwestern China. *Protist, 174*(2), Article 125948. Available at: https://doi.org/10.1016/j.protis.2023.125948.

Zargar, S. M., et al. (2017). Aquaporins as potential drought tolerance inducing proteins: Towards instigating stress tolerance. *Journal of Proteomics, 169*, 233–238. Available at: https://doi.org/10.1016/j.jprot.2017.04.010.

Zhang, A., et al. (2016). Global proteomic mapping of alkali stress regulated molecular networks in *Helianthus tuberosus* L. *Plant and Soil, 409*(1), 175–202. Available at https://doi.org/10.1007/s11104-016-2945-7.

Zhang, J., et al. (2017). Long-term grazing effects on vegetation characteristics and soil properties in a semiarid grassland, northern China. *Environmental Monitoring and Assessment, 189*(5), 216. Available at https://doi.org/10.1007/s10661-017-5947-x.

Zhang, M., et al. (2020). Impacts of heat stress on meat quality and strategies for amelioration: A review. *International Journal of Biometeorology, 64*(9), 1613–1628. Available at https://doi.org/10.1007/s00484-020-01929-6.

Zhang, X., Han, L., Gui, L., et al. (2022). Metabolome and microbiome analysis revealed the effect mechanism of different feeding modes on the meat quality of black Tibetan sheep. *Frontiers in Microbiology, 13*, Article 1076675. Available at: https://doi.org/10.3389/fmicb.2022.1076675.

Zhang, X., Han, L., Hou, S., et al. (2022). 'Metabolomics approach reveals high energy diet improves the quality and enhances the flavor of black Tibetan sheep meat by altering the composition of rumen microbiota. *Frontiers in Nutrition, 9*, Article 915558. Available at: https://doi.org/10.3389/fnut.2022.915558.

Zhao, G., et al. (2023). 'Effects of three different plant-derived polysaccharides on growth performance, immunity, antioxidant function, and cecal microbiota of broilers. *Journal of the Science of Food and Agriculture [preprint]*. https://doi.org/10.1002/jsfa.12988. Available at:.

Zheng, L., et al. (2022). Integrated transcriptomics, proteomics, and metabolomics identified biological processes and metabolic pathways involved in heat stress response in jojoba. *Industrial Crops and Products, 183*, Article 114946. Available at https://doi.org/10.1016/j.indcrop.2022.114946.

Zhou, Y., Mao, S., & Zhou, M. (2019). Effect of the flavonoid baicalein as a feed additive on the growth performance, immunity, and antioxidant capacity of broiler chickens. *Poultry Science, 98*(7), 2790–2799. Available at https://doi.org/10.3382/ps/pez071.