

TITLE PAGE
- Food Science of Animal Resources -
Upload this completed form to website with submission

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title	Comparative evaluation of the nutrient composition and lipidomic profile of different parts of muscle in the Chaka sheep
Running Title (within 10 words)	The nutrient composition and lipidomic profile in the Chaka sheep
Author	Xianli Xu 1,2, Tongqing Guo 1,2, Qian Zhang 1, Hongjin Liu 1, Xungang Wang 1, Na Li1,2, Yalin Wang1,2, Lin Wei1,2, Linyong Hu 1,* and Shixiao Xu1,*
Affiliation	1 Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810008, China 2 University of Chinese Academy of Sciences, Beijing 100049, China
Special remarks – if authors have additional information to inform the editorial office	
ORCID (All authors must have ORCID) https://orcid.org	Xianli Xu (https://orcid.org/0000-0002-5197-0299) Tongqin Guo(https://orcid.org/0000-0003-3876-325X) Xungang Wang(https://orcid.org/0000-0001-8593-7844) Qian Zhang(https://orcid.org/0009-0002-5554-7516) Hongjin Liu (https://orcid.org/0000-0001-5625-2395) Na Li(https://orcid.org/0009-0002-8642-8480) Yalin Wang(https://orcid.org/0009-0007-0914-4157) Lin Wei(https://orcid.org/0000-0003-1256-3698) Linyong Hu(https://orcid.org/0000-0002-6149-4636) Shixiao Xu(https://orcid.org/0000-0003-4100-7017)
Conflicts of interest List any present or potential conflicts of interest for all authors. (This field may be published.)	The authors declare no potential conflict of interest.
Acknowledgements State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available. (This field may be published.)	This research was supported by the Strategic Priority Research Program of Chinese Academy of Sciences (XDA23060603), the Natural Science Foundation of Qinghai Province (2023-ZJ-981Q), the CAS “Light of West China” for Interdisciplinary Innovation Team (CASLWC-2021), the Second Comprehensive Scientific Expedition to the Qinghai-Tibet Plateau (2019QZKK040104), Qinghai Province Science and Technology Achievement Transformation Special Project (2020-NK-166), and the Joint Special Project of Sanjiangyuan National Park (LHZX-2022-02).
Author contributions (This field may be published.)	Conceptualization: Xu XL. Data curation: Guo TG , Zhang Q. Formal analysis: Wang XG, Li N. Methodology: Wang YL, Wei L. Software: XuXL, Liu HJ. Validation: Hu LY. Investigation: Xu SX. Writing - original draft: Xu XL.. Writing - review & editing: Xu XL, Guo TG , Zhang Q, Liu HJ, Wang XG, Li N, Wang YL, Wei L, Hu LY, Xu SX.
Ethics approval (IRB/IACUC) (This field may be published.)	The animal procedures used in this study were performed according to the Guidelines for the Care and Use of Laboratory Animals in China. This study

	was approved by the Experimental Animal Use Ethics Committee of the Northwest Institute of Plateau Biology, CAS (Permit No. NWIPB20160302).
--	---

CORRESPONDING AUTHOR CONTACT INFORMATION

For the <u>corresponding</u> author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Shixiao Xu
Email address – this is where your proofs will be sent	ucasxianlixu@163.com
Secondary Email address	sxxu@nwipb.cas.cn
Postal address	Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810008, China
Cell phone number	13369766778
Office phone number	
Fax number	

ACCEPTED

Comparative evaluation of the nutrient composition and lipidomic profile of different parts of muscle in the Chaka sheep

Abstract

Mutton is one of the most popular meats among the public due to its high nutritional value. In this study, we compared and analyzed the nutritional composition and volatile flavor substances in *longissimus dorsi* (LD), *psoas major* (PM), and *biceps femoris* (BF) of the Chaka sheep, and then analyzed the lipid composition using the technique of UHPLC-Q-Exactive Orbitrap MS/MS. Our results indicated that the LD had the highest crude protein (CP) content (22.63%), the highest levels of aspartic acid (5.72%) and histidine (2.76%), the BF had the highest contents of glycine (3.40%) and Proline (2.88%), the PM had the highest abundance of ω -6 polyunsaturated fatty acids (PUFA) (7.06%), linoleic acid (C18:2n6c) (5.03%), and volatile flavor compounds (alcohols, ketones, and esters). Moreover, our study detected 2639 lipid molecules classified into 42 classes, among which phospholipids were the major lipids, accounting for nearly half of the total lipids. Among them, PE(18:2/18:2) and PC(25:0/11:3) were the characteristic lipids in LD. PS(20:3e/20:4), LPC(18:3), PE(8:1e/12:3), TG(18:0e/16:0/18:1), TG(18:0/18:0/18:0), TG(18:0e/18:0/18:1), and TG(18:0e/18:1/18:1) were marker lipids in PM. LPC(16:0), LPC(18:1), LPE(18:1), PC(15:0/22:6), PE(18:1/18:1), Hex1Cer(d24:1/18:1), and PC(10:0e/6:0) were representative

lipids in BF. Intermolecular correlations between PC, PE, Hex1Cer, PS, TG, DG, and CL were revealed by correlation analysis. In conclusion, this study provided the interpretation of the specific nutritional indicators and lipid profile in the tripartite muscle of Chaka sheep, which can be used as a guidance for future research on the nutritional qualities and economic benefits of mutton.

Keywords: Chaka sheep; Amino acids; Fatty acids; Volatile flavors; Lipids profiles

1 Introduction

Mutton is gradually becoming a superior substitute for the domestic market, as it is a good source of essential nutrients, high in protein and low in fat (Ramos et al., 2021). In the last few years, the consumption of high quality mutton products has risen dramatically. Chaka sheep as Qinghai Plateau wool and meat with semi-fine wool sheep is a breed of Tibetan sheep, which grows in the area of Chaka Salt Lake in Wulan County, is a cultivar of Chaka sheep (Chen et al., 2019). In 1971, Wulan County introduced the British and New Zealand Romney-Marsh long-wool type of breeding sheep from Hudong Original Breeding Field of Qinghai Province and Daliuliu Original Breeding Field of Anhui Province to be cross-bred (Chen et al., 2019). As the breed was improved and bred in the Chaka area, it was named "Chaka sheep", which has been granted the protection of the Registration of Geographical Indications for agricultural products (Guo et al., 2022). Studies have shown that there are some differences in the nutritional composition, sensory quality, and processing quality of different parts of the muscle (Mohanty et al., 2010). Different parts of muscle have unique collagen content, structure, and properties (Zuo et al., 2016). Kohn et al. (2023) found significant differences in muscle fiber types and metabolic profiles in different parts of African black ostrich muscles.

Lipids, an important nutritional component in lamb, are critical biological small molecules that act as components of cell membranes, signal transduction, energy sources, and signaling pathway intermediates (Lugo Charriez et al., 2021). The lipid profile of meat is influenced by many factors, one of which is the type of muscle (Boselli et al., 2008). The lipid composition of tissues varies in different parts of the body (Yin et al., 2023). Li et al. (2022) identified seventy-nine of 842 lipids in different muscles of Hu lambs. The content and type of lipids vary significantly in different meat products, and potential markers of lipids have gradually become one of the methods to analyze the quality and type of meat products. However, studies on the lipid profile and characteristics of different parts of the muscles of Chaka sheep are still incomplete. Thus, the lipidomic profile and its components of Chaka sheep need to be elucidated.

Therefore, this study determined and analyzed the nutrient composition, lipid content, and differential lipid molecules in *the longissimus dorsi* (LD), *psoas major* (PM), and *biceps femoris* (BF) of the Chaka sheep (**Figure 1**). The aims of this research are to define the basic data about the composition and nutritional quality of three different muscles of the Chaka sheep and to provide insight into the utilization of lipidomics in the animal products industry.

2 Materials and methods

2.1 Sample collection

The samples were obtained from Jintai Ranch, Chaka Town, Wulan County, Haixi Mongolian and Tibetan Autonomous Prefecture, Qinghai Province, China. We selected nine experimental sheep, all 1 year old, weighing about 45 kg, female. They were identified by experts as Chaka sheep. The Chaka sheep were manually slaughtered by puncturing the occipital atlantoaxial joint of the sheep with a sharp knife, severing the spinal nerves, then cutting the carotid artery to bleed out the blood, and then skinning the sheep, and after removing the head and hooves, 9 replicates each of LD (muscles of the back of the sheep between the 9th and 11th ribs), BF, and PM were collected. The LD, PM and BF of Chaka sheep were cut and packed into lyophilized vessels and then immediately stored in liquid nitrogen for lipidomic study, and the remaining meat samples were wrapped in cling film and placed in a thermal box and returned to the laboratory for nutrient analysis. Three replicates were set up for nutritional quality determination and six replicates for lipidomics.

2.2 Determination of the chemical components and characterization of the muscle fibers

An OPTO-LAB meat color analyzer (Konica Minolta, Tokyo, Japan) was employed to measure the color of the muscle. A PH-STAR carcass muscle pH direct tester (MATTHAUS GmbH, Bavaria, Portmeuse, Germany) was utilized for pH measurement of all samples. Crude protein (CP), total ash (Ash), ether extract (EE), and moisture content (MSTR) were measured according to Cohen's methods (Cohen, 1971).

Conventional hematoxylin and eosin (H&E) staining was used to measure muscle fiber characteristics. Samples were scanned using a Leica RM-235 slide scanner (Leica RM-235, Nussloch, Germany) and the images were acquired by using CaseViewer 6.0 software (3D-Histech, Ltd., Budapest, Hungary) (Xu et al., 2023).

2.3 Determination of amino acids and fatty acids

A Sykam S-433D amino acid analyzer (Sykam, Munich, Germany) was used for amino acid determination. After weighing 50 mg of dry meat powder from a single sample and hydrolysis with hydrochloric acid solution (36% hydrochloric acid: water = 1:1, v/v) for 24 h, 2 mL of the diluted specimens were transferred to the vial condenser. 10 mL of sample diluents were poured in and stirred. The compounded sample was withdrawn with a hypodermic syringe, filtered using a needle filter, and injected into the vial to assay (Ma et al, 2021).

The fatty acids were determined by gas chromatography-mass spectrometry (GC-MS). 5 g of the sample was taken, drawn and filtered into the injection vial with a stirred syringe. After adding 8 mL of a sodium hydroxide-methanol solution at 20 g/L relative density, a condenser for fat degradation and fatty acid methyl esterification was attached, n-heptane was poured in, shaken for 2.5 min, and a sodium chloride aqueous buffer solution was poured in. Supernatant was collected, a volume of sodium sulfate hydroxide was added, stirred thoroughly, and supernatant was transferred to vials for automated analysis (Schiavon et al., 2016).

2.4 Determination of volatile flavor compounds

The combined technology of solid phase microextraction (SPME) and GC-MS was applied to determine the volatile aroma compounds. The muscle was minced and ground, 4 g meat was added to a 20 mL headspace bottle, 20% NaCl solution and 5 μ L of 2-methyl-3-heptanone solution was mixed, stirred thoroughly, and water bath (85°C) for 55 min, then chilled to 55°C. The SPME fiber probe was inserted into the GC-MS injector connection, and the adsorber head was in place for extraction for 40 min. The SPME fiber probe was inserted into the GC-MS injector connection, and the adsorber head was in place during extraction for 40 min, and the fiber was withdrawn for desorbing into the GC-MS insertion port for 3 min

by GC-MS. The chromatographic parameters were as follows: the carrier gas was helium, the flow rate was 1.0 mL/min, the injection port temperature was 250°C, the column temperature was started at 40°C, maintained for 5 min, and then increased to 230°C (5°C/min) for 8 min. The mass spectrometry conditions were as follows: the ion source temperature was 200°C, the ionization mode was EI, the electron energy was 70 eV, and the scanning mass range was 35-500 amu (He et al., 2020).

2.5 Lipid extraction

The samples were spiked with the appropriate amount of internal lipid standard, and then homogenized with 200 µL water and 240 µL methanol. Then 800 µL of methyl t-butyl ether was added to the mixture. The mixture was sonicated for 20 min at 55°C and then allowed to stand for 30 min at room temperature. The upper layer of organic solvent was removed by centrifugation at 14,000 g for 15 min. The samples were dried under nitrogen.

2.6 LC-MS/MS for the analysis of the lipidome in muscle

A CSH C18 column (1.7 µm, 2.1 mm×100 mm, Waters) was applied for LC separation. Lipid extracts were dissolved in 200 µL of 90% isopropanol-acetonitrile and incubated at 14,000 g for 15 min. Finally, 3 µL

of the specimen was injected. The first mobile phase was 40% acetonitrile-isopropanol (1:9, v/v) at a flow rate of 300 μ L per minute. This was maintained for 3.5 minutes, followed by a linear increase to 75% acetonitrile-isopropanol (1:9, v/v) in 9 minutes. A linear increase to 99% acetonitrile-isopropanol for 6 minutes and then equilibration at 40% acetonitrile-isopropanol for 5 minutes.

The mass spectra were collected by the Q-Exactive Plus in the positive mode and in the negative mode, respectively. The ESI parameters were adjusted and optimized for all experiments. The experimental parameters were as follows: the source temperature was 300°C, the capillary temperature was 350°C, the ion spray voltage was 3000 V, and the instrument scan range was 200-1800 m/z (Tang et al., 2023).

2.7 Statistical analysis

Results of the study were presented as mean \pm standard deviation in this assay with three replicates for each sample. SPSS 22.0 was used to analyze the regular nutritional composition data statistically. OPLS-DA, VIP>1, p -value<0.05) was defined as the criterion for significant differences.

3 Results and discussion

3.1 Analysis of chemical components and muscle fiber parameters

There was no significant difference in the results of routine nutrient content measurements (**Table 1**) at $\text{pH}_{45\text{min}}$, moisture, ash, and ether extract, but the redness (CIE a^*), yellowness (CIE b^*), and brightness (CIE L^*) of PM were significantly higher than LD and BF ($P < 0.05$). The CIE a^* is associated with meat hemoglobin content, CIE b^* is negatively correlated with muscle freshness, and CIE L^* is related to muscle whiteness (Liang et al., 2023). Therefore, we hypothesized that PM has the highest hemoglobin content and LD has the best freshness, which may be more popular with consumers in terms of meat color. The rank order of CP levels in three parts of Chaka sheep was: LD>BF>PM.

Previous studies showed that muscle fiber properties, such as type, size and total number of fibers in different parts were different, the higher muscle fiber density, the higher economic value of meat (Qin et al., 2020). The results of myofiber measurements are shown in **Figure 2**, the total area of myofibers did not differ significantly between LD, PM, and BF, the total number of myofibers was ranked from most to least: PM>LD>BF, the density of myofibers was ranked: PM>LD>BF, the diameter of myofibers was the largest in BF. Our study demonstrated significant differences in total myofiber number, myofiber density, and myofiber diameter in LD, PM, and BF ($p < 0.05$). The myofiber density in LD (1557.93 n/mm^2) and PM

(1588.74 n/mm²) were significantly higher than in BF (781.11 n/mm²), so it may be suggested that LD and PM have a higher commercial value than BF.

3.2 Determination of amino acids and fatty acids

The results of amino acid measurement are presented in **Table 2**, the total amino acid and essential amino acid levels in different parts of Chaka sheep were not significant, but our previous study has indicated that there were differences in crude protein content. This inconsistency may be caused by calculating CP based on nitrogen content. However, in addition to protein, meat also includes other nitrogenous compounds such as phospholipids (Gao et al., 2022), so the level of CP was not exactly equal to TAA. There were significant differences in the levels of aspartic acid, glycine, histidine, and proline in different parts of the muscle. The contents of aspartic acid and histidine were the highest in LD, followed by BF, and the lowest in PM. The contents of glycine and proline were in the following order high to low: BF>LD>PM. Studies have indicated that the free precursor amino acids affect the flavor of the meat, which is essential in evaluating the quality of the meat (Tian et al., 2021), glycine often imparts a sweet flavor to mutton, aspartate gives the meat an umami taste, and is favored by consumers (Ribeiro de Araujo Cordeiro et al., 2020). Histidine is essential for producing histamine, a chemical messenger essential for

immune reactions, digestion, and sleep/wake cycles (Zhang et al., 2022). LD had the highest levels of aspartic acid and histidine, and BF had the highest levels of total glycine and proline, from which we hypothesized that the flavors of LD and BF might be more likely to be preferred by consumers.

Fatty acids are closely related to the eating quality of meat by determining the melting point and quality of meat fats (Dai et al., 2024). The contents of fatty acids in different parts of the muscle are shown in **Table 3**. A study has shown that intramuscular fat content was positively correlated with SFA (Zhang et al., 2019), which was consistent with the composition of crude fat in PM, LD, and BF in our findings. The content of linoleic acid (C18:2n6c) was, in decreasing order, ranked as follows: PM>BF>LD. Haag's study found that eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are metabolic byproducts of linoleic acid and linolenic acid, which are vital to the human body in the maintenance of cardiovascular health, brain function, and the reduction of pain in rheumatoid arthritis (Haag, 2003; Oh, 2005). Therefore, we hypothesized that the higher linoleic acid in PM would favor the production and accumulation of EPA and DHA. The total of ω -6 PUFA was ranked as: PM>BF>LD. Studies have found that ω -6 PUFA cannot be produced by the body, but must be consumed in the diet, and meat is an essential source of ω -6 PUFA. It is recommended that

people should get these nutrients through a balanced diet to maintain the body's ω -6 PUFA level (Simopoulos, 2008). Overall, our results indicated that there were differences in the fatty acid content of muscles from Chaka sheep, the PM exhibited the highest nutritional value in the fatty acids profile.

3.3 Determination of volatile flavor compounds

As presented in **Table 4**, aldehyde, alcoholic, ketonic, carboxylic, esteric, and other volatile aroma compounds have been identified in Chaka sheep muscle. There were marked differences in the alcohols, ketones, and esters of the Chaka sheep, with PM having the highest levels of total alcohols, ketones, and esters. Alcohols are derived from the breakdown of lipids and have a high olfactory threshold and a generally clear odor similar to that found in fruits and vegetables. Ketones, a category of volatile organic compounds with floral, fruity, and creamy aromas, have an impact on the overall flavor of meat products (Ge et al., 2023). Ester compounds, most of which have an aromatic flavor, are formed by the interesterification of acids and alcohols (Yang et al., 2024). We found that the PM exhibited the highest nutritional value in the fatty acids profile, so we speculated that the higher fatty acid oxidation may lead to the accumulation of flavor substances in PM.

3.4 Lipid profiles analysis

3.4.1 The lipid composition analysis in different muscle parts

Lipids play an essential role in the production of the odor and the flavor of meat via autoxidation or thermal oxidation (Li et al., 2021). A detailed classification of the categories is crucial for the analysis of the metabolic pathways that underlie the transformation of lipids (Enser et al., 1999). A total of 2,639 lipid molecule species were detected in the Chaka sheep. The lipids were composed of 42 categories (**Figure 3**), and the percentages of content, in descending order, were: triacylglycerol (TG: 18.91%), phosphatidylcholine (PC: 18.34%), phosphatidylethanolamine (PE: 14.25%), cardiolipid (CL: 6.14%), diacylglycerol (DG: 5.99%), monosaccharide ceramide (Hex1Cer: 5.53%), phosphatidylserine (PS: 4.96%), sphingolipid (SM: 3.68%), ceramide (Cer: 3.64%) and others. Intramuscular fat is composed of phospholipids (PLs), TGs, and cholesterol, and the balance between triglyceride synthesis, breakdown, and absorption is reflected in IMF content (Roy et al., 2024). The effect of the lipid content on aroma retention is greater than the influence of the lipid type (Ayed et al., 2018). TGs and PLs have been shown to be the predominant lipids for predominant lipids for binding and generating flavor compounds (Fu et al., 2022). Liu 'study suggested that the highest types and concentrations of TG may

contribute primarily to aroma retention in roasted mutton (Liu et al., 2022). Therefore, our hypothesis was that the high TG levels in Chaka sheep might be correlated with the taste of the meat. PC, PE, and CL being the major components of PLs. Zhou's study found that the different PL forms (PC and PE) not only can regulate membrane fluidity and permeability but also are important components of pLs (Zhou et al., 2014). PLs, which accounted for almost half of the total number of molecules of lipid classes, were predominant in these three samples in our study. A study showed that PLs are abundant in PUFAs and are an essential resource of PUFAs (Zhou et al., 2013), and the flavor of meat is associated with the composition of PLs (Wu and Wang, 2019), Wang Daoying et al. (Wang et al., 2009) found that the PLs were the main factor in forming the flavor of duck meat products, which also was one of the major determinants of the taste of duck meat products. Therefore, we hypothesized that the flavor of Chaka sheep would be associated with the levels of PLs.

The total number of lipid molecules in the different parts of the Chaka sheep is summarized in **Figure 4**. The comparative results were as follows: PM > BF > LD. The difference between the groups was not significant. The composition of lipid subclasses in different parts of the Chaka sheep is shown in **Figure 5**. PC, PE, PI, PS, SPH, TG, and WE were major

subclasses lipids in Chaka , and all of them were present at levels >1000 µg/g. TG levels varied significantly among the three comparison groups, with the highest levels in the BF group, followed by the PM group, and the lowest levels in the LD group ($p<0.05$). PC, PE, PI, PS, and SPH were belong to PLs. PC and PE are the major PL classes in porcine intramuscular fat, and PEs are the predominant glycerophospholipid (GPL) in ruminant cell membranes (Fernandez et al, 1999). In conclusion, PLs were the highest total percentage of Chaka sheep, and PLs are also essential constituents of cell membranes. Hazel's study indicated that long-chain PUFA is more highly mobilized at low temperatures, and the higher the phospholipid (PL) content, the higher the long-chain PUFA content, which may correlate with better plateau adaptation in yak (Hazel and Williams, 1990). Lin's study showed that fish muscle PLs were sensitive to decreasing temperature (Lin et al., 2019). Therefore, we hypothesized that the higher levels of PLs molecules in the muscles of Chaka sheep would facilitate their adaptation to the extreme environment of the Tibetan Plateau, which is characterized by high levels of cold and hypoxia.

3.4.2 The differential lipid molecular analysis

To study the significant differences in lipids between different parts of Chaka sheep, OPLS-DA model was used to evaluate differential lipids. The

OPLS-DA model maximizes the between-group differences in the horizontal coordinate, while the within-group variation is reflected in the orthogonal principal components in the vertical coordinate, and it is clear in **Figure 6** that the OPLS-DA model can distinguish the two groups of samples, and the model evaluation parameter $Q^2 < 0.5$, indicating that the model has a great deal of stability, and the test results are real and reliable (Zhang et al., 2022).

The significantly different lipid molecules ($VIP > 1$, $P < 0.05$) screened in this experiment were visualized in the form of bubble plots in **Figure 7**. Bubbles in graph represent significantly different lipid species, and the vertical coordinates show each subclass of lipid, marked by different colors, with the size of the bubbles representing the significance of the differences. In BF, TG, PS, PI, PG, PE, PC, LPE, LPC, and Hex1Cer were the highly significantly different lipid molecules compared with LD. In BF vs. PM, TG, PS, PE, PC, LPE, LPC, and Hex1Cer were the main significantly different lipid molecules. In PM vs. LD, TG, SM, PS, PI, PC, LPC, Hex1Cer, and DG were the main lipids with highly significant differences. PS, PE, PC, LPC, LPE in phospholipids, TG in triglycerides, and Hex1Cer in sphingolipids were the three major co-differentiating lipid molecules. In Sun's study, phospholipids were found to be the main factor distinguishing the muscle

lipidome between cattle-yak and yak (Sun et al., 2018; Vahmani et al., 2020), and our findings were consistent with these results. Jia's study mainly detected AcCa, Cer, DG, LPC, PC, PE, SM, and TG, less ChE, LPE, MG, PI, and SPH, and no Co, Hex1Cer, Hex2Cer, LSM, PG in Tan sheep meat (Jia et al, 2021). However, in our study, we detected lipid molecules such as Co, Hex1Cer, Hex2Cer, LSM, and PG. These differences may be related to diet, feeding habits, and other factors (Dannenberger et al, 2017). The top 20 differential lipids in the different comparison groups are shown in the **Supplementary Table S1**.

We used venn diagrams (**Figure 8**) to show specific differential lipid molecules, and the results of the study showed that the numbers of significantly different lipid molecules between the different comparison groups were: 30 (BF vs. LD), 38 (BF vs. PM), and 88 (PM vs. LD). Among them, PE(18:2/18:2) and PC(25:0/11:3) were LD group-associated differential lipids. PS(20:3e/20:4), LPC(18:3), PE(8:1e/12:3), TG(18:0e/16:0/18:1), TG(18:0/18:0/18:0), TG(18:0e/18:0/18:1), and TG(18:0e/18:1/18:1) were PM group-associated differential lipids. LPC(16:0), LPC(18:1), LPE(18:1), PC(15:0/22:6), PE(18:1/18:1), Hex1Cer(d24:1/18:1), and PC(10:0e/6:0) were BF group-associated differential lipids. Dashdorj et al. found that PE(16:0/20:4), PE(18:0/19:1),

and PE(18:1/22:5) were the top three lipid species, suggesting that they are the major contributors to shank/flank muscle liposome differentiation (Dashdorj et al, 2015). In our study, PE(18:2/18:2) was mainly found in LD, PE(8:1e/12:3) was predominantly detected in PM, and PE(18:1/18:1) was mostly identified in BF. It can be concluded that PE is an important biomarker for the differentiation of the different parts of the muscle. Li et al. (2022) showed that phospholipids containing PC and PE may be key lipids in the formation of aromatic substances in roasted lamb. Zhou et al. (2014) showed that PC is more easily oxidized to aldehydes due to its high content of unsaturated fatty acids. Guo's study showed that PC and PE are prone to the formation of the aroma compounds (E,E)-2,4-decadienal, 1-octen-3-one and (E)-2-decene (Guo et al, 2021). Our study found significant differences in the content of the flavor compounds alcohols, ketones and esters among the three comparison groups. Therefore, we hypothesized that the cause of the differences in flavor compounds is related to the lipids PE(18:2/18:2), PE(8:1e/12:3), PE(18:1/18:1), PC(25:0/11:3), and PC(15:0/22:6). In addition, TG had a distinct influence on the volatile components and on the oral release of the most important hydrophilic volatile components. The fatty acid species contained in TG were found to affect the oxidation of volatile compounds (Frank et al, 2017). We found that volatile flavor alcohols,

aldehydes, and esters were the most abundant in PM, while TG(18:0e/16:0/18:1), TG(18:0/18:0/18:0), TG(18:0e/18:0/18:1), and TG(18:0e/18:1/18:1) were the characteristic lipid molecules in PM. Alcohols have a higher taste threshold and less impact on the taste of meat compared to aldehydes (Huang et al., 2022). Studies have shown that aldehydes, usually derived from C18 PUFA such as linoleic and linolenic acids and C20 arachidonic acid, are the major products of lipid oxidation. Aldehydes have a low olfactory threshold and are important contributors to flavor (Varlet et al., 2007). Aldehydes were the highest percentage of flavor substances in our study and were significantly higher in the PM group than in the LD and BF groups. Therefore, we hypothesized that TG-like lipids are associated with the formation of aldehydes.

3.4.3 The cluster analysis and correlation of differential lipid molecules

To evaluate the plausibility of differential lipids and to visualize more clearly the association between specimens and the differential expression patterns of lipids in different samples, we conducted hierarchical clustering (**Figure 9**) using the expression levels of highly differential lipids (VIP > 1, p-value < 0.05) for each group of specimens. Differential lipids were analysed by clustering and heatmaps demonstrated significant differences in

lipid profiles between the BF, LD and PM, indicating significant differences in lipid molecules in different parts of the muscle of Chaka sheep.

To show the relationship of lipid coregulation more intuitively, the chord diagram (**Figure 10**) was utilized to show the correlation between lipid sub-classes, where the beginning point of the link in the inner circle represented the significantly different lipid molecules, and the bows on the outer circles indicated the lipid sub-classes. In BF vs. LD, our study reflected a strong correlation within lipid subclasses PC, PE, and Hex1Cer. By comparing the BF group with the PM group, we obtained some intramolecular correlations of lipid subclasses such as PC, PE, PS, TG, and CL. By comparing PM and LD, our results suggested that there were strong intramolecular correlations between the glycerol ester lipid subclass molecules TG and DG. McMaster's study showed that the majority of PE is synthesized from ethanolamines, while PE is a choline source. After the synthesis of PE, it can be converted to PC via sequential methylation steps (McMaster, 2018), which further validates the correlation between PC and PE in this study. Zhou's study found that there was a strong correlation between PS and PI (Zhou et al., 2021). Hou et al. found a correlation between the expression of PSs and PIs in subcutaneous adipose tissue (Hou et al., 2020). However in our study, we did not find a strong correlation

between PI and other lipid molecules, the relevance of other lipid molecules needs to be further verified.

4 Conclusions

In our study, we found differences in the composition of nutrients and lipid profile in three parts of the muscles of the Chaka sheep. Specifically, the LD had the highest CP content and the highest levels of aspartic acid and histidine, the BF had the highest contents of glycine and Proline, and the PM had the highest abundance of ω -6 PUFA, linoleic acid, and volatile flavor compounds. Phospholipids were the predominant lipids, which accounted for almost half of the total lipids. The lipid profiles were different in the LD, PM and BF of the Chaka sheep. There were obvious intermolecular relationships between PC, PE, Hex1Cer, PS, TG, DG, and CL. This work is aimed at characterizing the nutritional composition and lipid profile of the Chaka sheep. Our findings may open new horizons in the utilization of the Chaka sheep for nutritional or biomedical products, thus enhancing the commercial value of these products.

References

- Ayed C, Martins SIFS, Williamson AM, Guichard E. 2018. Understanding fat, proteins and saliva impact on aroma release from flavoured ice creams. *Food Chem* 267: 132-139.
- Boselli E, Pacetti D, Curzi F, Frega NG. 2008. Determination of phospholipid molecular species in pork meat by high performance liquid chromatography–tandem mass spectrometry and evaporative light scattering detection. *Meat Sci* 78: 305-313.
- Chen H, Cheng J, Cao X, Hu L, Huang Y, Lan X. 2019. Detecting copy number variation (CNV) marker related to growth trait of Chaka sheep used in molecular marker assisted selection breeding of Chaka sheep includes amplifying lysine methyltransferase 2D (KMT2D) gene and identifying. *Univ Northwest a & F*.
- Chen H, Yang Z, Hu L, Lan X, Huang Y, Hu S. 2019. Detecting CNV marker of Chaka sheep BAG4 gene useful in molecular marker assisted selection breeding of Chaka sheep comprises e.g. using real-time fluorescent quantitative PCR, using genomic DNA of Chaka sheep. *Univ Northwest a & F*.

Cohen EH. 1971. Comparison of official AOAC method with rapid methods for analysis of moisture in meats. *J Association Official Analytical Chemi* 54: 1432.

Dai ZC, Feng MW, Feng CG, Zhu HX, Chen Z, Guo BB, Yan LY. 2024. Effects of sex on meat quality traits, amino acid and fatty acid compositions, and plasma metabolome profiles in White King squabs. *Poultry Sci* 103: 103524.

Enser M, Scollan N D, Choi N J, Kurt E, Hallett K, Wood JD. 1999. Effect of dietary lipid on the content of conjugated linoleic acid (CLA) in beef muscle. *Animal Sci J* 69: 143-146.

Fernandez X, Monin G, Talmant A, Mourot J, Lebret B. 1999. Influence of intramuscular fat content on the quality of pig meat-:1.: Composition of the lipid fraction and sensory characteristics of *m. longissimus lumborum*. *Meat Sci* 53: 59-65.

Frank D, Kaczmarska K, Paterson J, Piyasiri U, Warner R. 2017. Effect of marbling on volatile generation, oral breakdown and in mouth flavor release of grilled beef. *Meat Sci* 133: 61-68.

Fu Y, Cao S, Yang L, Li Z. 2022. Flavor formation based on lipid in meat and meat products: A review. *J Food Biochem* 46: 12.

- Gao H, Zhang Y, Liu K, Fan R, Li Q, Zhou Z. 2022. Dietary sodium butyrate and/or vitamin D3 supplementation alters growth performance, meat quality, chemical composition, and oxidative stability in broilers. *Food Chem* 390.
- Ge Y, Gai K, Li Z, Chen Y, Wang L, Qi X, Sheng X. 2023. HPLC-QTRAP-MS-based metabolomics approach investigates the formation mechanisms of meat quality and flavor of Beijing You chicken. *Food Chem X* 17: 100550.
- Guo D, Wan P, Liu J, Chen D. 2021. Use of egg yolk phospholipids to boost the generation of the key odorants as well as maintain a lower level of acrylamide for vacuum fried French fries. *Food Control* 121.
- Guo T, Wang X, Zhang Q, Wei L, Liu H, Zhao N, Xu S. 2022. Comparative Analysis of the Composition of Fatty Acids and Metabolites between Black Tibetan and Chaka Sheep on the Qinghai-Tibet Plateau. *Animals (Basel)* 12.
- Haag M. 2003. Essential Fatty Acids and the Brain. *Can J Psychiat* 48: 195-203.
- Hazel J R, Williams EE. 1990. The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. *Prog Lipid Res* 29: 167-227.
- He YX, Zhou MY, Xia CL, Xia Q, He J, Cao JX, Sun YY. 2020. Volatile flavor changes responding to heat stress-induced lipid oxidation in duck meat. *Anim Sci J* 91.

- Hou B, Zhao Y, He P, Xu C, Ma P, Lam SM, Du G. 2020. Targeted lipidomics and transcriptomics profiling reveal the heterogeneity of visceral and subcutaneous white adipose tissue. *Life Sci* 245.
- Huang Q, Dong K, Wang Q, Huang X, Wang G, An F, Luo Z, Luo P. 2022. Changes in volatile flavor of yak meat during oxidation based on multi-omics. *Food Chem* 371:131103.
- Kohn TA, Anley MJ, Magwaza STN, Adamson L, Hoffman LC, Brand TS. 2023. Muscle fiber type and metabolic profiles of four muscles from the African black ostrich. *Meat Sci* 200.
- Li J, Yang Y, Tang C, Yue S, Zhao Q, Li F, Zhang J. 2022. Changes in lipids and aroma compounds in intramuscular fat from Hu sheep. *Food Chem* 383.
- Li J, Yang Y, Zhan T, Zhao Q, Zhang J, Ao X, Tang C. 2021. Effect of slaughter weight on carcass characteristics, meat quality, and lipidomics profiling in longissimus thoracis of finishing pigs. *Lwt-Food Sci Technol* 140.
- Li M, Ren W, Chai W, Zhu M, Man L, Zhan Y, Qin H, Sun M, Liu J, Zhang D, Wang Y, Wang T, Shi X, Wang C. 2022. Comparing the profiles of raw and cooked donkey meat by metabo-nomics and lipidomics assessment. *Front Nutr* 9: 851761.
- Liang Y, Jiao D, Du X, Zhou J, Degen AA, Ran F, Yang G. 2023. Effect of dietary *Agriophyllum squarrosum* on average daily gain, meat quality and muscle fatty acids in growing Tan lambs. *Meat Sci* 201: 109195.
- Lin Y, Wang H, Rao W, Cui Y, Dai Z, Shen Q. 2019. Structural characteristics of dietary fiber (*Vigna radiata* L. hull) and its inhibitory effect on phospholipid digestion as an additive in fish floss. *Food Control* 98: 74-81.
- Liu S, Shi L. 2021. UHPLC-Q-Orbitrap HRMS-based quantitative lipidomics reveals the chemical changes of phospholipids during thermal processing methods of Tan sheep meat. *Food Chem* 360.
- Liu Z, Zhao M, Wang X, Li C, Liu Z, Shen X, Zhou D. 2022. Investigation of oyster *Crassostrea gigas* lipid profile from three sea areas of China based on non-targeted lipidomics for their geographic region traceability. *Food Chem* 386.
- Lugo Charriez K, Soledade Lemos L, Carrazana Y, Rodriguez-Casariego JA, Eirin-Lopez JM, Hauser-Davis RA, Quinete N. 2021. Application of an Improved Chloroform-Free Lipid Extraction Method to Staghorn Coral (*Acropora cervicornis*) Lipidomics Assessments. *B Environ Contam Tox* 107: 92-99.
- Ma C, Li X, Zheng C, Zhou B, Xu C, Xia T. 2021. Comparison of characteristic components in tea-leaves fermented by *Aspergillus*

- pallidofulvus PT-3, *Aspergillus sesamicola* PT-4 and *Penicillium manginii* PT-5 using LC-MS metabolomics and HPLC analysis. *Food Chem* 350.
- McMaster CR. 2018. From yeast to humans - roles of the Kennedy pathway for phosphatidylcholine synthesis. *Febs Lett* 592: 1256-1272.
- Mohanty TR, Park KM, Pramod AB, Kim JH, Choe HS, Hwang IH. 2010. Molecular and biological factors affecting skeletal muscle cells after slaughtering and their impact on meat quality: a mini-review. *J Muscle Foods* 21: 51-78.
- Oh R. 2005. Practical applications of fish oil (Omega-3 fatty acids) in primary care. *J Am Board Fam Pract* 18: 28-36.
- Qin X, Zhang T, Cao Y, Deng B, Zhang J, Zhao J. 2020. Effects of dietary sea buckthorn pomace supplementation on skeletal muscle mass and meat quality in lambs. *Meat Sci* 166: 108141.
- Ramos LMG, Bezerra LR, de Oliveira JPF, de Souza MP, da Silva AL, Pereira ES, Oliveira RL. 2021. Effects of feeding growing-finishing lambs with cashew nut shell liquid on the growth performance, physicochemical attributes, lipid peroxidation and sensorial parameters of burger. *Small Ruminant Res* 202.
- Ribeiro de Araujo Cordeiro AR, de Medeiros LL, Alencar Bezerra TK, Bertoldo Pacheco MT, Galvao MdS, Madruga MS. 2020. Effects of thermal processing on the flavor molecules of goat by-product hydrolysates. *Food Res Int* 138.
- Roy BC, Coleman P, Markowsky M, Wang K, She YB, Richard C, Proctor SD, Bruce HL. 2024. Muscle Fiber, Connective Tissue and Meat Quality Characteristics of Pork from Low Birth Weight Pigs as Affected by Diet-Induced Increased Fat Absorption and Preferential Muscle Marbling. *Food Sci Anim Resour* 44: 51-73.
- Schiavon S, Pellattiero E, Cecchinato A, Tagliapietra F, Dannenberger D, Nuernberg K, Bittante G. 2016. The influence of different sample preparation procedures on the determination of fatty acid profiles of beef subcutaneous fat, liver and muscle by gas chromatography. *J Food Compos Anal* 50:10-18.
- Simopoulos AP. 2008. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp Biol Med* 233: 674-688.
- Sun N, Chen J, Wang D, Lin S. 2018. Advance in food-derived phospholipids: Sources, molecular species and structure as well as their biological activities. *Trends Food Sci Tech* 80: 199-211.

- Tang J, Zhang B, Liu D, Gao K, Dai Y, Liang S, Hou S. 2023. Dietary riboflavin supplementation improves meat quality, antioxidant capacity, fatty acid composition, lipidomic, volatilomic, and proteomic profiles of breast muscle in Pekin ducks. *Food Chem X* 19.
- Tian Y, Xu T, Li Y, Liu Y, Liu J. 2021. An untargeted LC-MS metabolomics approach to the metabolic profiles of bottom cultured scallops (*Mizuhopecten yessoensis*) subjected to mechanical shock in early post-harvest handling. *Aquaculture* 533.
- Vahmani P, Ponnampalam EN, Kraft J, Mapiye C, Bermingham EN, Watkins PJ, Dugan MER. 2020. Bioactivity and health effects of ruminant meat lipids. Invited Review. *Meat Sci* 165.
- Varlet V, Prost C, Serot T. 2007. Volatile aldehydes in smoked fish: analysis methods, occurrence and mechanisms of formation. *Food Chem* 105: 1536–1556.
- Wang DY, Zhu YZ, Xu WM. 2009. Comparative Study of Intramuscular Phospholipid Molecular Species in Traditional Chinese Duck Meat Products. *Asian Austral J Anim* 22: 1441-1446.
- Xu X, Liu H, Wang X, Zhang Q, Guo T, Hu L, Xu S. 2023. Evaluation of the *Longissimus Thoracis et Lumborum* Muscle Quality of Chaka and Tibetan Sheep and the Analysis of Possible Mechanisms Regulating Meat Quality. *Animals* 13.
- Yang ZH, Hou YR, Zhang M, Hou PX, Liu C, Dou L, Chen XY, Zhao LH, Su L, Jin Y. 2024. Unraveling proteome changes of Sunit lamb meat in different feeding regimes and its relationship to flavor analyzed by TMT-labeled quantitative proteomic. *Food Chem* 437: 137657.
- Yin M, Chen M, Matsuoka R, Song X, Xi Y, Zhang L, Wang X. 2023. UHPLC-Q-Exactive Orbitrap MS/MS based untargeted lipidomics reveals fatty acids and lipids profiles in different parts of capelin (*Mallotus villosus*). *J Food Compos Anal* 116.
- Zhang X, Han L, Hou S, Raza SHA, Wang Z, Yang B, Al Hazani TMI. 2022. Effects of different feeding regimes on muscle metabolism and its association with meat quality of Tibetan sheep. *Food Chem* 374: 131611.
- Zhang Y, Rui X, Vaugeois R, Simpson BK. 2022. Seal meat enzymatic hydrolysates and its digests: A comparison on protein and minerals profiles. *Lwt-Food Sci Technol* 157.
- Zhang Y, Zhang J, Gong H, Cui L, Zhang W, Ma J, Yang B. 2019. Genetic correlation of fatty acid composition with growth, carcass, fat deposition and meat quality traits based on GWAS data in six pig populations. *Meat Sci* 150: 47-55.

- Zhou J, Zhang Y, Wu J, Qiao M, Xu Z, Peng X, Mei S. 2021. Proteomic and lipidomic analyses reveal saturated fatty acids, phosphatidylinositol, phosphatidylserine, and associated proteins contributing to intramuscular fat deposition. *J Proteomics* 241.
- Zhou L, Khalil A, Bindler F, Zhao M, Marcic C, Ennahar S, Marchioni E. 2013. Effect of heat treatment on the content of individual phospholipids in coffee beans. *Food Chem* 141: 3846-3850.
- Zhou L, Zhao M, Bindler F, Marchioni E. 2014. Comparison of the Volatiles Formed by Oxidation of Phosphatidylcholine to Triglyceride in Model Systems. *J Agric Food Chem* 62: 8295-8301.
- Zuo H, Han L, Yu Q, Niu K, Zhao S, Shi H. 2016. Proteome changes on water-holding capacity of yak longissimus lumborum during postmortem aging. *Meat Sci* 121: 409-419.

Table 1 The routine nutrient composition of different parts of muscles

Items	Groups			P-value
	LD	PM	BF	
pH _{45min}	6.24±0.055	6.23±0.148	6.31±0.038	0.507
CIE L*	31.67±1.155 ^b	51.33±0.577 ^a	50.00±3.606 ^a	<0.001
CIE a*	29.67±2.082 ^c	39.33±1.155 ^a	34.33±1.528 ^b	0.001
CIE b*	13.00±2.000 ^b	17.67±1.528 ^a	16.67±1.528 ^a	0.034
MSTR (%)	71.50±0.700	70.53±3.150	72.33±2.139	0.637
Ash (%)	1.99±0.138	2.07±0.172	1.99±0.137	0.797
EE (%)	11.95±0.574	13.17±0.477	13.04±1.101	0.125
CP (%)	22.63±0.379 ^a	20.03±0.764 ^c	21.20±0.625 ^b	0.006

Note: CIE L* indicates brightness; CIE a* indicates redness; CIE b* indicates yellowness; MSTR for moisture, Ash for ash, CP for crude protein, EE for ether extract; The MSTR, Ash, CP and EE are on a dry matter basis; LD indicates the *longest dorsal muscle*, PM indicates the *psaos major muscle*; BF indicates the *biceps femoris*. a-c means within a row with different subscripts differ when p -value < 0.05.

Table 2 The amino acid content in different parts of Chaka sheep

Items (%)	Groups			P-value
	LD	PM	BF	
Asparticacid	5.72±0.265 ^a	5.23±0.172 ^b	5.34±0.119 ^b	0.049
Threonine	2.95±0.173	2.70±0.092	2.93±0.258	0.257
Serine	2.32±0.170	2.12±0.113	2.29±0.369	0.581
Glutamicacid	11.53±0.644	10.61±0.332	11.58±1.324	0.373
Glycine	3.05±0.040 ^a	2.66±0.104 ^b	3.40±0.358 ^a	0.015
Alanine	3.79±0.156	3.46±0.053	3.94±0.290	0.056
Cystine	0.34±0.074	0.44±0.093	0.46±0.198	0.519
Valine	3.32±0.159	3.09±0.035	3.35±0.178	0.118
Methionine	1.16±0.150	1.02±0.163	0.97±0.244	0.564
Isoleucine	3.32±0.140	3.07±0.047	3.29±0.156	0.097
Leucine	5.38±0.250	5.00±0.100	5.33±0.333	0.206
Tyrosine	2.13±0.270	1.94±0.083	1.99±0.404	0.725
Phenylalanine	3.56±0.255	3.20±0.042	3.19±0.183	0.081
Histidine	2.76±0.157 ^a	2.20±0.026 ^b	2.53±0.202 ^b	0.010
Lysine	5.73±0.253	5.20±0.137	5.53±0.397	0.147
Arginine	4.37±0.187	3.95±0.165	4.47±0.405	0.122
Proline	2.55±0.064 ^a	2.17±0.242 ^b	2.88±0.320 ^a	0.028
EAA	28.17±1.344	25.49±0.478	27.12±1.605	0.096
NEAA	35.80±1.540	32.57±0.958	36.34±2.740	0.100
TAA	63.97±2.873	58.06±1.400	63.46±4.327	0.106

Note: EAA indicates essential amino acids, NEAA indicates nonessential amino acids; TAA indicates total amino acids. a-c means within a row with different subscripts differ when p -value < 0.05. The content of amino acids (%) in this study is expressed as the percentage of amino acids per unit mass of the sample.

EAA=Threonine + Valine + Methionine + Isoleucine + Leucine + Phenylalanine + Histidine + Lysine + Arginine;

NEAA=TAA-EAA

Table 3 The fatty acid content in different parts of Chaka sheep

Items(%)	LD	PM	BF	P-value
∑SFA	44.83±2.272 ^b	49.19±1.003 ^a	48.97±0.336 ^a	0.021
C4:0	0.03±0.008	ND	0.02±0.004	-
C6:0	0.01±0.002	0.01±0.002	0.02±0.001	0.560
C8:0	0.01±0.008	0.01±0.004	0.01±0.000	0.847
C10:0	0.15±0.006	0.17±0.012	0.16±0.055	0.767
C11:0	ND	0.003±0.000	0.003±0.000	-
C12:0	0.06±0.001	0.09±0.006	0.08±0.017	0.066
C13:0	0.01±0.001	0.01±0.002	0.02±0.002	0.437
C14:0	2.07±0.112	2.37±0.052	2.42±0.363	0.198
C15:0	0.28±0.006 ^c	0.44±0.045 ^a	0.36±0.046 ^b	0.005
C16:0	21.49±0.855	27.14±1.521	29.40±4.087	0.471
C17:0	1.04±0.044 ^b	1.45±0.097 ^a	1.29±0.209 ^a	0.025
C18:0	20.21±2.357	16.82±1.009	13.72±0.566	0.125
C20:0	0.06±0.003 ^b	0.12±0.016 ^a	0.04±0.018 ^c	0.011
C21:0	0.25±0.017	0.34±0.079	0.28±0.050	0.184
C22:0	0.11±0.021	0.12±0.019	0.09±0.028	0.386
C23:0	0.05±0.006	0.06±0.010	0.05±0.018	0.317
C24:0	0.02±0.006	0.03±0.006	0.02±0.008	0.456
∑MUFA	1.29±0.094	1.41±0.037	1.50±0.070	0.107
C14:1	0.11±0.003 ^c	0.17±0.007 ^a	0.13±0.026 ^b	0.016
C15:1	0.16±0.007 ^c	0.22±0.003 ^a	0.17±0.024 ^b	0.005
C16:1	1.23±0.207	1.26±0.134	1.56±0.303	0.278
C17:1	0.48±0.057	0.58±0.043	0.63±0.093	0.095
C18:1n9t	ND	ND	ND	-
C18:1n9c	42.41±3.328	40.08±1.079	44.70±1.840	0.120
C22:1n9	0.02±0.003	0.02±0.003	0.01±0.005	0.969
C20:1	0.11±0.001 ^a	0.15±0.022 ^a	0.08±0.003 ^b	0.036
C24:1	0.14±0.021 ^a	0.10±0.012 ^b	0.15±0.007 ^a	0.012
∑PUFA	0.17±0.022	0.27±0.035	0.22±0.040	0.067
C18:2n6t	ND	ND	ND	-
C18:2n6c	3.32±0.439 ^b	5.03±0.770 ^a	3.90±0.629 ^b	0.040
C18:3n6	0.54±0.015	0.83±0.048	0.76±0.015	0.064
C18:3n3	0.15±0.018	0.16±0.019	0.15±0.041	0.840
C20:2	ND	ND	ND	-
C20:3n3	0.26±0.020	0.24±0.055	0.27±0.047	0.710
C20:3n6	0.87±0.037	1.10±0.154	0.92±0.068	0.063
C20:4n6	0.08±0.002	0.10±0.009	0.07±0.001	0.060
C20:5n3	0.65±0.010	0.66±0.086	0.70±0.014	0.865
C22:2	0.02±0.002	0.02±0.008	0.02±0.005	0.207
C22:6n3	0.11±0.036	0.10±0.001	0.09±0.002	0.465
∑UFA	50.71±2.990	50.82±1.004	54.32±0.985	0.099
SFA/UFA	0.97±0.103	1.00±0.039	0.83±0.011	0.057
PUFA/SFA	0.12±0.006	0.17±0.024	0.15±0.024	0.080
ω-3PUFA	1.17±0.153	1.16±0.148	1.21±0.213	0.938
ω-6PUFA	4.81±0.553 ^c	7.06±0.955 ^a	5.65±0.849 ^b	0.037
ω-6/ω-3PUFA	4.18±0.769 ^c	6.07±0.170 ^a	4.69±0.194 ^b	0.006

$\sum \text{SFA} = \text{C4:0} + \text{C6:0} + \text{C8:0} + \text{C10:0} + \text{C11:0} + \text{C12:0} + \text{C13:0} + \text{C14:0} + \text{C15:0} + \text{C16:0} + \text{C17:0} + \text{C18:0} + \text{C20:0} + \text{C21:0} + \text{C22:0} + \text{C23:0} + \text{C24:0};$

$\sum \text{MUFA} = \text{C14:1} + \text{C15:1} + \text{C16:1} + \text{C17:1} + \text{C18:1n9t} + \text{C18:1n9c} + \text{C22:1n9} + \text{C20:1} + \text{C24:1};$

$\sum \text{PUFA} = \text{C18:2n6t} + \text{C18:2n6c} + \text{C18:3n6} + \text{C18:3n3} + \text{C20:2} + \text{C20:3n3} + \text{C20:3n6} + \text{C20:4n6} + \text{C20:5n3} + \text{C22:2} + \text{C22:6n3};$

$\sum \text{UFA} = \sum \text{MUFA} + \sum \text{PUFA};$

$\omega\text{-6 PUFA} = \text{C18:2n6t} + \text{C18:2n6c} + \text{C18:3n6} + \text{C20:3n6} + \text{C20:4n6};$

$\omega\text{-3 PUFA} = \text{C18:3n3} + \text{C20:3n3} + \text{C20:5n3} + \text{C22:6n3}.$

SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

ND indicates not detected. In the same row, a-c means within a row with different subscripts differ when $p\text{-value} < 0.05$.

ACCEPTED

Table 4 The content of volatile flavor substances in three parts of muscle

Items (%)	Groups			P-value
	LD	PM	BF	
Aldehydes	62.94±5.997	64.39±0.428	57.33±8.095	0.356
Pentanal	0.24±0.051	0.64±0.333	0.36±0.117	0.131
2-Thiophene formaldehyde	0.21±0.059 ^b	0.75±0.095 ^a	0.32±0.656 ^a	<0.001
Hexanal	4.28±0.144 ^c	7.30±0.460 ^a	6.13±0.254 ^b	<0.001
Heptanal	4.67±0.879	5.09±0.511	3.84±0.421	0.124
Octanal	3.65±0.445 ^a	3.43±0.235 ^a	2.85±0.202 ^b	0.049
Nonanal	13.78±0.677 ^a	9.62±0.652 ^b	12.37±0.928 ^a	0.002
Methylthiopropionaldehyde	ND	0.26±0.046	0.43±0.252	-
Decanal	1.39±0.300 ^a	0.66±0.055 ^b	0.51±0.060 ^c	0.002
Benzaldehyde	21.24±3.623 ^b	26.89±0.206 ^a	21.40±1.003 ^a	0.031
2-Decenal,(E)	2.17±0.089 ^c	3.57±0.115 ^b	3.65±0.061 ^a	<0.001
2-Undecenal	3.72±0.498 ^a	2.59±0.500 ^b	3.11±0.245 ^a	0.049
2-Phenyl-2-butylenaldehyde	0.79±0.055	ND	0.77±0.131	-
(E,E)-2,4-heptadienal	0.73±0.305 ^b	0.58±0.036 ^b	0.97±0.060 ^a	0.001
Pentadecanal	1.26±0.350 ^a	0.54±0.120 ^b	0.68±0.064 ^b	0.014
Hexadecanal	9.35±0.291 ^a	2.53±0.325 ^b	3.86±0.127 ^c	<0.001
Alcohols	6.60±0.801 ^b	9.11±0.367 ^a	6.53±0.332 ^b	0.002
1-Pentanol	0.78±0.078 ^b	1.02±0.076 ^a	0.41±0.031 ^c	<0.001
1-Hexanol	0.44±0.099 ^b	0.72±0.066 ^a	0.41±0.031 ^b	0.003
2-Furanmethanol	0.37±0.097	1.34±0.061	ND	-
1-Octen-3-ol	1.55±0.387 ^c	2.21±0.125 ^b	2.38±0.097 ^a	0.012
Heptanol	0.93±0.119 ^b	1.31±0.070 ^a	0.75±0.157 ^c	0.004
Octanol	1.86±0.211 ^a	1.96±0.060 ^a	0.75±0.157 ^b	<0.001
(E)-2-Octene-1-ol	0.67±0.721	0.55±0.047	0.53±0.096	0.117
Ketones	1.72±0.180 ^c	2.51±0.165 ^a	2.33±0.110 ^b	0.002
2-Pentadecanone	0.58±0.085 ^b	0.53±0.057 ^b	0.74±0.101 ^a	0.044
3-Hexanone	0.13±0.045 ^c	0.36±0.081 ^b	0.44±0.049 ^a	0.002
2,3-Octanedione	0.78±0.082 ^b	1.45±0.096 ^a	0.90±0.144 ^a	0.001
6-Methyl-5-hepten-2-one	0.23±0.061	0.29±0.060	0.25±0.035	0.426
Hydrocarbons	5.78±0.700	5.82±0.180	5.63±0.474	0.331
Heptylbenzene	1.99±0.125 ^a	1.02±0.026 ^b	1.41±0.248 ^a	0.035
Xylene	1.76±0.371 ^a	1.25±0.060 ^b	0.70±0.099 ^c	0.004
Octylbenzene	1.17±0.154 ^b	1.14±0.072 ^b	1.85±0.901 ^a	0.001
Nonylbenzene	0.86±0.146	1.14±0.072	1.07±0.140	0.073

Hexadecane	ND	1.03±0.176	0.60±0.056	-
Esters	0.47±0.129 ^c	1.03±0.110 ^a	0.86±0.061 ^b	0.007
2-Ethyl ethyl acetate	ND	0.12±0.050	0.27±0.064	-
Methyl diethyldithiocarbamate	0.47±0.129 ^b	0.91±0.131 ^a	0.60±0.012 ^a	0.006
Others	9.94±1.023	9.34±0.793	8.15±0.475	0.081
2- (pentenyl) furan	1.50±0.280 ^b	5.41±0.720 ^a	4.03±0.243 ^a	<0.001
2-Acetyl thiazole	0.15±0.0252 ^c	0.45±0.111 ^b	0.48±0.090 ^a	0.006
trans-2-(Pentenyl)furan	1.40±0.627	1.23±0.326	1.20±0.247	0.832
2-Acetyl pyrrole	1.49±0.148	1.30±0.303	1.50±0.295	0.594
Acetoin	3.60±0.246 ^b	0.64±0.061 ^a	0.64±0.180 ^a	<0.001
Dimethyl disulphide	0.18±0.030 ^a	0.13±0.031 ^b	0.12±0.021 ^b	0.048
Dimethyl trisulfide	0.27±0.021 ^a	0.18±0.012 ^b	0.21±0.558 ^a	0.015

Notes: ND indicates not detected. a-c means within a row with different subscripts differ when *p*-value < 0.05.

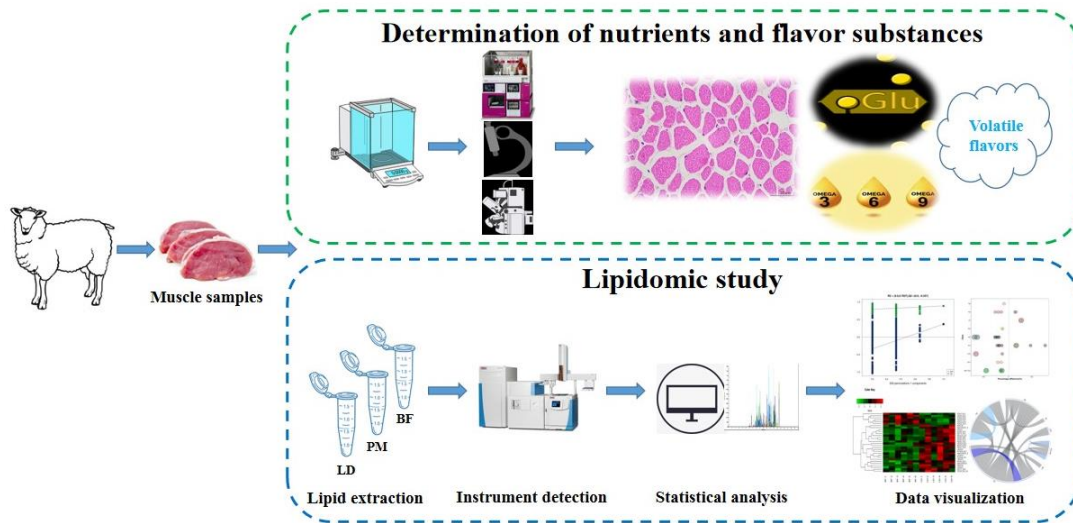


Figure 1 Experimental and analytical processes followed in this study

ACCEPTED

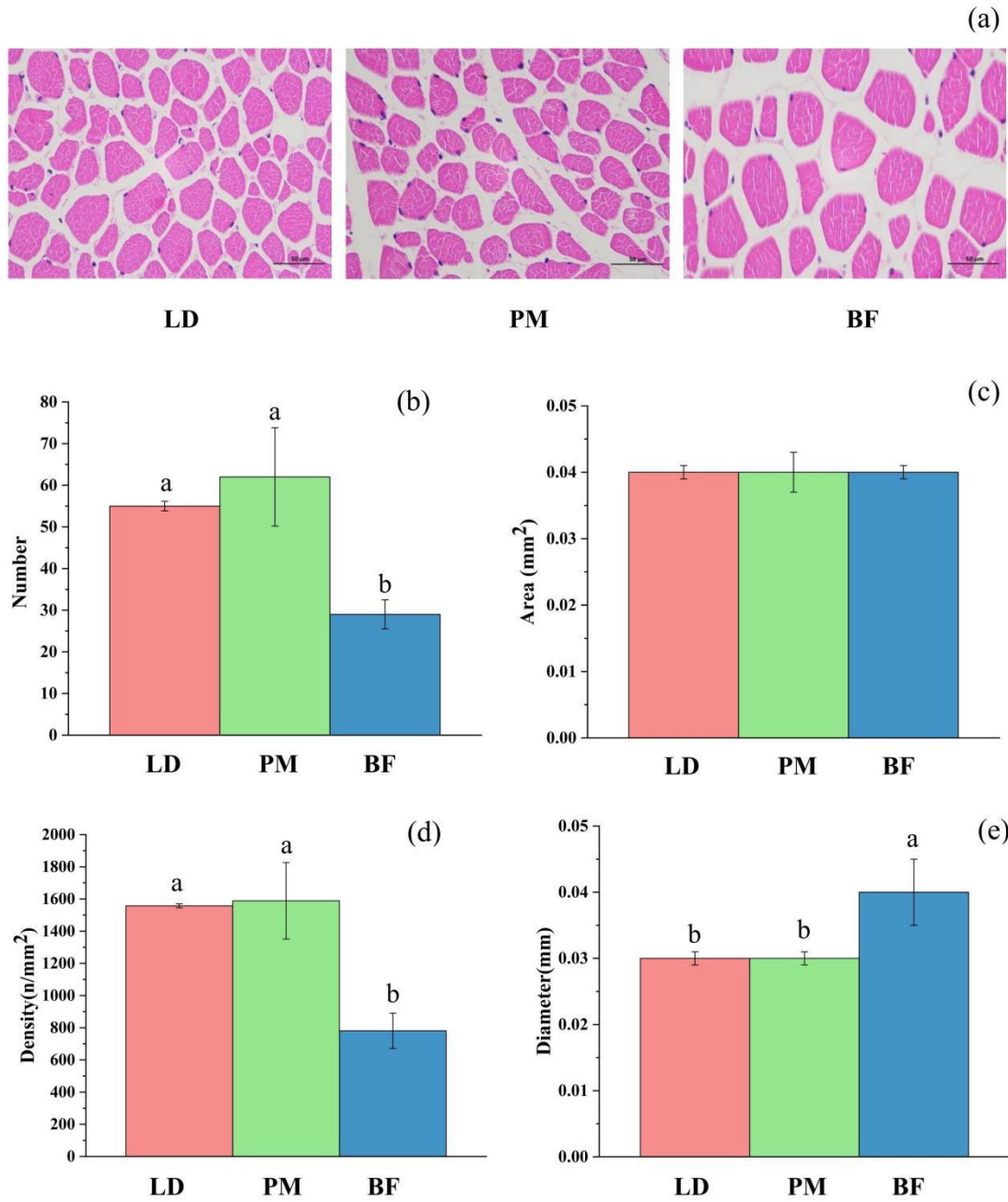


Figure 2 Characteristics of muscle fibers in different parts of the muscles

Notes: a-c means within a row with different subscripts differ when p -value < 0.05 .

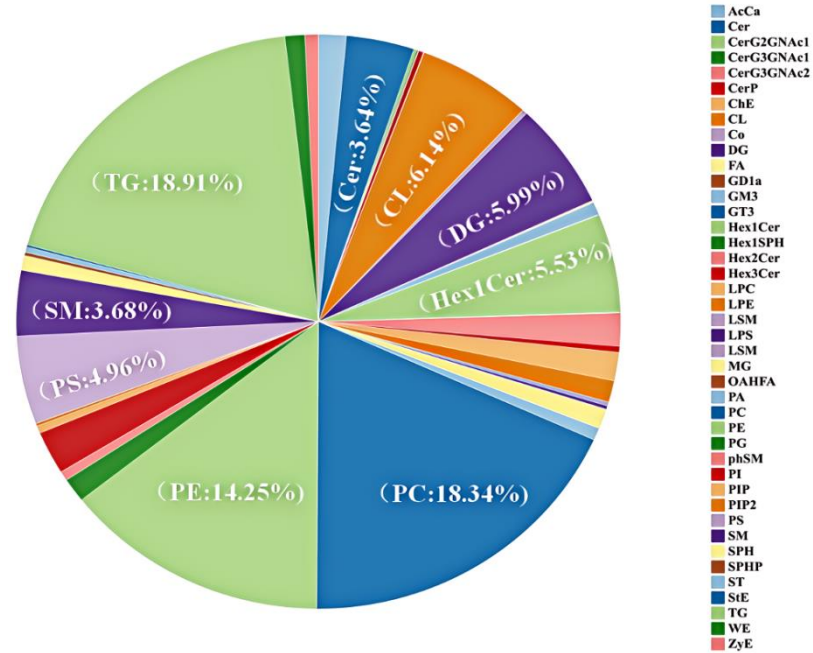


Figure 3 The molecular distribution of total lipids in Chaka sheep

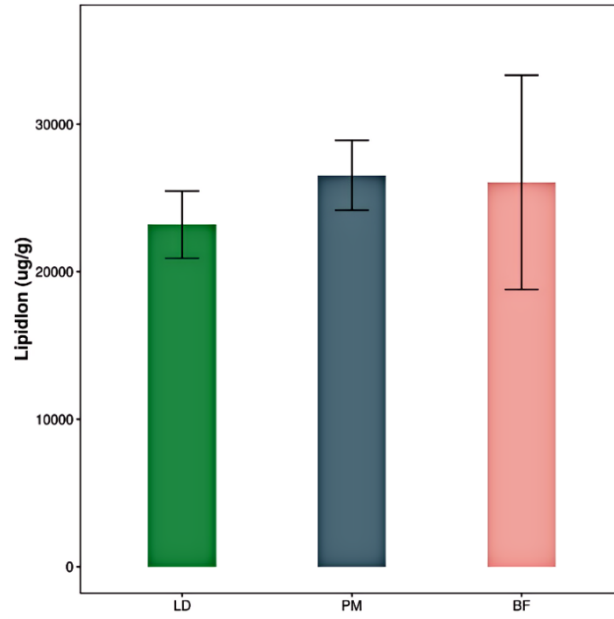


Figure 4 The total content of lipid molecules in different parts of Chaka sheep

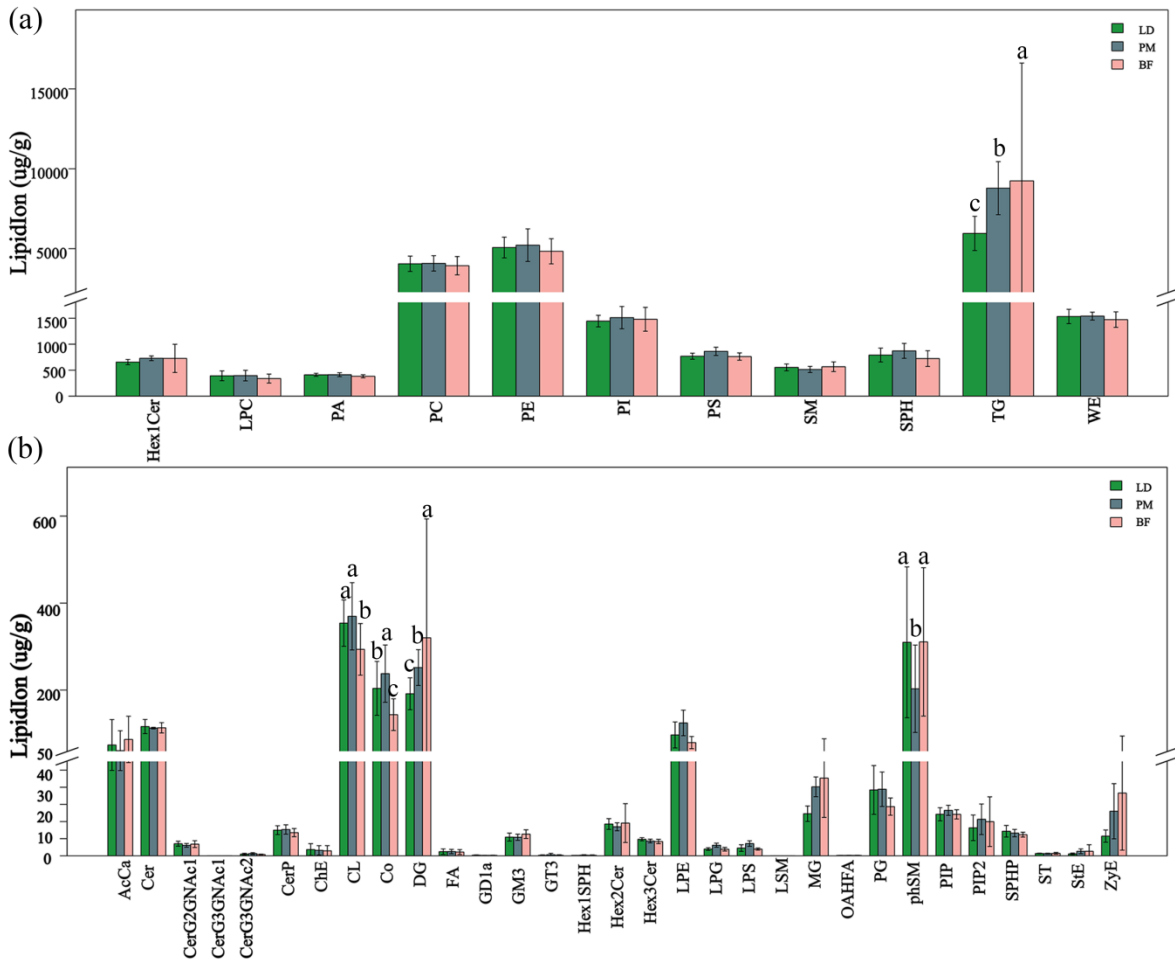


Figure 5 The composition of lipid subclasses in different parts of the Chaka sheep

Notes: a–c indicate significant differences between the groups compared in the figure ($p < 0.05$).

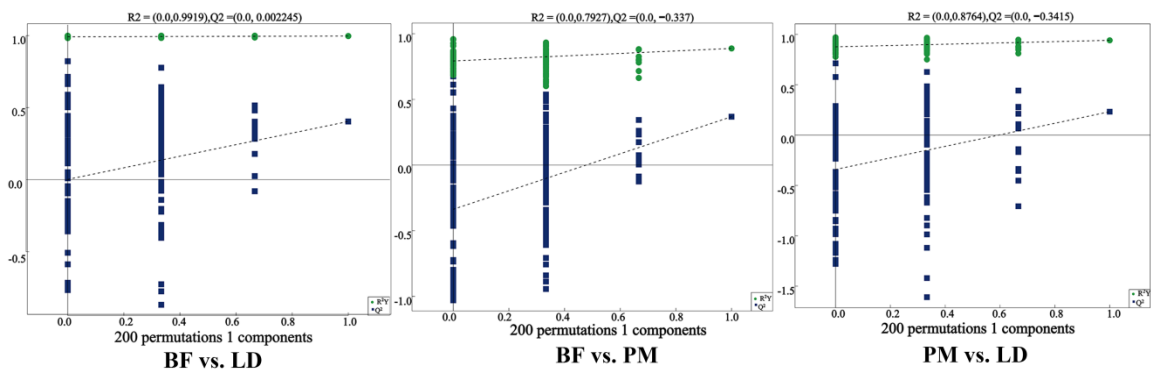


Figure 6 The OPLS-DA displacement test in positive and negative ion mode

ACCEPTED

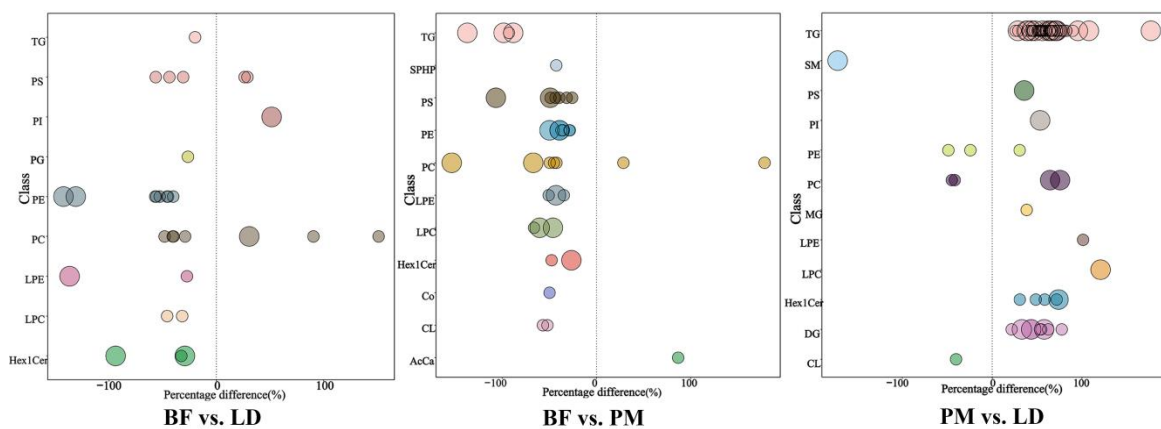


Figure 7 The molecular bubble diagrams of differential lipids in different parts of Chaka sheep

ACCEPTED

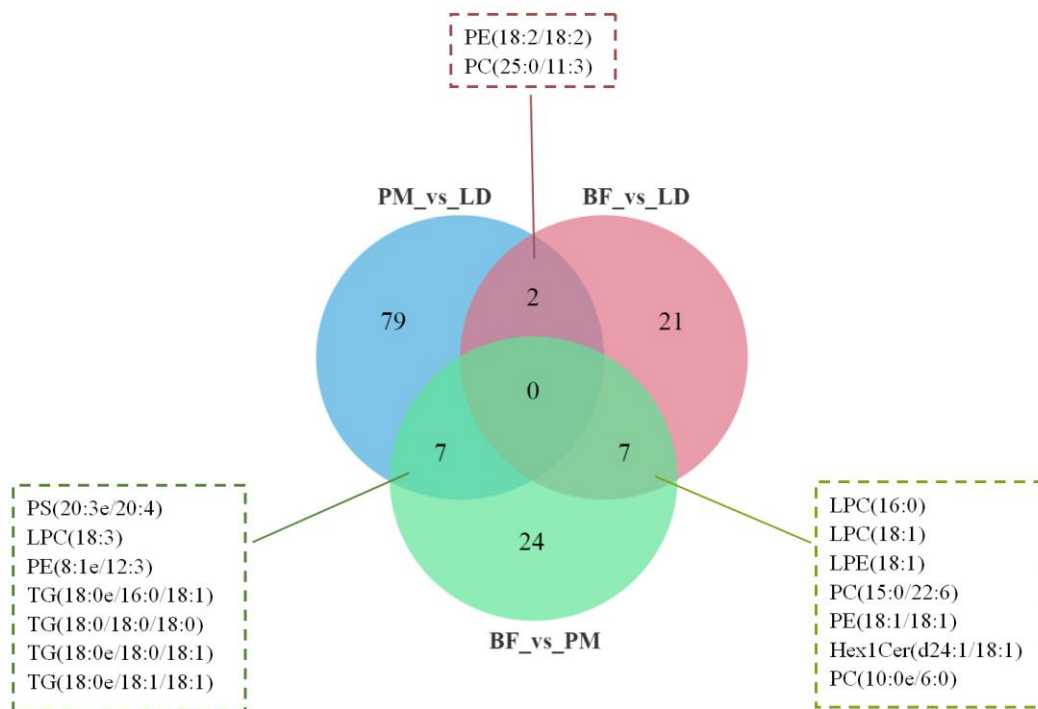
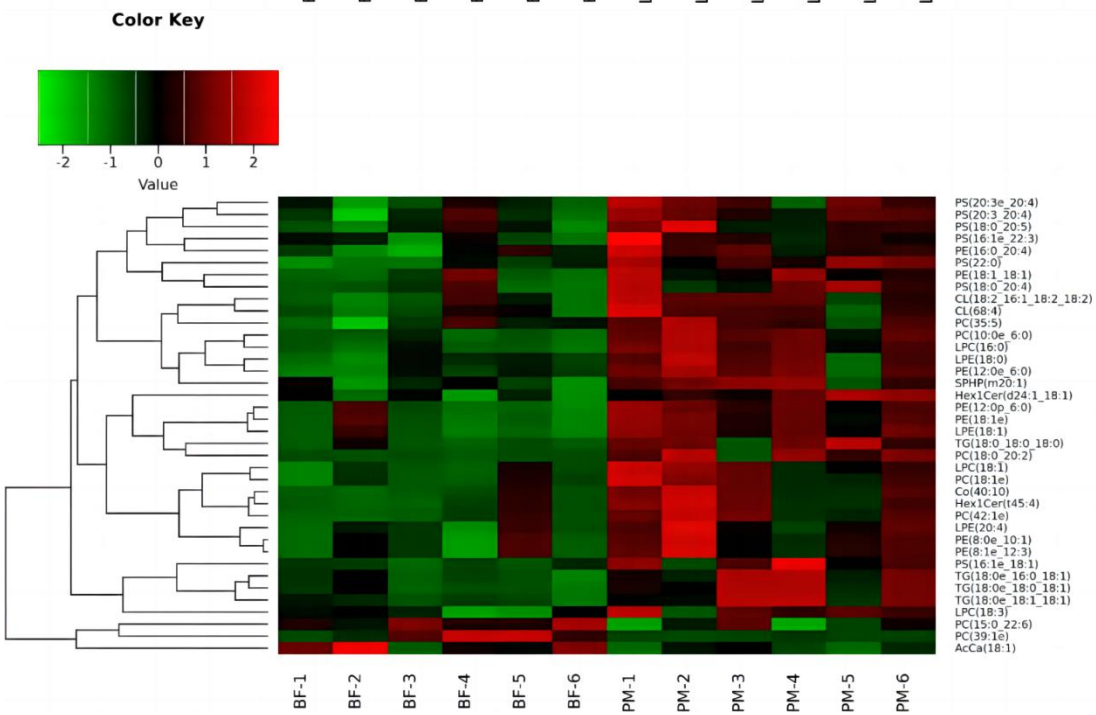
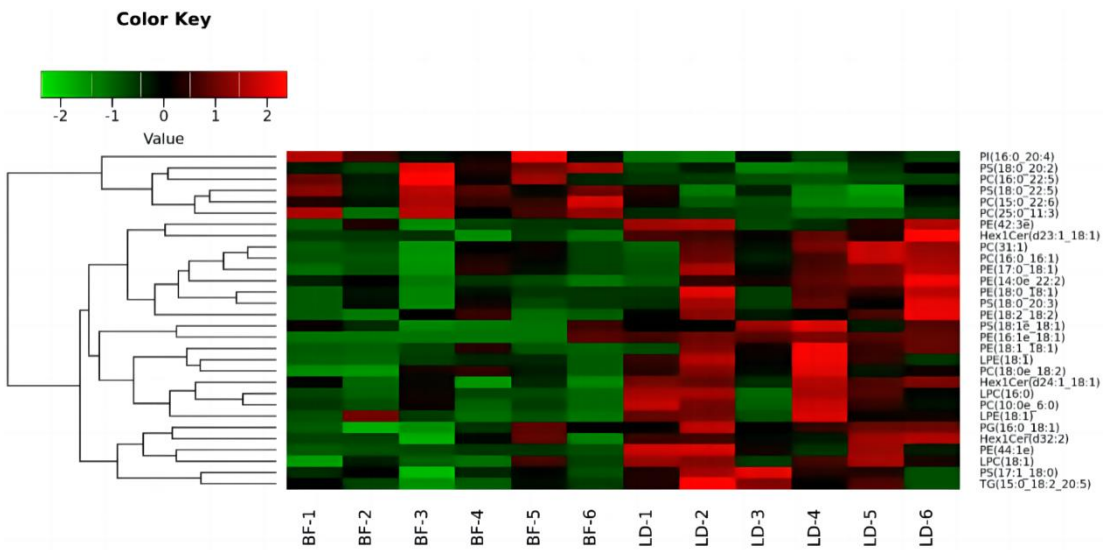


Figure 8 The Venn diagram of differential lipid molecules in different parts of Chaka sheep



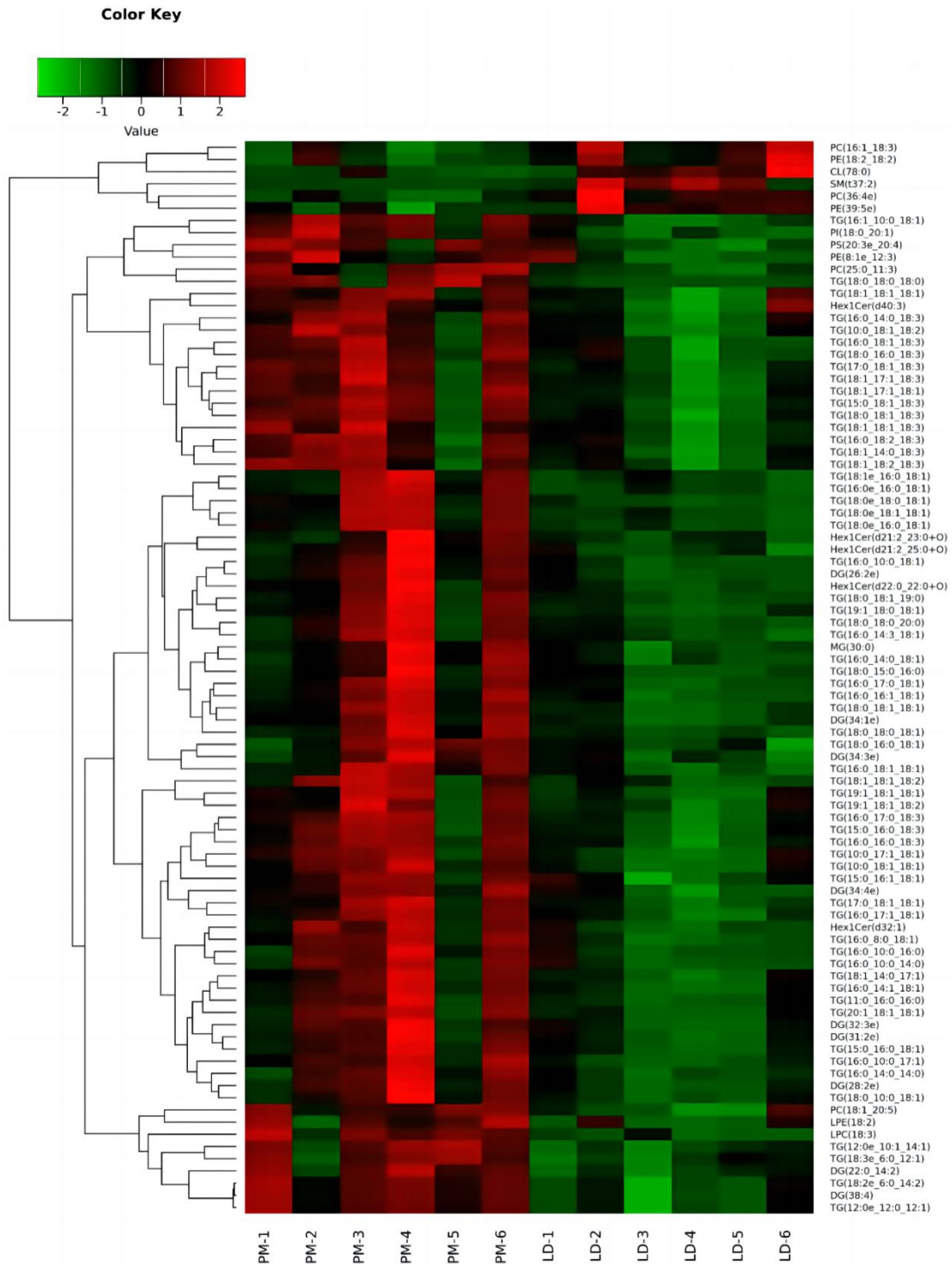
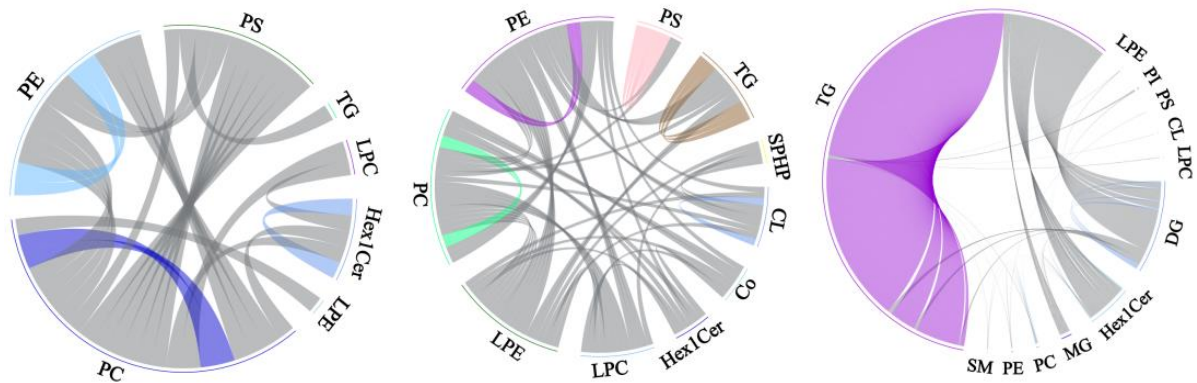


Figure 9 The heat map for clustering analysis of differential lipid molecules



BF vs. LD

BF vs. PM

PM vs. LD

Figure 10 The plot of the differential lipid molecule correlation analysis

ACCEPTED

Supplementary Table S1. The detailed results of differential lipid molecules in BF vs. PM, PM vs. LD and LD vs. BF (OPLS-DA VIP > 1 and *P*-value < 0.05)

Lipid molecules	Formula	RT (min)	CalMz	FC	<i>P</i> -value	VIP
BF vs. PM						
PC(16:0/16:1)	C39H75O8N1P1	9.968	716.523	0.662	0.045	2.270
PC(18:0e/18:2)	C45H87O9N1P1	10.409	816.612	0.746	0.048	1.501
PS(17:1/18:0)	C41H77O10N1P1	9.148	774.529	0.732	0.041	1.104
PS(18:1e/18:1)	C42H79O9N1P1	10.179	772.550	0.559	0.034	1.618
PS(18:0/20:3)	C44H79O10N1P1	11.100	812.545	0.641	0.044	2.432
PS(18:0/22:5)	C46H79O10N1P1	8.687	836.545	1.339	0.013	1.735
Hex1Cer(d32:2)	C39H72O10N1	9.059	714.516	0.720	0.035	1.026
LPC(18:1)	C27H53O9N1P1	1.947	566.346	0.725	0.013	2.167
LPE(18:1)	C23H45O7N1P1	2.076	478.294	0.759	0.048	1.565

PC(15:0/22:6)	C46H79O10N1P1	8.687	836.545	1.362	0.005	1.350
PC(16:0/22:5)	C47H83O10N1P1	9.178	852.576	7.202	0.012	5.603
PE(17:0/18:1)	C40H77O8N1P1	10.536	730.539	0.626	0.025	1.427
PE(18:0/18:1)	C41H79O8N1P1	11.102	744.555	0.582	0.026	5.759
PE(18:1/18:1)	C41H77O8N1P1	10.071	742.539	0.563	0.016	4.221
PE(18:2/18:2)	C41H73O8N1P1	8.340	738.508	0.556	0.031	1.036
PG(16:0/18:1)	C40H76O10N0P1	8.629	747.518	0.766	0.017	1.757
PI(16:0/20:4)	C45H78O13N0P1	7.339	857.519	1.694	0.009	1.004
PC(25:0/11:3)	C44H82O8N1P1Na1	7.805	806.567	2.653	0.014	1.789
PE(16:1e/18:1)	C39 H76 O7 N1 P1 Na1	9.657	724.525	0.168	0.001	7.808
PC(31:1)	C39H77O8N1P1	9.991	718.538	0.611	0.028	1.011
			PM vs. LD			
PC(36:4e)	C44H83O7N1P1	8.422	768.590	0.633	0.012	1.587

PE(8:1e/12:3)	C25H45O7N1P1	1.619	502.293	1.362	0.034	1.029
SM(t37:2)	C42H84O7N2P1	9.646	759.601	0.074	0.009	1.085
TG(12:0e/12:0/12:1)	C39H74O5Na1	12.953	645.542	1.558	0.002	1.068
TG(12:0e/10:1/14:1)	C39H72O5Na1	11.939	643.527	1.902	0.002	1.076
TG(18:3e/6:0/12:1)	C39H69O5	11.87	617.514	1.571	0.014	1.062
TG(18:2e/6:0/14:2)	C41H73O5	12.953	645.545	1.565	0.002	1.076
TG(16:0/10:0/14:0)	C43H86O6N1	5.09	712.644	1.952	0.019	2.263
TG(16:0/10:0/16:0)	C45H90O6N1	15.534	740.676	1.937	0.016	2.889
DG(26:2e)	C29H55O4	15.540	467.409	2.264	0.010	1.196
DG(28:2e)	C31H59O4	16.028	495.441	1.908	0.020	1.048
DG(32:3e)	C35H65O4	16.019	549.488	1.725	0.036	1.026
DG(34:1e)	C37H72O4Na1	17.019	603.532	1.554	0.006	1.593
DG(34:3e)	C37H69O4	16.701	577.519	1.244	0.031	1.304

DG(34:4e)	C37H67O4	16.474	575.503	1.396	0.009	1.416
DG(22:0/14:2)	C39H76O5N1	11.938	638.572	1.815	0.004	1.149
Hex1Cer(d32:1)	C38H77O8N2	14.177	689.567	2.172	0.009	1.385
Hex1Cer(d40:3)	C46H89O8N2	15.556	797.661	1.364	0.027	1.589
Hex1Cer(d22:0/22:0+O)	C50H103O9N2	14.798	875.765	2.123	0.028	1.398
Hex1Cer(d21:2_23:0+O)	C50H99O9N2	15.103	871.734	1.828	0.047	1.624
LD vs. BF						
CL(18:2/16:1/18:2/18:2)	C79H139O17P2	14.809	1421.949	1.742	0.017	4.765
CL(18:3/18:2/16:1/18:2)	C79H137O17P2	14.308	1419.933	0.891	0.026	3.343
LPC(16:0)	C25H51O9N1P1	1.957	540.330	1.341	0.001	1.106
PE(18:0/18:1)	C41H79O8N1P1	11.101	744.554	1.172	0.036	3.378
PI(16:0/20:4)	C45H78O13N0P1	7.338	857.518	0.986	0.011	2.607
PI(18:0/20:1)	C47H88O13N0P1	10.444	891.596	0.685	0.024	1.072

Co(Q10)	C59H94O4N1	15.460	880.717	1.639	0.032	5.571
PI(18:0/22:1)	C49H92O13N0P1	11.412	919.628	2.315	0.022	1.427
PI(18:0/24:1)	C51H96O13N0P1	12.370	947.659	0.779	0.047	2.607
PI(18:0/17:0)	C44H84O13N0P1	9.691	851.565	0.985	0.012	1.072
CL(18:3/16:1/20:4/16:1)	C79H135O17P2	13.773	1417.918	1.441	0.042	3.421
CL(18:3/18:2/20:3/15:0)	C80H139O17P2	14.653	1433.949	1.726	0.037	1.025
PI(18:0/23:1)	C50H94O13N0P1	11.887	933.643	1.662	0.008	3.915
CL(82:4)	C91H171O17P2	10.787	1598.198	1.448	0.049	1.103
CL(82:6)	C91H167O17P2	9.771	1594.167	2.567	0.025	2.341
SM(t36:0)	C41H86O7N2P1	8.975	749.616	2.317	0.012	1.892
SM(t37:2)	C42H84O7N2P1	9.646	759.601	1.809	0.005	5.351
SM(t38:4)	C43H82O7N2P1	8.722	769.585	1.646	0.016	1.037
SM(t38:5)	C43H80O7N2P1	9.45	767.569	0.985	0.033	4.647

SM(t41:6)

C46H84O7N2P1

9.646

807.601

1.758

0.032

1.327

RT, retention time; FC, Fold Change; VIP, variable importance in projection

ACCEPTED

ACCEPTED