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(Unstructured) Abstract (up to 350 words)

Lamb meat is consumed in various cultures worldwide, with lipids contributing to different attributes, including appearance, color, flavor, and tenderness. However, the impacts of various feeding regimes on fatty acid (FA) composition within lamb meat are not well elucidated. Hence, this study was aimed at elucidating the impacts of different feeding regimes—grazing (G), semi-grazing (SG), and stall feeding (SF) systems—on FA composition in the *longissimus dorsi* muscle (LDM) of Tibetan sheep by analyzing lipid profile and related pathways through untargeted lipidomics. Notably, LDMs of lambs under G and SG systems exhibited higher beneficial n-3 polyunsaturated FAs (PUFA) contents and more favorable n-6/n-3 and PUFA/saturated FA ratios than LDMs of lambs under the SF system ($p < 0.05$). Furthermore, 16 lipids closely associated with significant FAs were further analyzed ($p < 0.05$). Notably, C18:3n3 and c9t11-conjugated linoleic acid showed positive relation to ceramide (d18:1/25:0), and C20:5n3 positively correlated with phosphatidylcholine (16:0/14:0), phosphatidylserine (18:0/22:6), and sphingomyelin (d18:1/18:0) ($p < 0.05$). According to Kyoto Encyclopedia of Genes and Genomes analysis, n-3 essential FAs were closely linked to sphingolipids, glycerophospholipids, linoleic acid, glycerolipids, and alpha-linolenic acid metabolisms ($p < 0.05$). Altogether, the findings in this study highlight the metabolic mechanisms underlying diet-mediated changes in the FA compositions of LDM and identify specific lipid biomarkers associated with essential FAs, suggesting their potential in future studies and practical applications in the meat production industry.

Keywords (3 to 6):

n-3 essential fatty acids, Feeding regimes, Lipid profile, *Longissimus dorsi* muscle, Tibetan lamb

Introduction

Lamb meat is a part of the dietary practices of various cultures, and owing to its unique flavor, palatability, and nutritional value, it is widely consumed worldwide. Recently, an increase in lamb

meat consumption because of economic development-driven enhancement of living standards has notably heightened the interest of consumers in the quality of lamb meat [1]. Fatty acid (FA) composition considerably affects the lamb meat quality and has substantial implications for human health, particularly because of saturated FA (SFA)–unsaturated FA (UFA) balance and the presence of essential FAs such as omega-3 and -6 FAs, which are vital for cardiometabolic health, anti-inflammatory effects, and overall well-being [2]. Furthermore, ruminant-produced products such as raw milk or meat can provide FAs that are not synthesized endogenously in humans, such as conjugated linoleic acid (CLA), which exhibits numerous potential health benefits, including reduction of body fat deposits, enhanced immunity, and the prevention of asthma, various cancer types, and cardiovascular diseases [3].

Lipids are essential constituents of meat and notably influence consumer acceptance by contributing to various desirable attributes, including appearance, color, flavor, and tenderness [4]. Lipase-catalyzed hydrolysis breaks down lipids into free FAs and glycerol [5], with lipids serving as the primary source of both SFAs and UFAs in the meat. Lipids can be divided into the following eight classes: fatty acyls (FAC), glycerides (GL), glycerophospholipids (GPs), sterol lipids (ST), sphingolipids (SP), saccharolipids, prenol lipids, and polyketides [6]. Factors such as breed, diet, and feeding regimes can alter FA composition of ruminant meat, and therefore, its lipid profile. Lipidomics, a subdiscipline of metabolomics, is the comprehensive analysis of lipids and lipid-like molecules across different classes under specific conditions and has garnered considerable attention in nutrition and food research [7-9]. Lipidomics-based analysis of lipid compositions under specific treatments is both feasible and scientifically valid and can provide insights into the effect of different treatments on FA profiles.

Overgrazing causes a pronounced degradation of the environmental integrity of grasslands [10], and the Chinese government has performed the "Control Grazing for Grassland Recovery" policy to alleviate this degradation and introduced semi-grazing (SG) and stall feeding (SF) systems [11]. The impacts of different feeding strategies on FA composition in lamb meat have been reported. For instance, the traditional grazing (G) system generally increases the levels of beneficial UFAs, whereas concentrated feeding increases that of SFAs [12,13]. Tibetan sheep (>50 million) are among the three

primordial sheep populations in China and widely distributed in the Qinghai–Tibetan Plateau, including Qinghai, Tibet, and Gannan of Gansu [14], and being the predominant livestock species, they substantially affect the livelihoods of Tibetan herders [15]. Zhang et al. [16] indicated that n-3 poly-UFAs (PUFAs) were markedly increased in meat samples of Tibetan sheep under the traditional G system compared with those reared in SF regimes.

However, lipidomics-based studies on FA composition of Tibetan lamb meat reared under various feeding regimes are lacking. As a result, this study was aimed at elucidating the impacts of different feeding regimes on FA composition in lamb meat by employing lipidomics and identifying specific lipids and associated pathways that can serve as biomarkers for significant FAs. It was hypothesized that sheep subjected to the G regime would present a more advantageous FA profile, with increased UFA content, compared to those under a concentrated feeding regime. Results in this study may contribute to the advancement of meat science, facilitating the optimization of feeding strategies to enhance the FA profiles of the ovine meat by regulating relevant lipids and pathways, thereby improving meat quality and promoting superior health outcomes.

Materials and Methods

Experimental design and sample collection

In the present research, the Animal Ethics Committee of the Chinese Academy of Agricultural Sciences approved our animal welfare and experimental procedure (No. AEC-CAAS-1610332022011). Gangba sheep, as a famous breed of Tibetan sheep, are an important source of food and income for the local residents. Therefore, improving Gangba sheep meat quality is essential for the improvement of human health and economic growth. Herein, 27 male Gangba lambs (3 months old, the initial body weight of the experiment), with an identical genetic background and similar weight (14.49 ± 0.02 kg; mean ± standard error of the mean), were randomly classified into the following groups: G (n = 9), SG (n = 9), and SF (n = 9). All lambs were raised in separate units (1.5 m × 2.0 m), and those in G group grazed (08:00–18:00) at the desertification grassland in the Gangba county, Tibetan Autonomous Region, China (88°8′20″–88°56′47″E, 27°56′32″–28°45′27″N). This

region is abundant in *Artemisia minor Jacquem*.ex Besser, *Iris collettii* Hook.f., *Festuca wallichanica* E. Alexeev, *Kobresia capillifolia* (*Decne.*) C. B. Clarke, and *Kobresia deasgi* C. B. Clarke. SG lambs grazed the same steppe from 10:00 to 15:00h, and were supplied with 400–450g of a mixed pellet feed and free access to oat hay when off-pasture, and 800–900g of the same mixed pellet feed as the SG was given to lambs in the SF group and free access to oat hay (twice/day at 08:00 and 18:00 for both oat hay and pellets). The supplemented oat hay was adjusted daily based on the previous day's intake, allowing refusals of 20%. Concentrate supplementation was increased in the SG and SF groups as body weight of experimental lambs increased, and the ratio of concentrate supplementation of lambs between the SG and SF groups was consistently maintained at 1:2. Tables 1 and 2 illustrate the ingredient composition of the basal diet and the nutrition of the basal diet and pasture, respectively. All animals were allowed to take water and food freely through the experimental duration of 95 days, which included a 15-day adaptation period and an 80-day experimental period. After the experiment was over, six Gangba lambs were randomly chosen from each group following 12 h of fasting. Thereafter, animal slaughter was completed by professionals at the local commercial abattoir. Immediately following slaughter, *longissimus dorsi* muscle (LDM) samples were harvested from from 11th and 12th ribs and promptly preserved within liquid nitrogen for further analysis.

FA analyses of LDM and feed

After collection, the pasture samples were quickly brought back to the laboratory, heated in an oven at 105°C for 2 hours to stop enzymatic activity, and then dried to a constant weight at 65°C for the determination of pasture fatty acid composition. The chloroform–methanol mixture (2:1, v/v) was adopted to extract total lipids from 1.0 g of lyophilized LDM and 2.0 g of dried feed powder (pasture and concentrate), as described by Folch et al. [17]. Subsequently, acid (5% methanolic HCl) or base (0.5 N sodium methoxide) catalysis was conducted to methylate lipid aliquots, as described by Kramer et al. [18]. Henedecanoic acid methyl ester (1 mg/mL; 1 mL, 11:0) served as the endogenous reference. The resultant FA methyl esters (FAMEs) were analyzed using the Varian 450-GC gas chromatograph (Varian Chromatography Systems, Walnut Creek, CA, USA) with the flame-ionization detector and fused silica capillary column (SP-2560; length, 100 m; film thickness, 0.20 μm; internal diameter, 0.25 mm; Supelco Inc., Bellefonte, PA, USA). Both detector and injector

temperatures were consistently kept under 260°C. Initially, we set oven temperature at 120°C for a 5-min duration, later elevated it to 230°C at 3°C/min, where it was held for another 3 min, and finally elevated it to 240°C at 1.5°C/min and held it for 13 min. Nitrogen at 1 mL/min was the carrier gas. In addition, 1 μL of sample was injected using the automated split injector configured at the 1:30 split ratio. Each FAME was detected by comparing obtained retention times (RTs) with authentic standards, including a FAME mix (Supelco Inc.) and c9, t11-CLA (Larodan Fine Chemicals, AB, Sweden). The quantification was performed as depicted by Vahmani et al. [19]. FA concentrations (mg/100 g dry matter) were determined by analyzing the peak areas in the chromatograms and converting them using the appropriate conversion factors for each FAME.

Lipidomics

Lipid extraction

In terms of lipid extraction, solid samples (50 mg) were taken in the 2-mL plastic microtubes and added methanol:water (2:5, v/v) mixture (280 μL) and methyl tertiary butyl ether (400 μL). Before extraction, all samples were homogenized at −10℃ using 6-mm grinding beads and the high-throughput tissue grinder (Wonbio-96c, Shanghai Wonbio Technology Co., LTD) at 50 Hz for a 6-min duration, prior to sonication for 30 min at 40 kHz and 5℃. Following standing at −20℃ for 20 min, samples were subject to centrifugation at 13000 ×g and 4℃ for 15 min. Each lipid extract from the upper phase (350 μL) was moved to a new tube for evaporation till dryness under a nitrogen atmosphere. For ultra-high-pressure liquid chromatography (UHPLC)-tandem mass spectrometry (MS/MS) analysis, samples were reconstituted into an isopropanol (IPA):acetonitrile (ACN) loading solution (100 μL, 1:1, v/v) before 5 min of sonication at 40 kHz while positioned in a 5°C-water bath. Finally, the extracts were subject to 10 min of centrifugation at 13000 ×g and 4°C with the bench-top centrifuge to collect supernatants into sample vials. Finally, samples (2 μL each) were loaded for UHPLC-MS/MS analysis.

Quality control (QC) sample

Samples at equivalent amounts (20 μL) were mixed to prepare the combined QC sample for QC and system conditioning. Notably, this QC sample was subjected to the same analyses as all tested samples, and it was injected regularly (every 10 samples) to monitor the analysis stability.

UHPLC-MS/MS technology

UHPLC-MS/MS technology was performed with the Thermo UHPLC-Q Exactive HF-X Vanquish Horizon system and Accucore C30 column (100 mm × 2.1 mm i.d., 2.6 μm; Thermo, USA) by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). Mobile phases contained ammonium acetate (10 mM) within ACN:H2O (1:1, v/v; 0.1% [v/v] formic acid; solvent A) and ammonium acetate (2 mM) within ACN:IPA:H2O (10:88:2, v/v/v; 0.02% [v/v] formic acid). A 5 μL reaction system was prepared under the parameters including column temperature, 40°C; flow rate, 0.4 mL/min; and chromatographic separation, 20 min. Additionally, the solvent gradient was defined as following: 35%–60% B, 0–4 min; 60%–85% B, 4–12 min; 85%–100% B, 12–15 min; 100% B, 15–17 min; 100%–35% B, 17–18 min; and 35% B after separation was completed. Every sample was preserved under 4°C throughout the analysis.

The Thermo UHPLC-Q-Exactive HF-X Bench-top Orbitrap Mass Spectrometer was used to obtain mass spectrometry data using the heated-electrospray ionization source under both positive- and negative-ion modes. Then, conditions were set below: Aus gas heater temperature, 370°C; Aus and sheath gas flow rates, 20 and 60 psi, respectively; ion-spray voltage floating, +3000 V and −3000 V under positive and negative modes, separately; normalized collision energy, 20–40–60 V rolling during MS/MS. Data were acquired using the Data Dependent Acquisition mode and detected within the 200–2000 mass-to-charge ratio (m/z) range.

Data pre-processing and interpretation

Following UPLC-MS/MS analysis, the raw data was inputted in LipidSearch (Thermo, CA) to detect, align, and identify the peaks. MS/MS fragments were adopted for identifying lipids. Mass tolerances for fragment and precursor were 10 ppm. Grades A–D were adopted in the ID quality filter, whereas the m-score threshold was 2.0. All pre-processing analyses produced the data matrix comprising peak intensity, lipid class, m/z, and RT.

The public web-based Majorbio cloud platform (cloud.majorbio.com) was adopted to analyze data. Lipidomic features measured from > 80% of each sample set were kept. Next, minimal metabolite levels were assigned for certain samples whose metabolite contents were less than the lower limit of quantitation, with all metabolic features being standardized through summation. For

reducing errors resulting from instrumental instability and sample preparation, sum normalization was completed to normalize response intensities of sample mass spectral peaks, thus, the normalized data matrix was acquired. Additionally, variables whose relative standard deviations were >30% of QC sample were eliminated. Meanwhile, log10 transformation was carried out to acquire the eventual data matrix in later analyses.

Statistical analyses

One-way analysis of variance (ANOVA) with the post hoc test of Games-Howell followed by bonferroni for multiple testing correction was used to analyze the difference of the FAs concentration and the lipids abundance in LDM within groups. The partial least square discriminant analysis (PLS-DA) of the lipidomic data obtained from LDM was conducted with 200 permutations using ropls package of the R software (Version 1.6.2). Correlations between different lipids and FAs were determined by Pearson correlation ($|r| > 0.6$; $P < 0.05$), and their associations were visualized using the Cytoscape software (Version 3.9.1). Additionally, the annotated lipidomic metabolites closely related to differential FAs in LDM were subjected to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis (www.kegg.jp/kegg/pathway). Considerably enriched KEGG pathways were screened through relative_betweenness centrality analyses, and differential abundance scores and KEGG pathway bubble maps were plotted. The network of annotated lipidomic metabolites closely related to differential FAs in LDM and corresponding pathways were visualized using Cytoscape software (Version 3.9.1).

Results

Comparison of FA concentrations in the diets and lamb muscle samples

Examination of FA concentrations in the feed (pasture and concentrate) of G, SG, and SF groups (Table 3) revealed that the C16:0 and C18:0 FA levels within the pasture decreased compared with those in the concentrate, and the C18:1n9c and C18:2n6c concentrations remarkably decreased within the concentrate. Conversely, the C18:3n3 level within the pasture substantially increased relative to that in the concentrate.

As indicated by the FA composition in LDM samples (Table 4), the dominant FAs in LDM of various groups were all C18:1n9c, C16:0, C18:0, C18:2n6c, and C14:0. The C14:0, C16:0, C17:0, C22:0, SFA, and n-6/n-3 concentrations within the LDM in G group considerably decreased relative to those in the SF group, whereas C18:3n3, C20:5n3, C22:6n3, n-3, and PUFA/SFA levels showed opposite results ($p < 0.05$). Furthermore, n-6/n-3 in G group apparently reduced relative to SG group ($p < 0.05$). C16:0, C17:0, SFA, and n-6/n-3 levels in the SG group substantially decreased compared with those in the SF group, whereas levels of c9t11-CLA, C22:6n3, and PUFA/SFA in the SG group tended to significantly increase relative to those in the SF group ($p < 0.05$).

Number and categories of detected lipids in the *longissimus dorsi* muscle

The lipids identified through different ion modes of UHPLC-MS/MS are listed in Table S1. In total, 1,538 raw lipid metabolites were detected in the LDM, with 951 under positive-ion and 587 under negative-ion modes. The origin data were obtained after filtering, filling the missing values, transforming, and normalizing the raw data, presenting 1,269 total lipid metabolites in the LDM, with 756 under positive-ion mode and 513 under negative-ion mode. These lipids were divided as FAC, glycerolipids (GL), GP, ST, and SP categories, and included 21 (1.65%), 314 (24.74%), 694 (54.69%), 233 (18.36%), and 7 (0.55%) lipids, respectively (Figs. 1A and 1B). Furthermore, four subclasses were identified in the FAC category (namely acylcarnitine, coenzyme, FA, and oacyl-[gamma-hydroxy]fa). Four subclasses were identified in the GL category (namely diglyceride (DG), monoglyceride, monogalactosyldiacylglycerol, and triglyceride (TG)). The GP category consisted 18 subclasses (namely bis-methyl phosphatidic acid, cardiolipin, lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysophosphatidylinositol, lysodimethylphosphatidylethanolamine, monolyso-cardiolipin, methyl phosphatidylcholine, phosphatidic acid, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylethanol, phosphatidylglycerol, phosphatidylinositol, phosphatidylmethanol, phosphatidylserine (PS), and dimethylphosphatidylethanolamine). The SP category consisted of nine subclasses (namely ceramides (Cer), N-acetylglucosamine monohexosyl ceramide, gangliosides, simple glc series monohexosylceramide, simple glc series dihexosylceramide, lysosphingomyelin, sphingomyelin (SM),

sphingosine, and sphingomyelin). Lastly, three subclasses were identified in the ST category (namely cholesterol ester, acylglcsitosterol ester, and acylglcstigmasterol ester) (Figs. 1A and 1B).

Differential lipids within groups

PLS-DA generated an overview of the similarities and differences within the lipid dataset, with score plots showing distinct lipid profiles in the LDM samples of the G, SG, and SF groups (Fig. 2A). In PLS-DA score plots, the first and second principal components interpreted 63.1% and 9.7% of overall variance, respectively, separately of the dataset, warranting the analysis of differences in lipid abundances within groups. Permutation testing was adopted for confirming the PLS-DA model (200 random permutations) and a Q2Y intercept value of <0.05 suggested the robustness and good predictability of the model for the lipidomics data (Fig. 2B). The identity of differential lipids within feeding regime groups was further validated through linear mixed models. Notably, 313 differential lipids were identified in LDM samples of all groups, including GP (177, 57%), GL (96, 31%), SP (36, 11%), FA (3, 1%), and ST (1, 0%) (Fig. 3A). Among them, 40 lipids in the LDM were annotated in the KEGG database and used for subsequent analysis (Fig. 3A and 3B).

Different feeding regimes considerably affected the lipids belonging to the GP, GL, and SP categories, and the various feeding regimes mainly affected the lipids in the GP category for LDM. Herein, the number of differential GP lipids (including PC, PS, and PE) accounted for 64% (LDM) of the total differentially expressed lipids. The most affected lipids within the GP category belonged to the PC subclass and accounted for 52% of the total differential lipid number within the LDM. Furthermore, DGs in the LDM accounted for up to 10% of the total differential lipids (Fig. 3A and 3B).

Notably, 40 significantly different lipids were detected in the LDM samples of groups (Fig. 4, bar charts). Detailed information is in Table S2. The abundances of PC(16:0/20:5), PC(18:3/20:5), PC(16:0/16:0), PC(15:0/18:1), PC(16:0/14:0), PC(15:0/16:0), PC(22:5/20:4), PC(20:1/20:4), PC(14:0/14:0), PC(20:0/16:0), PC(22:5/22:6), PC(18:0/18:0), PC(18:0/15:0) (Fig. 4A), PC(16:0/14:1), PC(15:0/16:1), PC(20:5/20:4), PC(14:0/20:4), PC(22:5/18:2), PC(18:4/16:0), PS(18:0/22:5), PS(18:0/18:1), PS(18:0/22:6) (Fig. 4C), DG(18:0/16:0), DG(18:0/20:1), DG(18:0/20:4), DG(16:0/20:4) (Fig. 4B), SM(d18:1/18:0), SM(d18:0/18:0), Cer(d18:1/22:0), Cer(d18:1/25:0), and

Cer(d18:1/20:0) (Fig. 4D) within LDM of the SG and SF groups, and that of SM(d18:1/12:0) (Fig. 5D) in the SG group markedly reduced relative to G group ($p < 0.05$). Furthermore, PC(18:1/18:1), PC(16:1/18:1), PE(18:0/20:0) (Fig. 5C), DG(16:0/18:1), DG(16:1/18:1), and TG(18:0/18:1/20:1) (Fig. 5B) in the SG and SF groups, PE (16:1/18:1) (Fig. 5C) in the SF group, and TG(18:0/18:1/20:0) (Fig. 5B) in the SG group showed evidently elevated abundances relative to G group ($p < 0.05$). Additionally, PC(18:1/18:1), PC(16:1/18:1), PE(16:1/18:1) (Fig. 5C), TG(18:0/18:1/20:1), DG(18:0/16:0) (Fig. 5B) and SM(d18:1/12:0) (Fig. 5D) levels markedly elevated in SF group relative to SG group ($p < 0.05$). Contrarily, PC(20:5/20:4), PC(14:0/20:4), PC(22:5/18:2), PC(18:4/16:0) (Fig. 5C), TG(18:0/18:1/20:0) (Fig. 5B), Cer(d18:1/22:0), and Cer(d18:1/25:0) exhibited opposite results (Fig. 5D) ($p < 0.05$).

Correlation analysis of differential lipids and FAs

The 40 differential lipids and various FA concentrations or indices previously mentioned were subjected to Pearson correlation analysis to identify significant lipids that could serve as biomarkers for key FA indices (Fig. 5). In total, the following 16 lipids were found to be significantly correlated with specific FAs: C22:0 was positively correlated to PC(22:5/22:6); C18:3n3 and c9t11-CLA were positively related to Cer(d18:1/25:0); C20:5n3 was positively correlated to PC(16:0/14:0), PS(18:0/22:6), and SM(d18:1/18:0); C22:6n3 showed a positive relationship to SM(d18:1/18:0), PC(18:3/20:5), PC(18:4/16:0), and PC(14:0/20:4); n-3 PUFA was positively correlated with PC(16:0/14:0), PC(16:0/20:5), SM(d18:1/18:0), and PC(18:3/20:5); n-6/n-3 exhibited negative relation with PC(20:5/20:4), PC(18:0/18:0), PC(16:0/14:0), PC(16:0/20:5), PS(18:0/22:6), SM(d18:1/18:0), PC(18:3/20:5), and PC(16:0/16:0), whereas it was positively correlated with PE(16:1/18:1), PC(18:1/18:1), and PC(16:1/18:1); and PUFA/SFA was positively related to PS(18:0/22:6), PC(18:3/20:5), and PC(18:4/16:0), whereas it showed negative relation to TG(18:0/18:1/20:1) (all $p < 0.05$).

Differential Kyoto Encyclopedia of Genes and Genomes pathways and mapped lipids

The 16 lipids that were closely related to FA concentrations were mapped to the KEGG database (http://www.kegg.jp/kegg/pathway.html). The bubble chart for KEGG topology for different lipids within the LDM across varying feeding regimes (Fig. 6A) showed that GP, SP, linoleic acid, GL, and

alpha-linolenic acid metabolisms were considerably affected by various feeding regimes. Figs. 6B, 6C, and 6D show the differential abundance score analysis of the KEGG pathways. The GP, linoleic acid, and alpha-linolenic acid metabolism pathways for SG versus G and SF versus G were downregulated, whereas the GL metabolism in SG versus G and SF versus G was upregulated. Additionally, the SP metabolism in SF versus G was downregulated. The GP, SP, GL, linoleic acid, and alpha-linolenic acid metabolisms in SF versus SG were downregulated. Notably, all lipids in the PC category were related to GP, linoleic acid, and alpha-linolenic acid metabolisms and those in the PE and PS category were mainly involved in the GP metabolism. Furthermore, lipids belonging to TG were only involved in the GL metabolism, those in the SM and Cer subclasses only participated in the SP metabolism (Fig. 7).

Discussion

Fatty acid composition in pasture and concentrate

FA compositions found in the pasture (fed under G and SG systems) and concentrate (fed under SG and SF systems) diets used in the current work are in consistent with the findings previously reported, indicating relatively consistent FA type and proportion patterns among these feeding regimes [13]. Herein, the pasture diet contained lower concentrations of SFAs, specifically C16:0 and C18:0, compared with those in the concentrate diet. Similarly, Nuernberg et al. [20] reported that grass-based diets contain lower SFA content than that in concentrate diets. The concentrate diet presented higher levels of C18:1n9c and C18:2n6c, conforming to the findings of Yang et al. [13]. Additionally, the characteristic high concentration of C18:3n3, an essential omega-3 FA, was found in the pasture diet, as reported previously [13,20]. The high levels of C18:3n3 in the pasture diet caused a more favorable n-6/n-3 ratio, causing more health benefits for animals.

FA composition in LDM under various feeding regimes

FA composition in LDM in lambs fed through different feeding regimes underscores the substantial impact of the diet on nutritional quality. Predominant FAs identified across all experimental groups included C18:1n9c, C16:0, C18:0, C18:2n6c, and C14:0, exhibiting similarity to the characteristic FA

profiles observed in ruminant muscle tissues [12]. Herein, the predominant FA in lamb muscle was C18:1n9c, constituting approximately 40% of the total FAs, consistent with the findings of Wang et al. [12] and Nuernberg et al. [20]. Notably, different feeding regimes mainly affected the concentrations of FAs and did not alter their composition. The levels of C14:0, C16:0, C17:0, C22:0, and total SFA in G group notably decreased relative to SG and SF groups, conforming to the findings of Nuernberg et al. [20] and Wang et al. [12], who reported low SFA content in the muscle of grass-based diet-fed lambs. The biohydrogenation process within the rumen, which is influenced by the dietary composition, exerts a crucial role in identifying the nutritional outcome. PUFA-rich pasture diets can lead to less extensive hydrogenation than concentrate-based diets, further resulting in lower SFA levels within the muscle tissue. Consistent with the findings of higher omega-3 content in the sheep fed with pasture diet than that in the concentrate diet [13], herein, omega-3 FAs, including C18:3n3, C20:5n3, and C22:6n3, presented markedly increased concentrations in the G group, underscoring the nutritional benefits of the pasture-based diet. This may be attributed to the microbial activity-driven biohydrogenation process within the rumen. In pasture-based diets, the microbial ecosystem of the rumen downregulates the hydrogenation of PUFA, particularly C18:3n3, resulting in their higher concentrations for absorption and subsequent incorporation into muscle tissues. Furthermore, pasture-based diets are abundant in secondary metabolites, including flavonoids, polysaccharides, tannins, and polyphenols, which considerably affect the microbial activity within the rumen, potentially inhibiting the biohydrogenation process, and thereby preserving the more significant proportion of PUFA from converting into less desirable SFA [21]. The increased omega-3 FA concentrations and decreased n-6/n-3 ratio observed in the G group were particularly significant, as the decreased n-6/n-3 ratio is associated with anti-inflammatory effects and improved overall health outcomes [22]. The c9t11-CLA content in the SG group tended to significantly increase compared with that in the SF group, indicating the partial biohydrogenation of C18:2n6c within the rumen. The equilibrium between forage and concentrate possibly created favorable conditions in the SG group for CLA synthesis, a FA recognized for its health benefits, including anti-carcinogenic properties [3]. The World Health Organization (WHO) recommends the n-6/n-3 ratio to be below 5:1 [23], and that in the G and SG groups were within the recommended limit, presenting a balanced consumption of both omega-6 and

omega-3 FAs. According to the WHO and other health authorities, the recommended PUFA/SFA ratio for the normal diet should be >0.4 [24]. Although the G group presented the highest PUFA/SFA (0.24) among all groups, it still does not meet the recommended standards, warranting further optimization of basal diets to meet dietary guidelines. Overall, the G and SG feeding regimes could improve the nutritional quality of lamb meat, making it a healthier option for consumers.

Differential lipids analysis in the LDM under different feeding regimes

Based on chemical structures and biosynthetic pathways, lipids are classified into eight categories by Lipid Metabolites and Pathways Strategy Consortium, namely FA, GL, GP, SP, ST, prenol lipids, polyketides, and saccharolipids [25]. Li et al. [26] detected FA, SP, GP, and GL lipids in the psoas major muscle of castrated and intact Hu sheep; however, unlike the findings of the present study, they did not detect ST lipids. Furthermore, herein, GP lipids exhibited the highest relative response (54.69%), followed by GL (24.74%), in the LDM of the Gangba lambs, and Li et al. [26] reported GL (58.96%) as the highest, followed by GP (23.78%). These differences were attributed to the difference in the animal breeds (Hu sheep versus Gangba sheep).

The dietary FA composition considerably affects the FA profile in lipids. Herein, differential 40 lipids affected by various feeding regimes were categorized into GP, GL, and SP categories. The most affected subclass was the PC subclass, which contained n-3 beneficial FAs (C18:4, eicosapentaenoic acid [EPA] [C20:5n3] and docosahexaenoic acid [C22:6]), such as PC(18:4/16:0), PC(16:0/20:5), PC(18:3/20:5), PC(20:5/20:4), and PC(22:5/22:6). Notably, they showed the highest abundance in the LDM of the G group, possibly because of the production of beneficial PUFAs through lipolysis during the early post-mortem period. Additionally, PS and DG subclasses lipids contained certain PUFAs such as C22:5, C22:6, and C20:4. Conversely, FAs in PE, TG, SM, and Cer subclasses were SFAs and mono-UFAs (MUFAs), which contributed to increased contents of free SFAs and MUFAs after lipidolysis. The close relations among these significant lipids and differential FAs in LDM were revealed by correlation analysis to identify the closely related lipids.

Lipids closely related to significant FAs

The correlation analysis between differential lipids and FAs revealed significant interactions, providing insights into associated complex metabolic processes of FAs in LDM under different

feeding regimes. In total, 16 lipids were identified to be significantly correlated with specific FAs, underscoring the intricate relationship between lipid metabolism and FA composition of the lamb meat. The positive correlation between C22:0 and PC(22:5/22:6) suggested that PC-related lipids may serve as biomarkers for the accumulation of long-chain SFAs in muscle tissues. Notably, this relationship highlights the role of PC-related lipids in regulating membrane fluidity and stability, which are critical factors for meat quality [16]. Additionally, the positive correlations of C18:3n3 and c9t11-CLA with Cer(d18:1/25:0) highlighted the importance of Cer metabolism in regulating essential FAs. Cer exerts crucial effects on cellular signaling and apoptosis, and their association with beneficial FAs such as CLA suggests potential pathways associated with dietary intervention-mediated enhancement of meat quality [27]. Notably, EPA positively correlated with multiple lipids, including PC(16:0/14:0), PS(18:0/22:6), and SM(d18:1/18:0). These findings suggest a robust interaction of n-3 PUFA with specific phospholipids, which may facilitate their anti-inflammatory properties. Moreover, SM involvement in these correlations underscores the role of SPs in modulating immune responses and maintaining cellular homeostasis. The n-6/n-3 ratio was negatively related to many PC species, indicating the association of an increased n-6/n-3 ratio to pro-inflammatory states, which may be counteracted by specific phospholipids. This finding aligns with those of previous studies indicating that a balanced n-6/n-3 ratio is crucial for optimal health and meat quality. The positive correlations between the PUFA/SFA ratio and lipids such as PS(18:0/22:6) and PC(18:3/20:5) highlighted the potential of these lipids as indicators of a healthier FA profile, which is desirable in meat production. Conversely, the negative correlation of TG(18:0/18:1/20:1) indicated that TGs might be less favorable in producing a beneficial PUFA/SFA ratio in meat.

The KEGG pathway analysis further elucidated the underlying mechanisms of diverse feeding regimes that affected lipid metabolism. The downregulation of GP, SP, linoleic acid, and alpha-linolenic acid metabolisms in the SG versus G group and SF versus G group comparisons suggested that more intensive feeding practices may suppress essential FA metabolism pathways, implicating the meat quality, particularly regarding the associated health benefits. Interestingly, the upregulation of GL metabolism in the abovementioned comparisons indicated a shift toward GL synthesis, which may affect the overall lipid profile and energy storage in muscle tissues. The exclusive involvement of

PC lipids in GP, linoleic acid, and alpha-linolenic acid metabolisms and the participation of SM and Cer in SP metabolism indicate a mechanistic link among lipids, the regulation of associated metabolic pathways, and FAs concentrations that regulates the FA composition and quality in meat. Similar to the finding of this study, Xiong et al. [8] also found linoleic acid, and alpha-linolenic acid metabolisms were significantly affected by feeding regimes (grazed feeding vs stall feeding).

Altogether, the significant correlations of specific lipids and FAs with differential modulation of KEGG pathways underscore the intricate interplay between diet, lipid metabolism, and meat nutrition quality. Based on these findings, the associated lipids may be utilized as potential biomarkers for optimizing lamb meat production through targeted dietary strategies to enhance the nutritional value and sensory attributes of the meat product.

Conclusion

Overall, the present study elucidates the significant impact of different feeding regimes on FA compositions and lipid metabolisms in lamb muscles, underscoring their importance regarding the meat nutritional quality. Lambs in the G and SG groups exhibited higher levels of beneficial n-3 PUFA and more favorable PUFA/SFA and n-6/n-3 ratios than those in the SF group. Untargeted lipidomics analysis revealed 16 lipids that are closely associated with beneficial FAs, showing positive correlations among different lipids such as Cer(d18:1/25:0), C18:3n3, and c9t11-CLA and PC(16:0/14:0), PS(18:0/22:6), SM(d18:1/18:0), and C20:5n3. Notably, C22:6n3 was strongly associated with SM(d18:1/18:0), PC(18:3/20:5), PC(18:4/16:0), and PC(14:0/20:4), and the n-3 essential FAs were closely linked to GP, SP, linoleic acid, alpha-linolenic acid, and GL metabolic pathways. In contrast, c9t11-CLA was specifically associated with SP metabolism. Altogether, the results of this study highlight the metabolic mechanisms underlying diet-mediated changes in the FA composition and provide potential biomarkers for optimizing feeding strategies in order to enhance the nutritional quality of the lamb meat. However, metabolomic and transcriptomic analyses of muscle tissues are recommended for future studies, as they would allow us to investigate the gene expression related to fatty acid metabolism under different feeding regimes at the molecular level.

Acknowledgments

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Tables and figures

Fig. 1. Lipids in the *longissimus dorsi* muscle of Gangba lambs under three feeding regimes. (A) Proportions of different lipid categories and subclasses. (B) Percentage composition of different lipid categories and subclasses. FAC, fatty acyls; GL, glycerolipids; GP, glycerophospholipid; SP, sphingolipid; ST, sterol lipid; AcCa, acyl carnitine; Co, coenzyme; FA, fatty acid; OAHFA, oacyl-(gamma-hydroxy)fa; DG, diglyceride; MG, monoglyceride; MGDG, monogalactosyldiacylglycerol; TG, triglyceride; BisMePA, bis-methyl phosphatidic acid; CL, cardiolipin; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPG, lysophosphatidylglycerol; LPI, lysophosphatidylinositol; LdMePE, lysodimethylphosphatidylethanolamine; MLCL, monolyso-

cardiolipin; MePC, methyl phosphatidylcholine; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEt, phosphatidylethanol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PMe, phosphatidylmethanol; PS, phosphatidylserine; dMePE, dimethylphosphatidylethanolamine; Cer, ceramide; CerG2GNAc1, N-acetylglucosamine monohexosyl ceramide; GM3, ganglioside; Hex1Cer, simple glc series monohexosylceramide; Hex2Cer, simple glc series dihexosylceramide; LSM, lysosphingomyelin; SM, sphingomyelin; SPH, sphingosine; phSM, sphingomyelin (phytosphingosine); ChE, cholesterol ester; AcHexSiE, acylglcsitosterol ester; AcHexStE, acylglcstigmasterol ester.

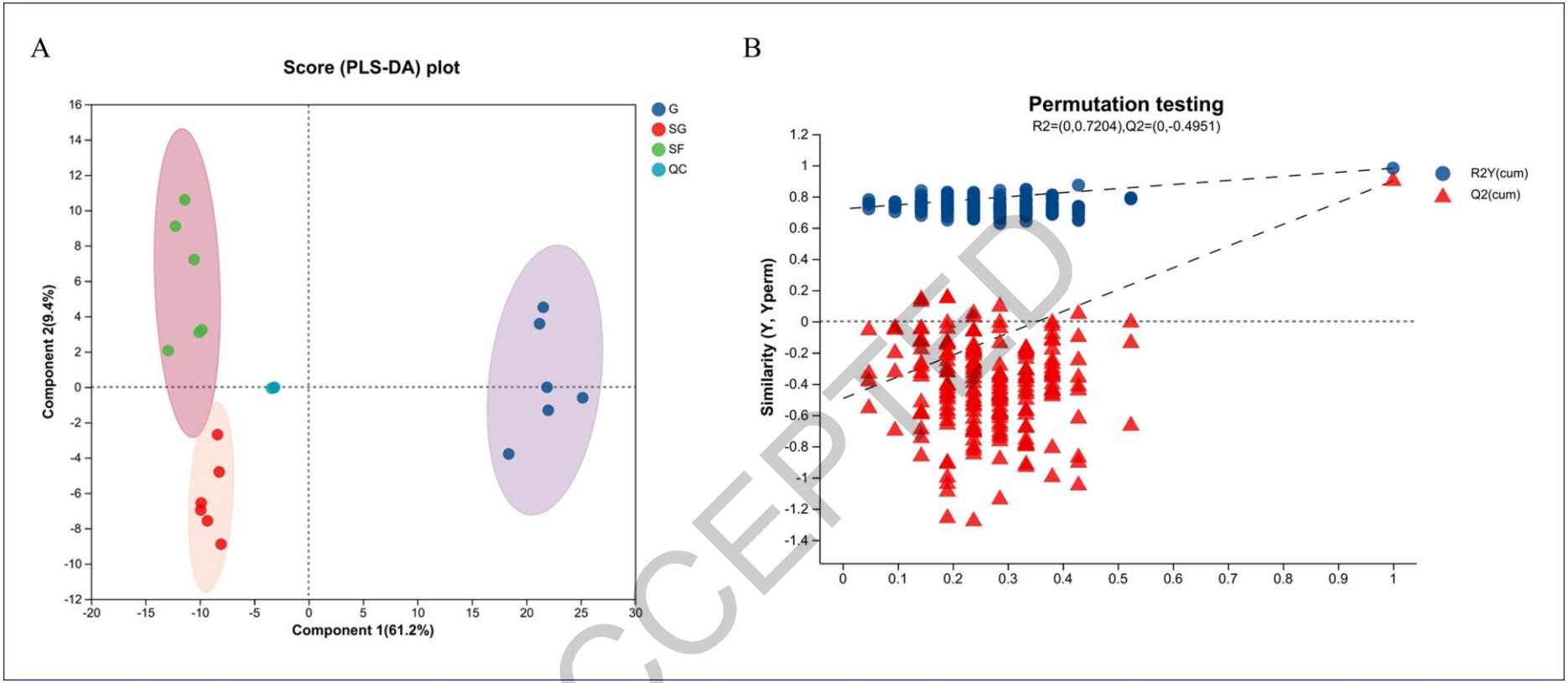

Fig. 2. Multivariate statistical analysis of lipids in the *longissimus dorsi* muscle of Gangba lambs within different feeding regimes. (A) The plot of partial least square discriminant analysis (PLS-DA) scores for different feeding regimes. (B) The Q2Y-intercept in the permutation test indicating the good fitness and predictive ability of the PLS-DA model (−0.495; <0.05). G, the grazing group; SG, the semi-grazing group; SF, the stall feedlot group; QC, the quality-control group.

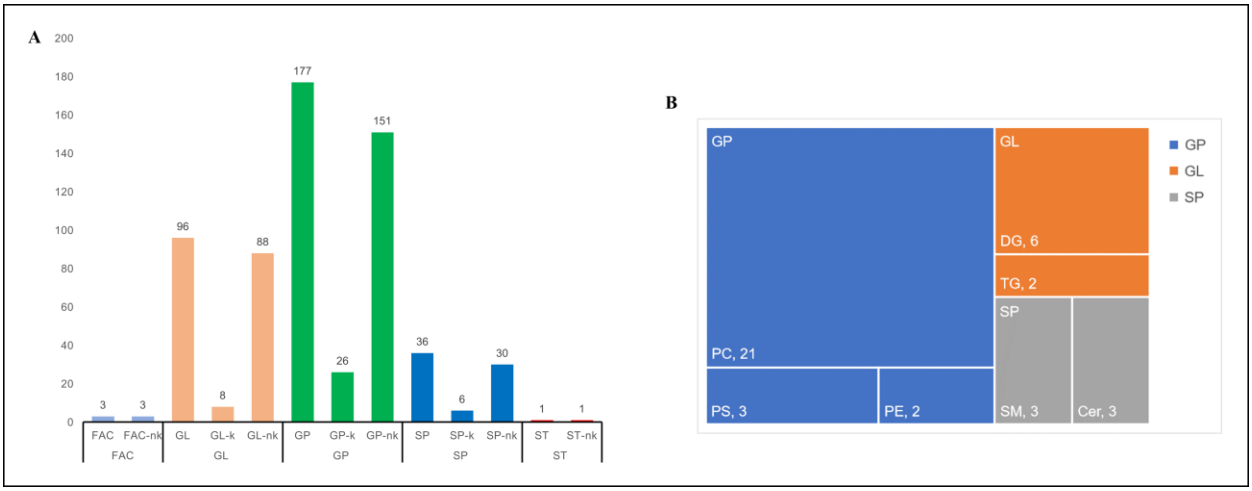

Fig. 3. Differential lipids profile: (A) Number of the significantly different lipids belonging to five differential classifications for the *longissimus dorsi* muscle. The bars with the category name, name-k, or with name-nk mean the various numbers of lipids of the specific category, and the different numbers of lipids of the particular category could be annotated to the KEGG database or not be annotated to KEGG database, respectively. FAC, fatty acyls; GL, glycerolipids; GP, glycerophospholipid; SP, sphingolipid; ST, sterol lipid; (B) The subclass and number of the differential compounds can be annotated to the KEGG database, which would be used for further analysis. PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; DG, diglyceride; TG, triglyceride; SM, sphingomyelin; Cer, ceramide.

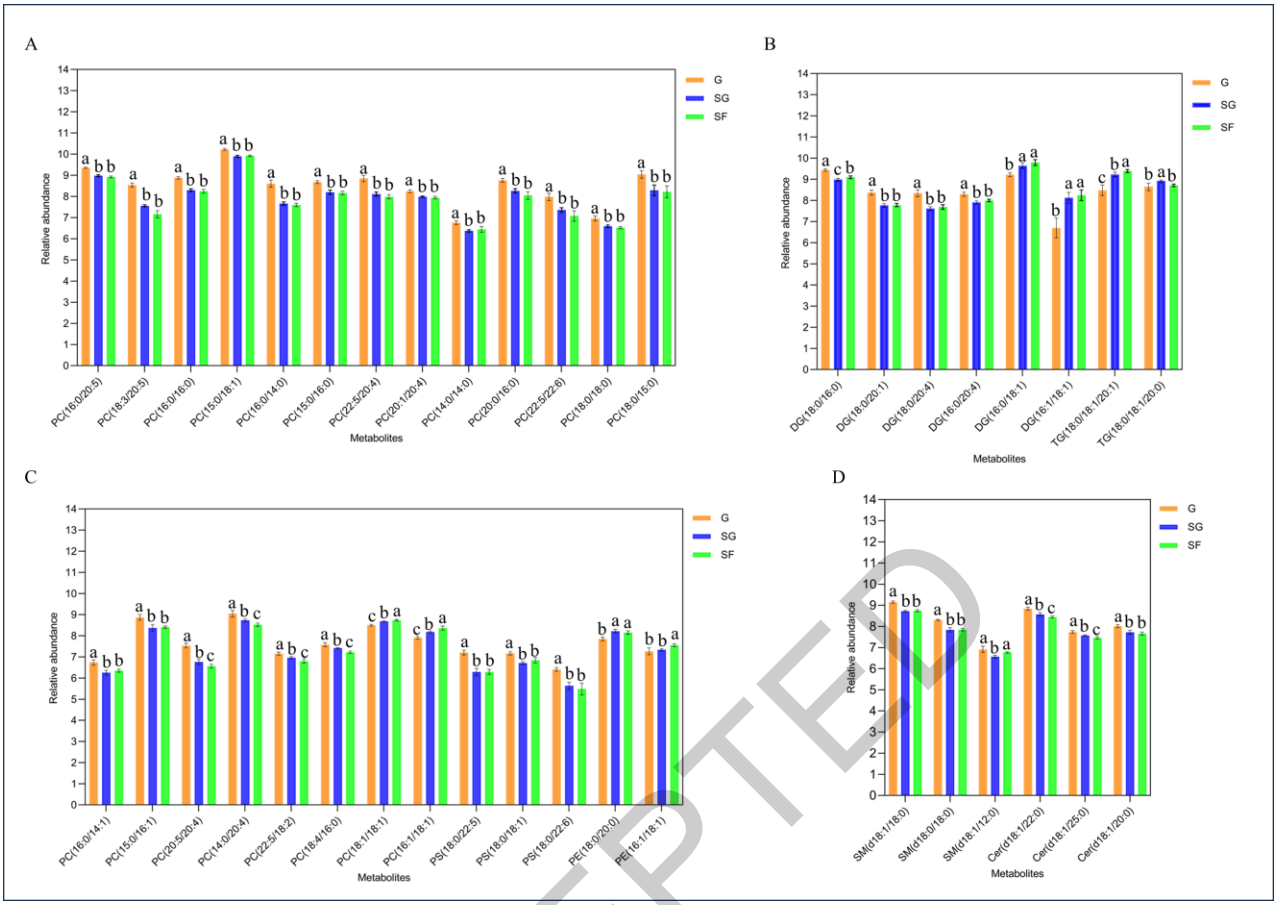

Fig. 4. The different lipids (the GP category, A and C; the SP and GL categories, B and D, respectively), determined in the *longissimus dorsi* muscle of Gangba lambs within three groups: G, the grazing group; SG, the semi-grazing group; SF, the stall feedlot group; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; DG, diglyceride; TG, triglyceride; SM, sphingomyelin; Cer, ceramide; The bars in a lipid, without significant differences share the same letters in graphs, whereas significantly different bars are indicated by diverse letters (a, b, and c).

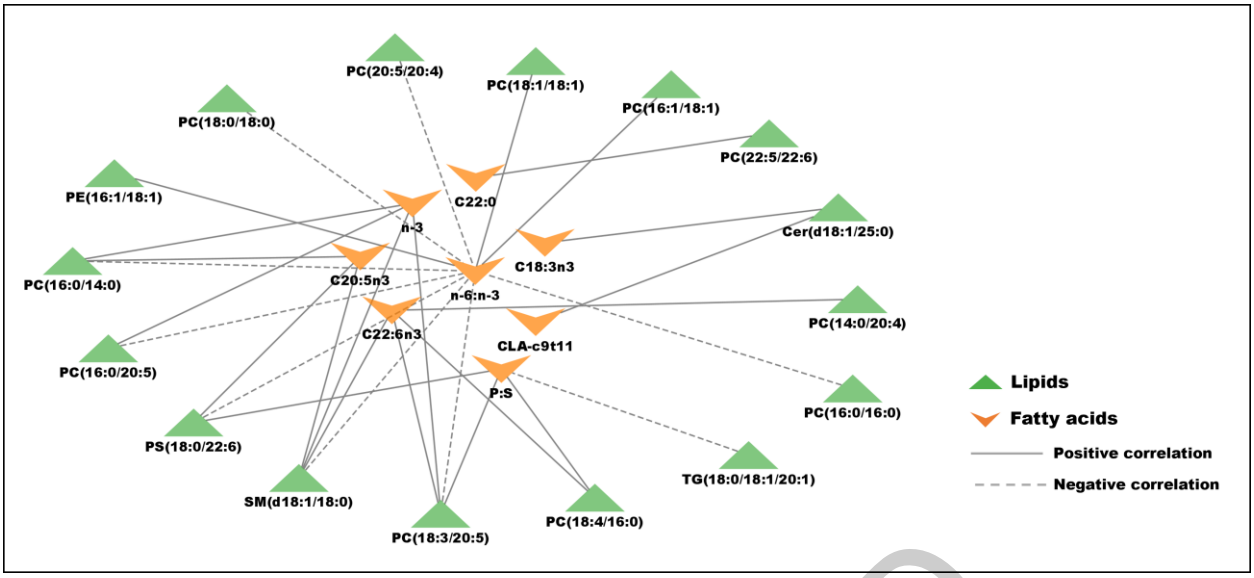

Fig. 5. Correlation analysis between differential lipids and fatty acids ($P < 0.05$; | R | > 0.6). PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; TG, triglyceride; SM, sphingomyelin; Cer, ceramide.

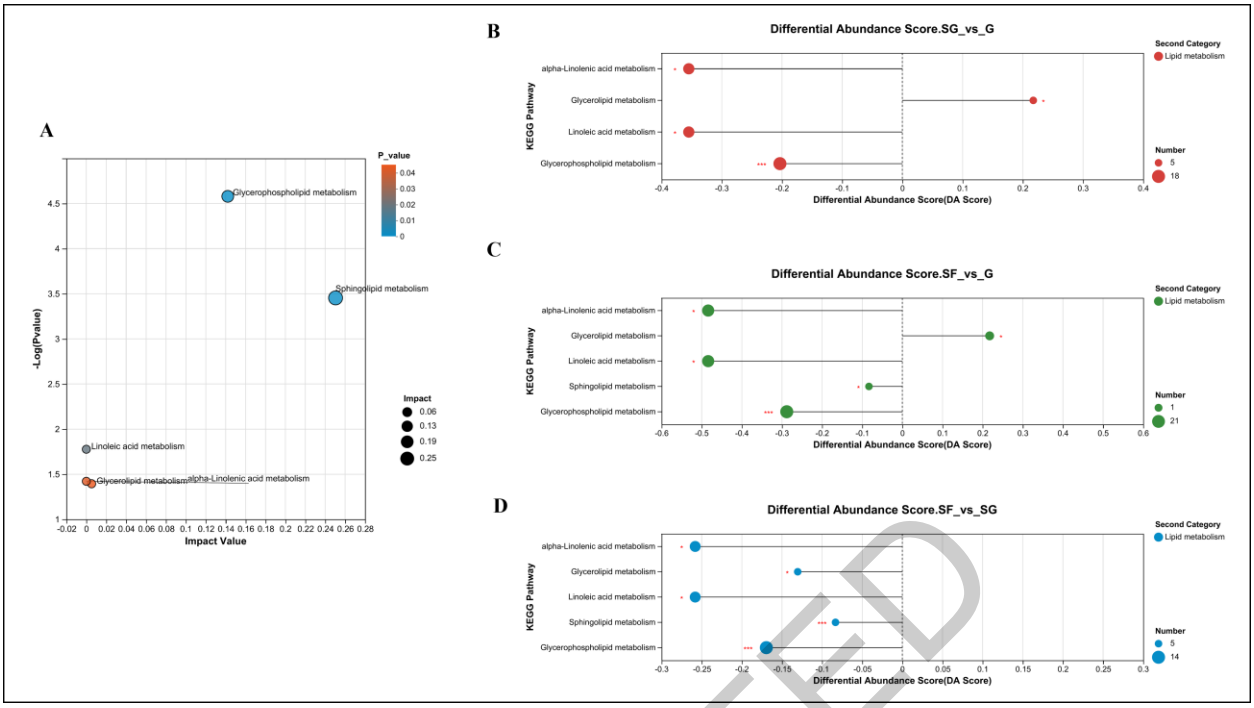

Fig. 6. Bubble chart of KEGG topology analysis of lipids in *longissimus dorsi* muscle of different feeding regimes (A); Differential abundance (DA) score analysis of KEGG pathway at SG versus G (B), SF versus G (C), and SF versus SG (D) of *longissimus dorsi* muscle, respectively. G, the grazing group; SG, the semi-grazing group; SF, the stall feedlot group.

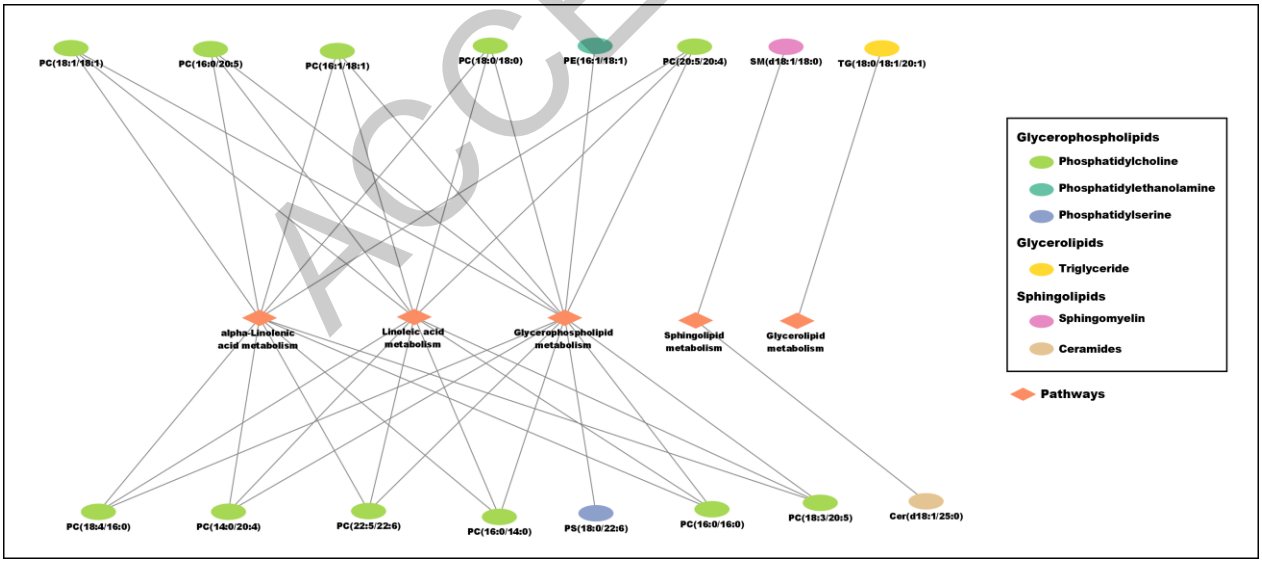

Fig. 7. Network of the 16 differential lipids and related metabolic pathways. PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; TG, triglyceride; SM, sphingomyelin; Cer, ceramide.

Table 1 Ingredients composition of the concentrate.

Ingredients	Content (%)
Chopped maize	55
Barley	10
Pea	23.5
Wheat bran	10
Limestone	1
Premix[1]	0.5

[1]Additive: Vitamin A, 10,500 IU; vitamin D3, 2,110 IU; vitamin E, 43 mg; Mn, 40 mg; Fe, 32mg; Zn, 95 mg; Cu 16 mg (per kilogram of dry matter provided).

Table 2 Nutrient level of the concentrate and pasture.

Nutrient level	Feed	
	Concentrate	Pasture
Dry matter	88.3	90
Digestible energy[1], MJ/kg	15.1	-
Crude protein	16.1	8.9
Neutral detergent fiber	8.7	59.8
Acid detergent fiber	6	48
Calcium	0.5	2.3
Phosphorus	0.4	0.1

[1]Digestible energy was calculated value.

Table 3 Fatty acids content of the concentrate and pasture.

Fatty acids (mg/100g)[1]	Feed	
	Concentrate	Pasture
C8:0 (octanoic acid)	1.44	1.96
C10:0 (decanoic acid)	1.94	4.23
C12:0 (dodecanoic acid)	3.39	8.17
C14:0 (myristic acid)	7.08	13.04
C15:0 (pentadecanoic acid)	2.26	5.74
C16:0 (palmitic acid)	435.53	351.48
C17:0 (heptadecanoic acid)	3.80	6.24
C18:0 (stearic acid)	57.03	47.87
C20:0 (arachidic acid)	13.16	28.34
C21:0 (heneicosanoic acid)	1.60	2.65
C22:0 (behenic acid)	14.52	32.34
C23:0 (tricosanoic acid)	2.11	3.34
C24:0 (lignoceric acid)	12.95	35.83
SFA	562.12	541.23
C16:1 (palmitoleic acid)	10.69	40.21
C18:1n9c (9-cis-Octadecenoic acid)	608.24	42.52
C20:1 (eicosenoic acid)	12.19	4.95
C22:1n9 (erucic acid)	5.19	4.19
C24:1 (nervonic acid)	3.02	4.53
MUFA	639.33	96.40
C18:2n6c (linoleic acid)	1,095	322.23
C18:3n3 (alpha-linolenic acid)	184.84	1,004
PUFA	1,280	1,326
P:S	2.28	2.45
n-6:n-3	5.92	0.32

[1] SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; CLA = conjugated linoleic acid; PUFA = polyunsaturated fatty acids.

Table 4 Fatty acid composition of the *longissimus dorsi* muscle of Gangba lambs under three feeding regimes.

Fatty acids (mg/100g)[1]	Groups[2]			SEM[3]	*p*-value
	G	SG	SF		
C8:0 (octanoic acid)	1.73	1.66	1.94	0.12	0.889
C10:0 (decanoic acid)	10.9	11.94	16.34	1.20	0.148
C12:0 (dodecanoic acid)	7.73	10.51	14.18	1.20	0.085
C14:0 (myristic acid)	172.76^{b}	185.48^{ab}	288.40^{a}	20.09	0.024
C15:0 (pentadecanoic acid)	29.97	30.26	42.8	2.65	0.062
C16:0 (palmitic acid)	1,937^{b}	2,031^{b}	3,111^{a}	188	0.008
C17:0 (heptadecanoic acid)	95.98^{b}	95.56^{b}	141.25^{a}	8.27	0.023
C18:0 (stearic acid)	1,540	1,664	1,979	96	0.159
C20:0 (arachidic acid)	16.07	14.92	13.94	1.04	0.792
C21:0 (heneicosanoic acid)	54.36	49.36	49.25	2.83	0.711
C22:0 (behenic acid)	8.42^{a}	7.24^{ab}	6.47^{b}	0.40	0.049
C23:0 (tricosanoic acid)	12.42	10.97	12.15	0.61	0.67
C24:0 (lignoceric acid)	10.85	9.07	8.36	0.54	0.106
SFA	3898^{b}	4122^{b}	5685^{a}	307	0.023
C14:1 (myristoleic acid)	5.96	5.61	9.39	0.85	0.166
C16:1 (palmitoleic acid)	149.43	129.83	210.85	14.77	0.054
C18:1n9c (9-cis-Octadecenoic acid)	3202	3239	4510	261	0.056
C20:1 (eicosenoic acid)	11.96	10.22	11.63	0.89	0.743
C22:1n9 (erucic acid)	4	3.34	2.53	0.25	0.055
C24:1 (nervonic acid)	8.71	7.74	6.71	0.48	0.344
MUFA	3,382	3,396	4,751	277	0.057
C18:2n6c (linoleic acid)	468.07	449.75	434.37	22.14	0.843
C20:3n6 (eicosatrienoic acid)	16.68	20.52	20.43	1.31	0.421
C20:4n6 (arachidonic acid)	212.04	170.75	169.01	10.86	0.193
CLA-c9t11 (Conjugated linoleic acid (C9, t11))	16.25^{ab}	20.30^{a}	13.56^{b}	1.20	0.042
CLA-t10c12 (Conjugated linoleic acid (T10, c12))	9.3	9.3	7.62	0.54	0.339
n-6	722.33	670.62	644.99	32.77	0.643
C18:3n3 (alpha-linolenic acid)	102.95^{a}	85.97^{ab}	69.45^{b}	5.76	0.049
C20:3n3 (eicosatrienoic acid)	2.76	2.13	2.42	0.17	0.278
C20:5n3 (eicosapentaenoic acid)	58.53^{a}	44.95^{ab}	32.62^{b}	3.97	0.019
C22:6n3 (docosahexaenoic acid)	17.34^{a}	14.07^{a}	10.31^{b}	1.08	0.02
n-3	181.58^{a}	147.12^{ab}	114.80^{b}	10.44	0.022
C22:2 (docosadienoic acid)	7.11	6.98	8.53	0.47	0.257
PUFA	911.02	824.73	768.32	41.82	0.397
P:S	0.24^{a}	0.21^{a}	0.14^{b}	0.01	0.004
n-6:n-3	4.00^{c}	4.56^{b}	5.79^{a}	0.24	0.001

[1] SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; CLA = conjugated linoleic acid; PUFA = polyunsaturated fatty acids; [2] G = the grazing system; SG = the semi-grazing system; SF = the stall-feeding system. [3] SEM = the standard error of the mean. [abc] Means within a row with different superscripts differ ($p < 0.05$).