

*Exploring the metabolomic landscape:  
Perilla frutescens as a promising enhancer  
of production, flavor, and nutrition in Tan  
lamb meat*

Article

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1 **Exploring the metabolomic landscape: *Perilla frutescens* as a promising enhancer**  
2 **of production, flavor, and nutrition in Tan lamb meat**

3  
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13 **ABSTRACT**

14 Addressing health-related concerns linked to the metabolite profile of lamb meat has  
15 become paramount, in line with the growing demand for enhanced flavor and taste. We  
16 examined the impact of *Perilla frutescens* seeds on Tan lamb growth, carcass traits, and  
17 metabolite profiles. Three diets were employed: a low-concentrate group (LC), a high-  
18 concentrate group (HC), and a PFS group (the LC diet supplemented with 3% *Perilla*  
19 *frutescens* seeds) on a dry matter basis. Forty-five male Tan-lambs (approximately six  
20 months) with similar body weights (25.1 kg ± 1.12 SD) were randomly assigned to one  
21 of these three groups for 84-day feeding, including an initial 14-day adjustment phase.  
22 The supplementation of PFS resulted in increased average daily gain ( $P < 0.01$ ) and  
23 improved carcass quality and meat color ( $P < 0.05$ ). Additionally, it led to an  
24 enhancement in omega-3 polyunsaturated fatty acids ( $P < 0.05$ ) and a reduction in the  
25 omega-6/omega-3 ratio ( $P < 0.05$ ). Using gas chromatography-mass spectrometry, 369  
26 volatile compounds were identified with enhanced levels of acetaldehyde and 1,2,4-  
27 trimethyl-benzene associated with PFS ( $P < 0.05$ ). Among the 807 compounds  
28 identified by ultra-high performance liquid chromatography-mass spectrometry, there  
29 were 66 significantly differential compounds ( $P < 0.05$ ), including 43 hydrophilic  
30 metabolites and 23 lipids. PFS supplementation led to significant alterations in 66  
31 metabolites, with three metabolites including 2,5-diisopropyl-3-methylphenol, 3-  
32 hydroxydecanoic acid, and lysophosphatidylcholine (15:0) emerging as potential PFS-  
33 related biomarkers. The study indicates that PFS supplementation can enhance Tan-  
34 lamb growth, feed efficiency, and meat quality, potentially providing lamb meat with  
35 improved flavor and nutritional characteristics.

36 **Keywords:** fatty acid, volatiles, lipids, lysophosphatidylcholine, flavor and taste  
37 precursors

## 38 1. Introduction

39 The cooked meat odor, flavor, and eating taste are closely related to the volatile,  
40 lipophilic, and hydrophilic metabolites, and even lipid oxidation compounds in raw  
41 meat (Munekata, Pateiro, López-Pedrouso, Gagaoua, & Lorenzo, 2021; Ramalingam,  
42 Song, & Hwang, 2019). The lipids and water-soluble compounds are also precursors of  
43 these volatile compounds (Khan, Jo, & Tariq, 2015). However, with the development  
44 of new techniques and data processing method, liquid chromatography coupled to  
45 accurate MS/MS with spectral entropy showed more accurate and helpful for both  
46 lipophilic and hydrophilic metabolites and new compounds identification (Li et al.,  
47 2021).

48 Diet is one of the most important factors affecting the flavor metabolites and  
49 precursors deposition in raw meat (Khan et al., 2015). The total oil content of *Perilla*  
50 *frutescens* seed can range from 30-45%, of which  $\alpha$ -linolenic acid accounts for 50-62%  
51 (ALA). Additionally, *Perilla frutescens* seed also contain functional components such  
52 as flavonoids, terpenes, polyphenols, amino acids, among others (Akriti, Rajni, &  
53 Meenakshi, 2019). In addition, it contains high concentration of essential oils that can  
54 act as preservatives in food systems (Al-Maqtari et al., 2022). Consumers nowadays  
55 are paying increasing attention to the relationship between food and health (De Smet &  
56 Vossen, 2016). Thus, foods enriched with omega-3 PUFAs (n-3 PUFAs) have gained  
57 worldwide acclaim, due to their antiviral, anti-inflammatory, immune-boosting and  
58 cholesterol-lowering effects (Kavyani et al., 2022), preventing cardiovascular diseases  
59 (Sunagawa et al., 2022), and even improving survival in patients with COVID-19  
60 infection (Hathaway et al., 2020). Meanwhile, research indicates that n-3 PUFAs  
61 supplementation during pregnancy reduces the risk of developing asthma or asthma  
62 symptoms during childhood. Thus, maintaining a moderate intake of n-3 PUFAs is  
63 considered crucial for optimal human health.

64 Despite prior studies on *Perilla frutescens* supplementation (Deng et al., 2018;  
65 Peiretti, Gasco, Brugiapaglia, & Gai, 2011) for carcass quality, organoleptic properties,  
66 and nutrition in meat, a comprehensive metabolomics analysis of its effect on Tan lamb

67 metabolism and diverse meat characteristics is lacking. In this study, two integrative  
68 untargeted metabolomics approaches were employed to uncover core metabolites and  
69 metabolic pathways in Tan sheep, aiming to elucidate the observed phenotypes. We  
70 aimed to examine the characteristics related to production (growth rates, feed  
71 efficiency), carcass and meat characteristics (classification and basic meat quality  
72 parameters), flavour and taste precursors (volatile, lipophilic, and hydrophilic  
73 metabolites) and nutritional quality (fatty acid profile, nutritionally-relevant  
74 metabolites) of raw meat from lambs fed with *Perilla frutescens* seed.

## 75 2. Materials and methods

### 76 2.1. Animals, diets, and samples preparation

77 All animal procedures in the present study were approved by the Animal Care  
78 Committee of China Agricultural University (Beijing, China; approval no.  
79 AW30901202-1-1). Forty-five male Tan-lambs (*Ovis aries*) (approximately six months  
80 of age) with an average bodyweight (BW) of 25.1 kg ( $\pm$  1.23 SD) were selected on a  
81 commercial Tan-sheep farm (Ningxia Hui Autonomous Region, China). The Tan-lambs  
82 were randomly divided into three groups with 15 animals in each group (each group  
83 has three blocks (pens); each block has five lambs) based on balanced BW using the  
84 RAND function in Excel. Each group was allocated in one of the following three  
85 experimental treatments: 1) a low-concentrate diet (LC; 45:55 forage: concentrate ratio,  
86 on a dry matter (DM) basis); 2) a high-concentrate control diet (HC; 20:80 forage:  
87 concentrate ratio, on a DM basis; 3) a LC diet supplemented with 3% *Perilla frutescens*  
88 seed (PFS; 3% of diet of Tan-lamb, on a DM basis). The addition of 3% perilla seeds  
89 in this study was based a previous study that found the addition of 1% perilla seeds  
90 extract of the feed subtract could mitigate the rumen methane production but with no  
91 effects on volatile fatty acids (Wang et al., 2016). The detailed ingredients and nutrient  
92 composition of the basal diet of are shown in Table 1. *Perilla frutescens* seeds  
93 (Purchased from an agricultural food e-commerce) exhibit a nutrient-rich profile with  
94 40.9% ether extract (EE), 23.5% crude protein (CP), 25.4% neutral detergent fiber

95 (NDF), 19.9% acid detergent fiber (ADF), and 3.9% ash (DM basis). In terms of fatty  
96 acids, they contain 57.8% C18:3n3 (ALA), 19.9% C18:1n9c (oleic acid), 11.9%  
97 C18:2n6c (linoleic acid), and 7.4% C16:0 (palmitic acid, % of total fatty acids) (Table  
98 2). The diets were fed as total mixed ration and the *Perilla frutescens* seed was  
99 uniformly mixed into the concentrates. The offered feed and refusals from each pen  
100 were weighed and recorded daily to determine the DM intake. The lambs were fed twice  
101 a day at 09:00 and 17:00. All the lambs had ad libitum access to feed and water  
102 throughout the experimental period. The experiment lasted for 84 d, including a 14 d  
103 adaptation period, plus 70 d of the feeding experiment.

104 An untargeted metabolomics was conducted for the botanical bioactive compounds  
105 for PFS based on the LC-MS/MS analysis, which was performed on an UHPLC system  
106 (Vanquish, Thermo Fisher Scientific) with a Waters UPLC BEH C18 column (1.7  $\mu$ m  
107 2.1\*100 mm). The detail protocol was following a previous study (Hou et al., 2019).

## 108 2.2. Carcass traits, basic meat quality and targeted fatty acid analysis

109 At the end of the feeding experiment (on the d 70), 6 lambs per group (2 lambs  
110 from each pen) were randomly selected for slaughter. The selection was performed  
111 using the RAND function in Excel. Slaughter was conducted following the outlined  
112 procedure: euthanasia; skinning; removal of the head at the atlas-occipital joint; cutting  
113 of the limbs at the carpo-metacarpal and tarso-metatarsal joints; removal of the heart,  
114 liver, spleen, lungs, kidneys, and testis; excision of the omental, perirenal, and tail fat  
115 deposits. The organs and carcass weights were measured and recorded immediately  
116 after slaughtering, and the organs' index were calculated based on their ratio to carcass  
117 weight. Immediately after slaughtering, the body fat (assessed as GR value) and loin-  
118 eye area were measured. The GR value was determined by using a vernier caliper to  
119 measure the thickness at the 12<sup>th</sup>/13<sup>th</sup> rib intersection, 11 cm away from the midline  
120 (Karim, Porwal, Kumar, & Singh, 2007). The loin-eye area was determined using a  
121 planimeter (QCJ-2000, Harbin Optical Instrument Factory, Harbin, China) at the  
122 interface of 12<sup>th</sup> and 13<sup>th</sup> ribs on both sides of the carcass (Karim, Porwal, Kumar, &

123 Singh, 2007). Then, meat samples taken from the 6<sup>th</sup> to 12<sup>th</sup> ribs of the left *longissimus*  
124 *lumborum* (LL) muscle were trimmed of fat. The determination of pH and meat colour  
125 were performed between 12<sup>th</sup> and 13<sup>th</sup> thoracic vertebrae. To measure the pH values of  
126 LL muscle samples at 45 min and 24 h postmortem, a pH meter with automatic  
127 temperature compensation was used after calibration with pH 4.6 and 7.0 buffers. Meat  
128 color parameters such as redness ( $a^*$ ), yellowness ( $b^*$ ), and lightness ( $L^*$ ) were  
129 measured in triplicate after 30 min of blooming at room temperature (approximately at  
130 20 °C) using an NS800 high-quality spectrophotometer (3NH Technology co., Ltd,  
131 Shenzhen, China). The measurements were conducted at 45 min and 24 h after slaughter,  
132 using illuminant D65 as the light source and a 10° observer with an 8 mm diameter  
133 measuring area and a 50 mm diameter illumination area (Honikel, 1998). The LL (from  
134 the 6<sup>th</sup> to 12<sup>th</sup> ribs) at 24 h post-mortem was used to assess instrumental meat quality  
135 characteristics including cooking rate and shear force (Ekiz et al., 2009). After thawing  
136 at 4 °C, the meat was cut into rectangular 2 × 2 × 1 cm samples weighing approximately  
137 30 ± 1 g and the connective tissue was removed. The muscle samples were placed in  
138 plastic vacuum packs (two steaming batch) and placed in a water bath at 75 °C for  
139 approximately 30 min until the final core temperature reached 70 °C. The samples were  
140 then cooled to room temperature, the surface of the samples was dried with paper towels,  
141 and cooking rate was measured and calculated as follows: (Weight of the samples after  
142 cooking / Weight of the samples before cooking) × 100%. After measuring the cooking  
143 rate, three sub-samples (cut parallel to the muscle fibres and 1 × 1 cm in cross-section)  
144 were removed from each cooked sample. Shearing perpendicular to the muscle fiber  
145 direction was performed using a TMS-PRO Texture Analyzer (FTC Co., Ltd., Virginia,  
146 USA) equipped with a WBSF (Warner-Bratzler Shear Force) device featuring a 250  
147 Newton load cell, employing an across-head speed of 60 mm/min.

148 The LL samples from the 12<sup>th</sup>/13<sup>th</sup> rib at 45 min were collected to freezing tubes  
149 and stored in liquid nitrogen for further metabolomics study. The LL samples taken  
150 from the 6<sup>th</sup> to 12<sup>th</sup> rib section at 24 h postmortem were stored in dry ice and then were  
151 used for targeted fatty acid composition via gas chromatography (Model 6890; Agilent  
152 Technologies, Santa Clara, CA, USA) equipped with a DB-23 capillary column (60.0

153 m×250 μm×0.25 μm) following our previous method (Zhang et al., 2022). The standard  
154 sample mixture (F.A.M.E. Mix, C4-C24 Unsaturates, Supelco-18919-1AMP, Sigma-  
155 Aldrich Trading Co.Ltd., Shanghai, China), which consisted of 40 free fatty acids, was  
156 used in the analysis. C11:0 (1.0 mg/mL) was employed as the internal standard. The  
157 fatty acid results expressed as mg/100 g of wet meat samples. The following  
158 combinations and ratios of fatty acids were calculated: saturated fatty acids (SFA),  
159 unsaturated fatty acids (UFA), total fatty acids (TFA), monounsaturated fatty acids  
160 (MUFA), conjugated linoleic acid (CLA), PUFA, SFA/UFA, and omega-6/omega-3  
161 ratio (n-6/n-3). The index of atherogenicity (IA) = (C12:0 + (4 × C14:0) + C16:0) /  
162 (MUFA + PUFA), and index of thrombogenicity index (IT) = (C14:0 + C16:0 + C18:0)  
163 / (0.5 × MUFA) + (0.5 × n-6) + (3×n-3) + (n-3:n-6) were calculated based on a previous  
164 study (Pretorius & Schonfeldt, 2021).

### 165 2.3. Volatile compounds identification based on GC-MS analysis

166 A 300 ± 5 mg LL sample was transferred into a 20 mL headspace vial, to which 10  
167 μL of a 2-octanol internal standard (at a concentration of 10 mg/L in deionized water)  
168 was added. Subsequent analysis was conducted using a gas chromatography-mass  
169 spectrometry (GC-MS) system, specifically during the Solid Phase Microextraction  
170 (SPME) cycle of the PAL rail system. The sample incubation temperature was set to  
171 60 °C, with a preheating duration of 15 min, followed by an incubation period of 30  
172 min. The desorption time was 4 min. The GC-MS analysis was performed on an Agilent  
173 7890 gas chromatograph system coupled with a 5977B mass spectrometer, utilizing a  
174 DB-Wax column. The sample was injected in splitless mode. Helium served as the  
175 carrier gas, with a front inlet purge flow of 3 mL/min and a column gas flow rate of 1  
176 mL/min. The initial oven temperature was set to 40 °C and maintained for 4 min, then  
177 incrementally increased to 245 °C at a rate of 5 °C/min, where it was held for an  
178 additional 5 min. The temperatures for the injection port, transfer line, ion source, and  
179 quadrupole were set at 250 °C, 250 °C, 230 °C, and 150 °C, respectively. Ionization  
180 was achieved using an energy of -70 eV in electron impact mode. Mass spectrometry

181 data acquisition was performed in scan mode, covering a mass-to-charge ratio (m/z)  
182 range of 20-400, with no solvent delay. Raw peak extraction, baseline data filtering and  
183 calibration, peak alignment, deconvolution analysis, peak identification, integration,  
184 and spectral matching of peak areas were conducted utilizing the Chroma TOF 4.3X  
185 software developed by LECO Corporation in conjunction with the NIST database  
186 (Garcia & Barbas, 2011).

#### 187 *2.4. Higher definition mix discovery metabolomics based on LC-MS/MS analysis*

188 Twenty-five mg of the LL sample was accurately weighed and transferred into a  
189 polypropylene microcentrifuge tube. Subsequently, 500  $\mu$ L of an extraction solution,  
190 composed of methanol and water in a 3:1 ratio with an isotopically-labelled internal  
191 standard mixture (250 nmol/L), was added to the sample. The samples underwent a  
192 homogenization process at a frequency of 35 Hz for a duration of 4 min, followed by a  
193 5-min sonication in an ice-water bath. This homogenization and sonication cycle was  
194 conducted thrice. Post these cycles, the samples were subjected to an incubation period  
195 of 1 hour at a temperature of -40  $^{\circ}$ C, followed by centrifugation at a rotational speed of  
196 13,800 g for 15 min at 4  $^{\circ}$ C. The supernatant resulting from the centrifugation was  
197 carefully transferred into a new, clean glass vial for further analysis. A quality control  
198 (QC) sample was prepared by amalgamating equal aliquots of supernatants from all  
199 samples.

200 The analytical assessments were conducted utilizing a high-definition (HD) mix  
201 Ultra-High-Performance Liquid Chromatography (UHPLC) system (Vanquish, Thermo  
202 Fisher Scientific) integrated with a UPLC HSS T3 column (2.1 mm  $\times$  100 mm, 1.8  $\mu$ m),  
203 linked to an Orbitrap Exploris 120 mass spectrometer (Orbitrap MS, Thermo) (UHPLC-  
204 OE-MS). The mobile phase comprised of 5 mmol/L ammonium acetate and 5 mmol/L  
205 acetic acid in water (A), alongside acetonitrile (B). The autosampler was maintained at  
206 a temperature of 4  $^{\circ}$ C, with an injection volume of 2  $\mu$ L. The Orbitrap Exploris 120  
207 mass spectrometer was employed due to its capacity for acquiring MS/MS spectra using  
208 information-dependent acquisition (IDA) mode under the supervision of the Xcalibur

209 acquisition software (Thermo). In this specified mode, the software incessantly assesses  
210 the complete scan MS spectrum. The ESI source conditions were preordained as  
211 follows: sheath gas flow rate at 50 Arb, auxiliary gas flow rate at 15 Arb, capillary  
212 temperature at 320 °C, full MS resolution at 60,000, MS/MS resolution at 15,000,  
213 collision energy at 10/30/60 in Normalized Collision Energy (NCE) mode, and spray  
214 voltage at 3.8 kV (positive) or -3.4 kV (negative). The raw data was converted into  
215 mzXML format utilizing ProteoWizard and subsequently processed with an in-house  
216 developed program, founded on XCMS. This program facilitated peak detection,  
217 extraction, alignment, and integration. Post-processing, an in-house MS2 database  
218 (BiotreeDB, V2.1) was utilized for metabolite annotation, with an annotation cut-off  
219 established at 0.3.

## 220 *2.5. Statistical analysis*

221 Variation in animal growth performance, carcass traits, meat quality, and targeted  
222 fatty acid analysis were described using linear mixed models by the IBM SPSS  
223 Statistics (version 26.0), with three treatments (LC, HC, and PFS) as a fixed effect and  
224 random terms for animal and pen. Predicted means and standard errors were obtained  
225 from the models, facilitating pairwise comparisons between means by calculating the  
226 least significant difference at a 5% critical value. The value of  $P < 0.05$  was considered  
227 statistically significant.

228 For the data from GC-MS and LC-MS/MS, the missing values were assumed to be  
229 half of the minimum value (Tiedt et al., 2020). Normalisation was implemented using  
230 the total ion current method. The consolidated dataset, inclusive of peak number,  
231 sample identifiers, and normalised peak areas, was transferred to the SIMCA16.0.2  
232 software package (Sartorius Stedim Data Analytics AB, Umea, Sweden) for  
233 comprehensive multivariate analyses. Data were scaled and logarithmically  
234 transformed to attenuate the influence of both background noise and significant  
235 variance across variables. Post-transformation, principal component analysis (PCA)  
236 was implemented as an unsupervised analytic method to condense the dimensionality

237 of the dataset, providing visual interpretation of sample distribution and clustering. A  
238 95% confidence interval within the PCA score plot was utilized as the criterion for  
239 potential outlier identification. Supervised orthogonal projections to latent structures-  
240 discriminant analysis (OPLS-DA) was employed for visualizing group differentiation  
241 and the identification of significantly altered metabolites. The validity and  
242 predictability of the model were assessed by executing a 7-fold cross-validation,  
243 yielding  $R^2$  and  $Q^2$  values.  $R^2$  quantifies the extent to which variation in a variable is  
244 elucidated, whereas  $Q^2$  denotes the predictability of a variable. The robustness and  
245 predictive capability of the OPLS-DA model were verified via a 200-iteration  
246 permutation test, from which the intercept values for  $R^2$  and  $Q^2$  were ascertained. In  
247 this context, a smaller  $Q^2$  intercept value is indicative of a robust model with low risk  
248 of overfitting and high reliability. The value of variable importance in the projection  
249 (VIP) of the first principal component in OPLS-DA analysis was obtained. It  
250 summarizes the contribution of each variable to the model. The metabolites with  $VIP >$   
251  $1$  and  $P < 0.05$  (student t test) were considered as significantly changed metabolites. In  
252 addition, commercial databases including KEGG (<http://www.genome.jp/kegg/>) and  
253 MetaboAnalyst (<http://www.metaboanalyst.ca/>) were used for pathway enrichment  
254 analysis.

255

### 256 **3. Results**

#### 257 *3.1. The plant secondary metabolites of PFS*

258 The top 5 categories of the plant secondary metabolites of PFS are flavonoids (37%),  
259 phenylpropanoids (28%), terpenoids (11%), quinones (11%), alkaloid (11%, Fig. S1A).  
260 For the specific compounds, luteolin (22%), 7-hydroxycoumarin (15%), and emodin  
261 (9%) are the most abundant bioactive compounds (Fig. S1B).

#### 262 *3.2. Growth performance and carcass characteristics*

263 As shown in Table 3, there was no significant effect of the dietary treatments on  
264 dry matter intake (DMI) ( $P > 0.05$ ). The HC exhibited the highest average daily gain  
265 (ADG, 194 g/d), followed by PFS (154 g/d), with LC (120 g/d) presenting the lowest  
266 value ( $P < 0.01$ ). The feed conversion rate (average DMI/ADG) was lower in HC (4.78)  
267 than in LC (8.28,  $P < 0.05$ ). The carcass traits, including live weight, carcass weight,  
268 and dressing percentage of HC and PFS, were higher than those of LC ( $P < 0.05$ ), with  
269 no significant differences observed between HC and PFS. The HC had the higher head  
270 weight compared to LC (2.72 kg vs 2.42 kg,  $P < 0.05$ ). The organ weight of the heart  
271 was higher in HC (145 g) and PFS (136 g) than in LC (120 g), with similar values  
272 between HC and PFS ( $P < 0.01$ ). The organ weights of the kidney in HC (101.2 g) were  
273 higher than those in LC (41.4 g) and PFS (46.2 g), with similar values between LC and  
274 PFS ( $P < 0.01$ ). The organ ratio of the heart was higher in LC than in PFS (8.52 vs 7.87  
275 g/kg carcass weight,  $P < 0.05$ ). The organ ratios of the lung and testis were higher in  
276 LC (21.2 and 22.4 g/kg carcass weight) than in HC (16.8 and 19.8 g/kg carcass weight)  
277 and PFS (16.6 and 18.3 g/kg carcass weight,  $P < 0.01$ ). The organ ratios of the kidney  
278 were higher in HC (5.62 g/kg carcass weight) than in LC (2.95 g/kg carcass weight)  
279 and PFS (2.65 g/kg carcass weight,  $P < 0.01$ ).

### 280 3.3. Meat quality

281 As shown in Table 4, the tail fat weight and the tail fat ratio were higher in HC than  
282 in LC ( $P < 0.05$ ). The perirenal fat weight and perirenal fat ratio were highest in the HC  
283 group, followed by PFS, with the lowest value found in LC ( $P < 0.01$ ). The omentum  
284 weight and omentum ratio were higher in HC and PFS than in LC ( $P < 0.01$ ). The eye  
285 muscle area was greater in HC than in LC ( $P < 0.05$ ). The value of shear force was  
286 higher in LC than in HC ( $P < 0.05$ ). The meat color of  $a^*$  (redness) after 24 hours was  
287 lower in PFS than in HC ( $P < 0.05$ ).

### 288 3.4. Targeted fatty acids composition

289 The raw meat DM content, intramuscular fat (IMF) content, and fatty acid profiles

290 in muscle are displayed in Table 5. The DM content in PFS was higher than in LC ( $P <$   
291 0.01). The HC had higher levels of C10:0, C16:1, and TFA compared to LC ( $P < 0.05$ ).  
292 Compared to LC, the concentrations of C22:0 and C23:0 were lower, but the  
293 concentrations of UFA, MUFA, and C18:1c9 were higher in LC and PFS ( $P < 0.05$ ).  
294 The PFS had higher concentration of C20:0 but lower C20:3n6 compared to LC. The  
295 PFS exhibited higher concentrations of  $\alpha$ -linolenic acid (C18:3n3, ALA), C20:5n3  
296 (eicosapentaenoic, EPA), C22:6n3 (docosahexaenoic acid, DHA) and the sum of n-3  
297 PUFA compared to LC and HC ( $P < 0.05$ ). Compared to PFS and LC, HC had higher  
298 levels of CLA-t10c12, C18:2n6, and the sum of n-6 PUFA ( $P < 0.05$ ). The SFA/UFA  
299 ratio was lower in HC and PFS than in LC ( $P < 0.01$ ). The ratio of n-6/n-3 was lowest  
300 in PFS (8.02), intermediate in LC (15.2), and highest in HC (21.3,  $P < 0.01$ ). The values  
301 of IA, IT, and C16:0/C18:1 were lower in PFS and HC than in LC ( $P < 0.05$ ).

### 302 3.5. Volatile compounds

303 In this experiment, a total of 434 peaks were detected, and 421 metabolites remained  
304 after relative standard deviation denoising based on GC-MS analysis. According to the  
305 HMDB and Biotree self-built databases, 369 of these metabolites were identified and  
306 categorized into 6 groups (Fig. 1A). The 6 categories are alcohols, aldehydes, ketones,  
307 esters, hydrocarbons, and others. Alcohols were the most abundant VOCs (Fig. 1A). A  
308 multivariate statistical analysis of volatile compounds (VOCs) was performed. The  
309 OPLS-DA revealed a clear separation of volatile compounds between the two groups  
310 ( $R^2X = 0.26$ ,  $R^2Y = 0.95$ ,  $Q^2 = 0.0703$ , Fig. 1B). The permutation plot and histogram test  
311 of the OPLS-DA model suggest that the original OPLS-DA model did not exhibit  
312 overfitting, indicating a relatively robust model (Fig. S2A-B).

313 Eight compounds were significantly different between these two groups, with 2,4-  
314 dihydroxybenzoic acid, 1,2,4-trimethylbenzene, and acetaldehyde being up-regulated  
315 by the PFS, and pyridine, 2-butanol, dl-isocitric acid lactone, 3,3-dimethylbutane-2-ol,  
316 and 4-ethylcyclohexanone down-regulated by the PFS (Fig. 1C). Meanwhile, 2,4-  
317 dihydroxybenzoic acid (AUC = 0.94), 3,3-dimethylbutane-2-ol (AUC = 0.94), and 4-

318 ethyl-cyclohexanone (AUC = 0.97) were selected as the potential VOCs biomarker to  
319 distinguish PFS from CON (Fig. 1D).

### 320 3.6. High definition mix discovery LC-MS/MS metabolome

321 Based on the high definition (HD) mix UHPLC-OE-MS discovery metabolomics,  
322 both hydrophilic substances and lipophilic metabolites can be detected. A total of  
323 22,630 peaks were detected in this experiment, and 17,985 peaks were retained after  
324 relative standard deviation de-noising. The Fig. S2C shows the score chart based on  
325 PCA analysis; all samples are located in the 95% confidence interval. The OPLS-DA  
326 model showed an apparent group separation between the two groups ( $R^2X= 0.215$ ,  
327  $R^2Y=1$ ,  $Q^2=0.275$ , Fig. S2D) and the model is robust based on permutations and  
328 interceptions (Fig. S2E-F). According to the HMDB database and self-built database, a  
329 total of 422 hydrophilic metabolites were identified, **belonging to various categories:**  
330 **organic acids and derivatives (25.1%); organoheterocyclic compounds (20.38%);**  
331 **phenylpropanoids and polyketides (13.03%); organic oxygen compounds (12.32%);**  
332 **benzenoids (10.9%); nucleosides, nucleotides, and analogues (7.35%); organic nitrogen**  
333 **compounds (4.03%); alkaloids and derivatives (1.18%); organosulfur compounds**  
334 **(0.71%); homogeneous non-metal compounds (0.47%); lignans, neolignans and related**  
335 **compounds (0.47%); hydrocarbons (0.24%); organic compounds (0.24%);**  
336 **organohalogen compounds (0.24%); and others (3.32%) (Fig. 2A). A total of 385 lipids**  
337 **were identified, belonging to various classes: phosphatidylcholine (PC, 16.88%); acyl**  
338 **carnitine (AcCa, 11.69%); free fatty acid (FFA, 10.91%); lysophosphatidylcholine**  
339 **(LPC, 5.45%); lysophosphatidylethanolamine (LPE, 3.12%); phosphatidylglycerol (PG,**  
340 **2.86%); phosphatidylinositol (PI, 2.34%); phosphatidylethanolamine (PE, 2.08%);**  
341 **sphingomyelin (SM, 1.82%); ceramides (Cer, 0.78%); phosphatidylserine (PS, 0.26%);**  
342 **triglycerides (TG, 0.26%); Coenzyme (Co, 0.26%); and others (41.30%) (Fig. 2B).**  
343 Among these lipids categories, the LPC in PFS was lower than that in LC, and Co was  
344 higher in PFS than that in LC (Fig. 2C).

345 In total, 66 compounds were screened out as **significantly different** metabolites by  
346 HD mix LC-MS/MS (VIP > 1,  $P < 0.05$ ). Among the hydrophilic metabolites, 43  
347 compounds showed statistically significant differences (Fig. 2D). **These included** the  
348 upregulated macrophorin A, hydroxypropyl-Isoleucine, sedoheptulose, rotenone, D-  
349 pantothenic acid, 5-hydroxyisourate, D-glutamine, xanthylic acid, S-adenosyl-L-  
350 methionine, uridine diphosphate glucuronic acid, 5'-inosinic acid, inosine 2'-phosphate,  
351 arginyl-alanine, aromadendrin, 3-ethyl-5-methylphenol, 2,5-diisopropyl-3-  
352 methylphenol, and N-desmethylvenlafaxine, and downregulated guanidinosuccinic  
353 acid, L-norleucine, 3,4-dihydroxybenzaldehyde, 1,6-dimethoxy pyrene, tectorigenin,  
354 2,4-dimethyloxazole, macrocarpal I, normicotine, 3-hydroxydecanoic acid, L-valine,  
355 adrenosterone, pelargonic acid, 2-hydroxybutyric acid, gyromitrin, guanosine-5'-  
356 triphosphate, 7-aminoflunitrazepam, zedoarondiol, 2-methylbutyrylcarnitine, and  
357 fexofenadine by PFS compared to LC. In lipids, there are 23 substances with different  
358 lipid molecular species affected by PFS (Fig. 2E). **In the PFS group, one species of**  
359 **AcCa, one species of Co, one species of PC, and two species of PG significantly**  
360 **increased. Conversely, nine species of LPC, one species of LPE, and eight species of**  
361 **PC decreased** (Fig. 2E). Meanwhile, 2,5-diisopropyl-3-methylphenol (AUC = 1), 3-  
362 hydroxydecanoic acid (AUC = 1), and LPC(15:0) (AUC = 1) were the potential PFS  
363 related biomarker in lamb meat based on ROC analysis (Fig. 2F).

### 364 3.7. Metabolic pathways analysis

365 Based on the KEGG enrichment analysis (Fig. 3A), we found the pathways of purine  
366 metabolism and choline metabolism in cancer were enriched ( $P < 0.05$ ). On the other  
367 hand, the metabolic pathways contributing to the metabolite differences of lamb meat  
368 were also conducted based on MetaboAnalyst (Fig. 3B). The bubble plot showed that  
369 the differential metabolites were mainly enriched in the ascorbate and aldarate  
370 metabolism, valine, leucine and isoleucine biosynthesis, pentose and glucuronate  
371 interconversions, and propanoate metabolism.

## 372 4. Discussion

373 *4.1. Growth performance and carcass characteristics*

374 Previous study found no significant improvement in the growth rate and carcass  
375 traits after the inclusion of 5%, 10% or 15% perilla seed in the diet, on a DM basis, of  
376 Hu-lambs (Deng et al., 2018). In this study, the HC diet, which has the highest dietary  
377 metabolic energy level, was set as a positive control. The results from the HC group  
378 indicated that the lambs fed with a higher energy diet exhibited increased growth  
379 performance but also higher fat deposition. The PFS significantly increased carcass  
380 weight and dressing percentage in Tan sheep compared to LC, which was similar to the  
381 carcass characteristics in HC. This improvement could be partly attributed to increased  
382 energy intake in the PFS group compared to the LC group, in line with previous  
383 observations that a higher growth rate can be achieved through oil supplementation in  
384 goats and sheep (Candyrine et al., 2018). In addition, feeding PFS increased the DM  
385 content of raw meat while reducing meat redness. Myoglobin interacts with oxygen to  
386 form the bright red oxymyoglobin, which along with myoglobin can be oxidized to the  
387 brown-colored high-iron content metmyoglobin, consequently affecting the perceived  
388 redness of the tissue (Brewer, 2004). **Flavonoids, known to prevent the production of**  
389 **free radicals (Zhu et al., 2022), are enriched in *Perilla* seeds in our study.** Consequently,  
390 the PFS might increase the activity of myoglobin reductase and delay the oxidation of  
391 myoglobin, potentially reducing meat redness when PFS was fed to lambs. The  
392 enhanced growth performance, increased carcass yield, and altered meat color observed  
393 in the PFS-fed group may be attributed to both the energy content of PFS and the  
394 presence of functional plant secondary metabolites. Thus, supplementing PFS in lamb  
395 diets, can synergistically improve the growth performance and carcass quality of lamb,  
396 when compared with diets with low concentrates; although the growth rates achieved  
397 by a HC diet would still be higher than a PFS diet.

398 *4.2. Meat fatty acid profiles*

399 This study mainly found that the PFS could increase the lamb meat UFA and n-3  
400 PUFA content and reduced the n-6/n-3, IA, and IT. The lamb fed with HC diet had

401 higher n-6 PUFA content which result in the highest n-6/n-3. The accumulation of n-3  
402 and n-6 PUFA in lamb meat are through direct consumption from diets or via  
403 desaturation and elongation processes from short-chain fatty acid precursors  
404 (Ponnampalam, Sinclair, & Holman, 2021). Hence, the elevated levels of n-6 PUFA in  
405 lamb meat from the HC group can be attributed to the common derivation of short-  
406 chain n-6 PUFA from grain-based and feedlot diets. The SFA may enhance lipid  
407 adhesion to immunological and circulatory system cells (pro-atherogenic), while UFA  
408 could inhibit plaque formation and reduce certain lipid levels, thereby decreasing the  
409 risk of coronary diseases (anti-atherogenic) (FAO, 2010). The firmness of adipose  
410 tissue is contingent upon the degree of fatty acid saturation, a factor that plays a critical  
411 role in determining the nutritional merit of meat products and their subsequent  
412 acceptance by consumers (Wood et al., 2004). It has been reported that n-3 PUFAs have  
413 properties that improve antioxidant capacity and nutritional value of meat, as well as  
414 playing a crucial role in health maintenance (Sunagawa et al., 2022). The diet's primary  
415 significant sources of preformed long-chain PUFAs such as eicosapentaenoic acid  
416 (C20:5 n3) and docosahexaenoic acid (C22:6 n3) are derived exclusively from ruminant  
417 meats and oily fish (Wyness et al., 2011). The consumption of lamb meat with reduced  
418 levels of n-6 PUFA and increased levels of n-3 PUFA had the potential to enhance both  
419 animal and human health, well-being, and resilience against diseases (Ponnampalam et  
420 al., 2021). The present study found higher concentrations of total n-3 PUFAs (+ 64%),  
421 C20:5n3 (+ 49%), and C18:3n3 (+ 83%) in PFS compared to LC, thus demonstrating a  
422 nutritionally improved fatty acid profile (Wood et al., 2004). Diet plays a pivotal role  
423 in shaping the fatty acid composition of lamb fat (Wood et al., 2004). Notably, the  
424 choice of dietary oil source can significantly impact the fatty acid content in lamb meat  
425 (Jeronimo, Alves, Prates, Santos-Silva, & Bessa, 2009). Furthermore, the presence of  
426 plant flavonoids has been shown to modify the fatty acid profiles of lamb meat (North,  
427 Dalle Zotte, & Hoffman, 2019). Thus, the reduction of SFA/UFA, n-6/n-3 PUFA, IA,  
428 and IT in the raw lamb meat from PFS group might be mainly explained by the high  
429 content of PUFA and plant flavonoids, and the production of PFS lamb meat can further

430 be regarded as beneficial effects from a public health and human nutrition perspective  
431 (Pretorius & Schonfeldt, 2021).

#### 432 *4.3. Compounds contribute to meat flavour and human health*

433 The VOCs and their respective precursors substantially influence the olfactory  
434 characteristic of ovine meat, or mutton, wherein the resultant odor profile is a complex  
435 interplay governed by their relative concentrations and perceptual thresholds (Zhan,  
436 Tian, Zhang, & Wang, 2013). Aldehydes typically possess a relatively low odor  
437 threshold, and as a result, they are regarded as having a vital impact on the distinct  
438 flavor of lamb meat (Zhang, Zhang, Liu, Zhao, & Luo, 2020), primarily originating  
439 from PUFAs (Hu et al., 2022). In this research, aldehyde serves as the primary aromatic  
440 active compounds based on VOC analysis. Acetaldehyde and acetal are types of  
441 aldehydes that have a fruity odor with sweet and astringent notes and play a role in  
442 forming the primary liquor aroma by assisting other flavor compounds (Wei, Zou, Shen,  
443 & Yang, 2020). 1,2,4-Trimethyl-benzene is thermal degradation **product** of  $\beta$ -carotene,  
444 which is produced in high amounts in the orange flesh color, imparting strong violet  
445 aromas (Wang & Kays, 2003). In addition, the pelargonic acid, an oily liquid with an  
446 unpleasant, rancid odor (Liu et al., 2022), was found to be decreased by PFS using HD  
447 mix LC-MS. 5'-Inosinic acid, identified as a taste-active component in the chicken meat  
448 extract (Fujimura et al., 1996), suggests that the taste of PFS lamb meat might be  
449 improved by increasing its 5'-inosinic acid content. **Therefore, the changes in VOCs**  
450 **and related hydrophilic metabolites in lamb meat suggest that feeding PFS may enhance**  
451 **the aroma, flavor, and taste of raw lamb.**

452 In addition, aroma compounds are mainly formed by lipids (Munekata et al., 2021).  
453 Thus, the lipids were further detected by a HD mix UHPLC-OE-MS. **Compared with**  
454 **normal LC-MS, HD LC-MS/MS has a mixed hydrophilic and lipophilic system,**  
455 **specifically the T3 chromatographic system, which can enhance the resolution and**  
456 **reliability in MS-oriented characterization of hydrophilic and lipophilic metabolites**  
457 **(Ding et al., 2022). Lipid subclasses (SM, Cer, LPC, PC, LPE, TG) in Tan sheep meat**

458 **can be significantly influenced by thermal processing** (Jia, Li, Wu, Liu, & Shi, 2021).  
459 We found the total LPC, 9 species of LPC, 7 species of PC, and 1 species of LPE were  
460 decreased by PFS, and 2 species of PG were increased by PFS. LPC(15:0) was also the  
461 potential biomarker to discriminate PFS from LC by the ROC analysis. LPC(15:0) was  
462 used to predict inflammatory response to TNF- $\alpha$  inhibitors in rheumatoid arthritis  
463 (Cuppen et al., 2016). In hepatic inflammation, there is a notable elevation in the  
464 concentrations of LPC and LPE, with particularly LPC as a potential biomarker in the  
465 diagnosis and monitoring of hepatic steatosis (Engel, Schiller, Galuska, & Fuchs, 2021).  
466 The addition of PG(18:1/18:1) and PG(18:2/18:2) can effectively reduce mitochondrial  
467 inflammation (Chen, Chao, Chang, Chan, & Hsu, 2018). Thus, the decreased LPC and  
468 increased PG(18:1/18:1) and PG(18:1/18:2) indicate the potentially desirable  
469 nutritional and safety characteristics of PFS lamb meat for consumers.

470 Furthermore, most of the potential detrimental hydrophilic metabolites were  
471 reduced by feeding PFS. For instance, the PFS reduced the relative abundance of  
472 muscular guanidinosuccinic acid that has been identified as a uraemic toxin (Duranton  
473 et al., 2012), and the hepatic guanidinosuccinic acid can be elevated in lambs under a  
474 high-energy diet induced immune response (Wang et al., 2023). 3-hydroxydecanoic  
475 acid is a potential negative biomarker associated with PFS. The tissue accumulation of  
476 3-hydroxydecanoic acid is associated with increased disease risk such as  
477 cardiomyopathy (Tonin et al., 2013). 2-Methylbutyrylcarnitine is an acylcarnitine, a  
478 group of compounds gaining recognition as crucial markers in metabolic investigations  
479 of various illnesses, such as metabolic disorders, cardiovascular diseases, diabetes,  
480 depression, neurological disorders, and some types of cancer (Dambrova et al., 2022).  
481 Thus, another potential benefit to human nutrition by consuming meat from lambs fed  
482 with PFS is the reduced concentrations of these compounds which are associated to  
483 various diseases.

484 Conversely, several metabolites that underwent substantial changes or potential  
485 biomarkers associated with PUFAs in lamb meat have yet to be thoroughly researched.  
486 Even though 2,5-diisopropyl-3-methylphenol was identified in the current investigation  
487 as a potential biomarker of PUFA-enhanced lamb meat, further investigations are

488 required to corroborate its association with PUFAs, understand its role in lamb  
489 physiology, and determine its impact on human nutrition and health.

## 490 **5. Conclusions**

491 The present study demonstrated that the dietary inclusion of *Perilla frutescens*  
492 seeds improved growth performance and simultaneously **improved** carcass quality and  
493 raw meat attributes in Tan-lambs. An increased n-3 PUFAs content and a decrease in  
494 the n-6/n-3, IA, and IT were observed in raw lamb meat as a result of feeding PFS.  
495 These changes are considered nutritionally desirable. Volatile compounds, including  
496 acetaldehyde and 1,2,4-trimethyl-benzene, were found in higher concentrations in PFS,  
497 which suggests an improved flavor profile for PFS raw lamb meat. In addition, several  
498 nutritionally beneficial lipids and hydrophilic metabolites were associated with PFS  
499 treatment, including PG(18:1/18:1), PG(18:2/18:2), and 5'-inosinic acid. Metabolites  
500 such as LPC, guanidinosuccinic acid, 3-hydroxydecanoic acid, and 2-  
501 methylbutyrylcarnitine, known to exert negative impacts on human health, were found  
502 in lower concentrations in PFS raw lamb meat. The present findings provide exhaustive  
503 understanding of the metabolome of raw lamb meat with improved n-3 PUFAs and  
504 corresponding volatile, lipidic, and hydrophilic metabolites achieved through the  
505 incorporation of *Perilla frutescens* seed. Additionally, the global alteration of  
506 compounds detected through HD-mix LC-MS/MS metabolomics proposes its utility as  
507 a replacement for lipidomics and hydrophilic metabolomics.

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## 513 **CRedit authorship contribution statement**

514 **Yue Yu:** Writing - original draft, Formal analysis. **Boyan Zhang:** Writing - review  
515 & editing, Resources, Conceptualization. **Xianzhe Jiang:** Writing - review & editing.  
516 **Yimeng Cui:** Investigation. **Hailing Luo:** Writing - review & editing, Resources.  
517 **Sokratis Stergiadis:** Writing - review & editing. **Bing Wang:** Project administration,  
518 Writing - review & editing, Supervision, Conceptualization.

## 519 **Declaration of Competing Interest**

520 The authors declare no conflict of interest.

## 521 **Data availability**

522 Data will be made available on request.

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650

651 **Tables**  
 652 **Table 1**

653 Ingredients and nutrient composition of the basal diet.

Item	LC <sup>1</sup>	HC <sup>1</sup>
Ingredient, % dry matter basis		
Corn grain	26.40	48.00
Soybean meal	12.80	14.20
Wheat bran	11.10	12.80
Corn silage	9.00	13.20
Alfalfa Hay	16.00	6.80
Caragana microphylla silage	20.00	0.00
NaHCO <sub>3</sub>	0.70	1.00
<i>Perilla frutescens</i> seed	0.00	0.00
Premix <sup>2</sup>	4.00	4.00
Nutrients		
Metabolic energy, MJ/kg	10.01	11.22
Crude protein, %	14.27	14.27
Neutral detergent fiber, %	37.84	22.39
Acid detergent fiber, %	24.56	11.16
Non-fiber carbohydrate, %	34.09	50.57
Ether extract, %	4.10	5.41
Ash, %	6.30	3.25
Calcium, %	0.81	0.51
Phosphorus, %	0.40	0.46

654 <sup>1</sup>LC: low-concentrate diet; HC: high-concentrate diet

655 <sup>2</sup>Formulated to provide (per kilogram of dry matter): 500,000 IU of vitamin A, 160,000  
 656 IU of vitamin D3, 650 IU of vitamin E, 150 g of NaCl, 20 g of Ca, 20 g of P, 1750 mg  
 657 of Zn, 15 mg of Se, 50 mg of I, 2000 mg of Fe, 20 mg of Co, 1500 mg of Mn, and 600  
 658 mg of Cu.

659 **Table 2**  
 660 The nutrient composition and fatty acid profiles of *Perilla frutescens* seeds (dry matter  
 661 basis).

Item	Composition	
Nutrients		
Crude protein, %		22.03
Neutral detergent fiber, %		23.82
Acid detergent fiber, %		18.67
Non-fiber carbohydrate, %		12.12
Ether extract, %		38.39
Ash, %		3.64
Fatty acids	mg/100g	% of total fatty acids
C8:0	3.48	0.01
C12:0	1.90	0.01
C14:0	8.19	0.03
C15:0	3.24	0.01
C16:0	2337	7.38
C16:1	32.55	0.10
C17:0	2.32	0.01
C18:0	698.45	2.20
C18:1n9c	6312	19.94
C18:2n6c	3767	11.90
C18:3n3	18292	57.78
C20:0	69.25	0.22
C20:1	55.91	0.18
C21:0	9.84	0.03
C20:2	8.18	0.03
C20:4n6	11.90	0.04
C22:0	17.54	0.06
C22:1n9	10.88	0.03
C24:0	17.10	0.05
Total fatty acids	31658	100.00

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663

**Table 3**

664

The growth performance and carcass characteristics of Tan-lamb

Items	Treatments <sup>1</sup>			SEM	P-value
	LC	HC	PFS		
Growth performance					
Initial BW, kg	25.4	25.0	25.3	0.30	0.674
DMI, g/d	934	961	869	41.0	0.345
ADG, g/d	120c	194a	157b	11.9	<0.001
FCR	8.28a	4.78b	5.44ab	1.848	0.061
Carcass traits					
Live weight, kg	31.1b	37.0a	35.4a	0.67	<0.001
Carcass weight, kg	14.1b	18.0a	17.3a	0.29	<0.001
Dressing percentage, %	45.6b	49.0a	48.8a	0.59	0.001
Head weight, kg	2.42b	2.72a	2.57ab	0.069	0.026
Hooves weight, kg	0.63	0.79	0.73	0.013	0.292
Pelage weight, kg	2.45	3.50	2.82	0.197	0.078
GR	6.03	7.98	6.99	1.020	0.458
The organ weight, g					
Heart	120b	145a	136a	3.7	0.001
Liver	507	660	523	33.8	0.083
Spleen	56.0	62.0	59.0	9.99	0.913
Lung	299	304	287	17.2	0.545
Kidney	41.4b	101.2a	46.2b	2.75	<0.001
Testis	316	358	315	13.6	0.100
The organ ratio, g/kg of carcass weight					
Heart	8.52a	8.07ab	7.87b	0.197	0.089
Liver	35.8	36.3	30.4	1.80	0.098
Spleen	3.79	3.36	3.54	0.522	0.842
Lung	21.2a	16.8b	16.6b	0.68	0.001
Kidney	2.95b	5.62a	2.65b	0.119	<0.001
Testis	22.4a	19.8b	18.3b	0.76	0.016

665

<sup>a-c</sup> Means within a row with different subscripts differ when *P*-value < 0.05.

666

<sup>1</sup> LC, low-concentrate diet; HC, high-concentrate diet; PFS: 3% *Perilla frutescens* seed

667

supplementation in LC; BW, body weight; DMI, dry matter intake; ADG, average daily

668

gain; FCR, feed conversion ratio (DMI/ADG); GR, the depth of muscle and fat tissue

669

from the surface of the carcass to the lateral surface of the 12th rib 110mm from the

670

midline.

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672

**Table 4**  
The fat distribution and raw meat quality of Tan-lamb

Items	Treatments <sup>1</sup>			SEM	P-value
	LC	HC	PFS		
Fat distribution					
Tail fat, g	1098b	1979a	1546ab	145.3	0.019
Tail fat ratio, g/kg	77.6b	110a	89.1ab	8.33	0.079
Perirenal fat, g	35c	287a	141b	26.4	<0.001
Perirenal fat ratio, g/kg	2.5c	15.9a	8.1b	1.50	<0.001
Omentum, g	154b	270a	322a	21.3	0.001
Omentum ratio, g/kg	10.9b	14.8a	18.7a	1.19	0.006
Meat quality					
Eye muscle area, cm <sup>2</sup>	15.6b	18.4a	17.7ab	0.89	0.098
Cooking rate, %	59.1	59.2	61.0	1.06	0.350
Shear force, N	63.7	47.0	52.2	5.00	0.081
pH 45 min	6.73	6.58	6.62	0.078	0.392
<i>a*</i> 45 min	8.5	8.3	9.0	0.45	0.519
<i>b*</i> 45 min	7.6	7.3	7.6	0.31	0.695
<i>L*</i> 45 min	34.3	34.2	34.2	0.69	0.987
pH 24 h	5.76	5.76	5.92	0.074	0.249
<i>a*</i> 24 h	12.0a	10.7ab	10.2b	0.49	0.050
<i>b*</i> 24 h	10.7	12.4	10.5	1.28	0.425
<i>L*</i> 24 h	38.1	41.5	38.1	0.70	0.106

673 <sup>a-c</sup> Means within a row with different subscripts differ when *P*-value < 0.05.

674 <sup>1</sup> LC, low-concentrate diet; HC, high-concentrate diet; PFS: 3% *Perilla frutescens* seed  
675 supplementation in LC; *a\**, redness; *b\**, yellowness; *L\**, lightness.

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**Table 5**  
The fatty acid composition (mg/100g tissue, wet fresh matter basis) and health index  
in the raw meat of Tan-lamb

Items	Treatments <sup>1</sup>			SEM	P-value
	LC	HC	PFS		
DM content of raw meat, %	22.9b	24.7a	25.9a	0.56	0.009
IMF, % FM	1.85	2.80	2.38	0.316	0.138
IMF, % DM	8.08	11.2	9.37	1.247	0.239
ΣSFA	879	1137	1128	128	0.152
C10:0	2.51b	3.57a	3.44ab	0.522	0.090
C12:0	3.30	3.12	3.82	0.896	0.570
C14:0	47.9	51.2	60.5	12.95	0.338
C15:0	7.02	9.71	7.24	1.894	0.294
C16:0	424	577	569	75.7	0.116
C17:0	20.9b	40.7a	21.3b	5.54	0.033
C18:0	346	427	429	35.0	0.187
C20:0	3.12b	3.44ab	3.93a	0.221	0.057
C21:0	11.6	10.0	11.4	1.23	0.200
C22:0	2.77a	2.19b	1.99b	0.231	0.005
C23:0	2.84a	2.11b	2.34b	0.275	0.011
C24:0	2.82	2.45	2.70	0.161	0.278
ΣUFA	916b	1310a	1271a	137.8	0.041
ΣMUFA	701b	1051a	1043a	126.3	0.037
C14:1	1.79	2.02	2.42	0.585	0.251
C16:1	27.8b	41.1a	39.1ab	6.19	0.074
C18:1c9	666b	1003a	996a	119.1	0.035
C20:1	2.54	2.41	2.45	0.263	0.939
C22:1n9	1.07	0.99	0.98	0.096	0.762
C24:1	2.87	2.53	2.52	0.184	0.334
ΣPUFA	207	252	218	15.3	0.121
CLA-c9t11	3.96	3.96	3.75	0.308	0.865
CLA-t10c12	1.16b	1.73a	1.13b	0.307	0.035
Σn-3	13.6b	11.5b	25.4a	1.51	<0.001
C18:3n3	7.53b	6.49b	15.7a	1.007	<0.001
C20:5n3	3.27b	2.67b	5.44a	0.415	<0.001
C22:6n3	2.61b	2.44b	3.96a	0.411	0.012
Σn-6	194b	241a	194b	14.5	0.053
C18:2n6	126b	171a	132b	11.6	0.028
C20:3n6	5.37a	4.79ab	4.53b	0.461	0.098
C20:4n6	61.9	64.4	57.2	3.04	0.262
ΣTFA	1795b	2447a	2399ab	265.3	0.077
SFA/UFA	0.96a	0.87b	0.88b	0.018	0.003
MUFA/PUFA	3.45	4.25	4.54	0.397	0.139
n-6/n-3	15.2b	21.3a	8.02c	1.127	<0.001

C16:0/C18:1	0.64a	0.58b	0.56b	0.012	0.001
IA	0.68a	0.60b	0.62b	0.017	0.020
IT	1.68a	1.56b	1.51b	0.039	0.017

679 <sup>a-b</sup> Means within a row with different subscripts differ when  $P$ -value < 0.05.

680 <sup>1</sup> LC, low-concentrate diet; HC, high-concentrate diet; PFS: 3% *Perilla frutescens* seed  
681 supplementation in LC; DM, dry matter; FM, fresh matter; IMF, intramuscular fat; SFA,  
682 saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids;  
683 PUFA, polyunsaturated fatty acids; CLA, conjugated linoleic acid; TFA, total fatty  
684 acids; IA, index of atherogenicity; IT, index of thrombogenicity.

685 **FIGURE CAPTIONS**

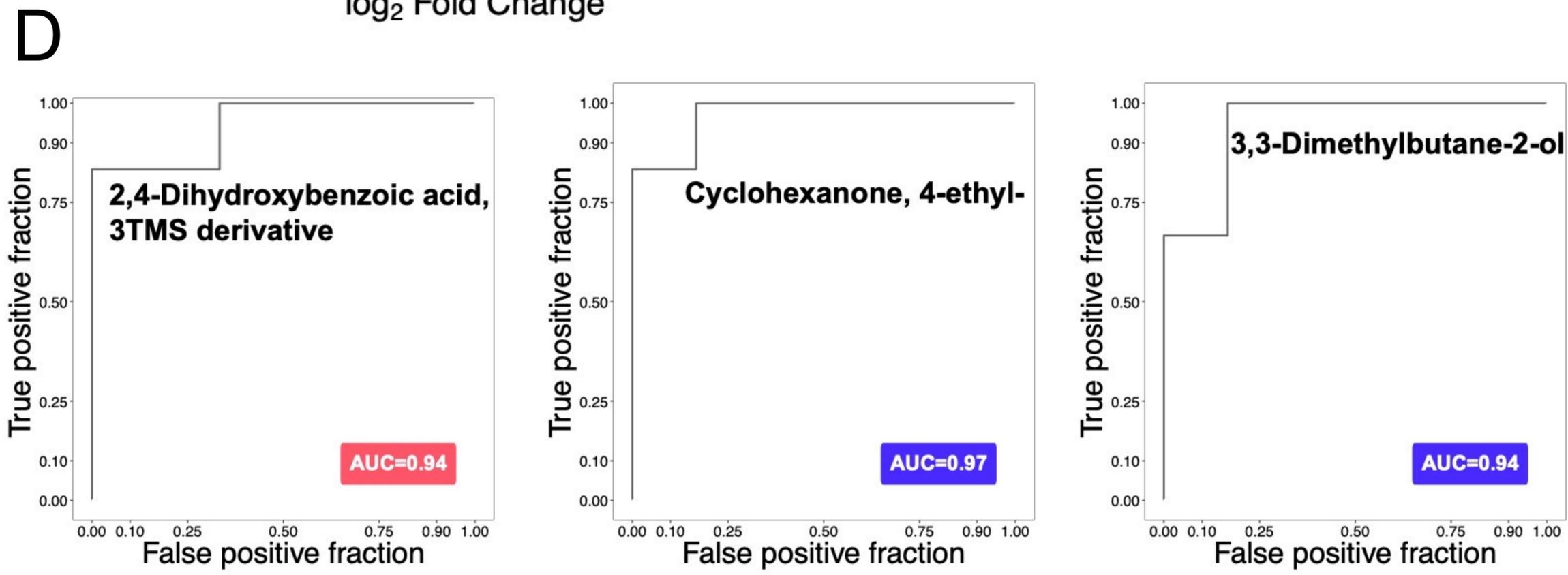
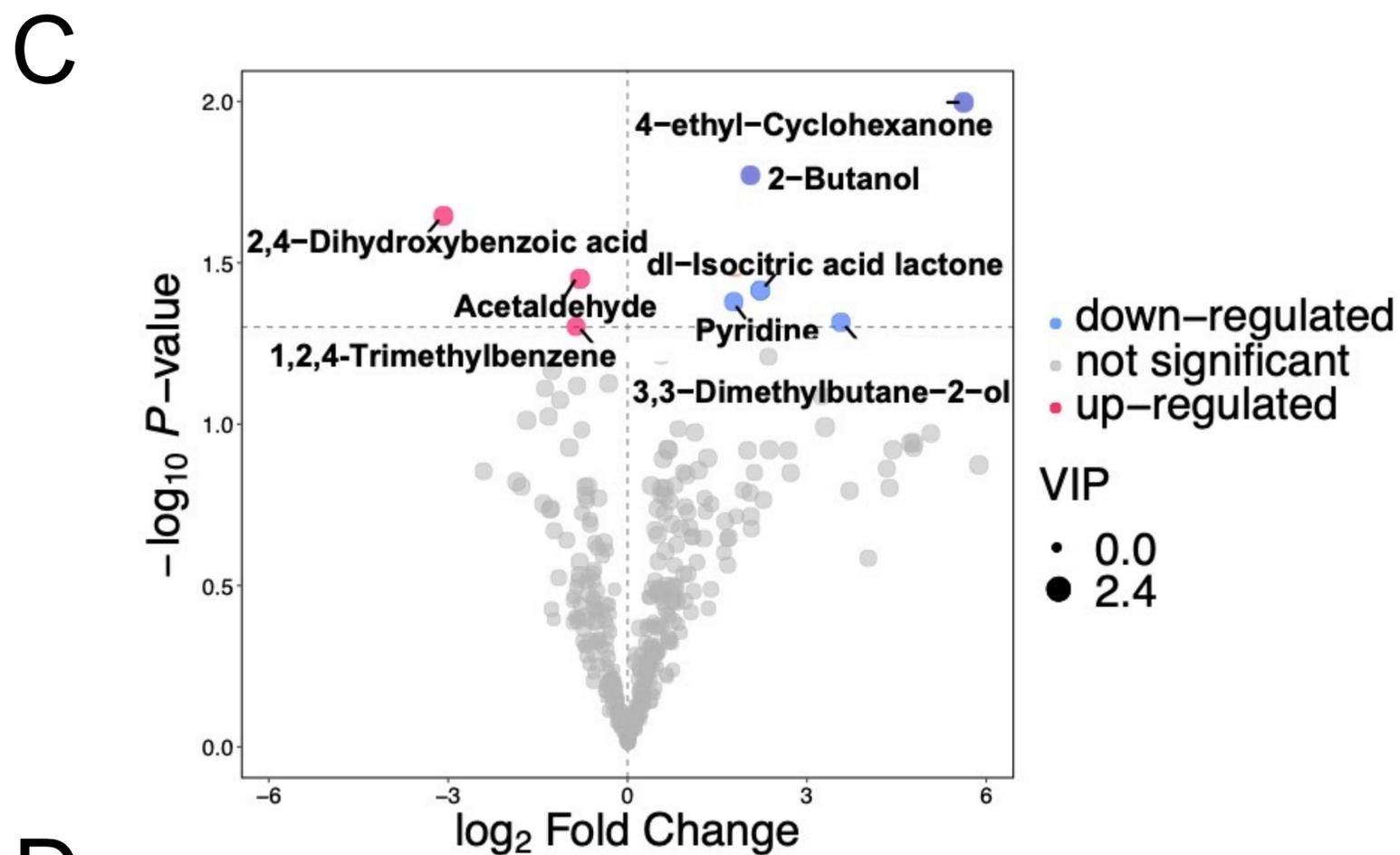
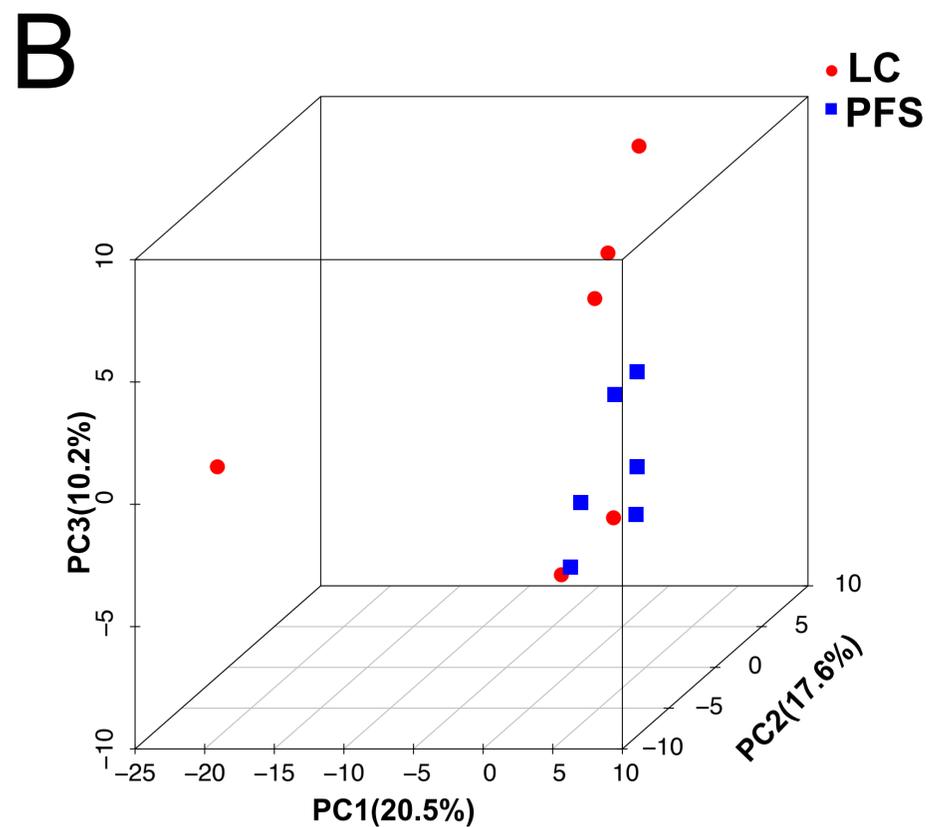
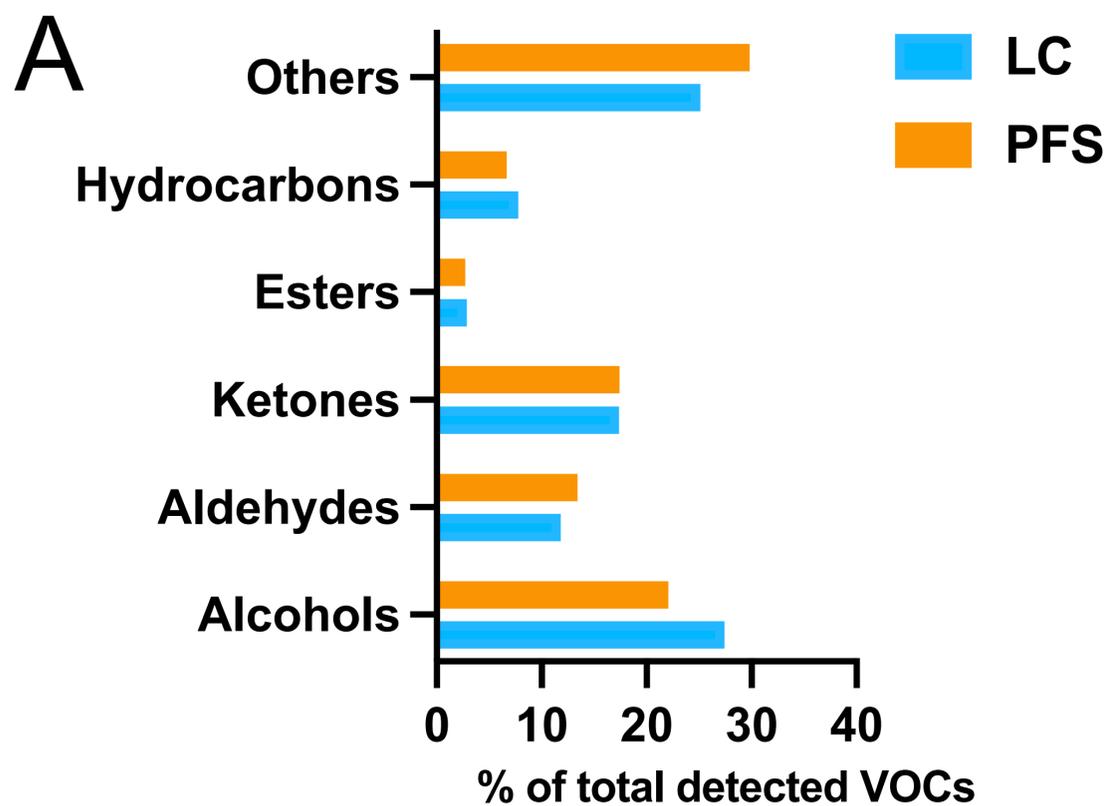
686 **Fig. 1.** The profiles of volatile compounds (VOCs) in *longissimus lumborum* based on  
687 GC-MS. (A) The relative proportion of volatile categories in the two groups. (B)  
688 Principal component analysis (PCA) score plots of volatile compounds. (C) The  
689 volcano plot and differential VOCs between LC and PFS. (D) Biomarker analysis  
690 results of VOCs (ROC curve view).

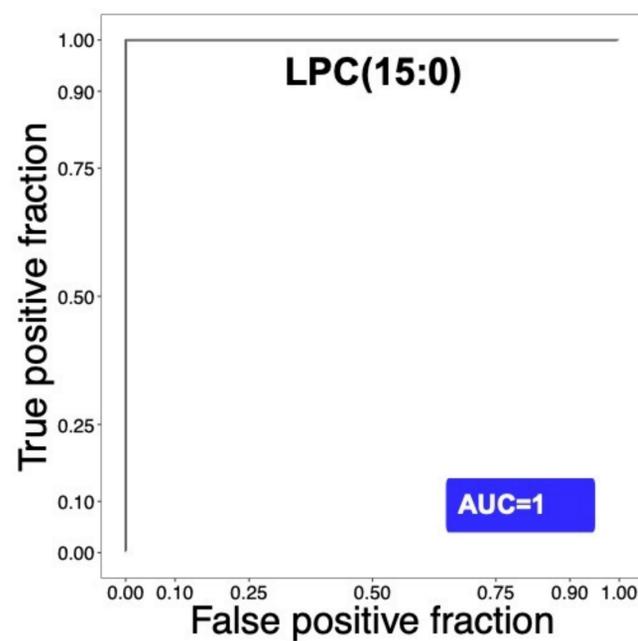
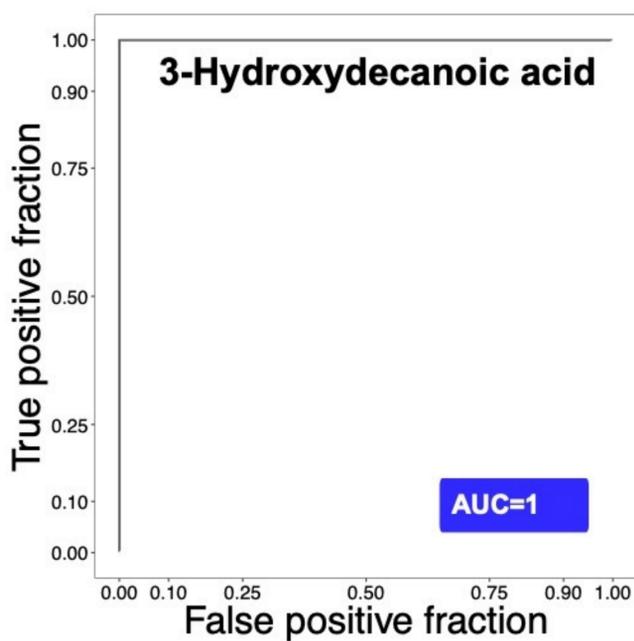
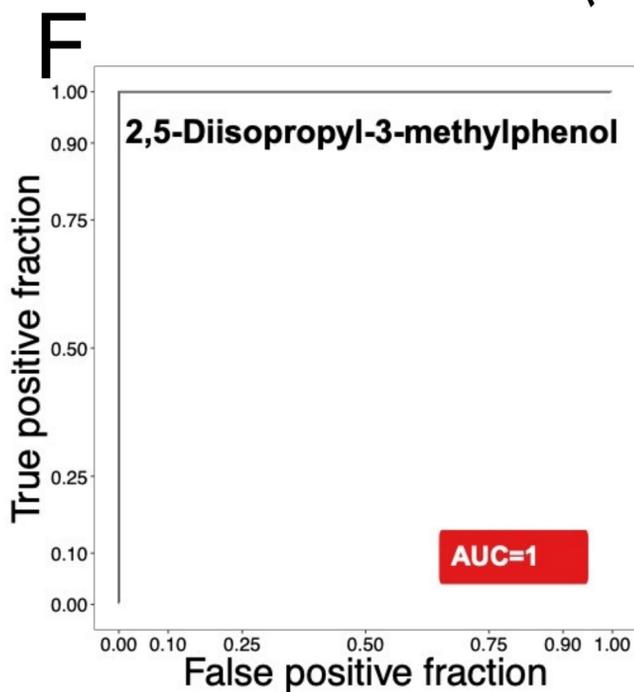
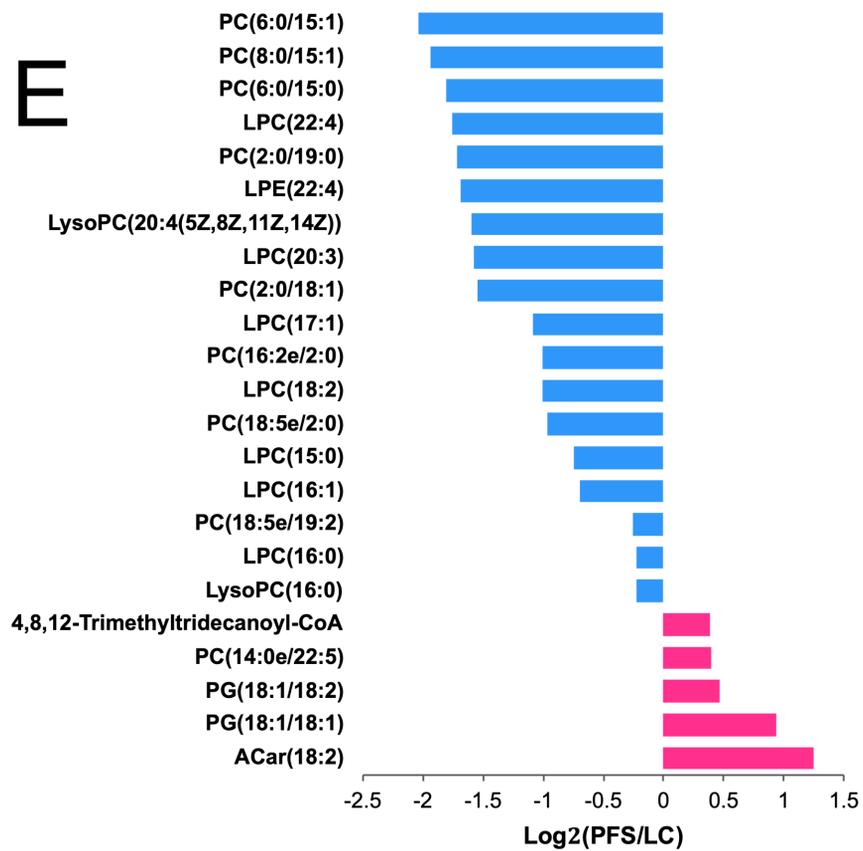
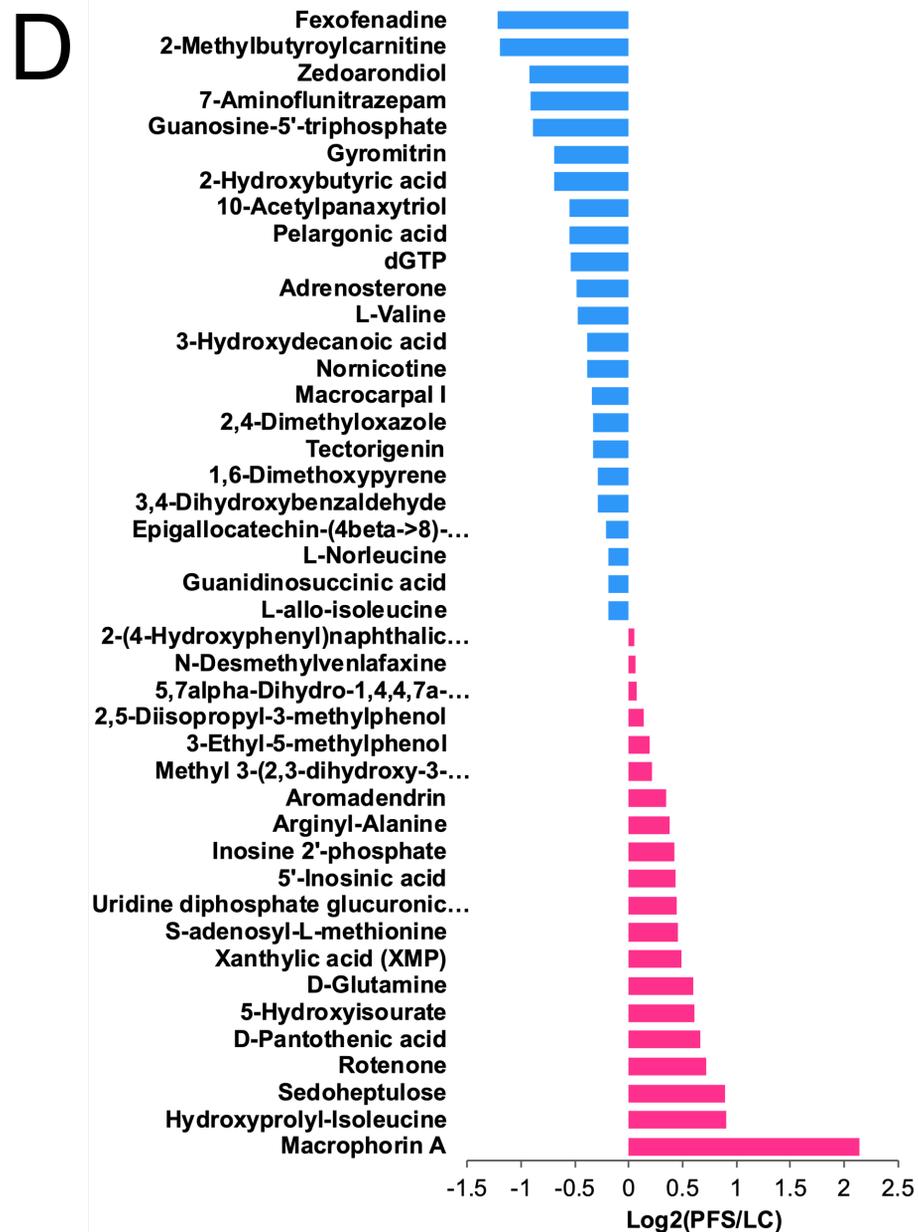
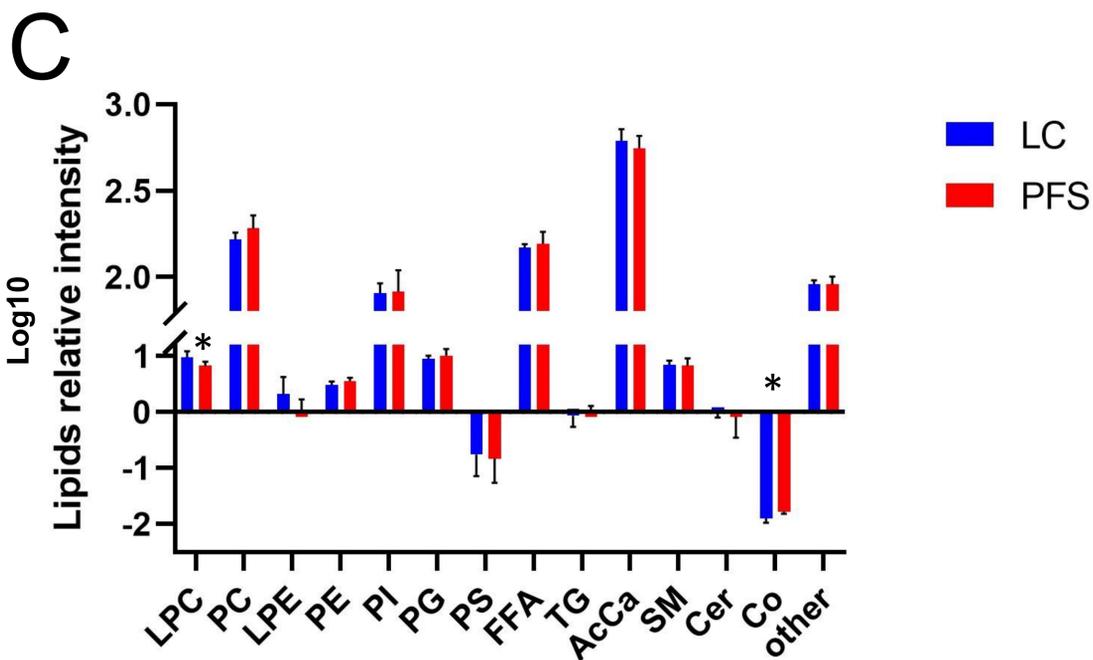
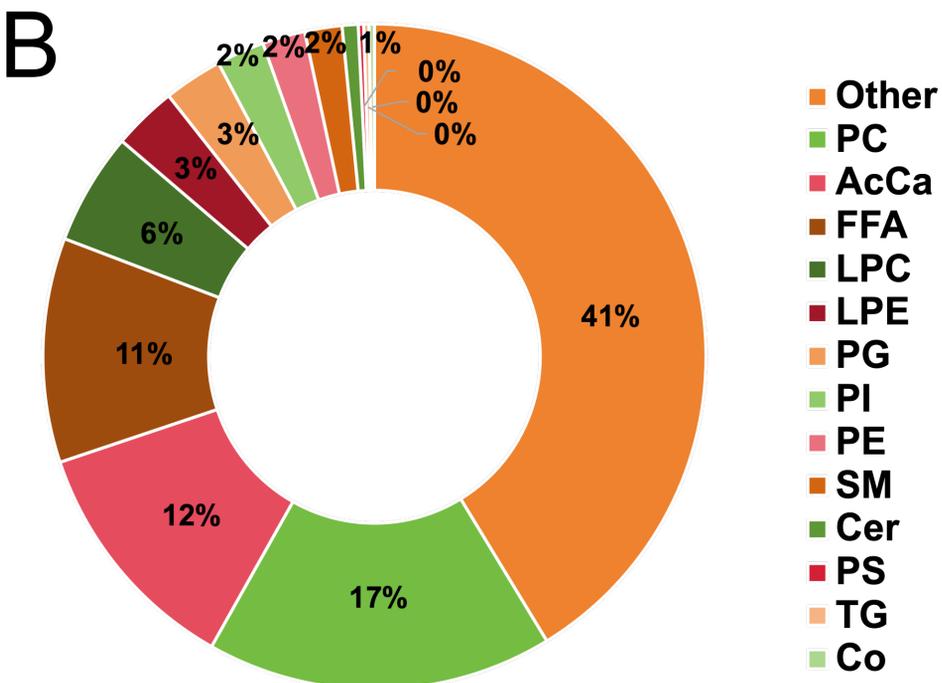
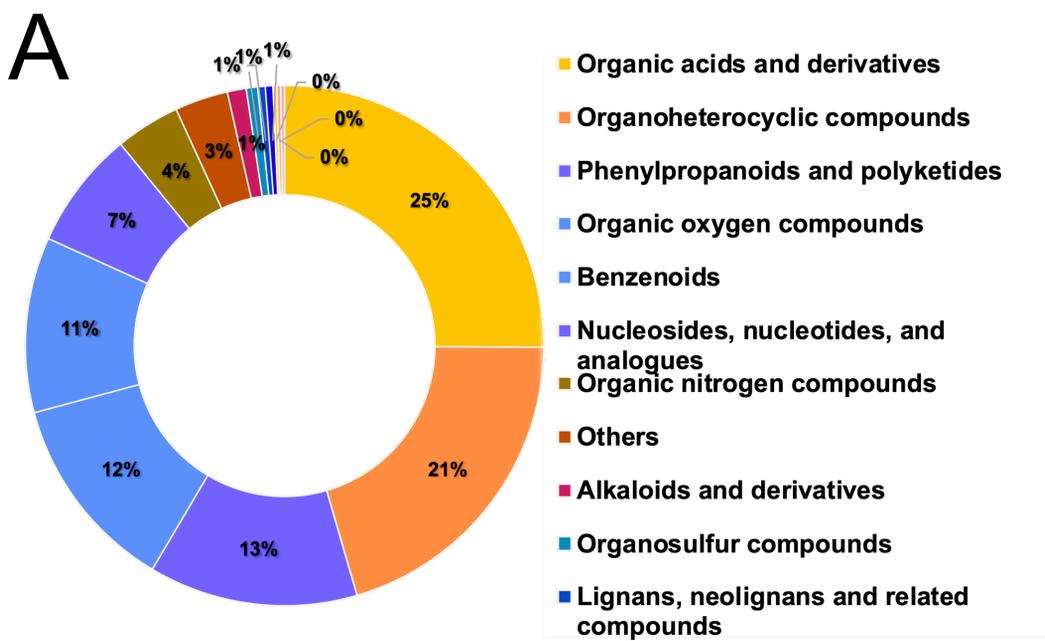
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692 **Fig. 2.** The profiles of lipids and hydrophilic metabolites in *longissimus lumborum*  
693 based on high definition mix LC-MS. (A) Percentages of categories of lipids and  
694 hydrophilic metabolites. (B) Percentages of numbers of lipid species. (C) Difference in  
695 lipid species between LC and PFS lamb. (D) Significant different hydrophilic molecular.  
696 (E) Significant different lipids molecular. (F) Biomarker analysis results of HD mix  
697 LC-MS metabolomics (ROC curve view). \*Represents significant differences using  
698 Student's two-tailed t-test. (\* $P < 0.05$ ). AcCa, acyl carnitine; Cer, ceramides; Co,  
699 coenzyme; FFA, free fatty acid; LPC, lysophosphatidylcholine; LPE,  
700 lysophosphatidylethanolamine; PC, phosphatidylcholine; PE,  
701 phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS,  
702 phosphatidylserine; SM, sphingomyelin; TG, triglyceride.

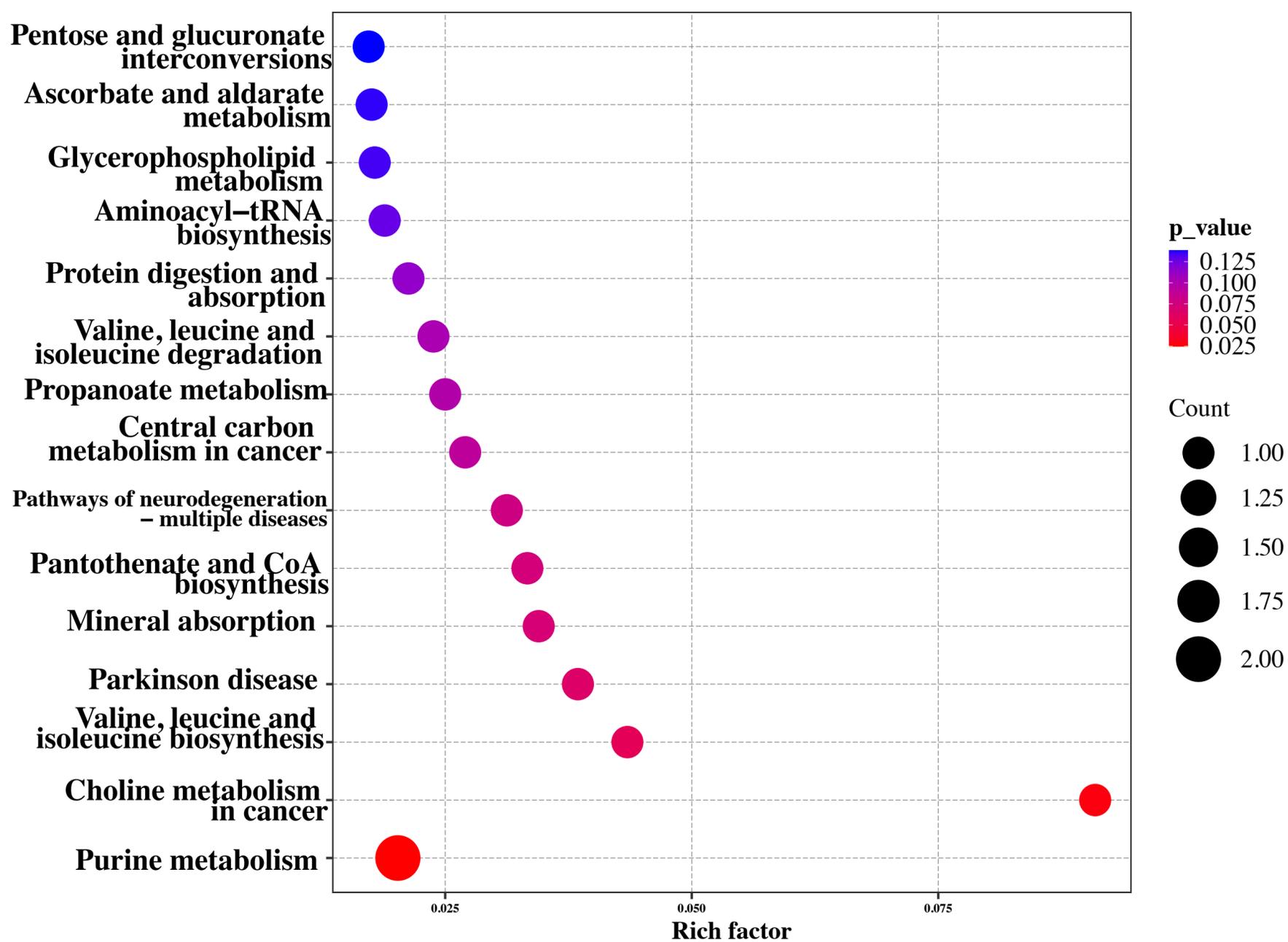
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704 **Fig. 3.** The enriched metabolic pathways and co-occurrence network analyses of the  
705 different volatile, lipophilic, and hydrophilic metabolites. (A) Metabolic pathways (top  
706 15) according to KEGG enrichment analysis of different metabolites (B) Overview of  
707 pathway analysis of significant metabolites using Metaboanalyst 5.0.

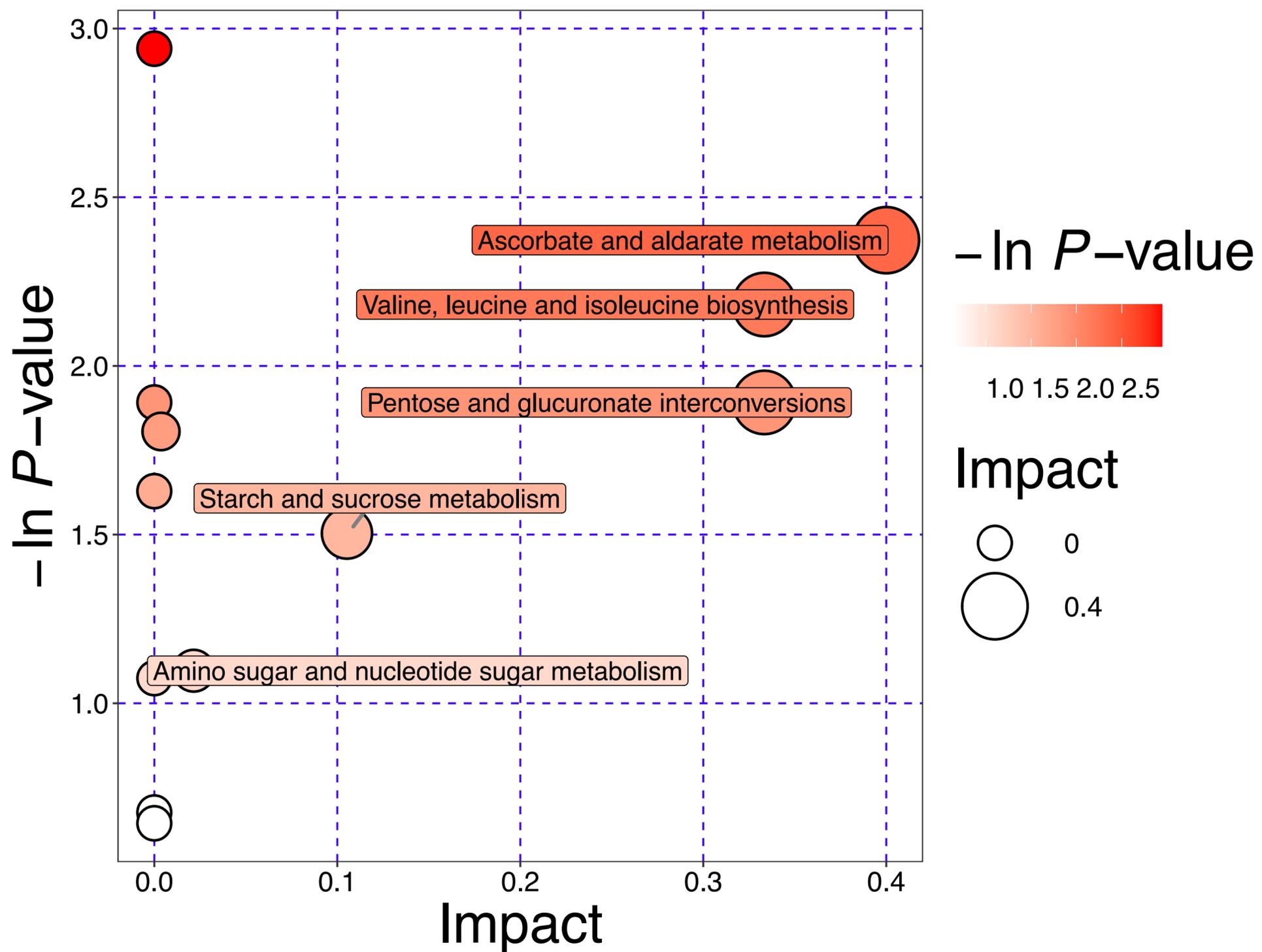




# A



# B



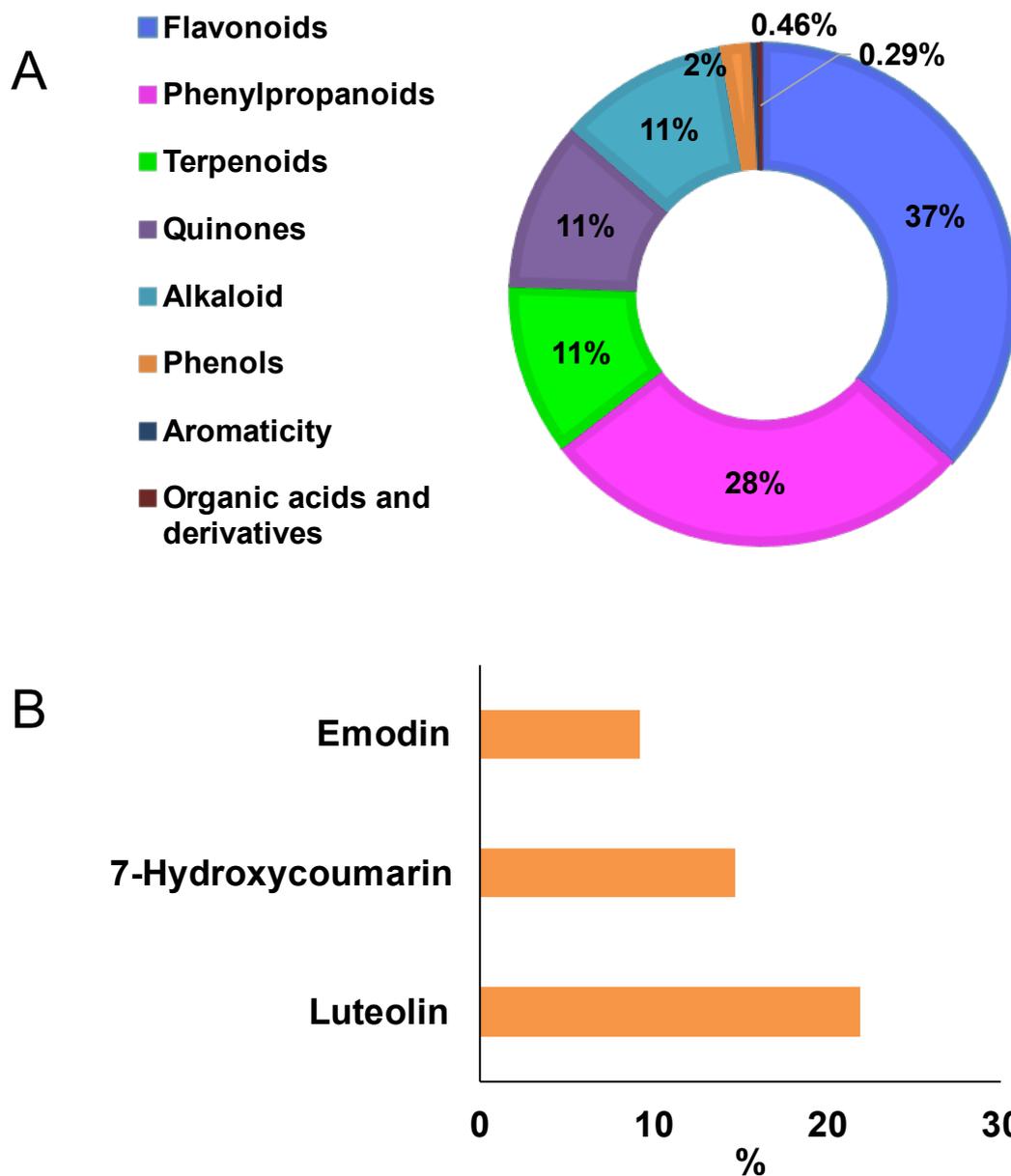
**Exploring the metabolomic landscape: *Perilla frutescens* as a promising enhancer  
of production, flavor, and nutrition in Tan lamb meat**

Yue Yu<sup>a</sup>, Boyan Zhang<sup>a</sup>, Xianzhe Jiang<sup>a</sup>, Yimeng Cui<sup>a</sup>, Hailing Luo<sup>a</sup>, Sokratis Stergiadis<sup>b</sup>, Bing Wang<sup>a,\*</sup>

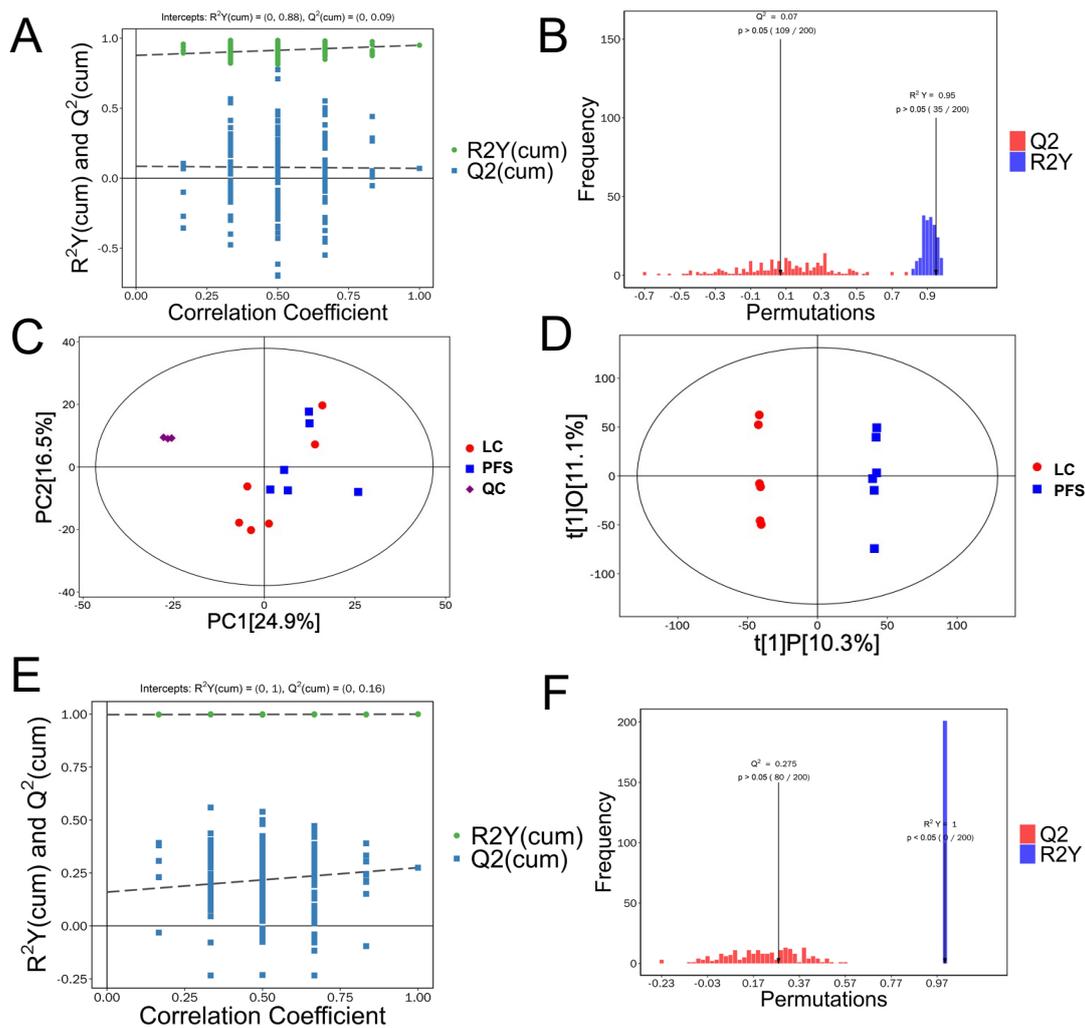
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*<sup>b</sup>University of Reading, School of Agriculture, Policy and Development, Department of Animal Sciences, Reading RG6 6EU, United Kingdom*

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**Fig. S1.** The plant secondary metabolites of *Perilla Frutescens* seeds. (A) the main categories of plant secondary metabolites. (B) the top three **compounds** of plant secondary metabolites.



**Fig. S2.** The principal component analysis (PCA) and supervised orthogonal projections to latent structures-discriminant analysis (OPLS-DA) plots. (A-B) the permutation plot and histogram test of the OPLS-DA model based on GC-MS. (C) PCA score plots of lipophilic and hydrophilic metabolites. (D) OPLS-DA score plots of lipophilic and hydrophilic metabolites. (E-F) the permutation plot and histogram test of the OPLS-DA model based on high-definition mix discovery LC-MS/MS.