

Assessment of components related to flavor and taste in Tan-lamb meat under different silage-feeding regimens using integrative metabolomics

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ABSTRACT

Two untargeted metabolomics approaches based on gas chromatography mass spectrometry and ultra-high-performance liquid chromatography quadrupole time-of-flight mass spectrometry were used to identify the effects of different feeding regimes (concentrate, corn silage, alfalfa silage, mulberry leaf silage) on the potential meat flavor and taste components of Tan-lamb. Among 31 identified volatiles, hexanal was affected by the alfalfa silage diet, and 3-hydroxydodecanoic acid was changed by the mulberry leaf silage diet. L-Pipecolic acid (area under the curve = 1, fold change = 0.18–0.48) and trimethylamine N-oxide (area under the curve = 1, fold change = 5.26–22.84) was the potential best discriminant biomarker under alfalfa silage and concentrate feeding, respectively. The hydrophilic components were more readily changed by feeding regimes than volatile flavor compounds. Our findings are helpful for the illustration of Tan-lamb meat chemistry and producing high-quality lamb meat with improved flavor and taste by corn silage, alfalfa silage, or mulberry leaf silage.

Introduction

Consumers' increasing demands for high-quality, safe, and good taste meat products require sustainable farming practices with good environmental, nutritional, and health value. Feeding regime is one of the most important factors determining the animal products quality (Álvarez-Rodríguez, Ripoll, Lobón, Sanz, Blanco, & Joy, 2018; Rocchetti, Gallo, Nocetti, Lucini, & Masoero, 2020). Tan sheep, a local Chinese sheep breed, is famous for its meat with low "off-flavor" and good taste (Gao et al., 2014; Wang et al., 2021b; Zhang, Zhang, Liu, Zhao, & Luo, 2020). However, in our recent study, we found that Tan-lamb meat quality and most beneficial meat compounds such as omega-3 polyunsaturated fatty acids and umami amino acids would be reduced by indoor feeding compared to grazing on pasture, even the indoor feeding improved the Tan-lamb growth performance (Wang et al., 2021b). One of the main reasons was the higher intake of

concentrates by lambs in the indoor feeding regime.

In recent years, there has been renewed interest in incorporating more high-quality forage within Tan-lamb indoor feeding systems, an efficient method to achieve synergistic improvement of growth performance and meat quality (Devincenzi, Delfosse, Andueza, Nabinger, & Prache, 2014). Corn plants, alfalfa, and mulberry leaf have highly palatable and digestible macronutrients to herbivorous animals (Doran, Laca, & Sainz, 2007; Jetana, Vongpipatana, Usawang, & Thongruay, 2011). These plants were also always used as silages in ruminants' diet, which could consistently provide high-quality green forage for livestock throughout the year (Muck & Shinnors, 2001). The inclusive of alfalfa in diet can improve the omega-3 fatty acids, conjugated linoleic acid, and α -tocopherol content in light lamb (Álvarez-Rodríguez et al., 2018). The inclusion of an appropriate proportion of mulberry leaf power has a positive role in improving the pork quality and the chemical composition of muscle (Liu et al., 2019). The silage-based diets could affect the

Abbreviations: CON, concentrate-based diet; CS, corn silage-based diet; AS, alfalfa silage-based diet; MS, mulberry leaf silage-based diet; IMF, intramuscular fat; GC-MS, gas chromatograph-mass spectrograph; SPME, solid-phase microextraction; RI, retention index; QC, quality control; UHPLC-QTOF-MS, ultrahigh-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry; ESI, electrospray ionization; IDA, information dependent acquisition; PCA, principal component analysis; PLS-DA, partial least squares discriminant analysis; DVCs, differential volatile metabolites; OPLS-DA, orthogonal partial least squares discriminant analysis; VIP, variable importance in the projection; FC, fold change; DFMs, differential metabolites; KEGG, Kyoto Encyclopedia of Genes and Genomes; TMAO, Trimethylamine N-oxide; AUC, area under the curve.

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aroma, tenderness, and flavor values of mixed-breed Santa Inés lambs' meat (Campos et al., 2017). Dietary nutritional strategies also affect the sensory, taste, and flavor of cooked lamb meat (Almela, Jordan, Martinez, Sotomayor, Bedia, & Banon, 2010), indicating the critical role of the feeding regime in the food industry.

The lamb flavor and taste compounds, including lipids, hydrophilic metabolites, and volatile compounds, were very complex and associated with lamb quality (Li et al., 2020). Metabolomics can comprehensively measure large numbers of small molecule metabolites deposited in animal foods (Goldansaz, Guo, Sajed, Steele, Plastow, & Wishart, 2017; Munekata, Pateiro, López-Pedrouso, Gagaoua, & Lorenzo, 2020). Thus, this technique can help us understand the meat chemistry and relevant compounds associated with Tan-lamb meat quality traits. Multi-metabolomics techniques have been used to identify suitable biomarkers of food integrity or characteristics, primarily for gas and liquid chromatography coupled to mass spectrometry-based metabolomics approaches for meat (Buttinger & Wenzl, 2020; Li et al., 2020; Rocchetti, Lucini, Gallo, Masoero, Trevisan, & Giuberti, 2018; Wang et al., 2021a). However, few studies did the Tan-lamb meat quality analysis under various silages' feeding by integrative metabolomics to the best of our knowledge. Our questions and hypothesis focused on how the three silage-based diets would change lamb meat's flavor and taste components. This study assessed the metabolome and potential biomarkers of raw Tan-lamb under different silage-based feeding regimes by two integrative metabolomics.

Materials and methods

Experimental design and sampling

The feeding and slaughter experiment was conducted in Tianyuan Liangzhong Sheep Farm (Ningxia Hui Autonomous Region, China). The animals used in this study were approved by the Animal Care Committee of China Agricultural University (Beijing, China; approval no. AW30129102-1-1). Twenty-four healthy castrated male Tan-lambs (*Ovis aries*) with an average age of 75 d (± 3), body weight of 15.3 kg (± 1.92 SD) were selected in the current study. The lambs were randomly allocated to 1 of 4 treatments (on a dry matter basis): 1) control group with concentrate-based diet (CON, $n = 6$); 2) silage I group with corn silage-based diet (CS, $n = 6$); 3) silage II group with alfalfa silage-based diet (AS, $n = 6$); 4) silage III group with mulberry leaf silage-based diet (MS, $n = 6$). The lambs in each group were fed individually with a basal total mixed ration diet. The dietary ingredients and nutrient composition (dry matter basis) are shown in Table S1. All the lambs were slaughtered after 80 d feeding. After slaughtering, the *longissimus lumborum* was collected. One meat sample was used for measuring meat crude protein, intramuscular fat (IMF), and ash after 24 h aging, and another meat sample was stored at liquid nitrogen immediately for subsequent analysis.

GC-MS based metabolomics procedure.

Longissimus lumborum samples were pretreated and determined by gas chromatograph-mass spectrograph (GC-MS) following a standard method from our previous study (Zhang et al., 2020). In general, 10 g of sample was added with 2.5 μ L internal standard solution (o-dichlorobenzene, 0.5 μ g/mL in methanol) in the extraction headspace flask with 2 g of sodium chloride heated at 120 °C for 30 min after sealing. Then, the system was cooled to room temperature and taken in a water bath at 60 °C. A solid-phase microextraction (SPME) injector with a 65 μ m polydimethylsiloxane vinylbenzene coated extraction head (Supelco, USA) was used to fully absorb the volatile flavor substances for 30 min after inserting them into the headspace flask. Then, the extracted SPME needle tube was inserted into the injection port of a gas chromatograph-mass spectrograph (GC-MS, 890B-5977B, Agilent, Palo Alto, CA, USA), thermally desorbed for 3 min, and then for later GC-MS analysis.

A DB-5 nonpolar column (60 m \times 0.32 mm I.D., 1 μ m film thickness,

J & W Scientific, Rancho Cordova, CA, USA) was used for metabolites separation. Helium was used as the carrier gas in a flow of 0.8 mL/min at 40 °C. The electron impact model was used with a 230 °C source temperature, 70 eV ionizing voltage, and a scan range from m/z 45 to m/z 350 at 2.76 scans/s. The identification of compounds was by first comparing their mass spectra with spectra from the Mainlib/NIST/Wiley7 Mass Spectral Database by comparing retention index (RI) with published RI values. The RIs were calculated based on the retention times of the substance following our previous study (Zhang et al., 2020). The compounds' approximate quantities were estimated by comparing their peak areas with that of the o-dichlorobenzene internal standard obtained from the total ion chromatograms. Quality control (QC) samples (10 g, $n = 4$), the mixed tested samples in equal quantities (approximately 1.67 g), were inserted into the sample queue to monitor and evaluate the stability of the system and the reliability of the experimental data.

UHPLC-QTOF-MS based metabolomics procedure

The *Longissimus lumborum* sample (80 mg) was pretreated and determined following a standard method from our previous study (Wang et al., 2021b). In general, 800 μ L methanol acetonitrile solution (1:1, vol/vol) was used to precipitate the protein. Then, the supernatant was used for the ultrahigh-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS) analysis. The samples were separated by UHPLC and analyzed by an Agilent 6550 mass spectrometer. Electrospray ionization (ESI) positive and negative ion modes were used for metabolites detection. Pooled QC samples (80 mg, $n = 3$) were generated by taking an equal aliquot (10 mg) of all the 24 samples included in the experiment. Then the QC samples are used to evaluate the stability of the system and the reliability of the experimental data.

The AB Triple TOF 6600 mass spectrometer was used to identify metabolites based on the collected primary and secondary spectra of QC samples. The secondary mass spectrum is obtained by information dependent acquisition (IDA) with high sensitivity mode. The data collected were used to identify the structure of metabolites using self-built MetDDA and LipDDA methods (Shanghai Applied Protein Technology Co. Ltd). The original data were converted into mzXML format by ProteoWizard, and then the XCMS program was used for peak alignment, retention time correction, and peak area extraction. The metabolite structure identification was based on accurate mass matching (< 25 ppm) and secondary spectrum matching methods and searches the laboratory's self-built commercial database (Shanghai Applied Protein Technology Co. Ltd).

Statistical analysis

For the univariate analysis, meat nutrients composition was analyzed by the one-way analysis of variance procedure of IBM SPSS 23 software with significance defined at $p < 0.05$, with trends were declared at $0.05 \leq p \leq 0.10$.

For the multivariate analysis, the raw GC-MS data were pre-processed by Pareto-scaling, then multi-dimensional statistical analysis including unsupervised principal component analysis (PCA) analysis and partial least squares discriminant analysis (PLS-DA) was performed. The volatile compounds were assessed by non-parametric tests (Wilcoxon rank-sum test) with p -value < 0.05 were set as differential volatile metabolites (DVCs). The statistical analyses were based on Metaboanalyst 5.0 (<https://www.metaboanalyst.ca/MetaboAnalyst/home.xhtml>) platform (Pang et al., 2021). The raw LC-MS data were pre-processed by Pareto-scaling, and then multi-dimensional statistical analysis including unsupervised PCA analysis and orthogonal partial least squares discriminant analysis (OPLS-DA) were performed. The first principal component of the variable importance in the projection (VIP) was obtained from OPLS-DA to refine this analysis. The volcano plot was

achieved based on fold-change (FC) and Student's *t*-test analysis (FC > 1.1 and *p*-value < 0.05). Then, the metabolites with VIP values exceeding 1 and the variables assessed by Student's *t*-test with *p*-value < 0.05 were set as differential metabolites (DFMs). The FC value of each metabolite was calculated by comparing the mean value between every two groups. The DFMs were further identified and validated by three databases, including Kyoto Encyclopedia of Genes and Genomes (KEGG, <http://www.kegg.jp/>), Human Metabolome Database, and Bovine Metabolome Database.

Results

Meat phenotype characteristics

Meat nutritional composition is shown in Table 1. The meat ash content was greater in the MS group than in the CS group (*p* < 0.05). Compared with the CON (*p* = 0.06) and AS (*p* = 0.08) groups, the meat ash content tended to be higher in the MS diet. The IMF content tended to be affected the four dietary treatments (*p* = 0.08). No significant differences were found in the protein content of meat.

Lamb meat volatile flavor compounds analysis based on GC-MS

The PCA analysis showed no apparent separation among the four groups. PLS-DA analysis was applied on the transformed dataset to investigate the possibility of clustering meat samples and illustrated an apparent separation between every two groups (Fig. 1). A total of 31 valid compounds in the muscles of lamb were identified and semi-quantified (Fig. 2a). These compounds were classified into five chemical families: alcohols (7), aldehydes (5), alkane (3), esters (12), and hydrocarbons (4) (Fig. 2b). Based on the cutoff (*p* < 0.05) for DVCS (Table 2), 14-octadecenal and dodecane were increased, and 2'-hexyl-1,1'-bicyclopropane-2-octanoic acid methyl ester was decreased in the CS compared to the CON. Hexanal was decreased in the AS compared to the CON. 3-hydroxydodecanoic acid was increased in the MS compared to the CON. 10,13-octadecadiynoic acid, methyl ester was decreased in the MS compared to the CS. Octaethylene glycol monododecyl ether was increased in the MS than it in the AS.

Lamb meat taste components analysis based on UHPLC-QTOF-MS

The PCA, OPLS-DA permutation, and volcano plots of the positive and negative ion modes for the four groups and QC samples are shown in Fig. S1-5. The parameters for the assessment of the PCA and OPLS-DA model quality are shown in Table S2. The R2X values of the PCA model that represent explained variance among the four groups in the positive and negative model were 0.54 and 0.53, respectively. The OPLS-DA permutation plot testing was used to cross-validate the OPLS-DA model quality, as shown in Table S2, Fig. S4, and Fig. S5.

Both positive ion mode and negative ion mode were used for later analysis. In the fetuses, 12,771 and 11,490 ion peaks were extracted in the positive and negative modes, respectively. In total, 225 and 168

Table 1

Effect of different feed regimes on meat nutritional composition in raw lambs.

Items, dry matter basis	Treatments				SEM	<i>p</i> -value
	CON	CS	AS	MS		
Crude protein, %	81.4	81.0	82.1	82.4	1.206	0.85
IMF ¹ , %	11.0	9.85	7.41	6.95	1.154	0.08
Ash, %	4.72ab	4.52b	4.75ab	5.04a	0.116	0.06

a-bMeans within a row with different subscripts differ when *p*-value < 0.05.

²CON, concentrate diet; CS, corn silage; AS, alfalfa silage; MS, mulberry leaf silage.

¹ IMF = intramuscular fat.

metabolites with qualitative names in the positive and negative modes, respectively. Based on the cutoff (VIP > 1 and *p* < 0.05) for DFMs (Table S3), 24 DFMs (12 upregulated and 12 downregulated DFMs in CS group) were found in the comparison between CS and CON, 44 DFMs (12 upregulated and 32 downregulated DFMs in the AS group) were found in the comparison between AS and CON, 45 DFMs (18 upregulated and 27 downregulated DFMs in the MS group) were found in the comparison between MS and CON, 35 DFMs (12 upregulated and 23 downregulated DFMs in the AS group) were identified in the comparison between AS and CS groups, 40 DFMs (18 upregulated and 22 downregulated DFMs in the MS group) were identified in the comparison between MS and CS groups, 41 DFMs (28 upregulated and 13 downregulated DFMs in the MS group) were identified in the comparison between MS and AS groups. Among them, 6 metabolites were CON group associated DFMs, including taurine, L-pyroglutamic acid, L-arginine, S-adenosylmethionine, trimethylamine *N*-oxide, and *N*-acetylneuraminic acid (Fig. 3a). 9 metabolites were CS group associated DFMs, including L-pipecolic acid, tyramine, phosphorylcholine, 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine, salicylic acid, L-leucine, dacarbazine, choline, and L-phenylalanine (Fig. 3b). 3 metabolites were AS group associated DFMs, including indoxyl sulfate, L-arginine, and 2-ethoxyethanol (Fig. 3c). 4 metabolites, including acetylcarnitine, 2-ethoxyethanol, benzylazanium, and L-palmitoylcarnitine, were MS group associated DFMs (Fig. 3d).

Based on the particular area under the curve (AUC = 1), 1, 7, 23, 3, 2, 11 metabolites were shown as the candidate biomarkers in discriminating CS with CON, AS with CON, MS with CON, AS with CS, MS with CS, and MS with AS, respectively. Then, using the Venn diagram, we found 2, 3, 4 metabolites were the most potential biomarker in discriminating the CON, AS, and MS from other feeding groups (Table 3). Trimethylamine *N*-oxide and S-adenosylmethionine were the CON-associated biomarkers. Dacarbazine, L-pipecolic acid, and salicylic acid were the AS-associated biomarker. L-Palmitoylcarnitine, L-carnosine, His-Ser, and Pro-Arg were the MS-associated biomarker.

Discussions

Feeding strategy is one of the most crucial factors in determining raw meat quality, especially for the meat fatty acids composition (Daley, Abbott, Doyle, Nader, & Larson, 2010). Lipids and hydrophilic metabolites, including small peptides, nucleotides, and amino acids, are the cooked meat taste components, which influence the taste and flavor, tenderness and juiciness of meat (Ritota, Casciani, Failla, & Valentini, 2012; Watkins, Frank, Singh, Young, & Warner, 2013). The volatile compounds detected in raw meat contribute to the raw lamb meat odor and cooked meat flavor (Almela et al., 2010; Zhang et al., 2020). Metabolomics is used to rapidly screen small molecular metabolites in certain conditions in tissues, fluid, and cells, which has been rapidly utilized to discriminate fraudulent activities in the food industry (Wang et al., 2021c).

Based on the UHPLC-QTOF-MS based metabolomics, we found several vital metabolites that showed not only potential biomarkers in discriminating the specific feeding regime from others but indicators of meat quality. Trimethylamine *N*-oxide (TMAO), constantly accumulating in the tissue of marine animals in high concentrations, can be generated by gut microbial metabolism from choline, betaine, lecithin, and carnitine (Velasquez, Ramezani, Manal, & Raj, 2016). We found the greater value of TMAO deposited in the lamb muscle of the three silage groups, indicating its potential biomarker role indicating silage feeding and the potential changed meat profile by silage feeding. Besides, glutamine, serine, and glutamate have umami or sweet taste (Watkins et al., 2013). Indoxyl sulfate was shown to be intestinal microbiota-derived uraemic toxins (Vanholder, Schepers, Pletinck, Nagler, & Glorieux, 2014). Thus, the much-decreased L-glutamine, L-serine, L-glutamate, cytosine, L-pyroglutamic acid, oleic acid, and cytidine and increased indoxyl sulfate in the CON compared to the CS group indicated the reduced raw meat quality and taste by CON feeding. Salicylic acid

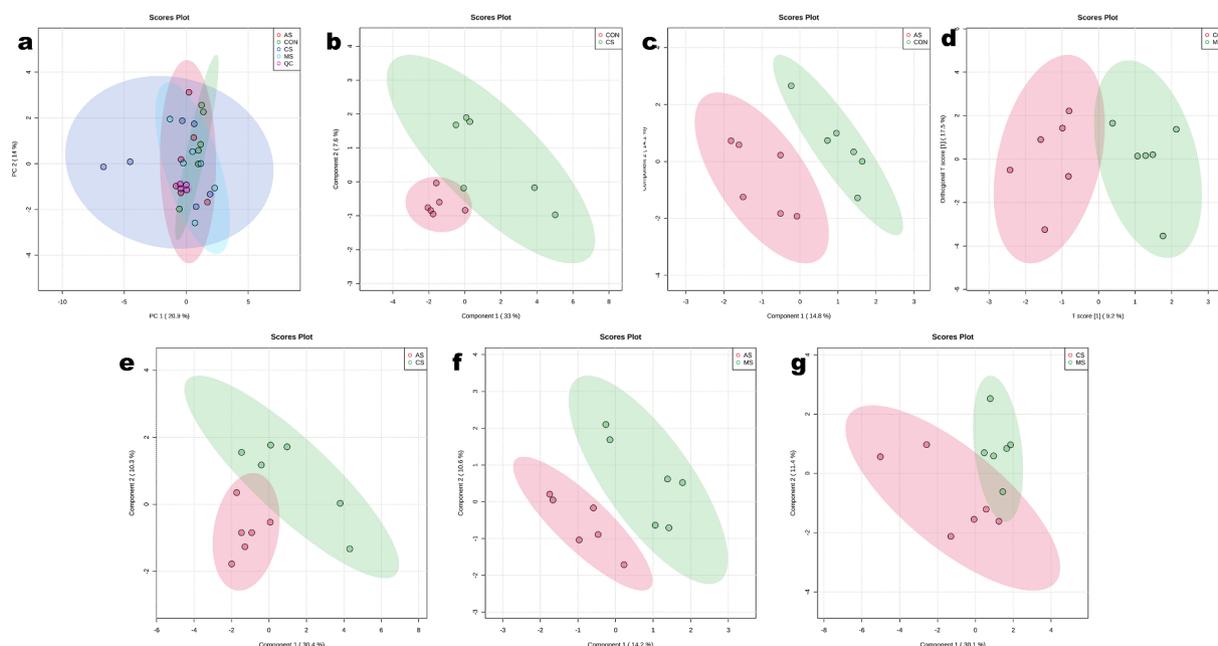


Fig. 1. Effect of different feed regimes on volatile compounds of raw lamb based on GC-MS. Principal component analysis (PCA) score plots of the individual metabolites from the four groups with the quality control (a). Partial least squares-discriminant analysis (PLS-DA) score plots of volatile compounds from every two groups' comparisons (b-g). CON, concentrate diet; CS, corn silage; AS, alfalfa silage; MS, mulberry leaf silage.

and L-pipecolic acid were AS-associated metabolites. In humans, more salicylic acid is excreted in the urine of vegetarians than in non-vegetarians, primarily because fruits and vegetables are important sources of dietary salicylates (Lawrence, Peter, Baxter, Robson, Graham, & Paterson, 2003). Besides, a high concentration of homostachydrine (pipecolic acid and pipecolic acid betaine) was reported in the extracts of alfalfa leaves (Servillo, Giovane, Balestrieri, Ferrari, Cautela, & Castaldo, 2012). Thus, in the current study, we estimated that L-pipecolic acid and salicylic acid might be the biomarkers for the lambs when they consume alfalfa or alfalfa silage. In addition, muscular pipecolic acid is a substrate for glutamate synthesis (Watanabe, Kobayashi, Shibata, Kubota, Kadowaki, & Fujimura, 2015). Pipecolic acid is a major intermediate of Lys metabolism in the mammalian (Takagi et al., 2003). Short-term feeding of a high-Lys diet could improve the taste of meat (Watanabe et al., 2015). Thus, the enhanced muscular pipecolic acid in the AS group might indicate the increased taste value of lamb meat. For the potential MS-associated biomarker, propyl cinnamate, enhanced in MS, is an amber, musty, and vine tasting compound (Thakur, Kumar, & Kanwar, 2012). Pro-Arg is a salt taste modulating peptide and His-Ser may have a bitter taste (Watkins et al., 2013). Both of them were decreased in MS. These changed MS-associated biomarkers indicated the improved lamb meat taste quality under MS feeding.

In recent research, meat from pasture-grazing sheep/goats had lower contents of methionine, carnosine, and anserine and a higher taurine content than meat from concentrate-fed sheep/goats metabolomic (Wang et al., 2021c). In our study, S-adenosylmethionine, a metabolic product of methionine, was significantly enhanced in the muscle of silage groups compared to CON. These results might be attributed to the more forage intake under silage feeding regimes. S-adenosylmethionine, a natural substance, has anti-inflammatory activity and was used in the treatment of chronic liver disease (Anstee & Day, 2012). Thus, the much lower value of S-adenosylmethionine in CON may indicate the decreased health status of lamb meat.

Nowadays, the detected volatile compounds in raw sheep meat have been received more and more attention due to their contribution to the special odor and flavor of sheep meat (Li et al., 2020; Zhang et al., 2020). Generally, meat from pasture-fed animals is regarded as a better flavor than the meat from concentrate-fed animals because the unpleasant

odor volatile compounds were less in pasture-fed animals (Strandvik, 2011; Wang et al., 2021c). Few studies focused on volatile compounds in lamb meat by different silage feeding regimes, especially for the alfalfa silage and mulberry leaf silage. 3-Hydroxydodecanoic acid is a medium-chain fatty acid associated with fatty acid metabolic disorders (Chickos, Way, Wilson, Shaharuzzaman, Laird, & Landt, 2002). The increased abundance of 3-hydroxydodecanoic acid in the MS and CON group using GC-MS based metabolomics might confirm the changed fatty acids metabolism based on the UHPLC-QTOF-MS based metabolomics study. The increased concentration of raw meat hexanal was due to β -oxidation of unsaturated fatty acids (Schuster, Franke, Silcock, Beauchamp, & Bremer, 2018). The much lower hexanal in AS group might indicate its higher thermal oxidative stability (Zhou, Zhao, Bindler, & Marchioni, 2014). Besides, hexanal plays a valuable role in meat flavor but may produce undesirable odor at higher concentrations (Wu & Wang, 2019). The current study found a much higher hexanal value in CON than AS, probably indicating the foul odor occurred by high concentrate feeding.

Different forage systems can affect the fatty acid and volatile profiles in meat from 12-month-old Merino lambs (Ye et al., 2020), and some aldehydes, ketones, and furans deriving from lipid oxidation in longissimus dorsi muscle of heifers were significantly affected by the pasture, pasture silage or cereal concentrate feeding regimes (Vasta et al., 2011). However, our study found that fewer changed meat volatile compounds are observed than the different hydrophilic metabolites, indicating that the three silage feeding regimes might more easily change the taste components than volatile flavor compounds in raw Tan-lamb meat. Further studies should be paid more attention to confirm these core changed components' essential roles and functions in meat chemistry.

Conclusions

We identify a whole picture of taste and flavor components for differentiating meat from different feed regimes using UHPLC-QTOF-MS and GC-MS based metabolomics approaches. More differential hydrophilic components were changed than the volatile compounds, demonstrating that the taste components were more easily changed than the volatile flavor compounds in raw lamb meat by feeding regimes. Dietary corn silage increased glutamate, glutamine, and serine, and decreased

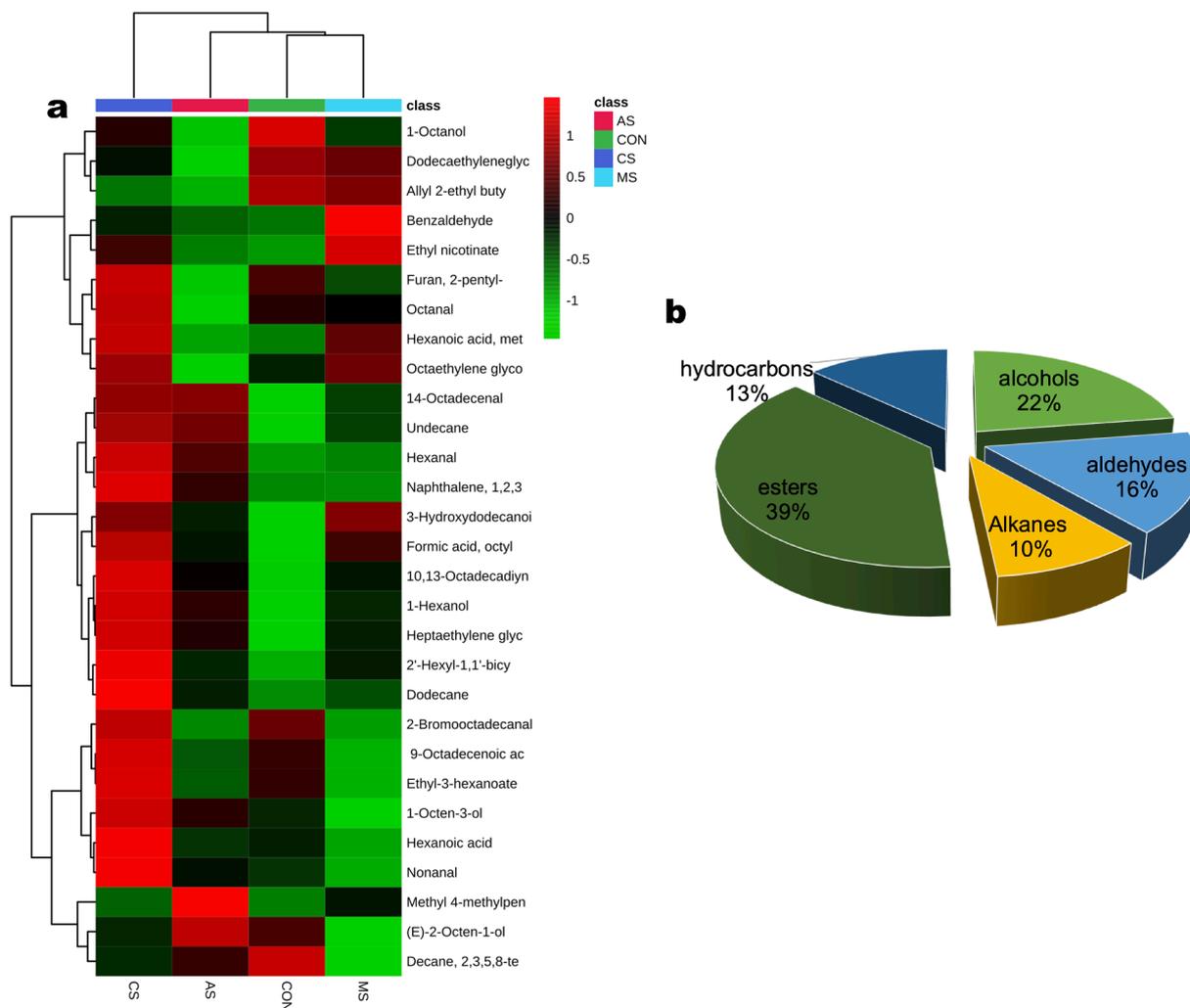


Fig. 2. The detected volatile compounds profiles of raw lamb muscle of Tan-lamb among the four groups (n = 6). Heatmap of the detected volatile compounds profiles from the four groups (a). Percentages of volatile categories identified in raw lamb (b). Heatmap of the significantly correlated volatile compounds (p -value < 0.01, c). CON, concentrate diet; CS, corn silage; AS, alfalfa silage; MS, mulberry leaf silage.

Table 2

Effect of different feed regimes on differential volatile compounds of raw lamb based on GC-MS.

Item ¹	Formula	Class	Retention Time	Relative abundance, $\mu\text{g}/\text{kg}$ meat		$\log_2\text{FC}$	p -value	FDR
CS-CON				CS	CON			
2'-Hexyl-1,1'-bicyclopropane-2-octanoic acid methyl ester	C21H38O2	Esters	20.98	0.87	3.82	-2.14	0.01	0.15
14-Octadecenal	C18H34O	Aldehydes	25.75	5.34	0.87	2.61	0.01	0.15
Dodecane	C12H26	Alkanes	9.2	27.30	19.18	0.51	0.02	0.15
AS-CON				AS	CON			
Hexanal	C6H12O	Aldehydes	6.31	8.91	165.51	-4.22	0.05	0.73
MS-CON				MS	CON			
3-Hydroxydodecanoic acid	C12H24O3	Alcohols	20.97	8.26	3.48	1.25	0.03	0.75
MS-CS				MS	CS			
10,13-Octadecadiynoic acid, methyl ester	C19H30O2	Esters	20.03	0.66	6.61	-3.32	0.02	0.47
MS-AS				MS	AS			
Octaethylene glycol monododecyl ether	C28H58O9	Esters	25.39	24.38	0.39	5.95	0.02	0.72

¹ CON, concentrate diet; CS, corn silage; AS, alfalfa silage; MS, mulberry leaf silage; FC, fold change; FDR, false discovery rate.

indoxyl sulfate in lamb meat, and dietary mulberry leaf silage increased propyl cinnamate and decreased Pro-Arg and His-Ser in lamb meat, indicating the potentially improved lamb meat quality under these feeding regimes. The increased L-pipecolic acid in AS and decreased trimethylamine N-oxide in CON were the potential best discriminant biomarker of the raw lamb meat under concentrate and alfalfa silage feeding regime, respectively. Our new findings provide comprehensive insights on the changes of flavor and taste components in raw Tan-lamb

meat by three silage-based feeding strategies compared to high-concentrate feeding, which is helpful for meat quality improvement using high-quality corn silage, alfalfa silage, or mulberry leaf silage in the diet.

CRediT authorship contribution statement

Bing Wang: Conceptualization, Methodology, Formal analysis,

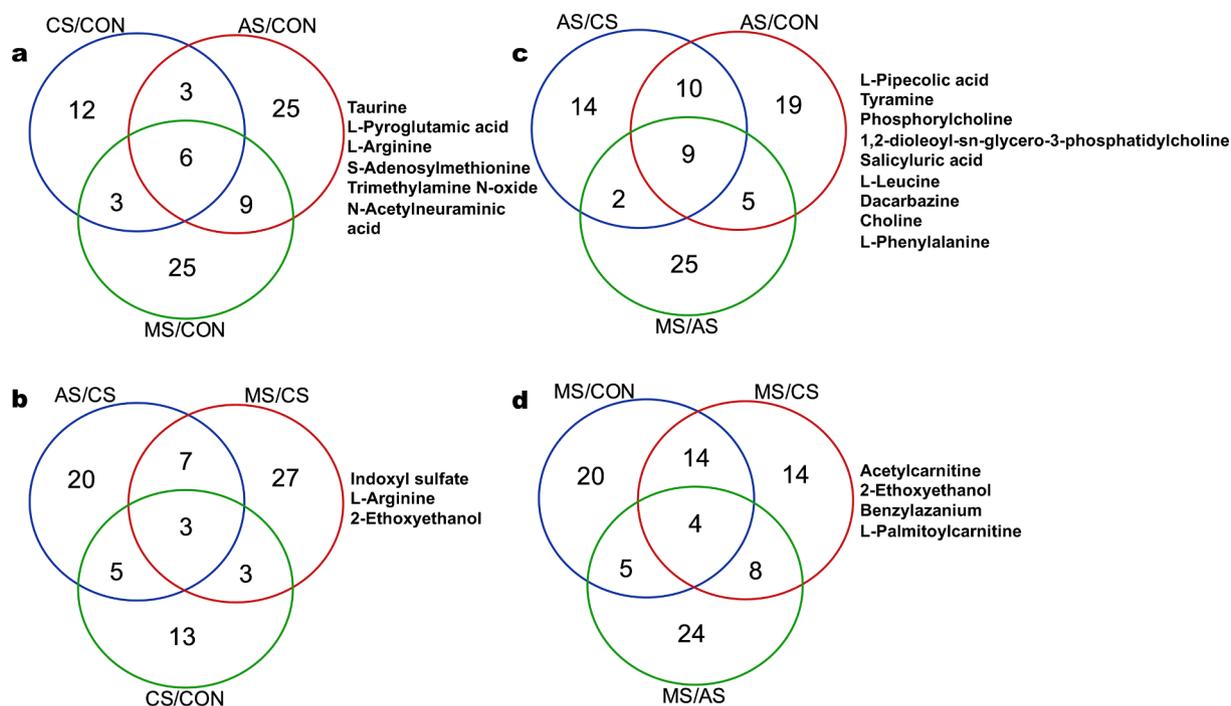


Fig. 3. Venn diagram illustrating overlap of the four group-associated differential metabolites (DFMs) (e.g., CS-associated DFMs means the mutual DFMs between CS and other three groups) based on LC-MS in the raw lamb. CON-associated DFMs (a); CS-associated DFMs (b); AS-associated DFMs (c); MS-associated DFMs. CON, concentrate diet; CS, corn silage; AS, alfalfa silage; MS, mulberry leaf silage.

Table 3

Overlap of the diet-associated potential biomarkers in discriminating it from others (e.g., AS associated biomarkers mean the mutual biomarkers between CS and other at least two groups) based on LC-MS in the raw lamb meat.

Item ¹	Comparisons	No.	Discriminant compounds
CON associated biomarkers	CON-AS, CON-MS	2	Trimethylamine N-oxide, S-Adenosylmethionine
AS associated biomarkers	AS-CON, AS-CS, AS-MS	2	L-Pipecolic acid Dacarbazine
	AS-CS, AS-MS	1	Salicylic acid
MS associated biomarkers	MS-CON, MS-CS	1	Pro-Arg
	MS-AS, MS-CON	2	L-Carnosine, His-Ser
	MS-AS, MS-CS	1	Propyl cinnamate

¹ CON, concentrate diet; CS, corn silage; AS, alfalfa silage; MS, mulberry leaf silage.

Investigation, Writing – original draft, Writing – review & editing, Visualization, Validation, Funding acquisition. **Xingang Zhao:** Formal analysis, Visualization. **Boyan Zhang:** Formal analysis, Methodology. **Yimeng Cui:** Formal analysis, Methodology. **Muzaipaier Nueraihemaiti:** Formal analysis, Methodology. **Qifang Kou:** Resources, Project administration. **Hailing Luo:** Conceptualization, Resources, Supervision, Funding acquisition.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fochx.2022.100269>.

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