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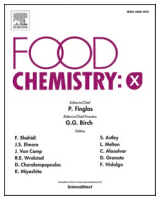

Metabonomic analysis of human and 12 kinds of livestock mature milk

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Maltitol (PubChem CID493591)
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ABSTRACT

Mature milk, as a nutrient-rich endogenous metabolite, has various beneficial effects on the human body. In order to investigate the specific nutrients provided by different dairy products to humans, we used UHPLC-Q-TOF MS to analyze the highly significantly differentially expressed metabolites in 13 species of mammalian mature milk, which were grouped into 17 major metabolite classes with 1992 metabolites based on chemical classification. KEGG shows that 5 pathways in which differentially significant metabolites are actively involved are ABC transporters, Purine metabolism, Pyrimidine metabolism, Phosphotransferase system, Galactose metabolism. The study found that pig milk and goat milk are closer to human milk and contain more nutrients that are beneficial to human health, followed by camel milk and cow milk. In the context of dairy production, the development of goat milk is more likely to meet human needs and health.

1. Introduction

Milk is a kind of complex body fluid with variable nutritional components based on the exclusive endocrine organ of mammals-mammary gland. At present, the global milk production is dominated by five kinds of animals, accounting for 83 % of the total milk production from cows, followed by buffalo, accounting for 13 %, goats, sheep, camels, accounting for 2 %, 1 %, 0.4 % (Foroutan, Guo, &.Wishart, 2019). From a nutritional point of view, milk is considered a nutritionally complete and high-quality food. Breast milk is the most suitable food for infants in many aspects, such as neurology, immunity, and nutrition (Andreas, Kampmann, & Mehring Le-Doare, 2015). However, due to irresistible factors such as the environment and health, it has become inevitable to use milk of other species as the basis for formula milk. Commonly used formula milk is made from cow milk and soy milk, milk from other mammals can also be used as formula milk base, but it has not been exploited in depth. Milk, a globally popular dairy product that contributes to bone calcium deposition, controls obesity, and prevents cardiovascular disease, has been deeply recognized for its biological activity and physiological effects. At the same time, in previous studies, it has been found that milk of different species has different functions. For example, buffalo milk has high fat content and low cholesterol content, which is very beneficial to patients with coronary heart disease (Selvaggi, Laudadio, & Tufarelli, 2014). Donkey milk can regulate human glucose and lipid metabolism, reduce the production of mitochondrial reactive oxygen species, and have a positive impact on cell

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metabolism and biological activity (Trinchese, Cavaliere, & Mollica, 2018). Horse milk has a good antioxidant effect on cell membrane (Musaev, Sadykova, & Kolbaev, 2021). Camel milk deficiency β-Lactoglobulin, which makes camel milk not prone to allergic reaction, and camel milk contains a large amount of lactoferrin and immunoglobulin, which plays an important role in anti-bacteria, anti-inflammation and anti-tumor (Swelum, El-Saadony, & El-Hack,2021). β-Lactoglobulin, a homologous protein of ruminants, has been detected in pig milk, but its structure seems to be different, which may prevent allergic reaction in patients with cow milk protein allergy (Kessler & Brew, 1970). However, due to the influence of pig milk production and people's consumption psychology, pig milk has not yet entered the market. There are few reports on the nutritional composition of pig milk, and the research on pig milk is still blank. The analysis of bioactive substances in pig milk is of great significance. In order to compare and analyze the nutrients better absorbed and utilized by humans in the emulsions of different species, we used metabolomics technology to analyze and compare milk samples of 13 species.

Metabolomics is a new technology proposed at the end of the 20th century to screen and analyze metabolites after genomics and proteomics. At present, metabolomics research on milk samples is being paid attention to over time. Metabolomics research in dairy products mainly focuses on the identification of molecular markers in different species of milk and the screening of differential metabolites, which is of great significance to promoting dairy quality identification and in-depth analysis of milk biological composition. Through metabonomics research, it is found that camel milk contains low concentrations of silver, thorium, uranium, molybdenum, strontium, vanadium and high concentrations of fatty acids and fructose, and these substances are within the safety limit of daily intake, which indicates that camel milk can be used as a safe and healthy high nutrition dairy product (Ahamad, Raish, & Shakeel, 2017). Shi et al. found that there are differences in metabolites among different buffalo breeds, and more suitable buffalo breeds for human can be screened through metabonomic (Shi, Yuan, & Li, 2021). The contents of metabolites in goat milk and cow milk have different expression characteristics, and goat milk contains more heterogeneous components, which means that goat milk and cow milk can be distinguished by metabolomics (Scano, Murgia, & Caboni, 2014).At present, the metabonomic comparison of donkey milk has not been completely reported, but there are some comparative studies on donkey colostrum and mature milk. The study of milk metabolites is an important way to study milk nutrients, in order to explore the differential metabolites and expression levels in different species of emulsions, compare them with human milk, and screen metabolites that are beneficial to the development of infants and young children, we therefore performed a comprehensive metabolomic study.

Metabolomics is still evolving and new technologies are emerging to push new substances to be observed. In recent years, metabolomics has gradually attracted the attention of researchers, but there are not many comparative studies between different species, which usually focus on the comparison between varieties. In this study, non-target metabolomics technology was used to study the differences in milk-derived metabolites among 13 species, and new explorations were made in species number and analysis accuracy. At the same time, the introduction of pig milk into the research scope fills the research gap of pig milk in dairy science to some extent. We hope to identify specific differential metabolites and special bioactive substances from 13 species and provide new insights for the development of the milk industry on the basis of filling some data gaps.

2. Materials and methods

2.1. Test sample collection

In 2021, we collected 6 forms each species of 78 normal milk samples were obtained from 13 species of healthy mammals as follows: Chinese human milk samples (CHP) were donated by Chinese mothers from Shenyang Maternal and Child Care Center. Holstein cow milk samples (HST) were obtained from Huishan Dairy Group. Buffalo milk samples (GXB) were obtained from Guangxi Buffalo Research Institute and Nanjing Agricultural University. Yak milk samples (QHY) were obtained from Qinghai province and Lanzhou institute of husbandry and pharmaceutical science of cass. DeZhou Donkey milk samples (DZD)were obtained from East Ajiao Donkey Farm in Fumeng County. Mongolian Horse milk samples (MGH)were obtained from Inner Mongolia XilinGol League Science and Technology Association. Alxa camel milk samples (ALC) were obtained from Alxa Alpaca Farm in Inner Mongolia. Saanen milk goat milk samples (SNG) and Toggenburg milk goat milk samples (TGB) and Nubian milk goat milk samples (NBY) were obtained from Liaoyang dairy goat farm. Liaoning Cashmere goat milk samples (LCG) were obtained from Liaoyang National Core Sheep Breeding Farm. Small Tail Han sheep milk samples (OAS) were obtained from Inner Mongolia Horinger County sheep farm. Pig milk samples (YXP) were obtained from Yangxiang Pig Group. In all species, milk samples should be collected after 2 weeks of birth, with a minimum of 50 mL of milk per dam. At the end of each milking, the milk sample is removed and placed in the same 10 mL plastic container and immediately placed in a refrigerator at −20 °C for a short period of time, not exceeding two weeks. The samples are then transported in undefrosted dry ice and placed in a freezer at −80 °C for long-term storage, but for no more than four months.

Ethical statements: The Animal Welfare and Ethics Committee of Shenyang Agricultural University approved the participation of people and animals in this study. Approval No.: 20210601.

2.2. Sample extraction

After the samples were slowly thawed at 4 °C, an appropriate amount of sample was added to a pre-chilled methanol/acetonitrile/water solution (2:2:1, v/v), vortexed and mixed, sonicated at low temperature for 30 min, left to stand at −20 °C for 10 min, centrifuged at 14,000 g for 20 min at 4 °C, and the supernatant was vacuum dried and added to 100 μL of aqueous acetonitrile (acetonitrile: water = 1:1, v/v) for mass spectrometry. The supernatant was centrifuged at 14,000 g for 15 min at 4 °C, and the supernatant was taken into the sample for analysis. The centrifugation process was performed to remove proteins and some lipids, using methanolic acetonitrile water extraction, targeting the hydrophilic metabolome.

2.3. Chromatography-mass spectrometry

This study is high-resolution untargeted metabolomics, which was performed by matching metabolite retention times in local databases, molecular mass (error within < 10 ppm), secondary fragmentation spectra, collision energies, etc., of metabolites in biological samples. The metabolites in the biological samples are structurally identified and the results are rigorously checked and confirmed manually (Zhang, Liu, & Hussain, 2021).

2.3.1. Chromatographic conditions

The samples were separated by Agilent 1290 Infinity LC ULTRA high Performance Liquid chromatography (UHPLC) HILIC column.

Column temperature 25 ℃; The flow rate was 0.5 mL/min. Injection volume 2 μL; Mobile phase composition A: water + 25 mM ammonium acetate + 25 mM ammonia water, B: acetonitrile; The gradient elution procedure was as follows: 0–0.5 min, 95 %B; 0.5–7 min, B changes linearly from 95 % to 65 %; 7–8 min, B changes linearly from 65 % to 40 %; 8–9 min, B remained at 40 %; 9–9.1 min, B changed linearly from 40 % to 95 %; 9.1–12 min, B maintained at 95 %; The samples were placed in an automatic sampler at 4℃ during the whole analysis. In order to avoid the influence of instrument detection signal fluctuation, random sequence is used for continuous analysis of samples. QC samples were

inserted into the sample queue to monitor and evaluate the stability of the system and the reliability of experimental data.

2.3.2. Q-TOF mass spectrometry conditions

AB Triple TOF 6600 mass spectrometer was used to collect the first and second order spectrograms of samples. The samples were separated by Agilent 1290 Infinity LC ULTRA high performance liquid chromatography (UHPLC) and analyzed by Triple TOF 6600 mass spectrometers (AB SCIEX). Electrospray ionization (ESI) positive and negative ion modes were used for detection. ESI source setting parameters are as follows: Atomizing gas auxiliary heating 1 (Gas1): 60, auxiliary heating 2 (Gas2): 60, Air curtain gas (CUR): 30PSI, ion source temperature: 600℃, spray voltage (ISVF) ± 5500 V (positive and negative modes); Primary mass charge ratio detection range: 60–1000 Da, secondary ion mass charge ratio detection range: 25–1000 Da, primary mass spectrometry scanning cumulative time: 0.20 s/ Spectra, secondary mass spectrometry scanning cumulative time: 0.05 s/ Spectra; The secondary mass spectrometry was obtained by data-dependent acquisition mode (IDA) and peak intensity value screening mode. The cluster removal voltage (DP) was ± 60 V (positive and negative modes), and the collision energy was 35 ± 15 eV. IDA was set as follows: dynamic exclusion isotope ion range was 4 Da, and 10 fragment profiles were collected in each scan.

2.4. Data analysis

Data analysis included univariate statistical analysis, multidimensional statistical analysis, differential metabolite screening, correlation analysis of differential metabolite, KEGG pathway analysis and other contents. Data analysis was completed by APPLIED PROTEIN TECHNOLOGY (https://bio-cloud.aptbiotech.com/).

The raw data of this study have been uploaded in the "MetaboLights" database. (URL: https://www.ebi.ac.uk/metabolights/MTBLS6777; Identifier:MTBLS6777).

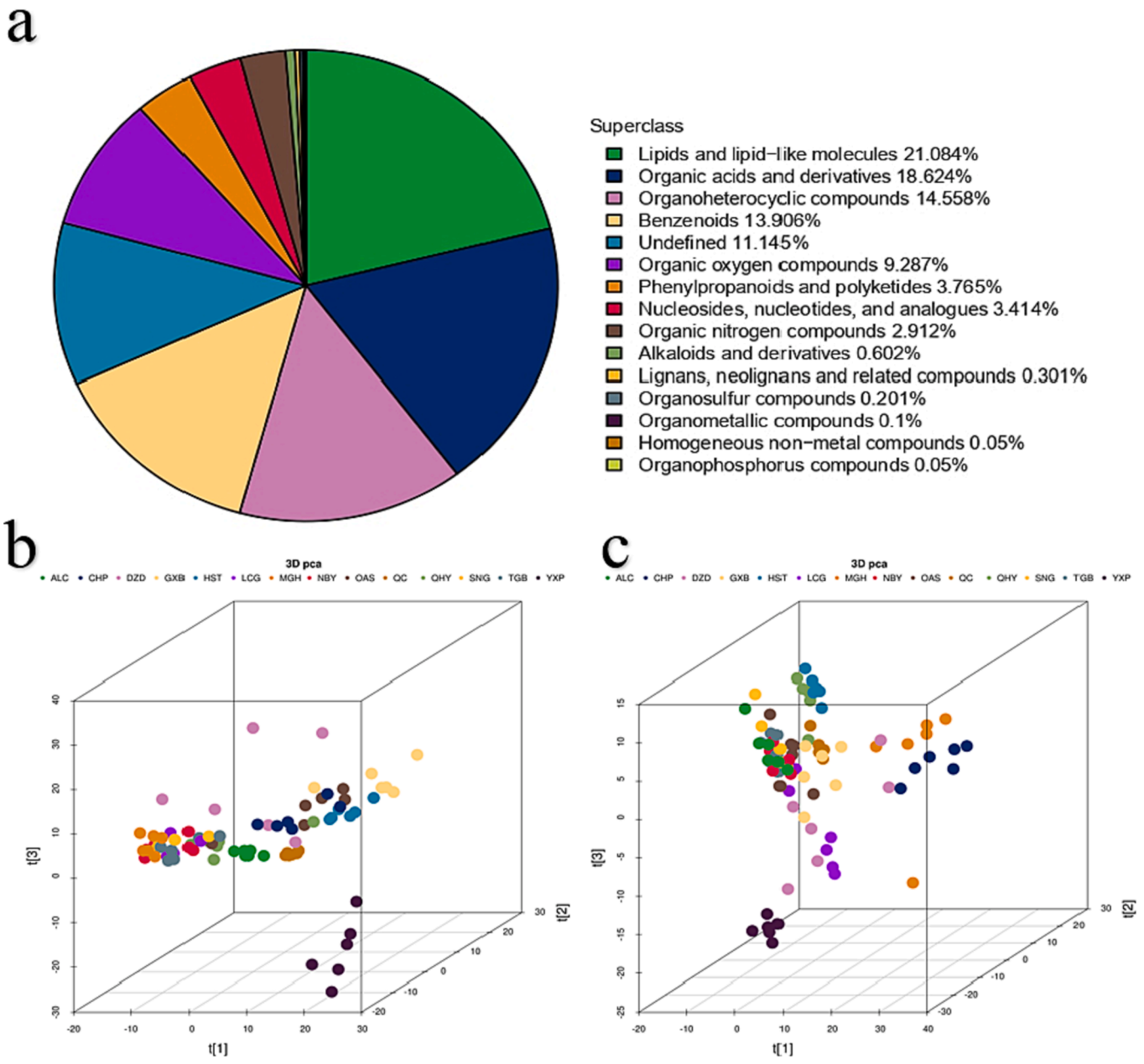

Fig. 1. a) The proportion of metabolites identified in each chemical classification; b) In Pos 3D PCA score plot; c) In Neg 3D PCA score plot.

3. Results

3.1. Identification results statistics and grade analysis

This project uses the in-house database (Shanghai Applied Protein Technology) search database. The structure of metabolites in biological samples was identified by matching with the retention time, molecular weight (molecular weight error is<10 ppm), secondary fragmentation spectrum and collision energy in the local database, and the identification results were strictly checked and confirmed by manual secondary verification. The identification level was above Level 2. The total ion current diagram of QC samples was compared for spectral overlap, as shown in Fig.S1a and Fig.S1b.

3.2. Metabolite identification quantity statistics

In this project, 1992 metabolites were identified in combined positive and negative ion mode, of which 1230 metabolites were identified in positive ion mode (Pos) and 762 metabolites were identified in negative ion mode (Neg).

3.3. Metabolite chemical classification attribution statistics

All the metabolites identified in this project (combined with those identified by positive and negative ions) were classified according to their chemical taxonomy attribution information, and the proportion of the number of each type of metabolite is shown in Fig. 1a. The detected metabolites were classified as Lipids and lipid molecules (21.084 %), Organic acids and derivatives (18.624 %), Organ heterocyclic compounds (14.558 %), Benzenoids (13.906 %), Organic oxygen compounds (9.287 %), Phenylpropanoids and polyketides (3.765 %), Nucleosides, nucleotides, and analogues (3.414 %), Organic nitrogen compounds (2.912 %), and other undefined metabolites (11.145 %).

3.4. Change trend of metabolites expression

To analyze the expression pattern of all qualitative metabolites in each group and characterize the trend of metabolite expression. The Mfuzz package of fuzzy c-means (FCM) algorithm was used for the analysis, and all metabolites were classified into different expression patterns according to their expression trends. The expression pattern and trend classification of this project, the results were shown in Fig. 2a/b.

In the figure, according to the expression trend of all metabolites, it is divided into 9 expression modules, and the expression trends of metabolites that can be clustered into each module are close, a cluster with a significant trend is regarded as an important object of interest, and the metabolites displayed by the cluster are searched in the relevant data, and the top 20 metabolites with significant differences are combined to screen out the data in Table S1a/b.

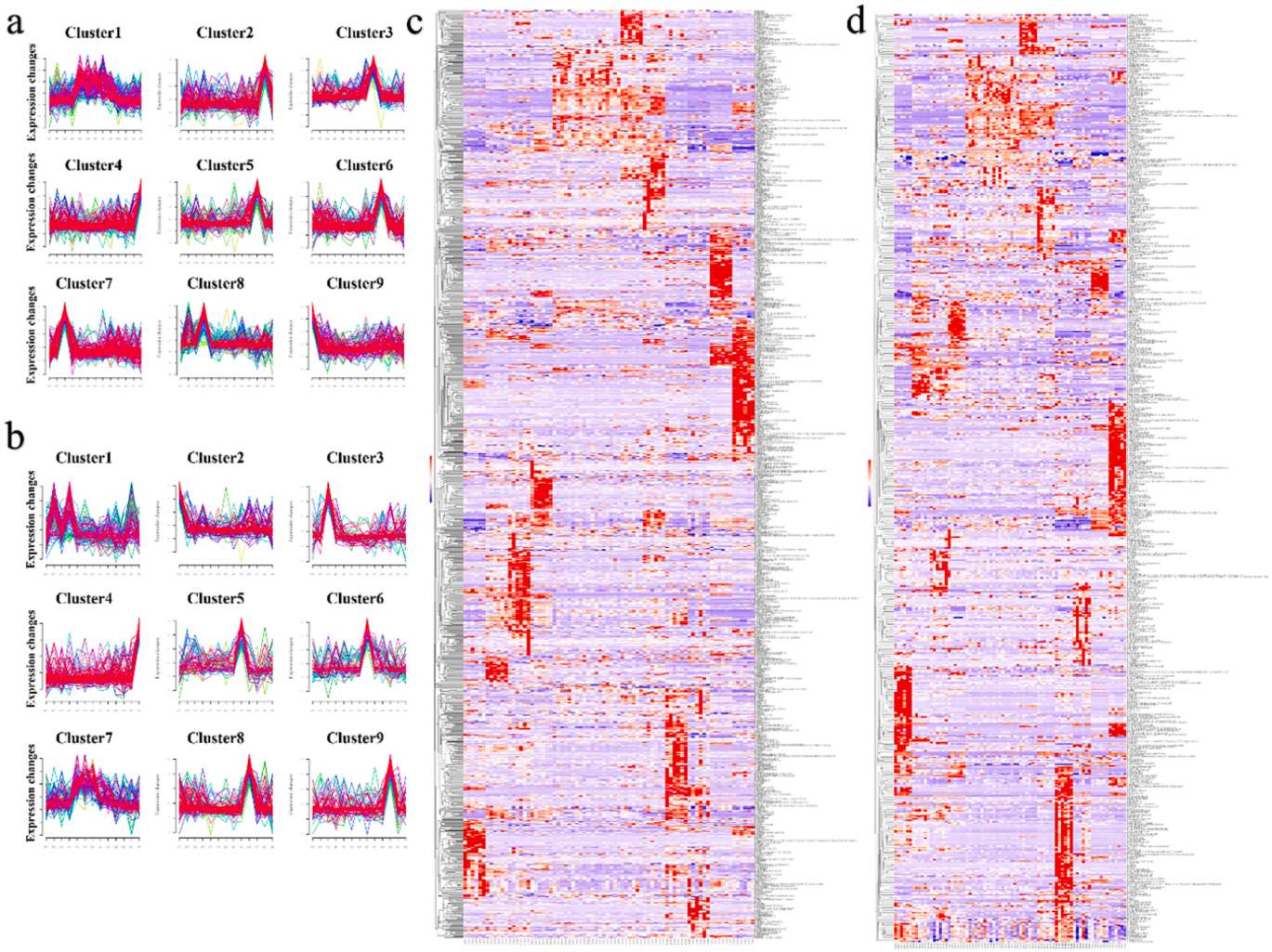

Fig. 2. a) the trend of metabolites identified by pos in different group; b) the trend of metabolites identified by neg in different group; c) Hierarchical clustering thermogram of distinct metabolites in Pos; d) Hierarchical clustering thermogram of distinct metabolites in Neg.

In Pos, the expression trends of metabolites in ALC, OAS, DZD, MGH, GXB and QHY were prominent in cluster2, cluster3, cluster5, cluster6, cluster7, and cluster8. After identification and analysis, each species expressed prominent metabolites, as shown in Table S1a.

In Neg, the expression trends of metabolites in GXB, OAS, LCG, MGH and DZD were prominent in cluster3, cluster5, cluster6, cluster8, and cluster9, respectively. After identification and analysis, each species expressed prominent metabolites, as shown in Table S1b.

3.5. Statistical difference analysis of single variable between groups

Based on univariate analysis, the differences of all metabolites (including unidentified metabolites) detected under Pos and Neg were analyzed. The differential metabolites with FC $>$ 1.5 or FC $<$ 0.67, p value $<$ 0.05 were visualized in the form of volcanic graphs, and the results were shown in Fig.S2.

In Pos, the number of up-regulated metabolites of all species were significantly higher than the number of down-regulated metabolites. All species are significantly different from CHP. In Neg, the number of up-regulated metabolites of MGH, GXB and OAS were significantly higher than the down-regulated metabolites; DZD, HST, LCG, NBY, SNG and YXP have the same number of up and down metabolites; but ALC, QHY and TGB have higher down-regulated metabolites than up-regulated ones. MGH, GXB, OAS, ALC, QHY, TGB have significant differences compared to CHP.

3.6. Principal component analysis

Principal component analysis of metabolites can also reflect the variation between groups and within groups in general. At the same time, it can not only reduce the dimension and speed up the operation, but also summarize the original data information. Therefore, in data analysis, PCA method is generally used to observe the overall distribution trend of inter-group samples and the different degree of inter-group samples. PCA analysis was performed for each comparison group, the PCA score diagram was shown in Fig. 1b/c.

It can be seen from the figures that in Pos and Neg, each species gathered together, and there were significant differences and clustering among different species. In Pos, QHY, LCG, ALC clustered into one class; OAS, GXB, HST clustered into one class; NBY, TGB, SNG, MGH clustered into one class; CHP and DZD, YXP into a class, as shown in Fig. 1b. In Neg, LCG, NBY, TGB, SNG and OAS clustered into one class; GXB, HST clustered into one class; MGH clustered into one class; ALC and DZD clustered into one class; CHP and YXP clustered into one class, as shown in Fig. 1c.

3.7. Screening differential metabolites

Metabonomics usually uses strict OPLS-DA VIP $>$ 1 and P value $<$ 0.05 as screening criteria for significant difference metabolites. A total of 1041 differential metabolites were identified in Pos. A total of 681 differential metabolites were identified in Neg. A total of 1722 differentially expressed metabolites were obtained, and the first 20 differentially expressed metabolites in Pos and Neg were shown in the Table 1.

In Pos, the first five metabolites with different expression levels were Valeroyl salicylate; 2,6-dihydroxy-4-methylbenzoic acid; 3-methoxytyramine; Benzophenone and *N*-acetyl-d-lactosamine. The first four substances were Benzenoids. The top 20 molecules were concentrated in 8 known classes and 1 unknown class. They are Benzenoids; Lipids and lipid-like molecules; Nucleosides, nucleotides, and analogues; organic acids and derivatives; organic oxygen compounds; Phenylpropanoids and polyketides; Organoheterocyclic compounds.

In Neg, the first five differentially expressed metabolites were Inosine; Pseudouridine; Maltitol; Adenosine 5'-phosphosulfate; Uridine 5'-diphosphoglucose, and 20 differentially expressed metabolites were concentrated in 5 known categories and 1 unknown category. They are Nucleosides, nucleotides, and analogues; Lipids and lipid-like molecules; Organic acids and derivatives; Organic oxygen compounds and Organoheterocyclic compounds. On the basis of OPLS-DA VIP $>$ 1 and P value $<$ 0.05, the differential metabolites were further screened by adding FDR (False Discovery Rate) correction (standard).

In Pos and Neg, in order to ensure that at least one metabolite under each chemical classification is screened, we selected a total of 12 highly expressed differentially metabolites. Among them, there are Valeroyl salicylate; *N*-acetyl-d-lactosamine; Adenosine 5′-diphosphoribose; *N*-glycolylneuraminic acid; 1-(4-piperidinyl)-1,3-dihydro-2 h-indol-2-one and Chrysosplenetin in Pos. Inosine; Maltitol; Adenosine 5′-phosphosulfate; Phe(benzoyl)-leu-arg; 3′-sialyllactose and Allantoin in Neg. (The expression amount of these metabolites can be seen with reference to Fig. 5).

In Pos, the content of Valeroyl salicylate in YXP was significantly higher than that in other species. The content of *N*-acetyl-d-lactosamine in CHP was significantly higher than that in other species. Adenosine 5 ' -diphosphoribose was significantly higher in ALC, DZD, GXB, HST and QHY than in other species. The content of 1,1,3-trimethylurea in ALC is very high, significantly higher than other species. The expression of *N*-glycolylneuraminic acid was higher in all sheep than in other species. The expression of 1- (4-piperidinyl) −1,3-dihydro-2 h-indol-2-one in CHP, MGH and YXP was higher than that in other species, and the difference was very significant. Chrysosplenetin was low expressed only in ALC, CHP, DZD and MGH.2,6-dihydroxy-4-methylbenzoic acid was highly expressed only in YXP.

In Neg, Maltitol was highly expressed in CHP, DZD and YXP. Adenosine 5′-phosphosulfate (aps) was only expressed in OAS and all goat, and the expression levels in other species were extremely low. 3′-sialyllactose was highly expressed in YXP, and Allantoin was low expressed in CHP, DZD, MGH and YXP. The expression of Inosine in CHP, DZD, GXB and QHY were significantly lower than that in other species. Phe (benzoyl) -leu-arg was highly expressed in CHP, DZD, GXB, MGH, QGY and YXP.

3.8. Differential metabolite bioinformatics analysis

Follow-up bioinformatic analysis was performed on the selected significantly different metabolites (metabolites should meet OPLS-DA VIP $>$ 1 and p value $<$ 0.05, with qualitative names), including cluster analysis, correlation analysis and pathway analysis.

3.8.1. Cluster analysis

3.8.1.1. Hierarchical clustering heat map of distinct metabolites. The hierarchical clustering analysis results of significant difference metabolites (VIP $>$ 1, p value $<$ 0.05) were shown in Fig. 2c/d. The metabolites clustered in the same cluster have similar expression patterns and may have similar functions or participate in the same metabolic process or cell pathway together.

Through the thermogram, we can intuitively see the overall expression pattern between different groups. Each species can express similar values on the same metabolites, and the repeatability of each sample is good. This shows that the data is reliable and logical. In Pos and Neg, each species has significantly up-regulated differential metabolites. In Pos, the differential metabolites of YXP and ALC showed more obvious differences. The differential metabolites of CHP, HST, GXB and QHY showed relatively obvious state. In Neg, the differential metabolites of CHP, MGH and YXP showed extremely obvious state, and the differential metabolites of ALC, QHY and LCG showed relatively obvious state.

3.8.2. Correlation analysis

Measuring the metabolic closeness between significantly different metabolites facilitates further understanding of the inter-regulatory

Table 1
Sample POS&NEG _ Qualitative-13 Top 20 Expressions in Species Classification.

Name	mode	ANOVA p value	FDR	ALC	CHP	DZD	GXB	HST	LCG	MGH	NBY	OAS	QHY	SNG	TGB	YXP
Valeroyl salicylate	POS	5.84E-35	2.38E-32	2.58E+07	3.59E+07	1.33E+07	2.15E+07	1.90E+07	6.28E+06	1.97E+07	8.70E+06	3.12E+06	1.82E+07	7.98E+06	6.90E+06	4.22E+08
2,6-dihydroxy-4-methylbenzoic acid	POS	1.35E-34	4.39E-32	8.11E+06	1.07E+07	4.22E+06	6.29E+06	5.75E+06	1.77E+06	6.74E+06	2.57E+06	8.75E+05	5.44E+06	2.30E+06	2.23E+06	1.34E+08
3-methoxytyramine	POS	1.09E-31	1.21E-29	2.68E+07	3.99E+07	3.52E+06	5.89E+06	8.44E+07	5.67E+07	3.61E+06	7.08E+07	3.47E+08	6.25E+07	7.78E+07	7.89E+07	8.12E+07
Benzophenone	POS	2.95E-31	2.94E-29	1.75E+08	8.87E+06	8.29E+07	4.08E+07	9.03E+07	1.98E+08	3.00E+08	2.20E+08	2.57E+08	2.54E+08	1.87E+08	2.19E+08	2.72E+06
N-acetyl-d-lactosamine	POS	1.38E-38	9.59E-36	1.55E+07	2.88E+09	4.99E+07	9.83E+06	2.25E+07	8.45E+06	1.05E+07	6.33E+06	1.32E+07	2.15E+07	4.71E+06	4.98E+06	3.36E+08
5-aminovaleric acid betaine	POS	8.90E-32	1.01E-29	1.19E+10	8.58E+08	2.38E+08	5.18E+10	2.81E+10	2.80E+09	8.47E+08	1.01E+10	2.99E+10	3.39E+09	9.51E+09	6.10E+09	4.56E+07
Echinocystic acid	POS	1.42E-31	1.54E-29	2.84E+06	2.54E+06	8.79E+07	7.30E+08	3.71E+08	1.81E+08	1.75E+08	1.89E+08	2.83E+08	4.10E+08	1.74E+08	2.03E+08	1.74E+06
Adenosine 5′-diphosphoribose	POS	4.04E-32	4.93E-30	7.05E+05	7.87E+07	6.79E+07	1.58E+08	1.11E+08	5.81E+06	8.37E+05	2.65E+06	1.48E+06	1.35E+08	3.41E+06	1.67E+06	1.81E+07
Uridine 5′-diphosphate	POS	3.28E-31	3.14E-29	1.58E+07	1.13E+07	3.95E+06	9.14E+06	8.29E+06	1.13E+09	1.51E+08	7.37E+08	1.10E+09	5.39E+06	6.49E+08	7.09E+08	9.17E+08
1,1,3-trimethylurea	POS	2.02E-38	1.23E-35	2.03E+07	2.70E+06	2.42E+06	2.20E+06	2.28E+06	2.39E+06	2.34E+06	2.50E+06	2.34E+06	2.29E+06	2.44E+06	2.36E+06	2.53E+06
N-glycolylneuraminic acid	POS	6.04E-45	9.83E-42	3.57E+05	2.27E+05	1.07E+06	1.88E+06	1.87E+06	9.68E+07	8.16E+05	3.95E+07	3.06E+08	1.78E+06	8.75E+07	6.21E+07	1.54E+07
Lnnt	POS	9.22E-36	4.50E-33	5.32E+06	1.34E+09	1.66E+07	6.70E+06	2.10E+06	2.84E+06	2.69E+06	1.32E+06	4.46E+06	3.87E+06	1.01E+06	1.53E+06	1.12E+08
N-acetylneuraminic acid	POS	8.49E-35	2.96E-32	1.63E+08	2.29E+08	8.00E+07	1.28E+08	1.20E+08	3.06E+07	1.25E+08	4.74E+07	6.89E+06	1.09E+08	4.18E+07	3.82E+07	2.91E+09
N-acetylneuraminic acid, 2,3-dehydro-2-deoxy	POS	3.58E-34	9.71E-32	2.94E+08	4.08E+08	1.45E+08	2.34E+08	2.14E+08	5.59E+07	2.28E+08	8.51E+07	1.26E+07	1.95E+08	7.53E+07	6.94E+07	5.28E+09
1-(4-piperidinyl)-1,3-dihydro-2 h-indol-2-one	POS	2.66E-35	1.18E-32	5.73E+05	7.87E+07	1.30E+06	6.38E+05	3.92E+06	9.53E+05	9.24E+07	1.93E+06	1.04E+07	2.82E+05	2.82E+06	2.16E+06	1.49E+08
3-methyladenine	POS	6.79E-34	1.58E-31	1.40E+07	2.13E+07	4.36E+06	1.40E+07	2.16E+07	3.15E+07	6.75E+06	3.21E+07	5.42E+07	2.45E+07	3.43E+07	3.15E+07	6.39E+07
(-)-riboflavin	POS	1.28E-32	1.74E-30	1.15E+07	3.69E+06	2.83E+06	1.92E+08	1.37E+08	2.09E+08	1.37E+06	1.49E+08	2.76E+08	1.40E+08	1.28E+08	1.85E+08	1.74E+08
Glutethimide	POS	3.41E-32	4.27E-30	3.15E+06	1.64E+06	3.23E+06	2.37E+06	3.43E+06	3.25E+06	1.29E+06	3.68E+06	3.67E+06	1.33E+08	3.39E+06	3.57E+06	3.24E+06
Chrysosplenetin	POS	2.44E-33	4.77E-31	5.94E+06	9.71E+05	5.42E+05	6.25E+07	5.13E+07	9.12E+07	2.42E+05	7.19E+07	1.25E+08	5.03E+07	7.29E+07	9.50E+07	1.52E+08
Methyl	POS	6.93E-33	1.13E-30	4.39E+06	1.01E+08	3.85E+06	2.34E+06	2.25E+05	2.23E+05	2.18E+06	3.46E+05	2.05E+05	1.93E+05	2.30E+05	5.19E+05	2.47E+07
Inosine	NEG	5.08E-31	4.94E-29	1.63E+09	3.19E+07	9.91E+07	4.64E+07	1.45E+08	4.07E+09	3.05E+08	2.56E+09	3.72E+09	1.81E+08	2.19E+09	3.06E+09	2.68E+09
Pseudouridine	NEG	4.05E-30	3.33E-28	4.01E+06	3.06E+07	4.11E+06	1.30E+07	5.57E+07	6.31E+06	3.97E+06	1.04E+07	1.08E+07	4.58E+07	1.51E+07	1.16E+07	7.85E+07
Maltitol	NEG	8.11E-30	6.57E-28	1.65E+06	1.56E+07	3.34E+07	4.79E+05	2.90E+06	1.55E+05	8.29E+06	4.16E+05	3.29E+05	2.14E+06	3.48E+05	3.76E+05	5.45E+07
Adenosine 5′-phosphosulfate (aps)	NEG	9.80E-38	5.93E-35	1.01E+06	8.21E+05	4.99E+05	1.25E+06	1.17E+06	2.59E+09	1.73E+06	1.16E+09	2.17E+09	3.64E+05	1.45E+09	1.42E+09	9.86E+05
Uridine 5′-diphosphoglucose	NEG	3.32E-33	6.12E-31	4.79E+07	3.76E+07	1.31E+07	2.23E+07	1.71E+07	7.49E+09	8.78E+08	4.29E+09	7.12E+09	1.76E+07	3.76E+09	4.39E+09	6.01E+09

(*continued on next page*)

Table 1 (*continued*)

Name	mode	ANOVA p value	FDR	ALC	CHP	DZD	GXB	HST	LCG	MGH	NBY	OAS	QHY	SNG	TGB	YXP
5′-phosphoribosyl-5-amino	NEG	3.70E-31	3.92E-29	5.37E+07	2.31E+07	1.96E+07	2.64E+06	4.24E+06	3.54E+06	5.17E+07	2.56E+06	5.93E+06	4.53E+06	1.72E+06	3.73E+06	1.45E+08
L-2-hydroxyglutaric acid	NEG	1.76E-31	2.19E-29	6.05E+08	2.46E+08	1.41E+08	3.45E+09	3.73E+09	1.01E+09	9.15E+08	9.91E+08	3.42E+09	1.34E+09	1.05E+09	1.27E+09	1.48E+08
Taurine	NEG	1.89E-31	2.29E-29	1.51E+08	1.85E+09	1.92E+08	1.12E+09	2.34E+08	2.65E+09	1.67E+08	2.54E+09	2.01E+09	7.18E+08	3.32E+09	2.05E+09	2.42E+09
Phe(benzoyl)-leu-arg	NEG	2.57E-31	3.05E-29	2.76E+05	2.07E+08	1.75E+08	1.42E+08	2.12E+06	3.59E+07	2.51E+08	6.26E+06	4.46E+05	1.78E+08	5.55E+06	3.33E+06	2.00E+08
3′-sialyllactose	NEG	9.15E-36	2.88E-33	9.91E+06	8.99E+07	2.94E+06	7.02E+06	2.17E+06	7.91E+05	2.58E+06	7.59E+05	1.95E+08	3.51E+06	1.19E+06	9.36E+05	9.50E+08
Lnt	NEG	5.13E-35	1.44E-32	7.36E+06	3.35E+09	3.38E+07	8.29E+06	3.17E+06	1.66E+06	5.44E+06	1.30E+06	8.49E+06	6.57E+06	1.24E+06	1.48E+06	2.03E+08
4.alpha.-mannobiose	NEG	5.09E-33	8.78E-31	1.29E+07	1.06E+06	1.31E+07	4.62E+07	6.92E+07	9.16E+06	1.66E+08	2.05E+07	1.49E+06	1.79E+06	2.22E+07	1.69E+07	4.61E+05
Erythritol	NEG	1.31E-32	2.00E-30	3.65E+05	1.91E+05	3.49E+06	2.89E+05	1.94E+05	3.89E+05	5.16E+07	4.53E+05	1.87E+05	2.35E+05	6.03E+05	3.26E+05	2.00E+08
Allantoin	NEG	1.49E-44	3.98E-41	9.14E+09	3.84E+08	2.07E+09	4.67E+09	1.99E+10	9.03E+09	1.06E+09	1.06E+10	1.00E+10	1.79E+10	1.09E+10	1.11E+10	3.26E+09
Uracil	NEG	2.74E-34	6.33E-32	2.25E+07	3.12E+07	4.25E+07	1.52E+09	2.30E+09	4.70E+08	3.73E+07	4.54E+08	8.55E+08	7.78E+08	4.10E+08	2.76E+08	1.89E+08
4-pyridoxic acid	NEG	1.27E-33	2.61E-31	2.02E+07	2.96E+07	5.38E+07	5.36E+08	5.11E+08	4.81E+08	2.28E+08	1.96E+09	4.70E+08	3.89E+08	1.45E+09	2.02E+09	1.69E+08
Hypoxanthine	NEG	2.18E-32	3.23E-30	6.98E+09	2.50E+07	1.31E+07	1.96E+07	2.26E+07	2.24E+07	1.77E+07	2.59E+07	4.35E+07	2.55E+07	3.39E+07	5.48E+07	8.52E+09
Mangiferin	NEG	8.36E-32	1.12E-29	9.56E+08	1.91E+09	5.37E+07	2.62E+07	1.24E+08	4.21E+08	8.43E+07	2.56E+08	6.32E+07	4.16E+07	2.84E+08	3.09E+08	5.95E+09
4,5-dihydro-4,5-dioxo-1 h	NEG	6.77E-31	6.35E-29	2.55E+06	2.07E+08	6.48E+07	3.47E+07	1.88E+08	1.97E+07	4.31E+07	2.68E+06	1.95E+08	1.11E+06	3.60E+06	1.26E+06	1.21E+06
Citrinin	NEG	9.13E-30	7.17E-28	2.28E+06	1.84E+06	1.51E+06	7.87E+06	4.26E+07	3.47E+07	8.16E+06	2.21E+08	5.76E+07	4.44E+07	1.13E+08	2.11E+08	3.29E+06

relationships between metabolites during changes in biological state. The results of the correlation analysis are presented visually in the form of a correlation heatmap, as shown in Fig. 3a. The screened important differential metabolite correlation heatmap are shown in Fig. 3b (Red indicates a positive correlation, blue indicates a negative correlation, and white indicates a non-significant correlation. The color depth is related to the absolute value of the correlation coefficient, that is, the higher the degree of positive or negative correlation, the darker the color. The size of the point is related to the significance of the correlation, the more significant, the smaller the p-value and the larger the point). The graph shows a clear correlation between the individual metabolites.

In order to reveal the co-regulatory relationships between the various metabolites more visually, the correlation matrix was converted into a chord diagram and a network diagram. As shown in Fig. 3c and Fig. 3d/e. Both chord and network plots show metabolite molecules with correlation coefficients $|r|>0.8$ and $p < 0.05$, and this criterion can be adjusted according to the actual situation. The chord diagram and the network diagram can better show the correlation between various metabolites, each has its own advantages. The results showed that there were different relationships among the metabolites, and the relationship was obvious.

3.8.3. KEGG pathway

Each bubble in the bubble diagram represents a metabolic pathway (the first 20 pathways with the highest significance are selected according to the p value), the horizontal coordinate of the bubble and the size of the bubble indicate the influence factor of the pathway in the topological analysis, the larger the size the greater the influence factor; the vertical coordinate of the bubble and the color of the bubble indicate the p value of the enrichment analysis (taking the negative common logarithm, i.e. -log10 p-value), the darker the color the smaller the p value, the more significant the degree of enrichment, the rich factor represents the number of differential metabolites in the pathway as a percentage of the number of metabolites annotated in the pathway. As shown in Fig. 4a.

The vertical axis in the bar graph represents each KEGG metabolic pathway, and the horizontal axis indicates the number of differentially expressed metabolites contained in each KEGG metabolic pathway. The color indicates the p-value of the enrichment analysis, the darker the color the smaller the p-value, the more significant the enrichment, the number on the bar represents the rich factor, the rich factor represents the number of differential metabolites in the pathway as a percentage of the number of metabolites annotated to the pathway. As shown in Fig. 4b.

The KEGG pathway showed that the five pathways with active involvement of differentially significant metabolites were ABC transporters (ko02010); Purine metabolism (ko00230); Pyrimidine metabolism (ko00240); Phosphotransferase system (PTS) (ko02060); Galactose metabolism (ko00052), as shown in Fig.S3. The expression of 10 metabolites in the 5 KEGG pathways in 13 different species of milk samples was shown in Table S2. The expression amount of these differential metabolites is shown in Fig. 5.

4. Discussion

In this study, we explored the metabolite profiles of milk samples from 13 species using a non-targeted LC/MS metabolomics approach. This study is also the first to describe metabolites from milk collected from 13 species simultaneously, and also identifies 13 species-specific differential metabolites and endemic substances. The detailed analysis of representative differential metabolites fills in the research on individual species in milk and also provides new insights for the development of the milk industry. In this study, the screened differential metabolites are discussed separately into organic oxygen compounds, nucleosides, nucleotides and analogs, organic heterocyclic compounds, lipids and lipid-like molecules, and other classes.

3′Sialyllactose, a type of oligosaccharide, is one of the main forms of sialic acid. Some studies have reported a positive correlation between 3′Sialyllactose and cognitive abilities in infancy, especially language development (Pisa, Martire, & Macrì, 2021). 3′Sialyllactose, which is important for intellectual development in infancy, is also expressed at high levels in breast milk, yet it is not present in infant formula (ten Bruggencate, Bovee-Oudenhoven, & Schoterman, 2014). This has negative effects on non-breastfed infants. However, in this study, we found that 3′-sialyllactos is highly expressed in YXP. In the future, we can consider adding pig milk to infant formula to improve its function and effect. Erythritol is a filler sweetener that is widely found in nature. It is usually considered as a substitute for sugar to provide sweetness to foods. Some studies have shown that the intake of erythritol, with low calorie content, also increased satiety, The biological function of erythritol is not simply as a sugar substitute, but it can have a positive protective effect on the mouth compared to sugar,and its unique metabolic pathway is friendly for diabetics (Teeuw, Gerdes, & Loos, 2010). In this study, the expression levels of Erythritol in YXP and MGH were higher, indicating that these two kinds of milk may be good choices for maintaining oral health and diabetes patients.*N*-glycolylneuraminic acid is one of the three basic forms of sialic acid. In humans, the absence of the *CMAH* gene prevents self-biosynthesis of structures containing *n*-glycolylneuraminic acid. Exogenous *N*-glycolylneuraminic acid can participate in metabolic binding to newly synthesized polysaccharides and appear on human tissue cells (Nguyen, Tangvoranuntakul, & Varki, 2005). However, the effects on the activity of the cells themselves do not seem to be compared in detail. The immunogenic potential of *N*-glycolylneuraminic acid has recently been mentioned, but whether it has a positive or negative effect on human autologous composition remains to be explored in depth (Yehuda & Padler-Karavani, 2020). Glucose 1-phosphate is mainly involved in glycogen metabolism, which is formed from glycogen by catalyzing the phosphorylation cleavage of glycogen phosphorylase. Glucose 1-phosphate can be isomerized into 6-phosphate Glucose, which is further processed through isomerization and participates in various biochemical reactions of the body. The physiological role of this substance in milk is still under discussion. D-mannose is a functional monosaccharide with excellent physiological properties and biological activity. At the same time, D-mannose is used in tumor suppression and anti-inflammation through immunomodulation (Zhou, Zheng, & Liu, 2021). Especially in the fight against urinary tract infections, D-mannose alone or in combination with several dietary supplements has a potential role in non-antibacterial prophylaxis or recurrent urinary tract infections in women (De Nunzio, Bartoletti, & Ficarra, 2021). In this study, D-Mannose was expressed at the highest level in YXP, which suggests that YXP may have higher anti-inflammatory and antibacterial effects.

Inosine is an endogenous purine nucleoside formed by the deamination of adenosine-by-adenosine deaminase and has been shown to have neuroprotective, anti-inflammatory and antioxidant properties (Ruhal & Dhingra, 2018). Meanwhile, a study by Mabley et al. found that inosine may have a role in preventing the development of diabetes and rejection of transplanted islets (Mabley, Rabinovitch & Szabó, 2003). Inosine was expressed at higher levels in the present study in the milk of ALC, YXP, OAS and all goat, which may be suitable for special populations such as diabetics. This is consistent with previous evidence that camel milk has a potent anti-diabetic effect. Adenosine 5′-phosphosulfate is an intermediate activation product of sulfated biomolecules, and aps plays multiple roles in this process as a sulfhydrylase reaction. It is also a potent product inhibitor of sulfhydrylases in many organisms. Adenosine 5′-phosphosulfate is thought to be a second messenger that activates TRPM2(Koch-Nolte, Haag, & Ziegler, 2009). It is involved in a variety of cellular signaling mechanisms by releasing intracellular calcium ions and activating the inward flow of calcium ions at the plasma membrane (Fliegert, Riekehr, & Guse, 2020). Therefore, the substance has an important role in the organism. In the present

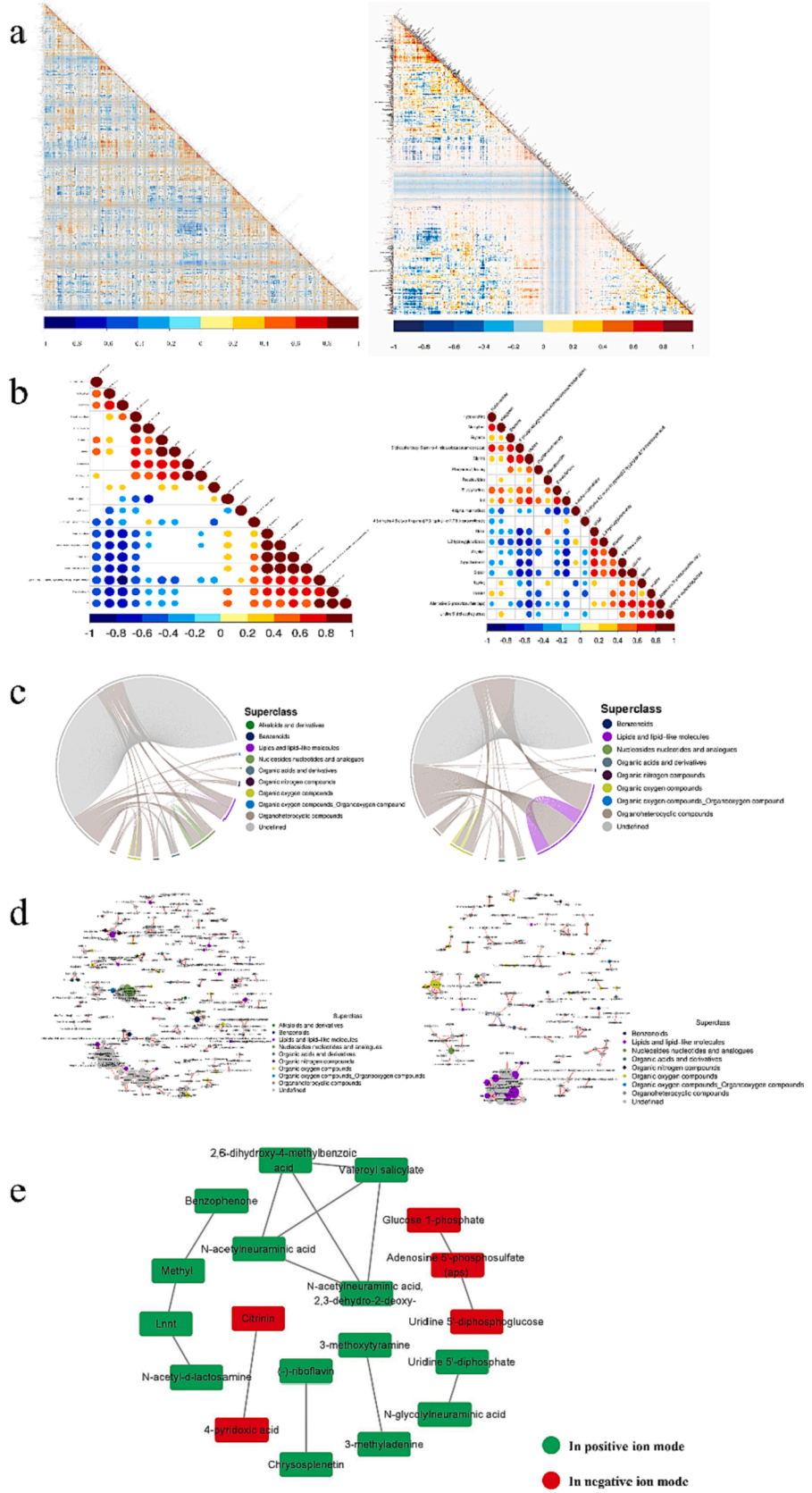

Fig. 3. a) Heatmap of the mutual regulation relationship between differential metabolites screened; b) Heatmap of the mutual regulation relationship between screened metabolites; c) Chord diagram; d) Network diagram; e) Network diagram of screened differential metabolites. (The left side represents Pos; the right side represents Neg.).

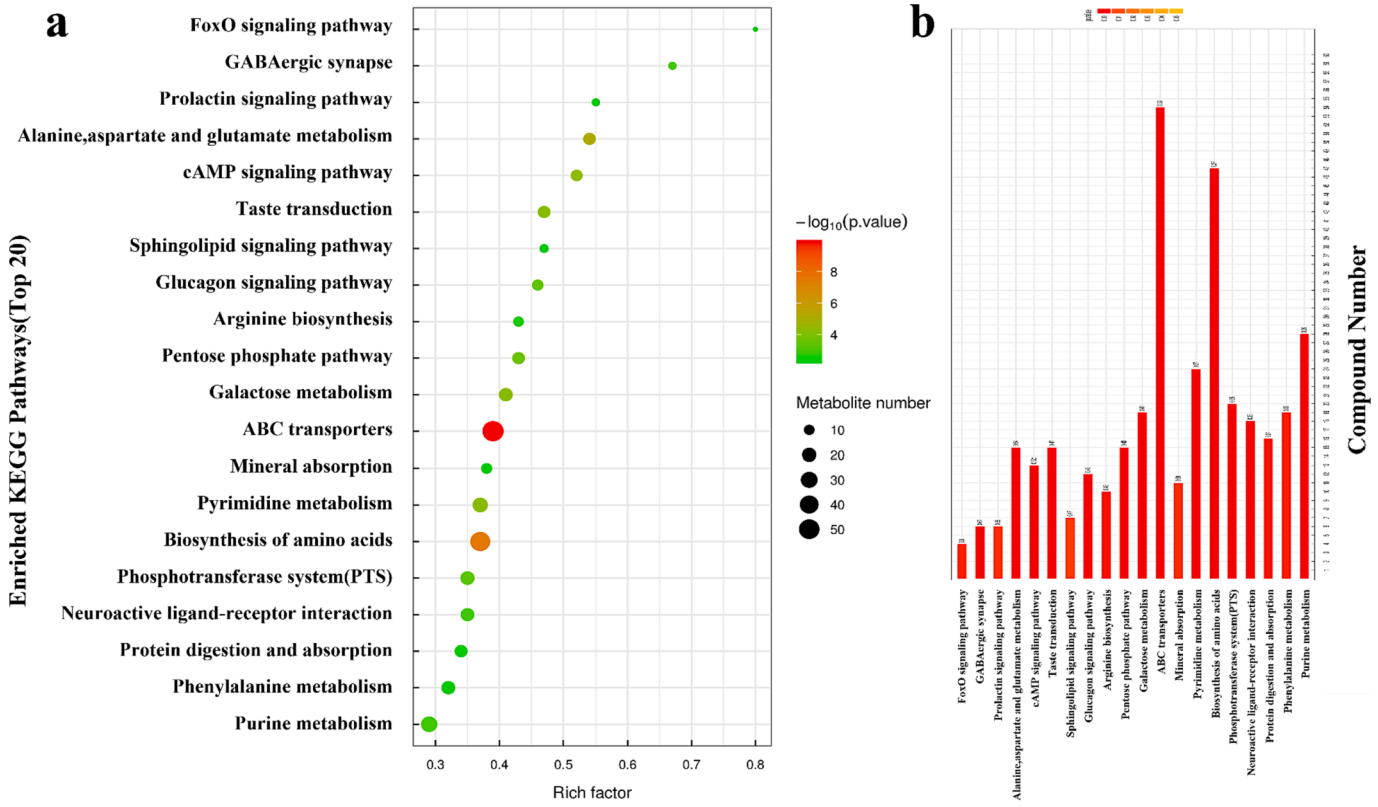

Fig. 4. a)KEGG pathway map (Bubble Chart); b) KEGG pathway map (Histogram).

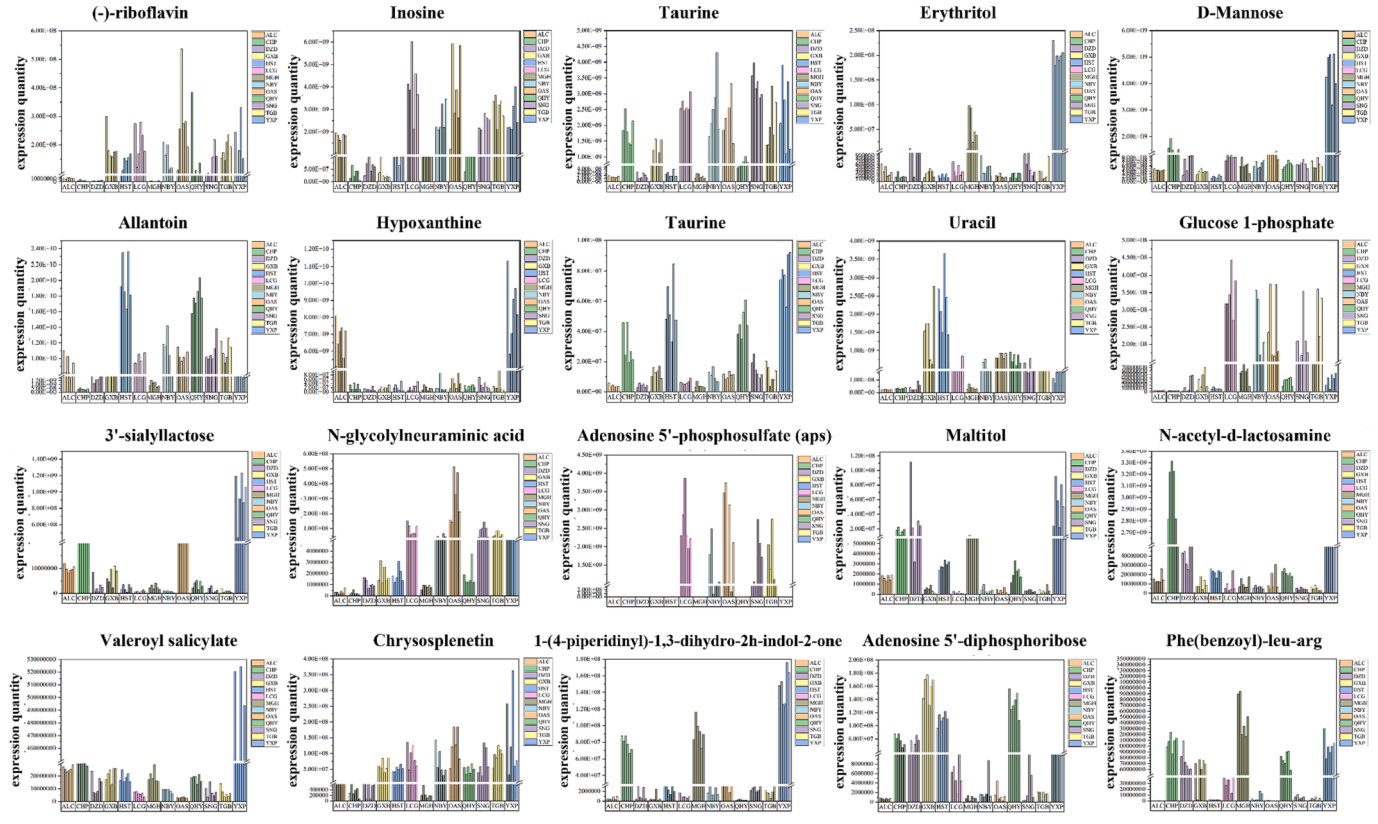

Fig. 5. Expression of 10 differential metabolites screened by kegg & Representative metabolites under each chemical classification.

study, CHP, DZD and all cattle breeds were highly expressed and this milk may have an impact on human growth and development.

Allantoin, also known as 5-ureidohydantoin or glyoxyldiureide, is well known for its antioxidant and anti-inflammatory effects (da Silva, Martins, & Alves Costa, 2018). In the present study, Allantoin was found to be highly expressed in the milk of all goats, OAS, HST, QHY, and ALC, and in the future it could be considered whether its milk has anti-inflammatory and antioxidant effects. Hypoxanthine is a naturally occurring purine compound that is a minor component of the nucleoside inosine in transit RNA (Lawal & Adeloju, 2012). Hypoxanthine has been shown to be a breakdown product of ATP during meat spoilage and is often used to judge the freshness of meat (Huang, Zheng, & Huang, 2021). Also in milk, high hypoxanthine content means that the ATP in the milk is broken down and produces a characteristic bitter taste that affects the taste. Therefore, the ability of milk to retain freshness can be reflected laterally by the hypoxanthine content, which is higher in YXP and ALC for the same transport cycle, meaning faster spoilage. Therefore, when handling both types of milk, the processing time needs to be minimized to keep the milk fresh. (-)-riboflavin is part of the functional group of the fat mononucleotide and fat adenine dinucleotide cofactors and is therefore required for many adiponectin-catalyzed reactions (Suwannasom, Kao, & Bäumler, 2020). In animal and human studies, riboflavin deficiency has been shown to impair the body's oxidative state, particularly in relation to lipid peroxidation states, such as stroke through redox reactions that prevent tissue ischemia and reperfusion injury. At the same time, some studies have shown that in the nervous system, riboflavin deficiency leads to the destruction of the myelin layer (Naghashpour, Majdinasab, & Hajinajaf,2013). Riboflavin is expressed at high levels in all goats, OAS, GXB, HST and YXP, which may mean that these milks have a protective effect on the nervous system. Uracil is a naturally occurring pyrimidine compound, which is the active component of RNA (Ramesh, Vijayakumar, & Kannan, 2020). Uracil participates in various biological activities through a complex mechanism of action in vivo. The discovery by members of the APOBEC family highlights the central role of uracil as a barrier against infectious pathogens, first as a diversified antibody participant in adaptive immunity, and second as an effective antiviral in its own right (Sire, Quérat, & Priet, 2008).

Maltitol is one of the polyol family with a sweet taste of about 75–90 % sucrose and is often listed as a substitute sweetener for sugar. Studies have shown that maltitol can affect animal intestinal epithelial cells, leading to increased intestinal calcium uptake and the highest active intestinal transport of calcium (Goda, Kishi, & Takase, 1998). This has an important role in bone growth in infants and children. Also, data from studies have shown that a single oral dose of 50 g of maltitol has significant hypoglycemic potential with significantly lower blood glucose and insulin response compared to the same amount of glucose or sucrose (Quílez, Bulló, & Salas-Salvadó, 2007). This shows that Maltitol can be used as a sweetener for diabetic patients. In this study, Maltitol was highly expressed in CHP, DZD and YXP, and the exploitation of DZD and YXP can be considered when targeting special populations such as infants and children at the skeletal development stage and diabetic patients. *N*-Acetyl-D-lactosamine is an oligosaccharide fraction present in breast milk as a free disaccharide, and most oligosaccharides cannot be hydrolyzed by human glycosyl hydrolases, which suggests that these structures have evolved into natural prebiotics that help establish a healthy gut microbiota in newborns (Arnold, Whittington & Bruno-Barcena,2021). At the same time, *N*-Acetyl-D-lactosamine can inhibit gal-3 proliferation to control disease treatment and has excellent performance in the treatment of hypoxic pulmonary hypertension and Chagas cardiomyopathy (Souza, Silva, & Soares ,2017). In this study, *N*-Acetyl-D-lactosamine has the highest expression in CHP, followed by YXP. This also shows that YXP is similar to CHP. Valeroyl salicylate is the main component of a non-steroidal anti-inflammatory drug. The latest research on anti-aging found that Valeroyl salicylate can extend the life span of Drosophila by 17 % (Danilov, Shaposhnikov, & Moskalev, 2015). The significantly higher content of this substance in YXP compared to other species may provide new ideas for the discovery of new healthy dairy products. Chrysosplenetin is an active O-methylated flavonol that has been shown to induce human osteoblasts via the Wnt/β-catenin pathway,and it selectively inhibits the proliferation of breast cancer cell lines MDS-MB-231, MCF-7 and T47D, and is relatively less toxic to normal non-tumor cells (such as MRC-5 and HUVEC)(Lan, Li, & Mu, 2021). However, Chrysosplenetin inhibits DNA topoisomerase I and II and therefore has some cytotoxicity (Şöhretoğlu, Barut, & Arroo, 2020). Chrysosplenetin is high in YXP, which has the potential to become a health product for the elderly due to the high incidence of osteoporosis and cancer in the elderly population. Taurine is a structurally simple sulfur-containing amino acid in animals and has a wide range of biological functions. It has strong biological activity in vision formation and tumor inhibition. Some studies have shown that taurine can cause human bone marrow stem cells to produce neural retinal cells or photoreceptor-like cells (Forouzanfar, Soleimannejad, & Asgharzade, 2021). Wang et al. showed that a combination of three candidate metabolites of taurine, glutamic acid and ethylmalonic acid was found to reflect tumor load, i.e., it could be used as a diagnostic marker in patients with breast cancer (Wang, Zhao, & Zhang, 2018). In addition, taurine can also have a positive impact on infant development, as it has been shown to play an important role in trophoblast turnover and cytoprotection in the human embryo (Holm, Kristiansen, & Michelsen, 2018). The high expression of taurine in CHP laterally reveals its important physiological functions. Therefore, mammalian milk with higher sieved taurine content may be more suitable for humans.

By integrating metabolomics data from 13 species, we screened 14 differential metabolites in Pos and Neg, and analyzed differentially significant metabolites in the KEGG pathway. Interestingly, some beneficial metabolites with high bioactivity were not significantly expressed in CHP, perhaps due to the fact that CHP is mainly tailored for infants. However, they are widely available in the milk of animals. Therefore, the possibility of producing suitable processed milk products for specific groups could be taken into consideration. The most unexpected aspect of this study was the astounding biological potential of YXP. There seems to be a large number of beneficial differential metabolites in YXP, which may be able to be strong evidence to bring YXP to the market, and there is still a lot of downstream potential for milk metabolomics research.

5. Conclusion

In this study, ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-Q-TOF MS) was used to analyze the differentially expressed metabolites in 13 mammalian mature milk. In Pos and Neg, the top 20 differentially expressed metabolites were screened out. A total of 1041 significant metabolites were detected in Pos ($P < 0.05$, VIP value > 1). The top 20 metabolites with high differential expression were assigned to 7 categories: Lipids and lipid-like molecules, Nucleosides, nucleotides, and analogues, Organic oxygen compounds, Organoheterocyclic compounds compounds, Benzenoids, Organic acids and derivatives, Phenylpropanoids and polyketides. The highest expressed metabolites in each category were Valeroyl salicylate (YXP), *N*-acetyl-d-lactosamine (CHP), Adenosine 5′-diphosphoribose (ALC, DZD and HST), 1,1,3-trimethylurea (ALC), *N*-glycolylneuraminic acid (LCG, NBY, SNG, TGB and OAS), 1-(4-piperidinyl)-1, 3-dihydro-2 h-indol- 2-one (CHP, MGH and YXP), Chrysosplenetin (HST, YXP, LCG, NBY, SNG, TGB and OAS). A total of 681 significa nt metabolites were detected in the Neg ($P < 0.05$, VIP value > 1). The top 20 differentially highly expressed metabolites were assigned to 4 categories, consistent with the top 4 categories in the Pos, and the highest expressed metabolites in each category were Maltitol (CHP, DZD and YXP), Adenosine 5′-phosphosulfate (LCG, NBY, SNG, TGB and OAS), 3′-sialyllactose (YXP), and Allantoin (HST, ALC and OAS). And the key metabolites were screened out in combination with

the KEGG metabolic pathway. The KEGG pathway showed that five pathways in which differentially significant metabolites are actively involved are ABC transporters: ABC transporter (ko02010); Purine metabolism (ko00230); pyrimidine metabolism (ko00240); phosphotransferase system (PTS) (ko02060); Galactose metabolism (ko00052); differential metabolites: inosine, creatinine, allantoin and D-mannose play important roles in metabolic pathways. The study found that compared with human milk, pig milk and goat milk contain nutrients that are more conducive to human health, followed by camel milk and milk. In the context of dairy production, developing goat milk is easier to meet human needs and health.

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CRediT authorship contribution statement

Zeying Wang: Conceptualization, Methodology, Funding acquisition. **Yinggang Sun:** Data curation, Writing – review & editing. **Yanzhi Wu:** Software. **Rui Chen:** Writing – original draft. **Yanan Xu:** Investigation. **Yafei Cai:** Resources. **Min Chu:** Resources. **Xingtang Dou:** Resources. **Yu Zhang:** Investigation. **Yuting Qin:** Investigation. **Ming Gu:** Investigation. **Yanjun Qiao:** Visualization. **Qiu Zhang:** Visualization. **Xi Li:** Visualization. **Xiaowei Wang:** Visualization. **Junrui Wu:** Supervision. **Rina Wu:** Project administration.

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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100581.

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