

Analysis of differential colostrum metabolism in high and low yielding Guanzhong dairy goats

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Abstract: In order to identify and analyze the metabolites of colostrum differences between high yielding and low yielding Guanzhong dairy goats from metabolomics point of view. Six high-yielding (milk yield (3.68 ± 0.57) kg/d) and six low-yielding (milk yield (1.17 ± 0.64) kg/d) Guanzhong dairy goats of similar age, litter size, body weight, and days of lactation (2nd day postpartum) were selected. The colostrum samples were examined by liquid chromatography-tandem mass spectrometry (LC-MS/MS), and the results were combined with orthogonal partial least squares discriminant analysis (OPLS-DA) and Student's *t*-test. The metabolites were screened for differential metabolites based on the principle of variable importance in the projection (VIP) > 1, *P* < 0.05, and the differential metabolites were subjected to cluster analysis, significant difference screening, and enrichment of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. The results showed that 95 differential metabolites were screened from the colostrum of Guanzhong dairy goats in the two groups, and the clustering heatmap showed that 9 of the top 50 differential metabolites were the most abundant in relative terms. The metabolites with significantly increased content in the colostrum of Guanzhong dairy goats in the high-yield group were *D*-alanine-*D*-serine, α -lactose, isoflavoprotein 2''-(6'-*p*-coumaroylglucoside), *D*-maltose, pravastatin, and diketoglucuronic acid, whereas the differential metabolites with relatively decreased content were taurocholate, *L*-carnitine, and thiamphenicol A. KEGG pathways enrichment analysis identified 8 potential metabolic pathways. The results of this experiment provide a theoretical basis for the subsequent analysis of the mechanism of milk production traits in dairy livestock and the selection and breeding of high-yielding dairy goats.

Keywords: metabolomics; Guanzhong milk goat; milk production; colostrum; differential metabolites

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1 Introduction

Milk production varies greatly among individuals of dairy goats with equal feeding conditions. Milk production is affected by multiple factors such as physiological stage, genetic improvement, feeding management, and ration composition^[1-3]; however, important differential metabolites related to lactation are one of the important factors affecting lactation performance of dairy goats^[4]. Studies have found that lactation stage, season, litter size, and calving season had different effects on lactation performance of dairy cows^[5]; furthermore, it was reported that milk content (fat, protein, and solid non-fat (SNF)) of Balkan goats showed different milk production performance as influenced by two factors: goat breed and lactation stage^[6].

Metabolomics is a technique to study the metabolic network of biological systems. The research targets are mainly endogenous small molecules with relative molecular mass less than 1 000 mol, and its detection of small endogenous metabolites in biological samples through high-throughput sequencing technology^[7]; liquid chromatography-tandem mass spectrometry (LC-MS/MS) technology is characterized by its high sensitivity, resolution, and automation^[8], has been widely used in screening animal tissue metabolite markers in recent years. Diet-induced metabolic alterations in the rumen metabolic profile and their effects on milk production-related traits in ruminants are examined by studying

potential metabolic biomarkers of disease or physiology^[9-10]. There have been many reports on the detection of milk composition and content in dairy goats at different lactation stages using metabolomics techniques^[11-12], but there was still limited research on the differences in metabolites during lactation in dairy goats at different milk production levels.

Colostrum is an important substance secreted by the mammary epithelial cells of dairy goats, and the study by Zhang et al.^[13] pointed out that the content of nucleotide nutrients, such as cytosine nucleotides, guanine nucleotides, and uridine, was significantly higher in goat's milk than that of cow's milk. Additionally, numerous studies have confirmed that colostrum contains a higher content of immunologically active substances, hormones, and growth factors, which is essential for maintaining the growth and development of young animals^[14]. For this reason, the present experiment analyzed the different metabolites in the colostrum of high-yielding and low-yielding dairy goats by LC-MS/MS, aiming to study the metabolomic characteristics of colostrum in high-yielding and low-yielding dairy goats and explore the metabolite characteristics of colostrum in high-yielding and low-yielding dairy goats from the metabolic point of view, which is helpful to provide theoretical basis for the subsequent development of feed nutritional control of milk-producing performance of dairy goats and selection of excellent dairy goat populations.

2 Materials and methods

2.1 Materials and reagents

Six high-yielding (milk yield (3.68 ± 0.57) kg/d) and six low-yielding (milk yield (1.17 ± 0.64) kg/d) Guanzhong dairy goats, each of which was 3 years of age and had given birth to its second litter, with similar days of lactation (2nd day after lambing) and body weights, were selected and numbered into high-yielding (H) and low-yielding (L) groups from the He's farm (Shaanxi, China). The dairy goats were free to drink and feed.

Test reagents: Methanol, formic acid, acetonitrile, and water were purchased from Thermo Fisher Scientific Technology (Shanghai, China). *L*-2-Chlorophenylalanine was purchased from Shanghai Hengchuang Biotechnology (Shanghai, China). All chemicals and solvents were analytically pure or chromatographic grade.

2.2 Test methods

2.2.1 Collection and processing of colostrum samples

Fresh milk samples were collected on the 2nd day after lambing before early morning feeding, and the outside of the udder was cleaned and the first two handfuls of milk were discarded before milking. Each sample was collected manually in 35–45 mL, and after collection, the colostrum samples were stored in 50 mL sterile centrifuge tubes and labeled. All the collected samples were put into liquid nitrogen (-196 °C) immediately after numbering, then transported to the laboratory within 6 hours by cold chain transportation, and then put into the refrigerator at -80 °C for storage.

2.2.2 Colostrum sample pretreatment

Take out colostrum samples stored at -80 °C, thaw at room temperature, and transfer 100 μ L samples, and 20 μ L of the internal standard (*L*-2-chlorophenylalanine, 0.06 mg/mL; prepared in methanol) was added and vortexed for 10 s to mix the sample and the internal standard. Add 300 μ L of protein precipitant (methanol-acetonitrile, 2:1, V/V), and vortex for 1 minute. The mixture was extracted by ultrasonic extraction in an ice-water bath for 10 min, and centrifuged for 10 min (13 000 r/min, 4 °C) after standing at -20 °C for 30 min. Taken 200 μ L supernatant, and transfer into an LC-MS injection vial and evaporate to dryness; and dissolve the milk samples again with 300 μ L of a mixture of methanol and water, while vortex for 30 s and sonicate for 3 min, then let stand at 20 °C for 2 h. After that centrifuge for 10 min (13 000 r/min, 4 °C). Transfer 150 μ L supernatant with a pipette, and filtered through a 0.22 μ m organic phase pinhole filter, then transferred to an LC injection vial and stored at -80 °C until analyzed by LC-MS/MS. The quality control (QC) sample was prepared by mixing the extraction solutions of all samples in equal volumes.

2.3 LC-MS detection and analysis

Chromatographic conditions: the specification of the chromatographic column used is ACQUITY UPLC HSS T3 (100 mm $\times$ 2.1 mm, 1.8 μ m). The phase A was water containing 0.1% formic acid and phase B was acetonitrile containing 0.1% formic acid. The column temperature was 45 °C; the flow rate was 0.35 mL/min; the temperature of the sample tray was 4 °C; injection volume was 2 μ L.

Mass spectrum conditions: select QE HFX mass spectrometer, and conduct positive and negative ion scanning mode for each sample by electrospray ionization (ESI). The positive ion spray voltage was 3 800 V; the negative ion spray voltage was 3 000 V; air curtain was 40 psi; collision induced ionization (CAD) parameters was medium; capillary temperature was 320 °C. The full MS resolution was 7 000; MS-MS resolution was 17 500; spray gas (Gas1) was 60 psi; auxiliary heating gas (Gas2) was 55 psi.

2.4 Statistical analysis

Before pattern recognition, data preprocessing was performed using Progenesis Q1 v2.3 software (Non linear Dynamics, Newcastle, UK) to perform baseline filtering, peak recognition, integration, retention time correction, peak alignment, and normalization on the raw data. Then, the Human Metabolome Database (HMDB), Lipidmaps (v2.3), METLIN database, and database were used for qualitative analysis, with a screening criterion of 36 points (out of 60 points).

Perform multivariate and unit statistical analysis on the obtained data to test the differences between two groups of colostrum samples. Input the obtained data matrix into the SIMCA 14.0 software system (Umetrics AB company) to complete pattern recognition, and use multivariate statistical analysis methods, including principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA). PCA is used to identify trends in intra group aggregation and inter group separation, while OPLS-DA is used to determine differences and calculate variable importance in the projection (VIP). To evaluate the fit and quality of the model, the OPLS-DA model is used to prevent overfitting, with 7-fold cross validations and 200 response permutation tests (RPT) to assess the quality of the model. Then, the effectiveness of the model is evaluated using the R^2_Y (interpretability of the model for categorical variable *Y*) and Q^2 (predictability of the model) obtained from cross validation. When both R^2_Y and Q^2 are ≥ 0.5 , the model is considered to have good robustness. Use univariate statistical analysis to analyze the differences in metabolite abundance between two groups, including Student's *t*-tests and fold change (FC) analysis. Use the Fiehn database to allocate metabolites and calculate the FC values of the two groups of differential metabolites. Use $\log_2$ FC to represent them. When $\log_2$ FC > 0 , it indicates an up regulation; when $\log_2$ FC < 0 , it indicates a decrease. There is a significant difference in metabolites with a VIP value greater than 1, and when $P < 0.05$, it indicates that metabolites are significantly different.

Cluster analysis of differential metabolites was performed using the BioTech Cloud Analytics Platform (<https://www.bioincloud.tech>). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of differential metabolites was performed using MetaboAnalyst 5.0 (<https://www.metaboanalyst.ca/>) online platform.

3 Results and analysis

3.1 Multivariate statistical analysis based on LC-MS/MS detection results

The results of multivariate statistical analysis indicate that the model established in this study has high predictive ability and good stability. According to the results, R^2_X , R^2_Y , and Q^2 were all greater than 0.5 and tended towards 1, indicating that the model could

accurately predict the differences between samples and has good predictive ability. In addition, the PCA results showed that the colostrum samples within the 95% confidence interval could be well separated into two groups, indicating significant differences in the metabolism of the samples (Figure 1A). This experiment used PLS-DA to establish a relationship model between metabolite expression levels and sample differences. The PLS-DA results showed that the model could well express and predict the significant differences between the high-yield sheep group and the low-yield sheep group samples, further verifying the predictive ability and stability of the model (Figure 1B). The OPLS-DA score results show that the explanatory power (R^2_X) of the model in the X-axis direction was 0.809, and the predictability (Q^2) was 0.995, further enhancing the reliability and accuracy of the model. By analyzing the OPLS-DA model, it is possible to effectively explain and predict the differences between two groups of Guanzhong milk goat colostrum samples (Figure 1C, Table 1). To verify the quality of the model, seven rounds of cyclic cross validation and

200 rounds of RPT were used. Through these verification methods, the intercept R^2 obtained was 0.809, and Q^2 was -0.329 (Figure 1D). These results indicate that the established model was effective, and the OPLS-DA model based on LC-MS/MS could reliably detect metabolic differences between two groups of Guanzhong milk goat colostrum samples.

3.2 Univariate statistical analysis based on LC-MS/MS detection results

Using the P -value and FC of the t -test, a volcano plot of the preprocessed data was shown in Figure 2. The red part represents upregulation of metabolite expression, the blue part represents downregulation of metabolite expression, and the gray part shows no significant difference in metabolite expression. The results showed that a total of 452 metabolites were identified from the colostrum samples of Guanzhong dairy goats in groups H and L, of which 85 metabolites were upregulated, 58 metabolites were downregulated, and 309 metabolites remained unchanged.

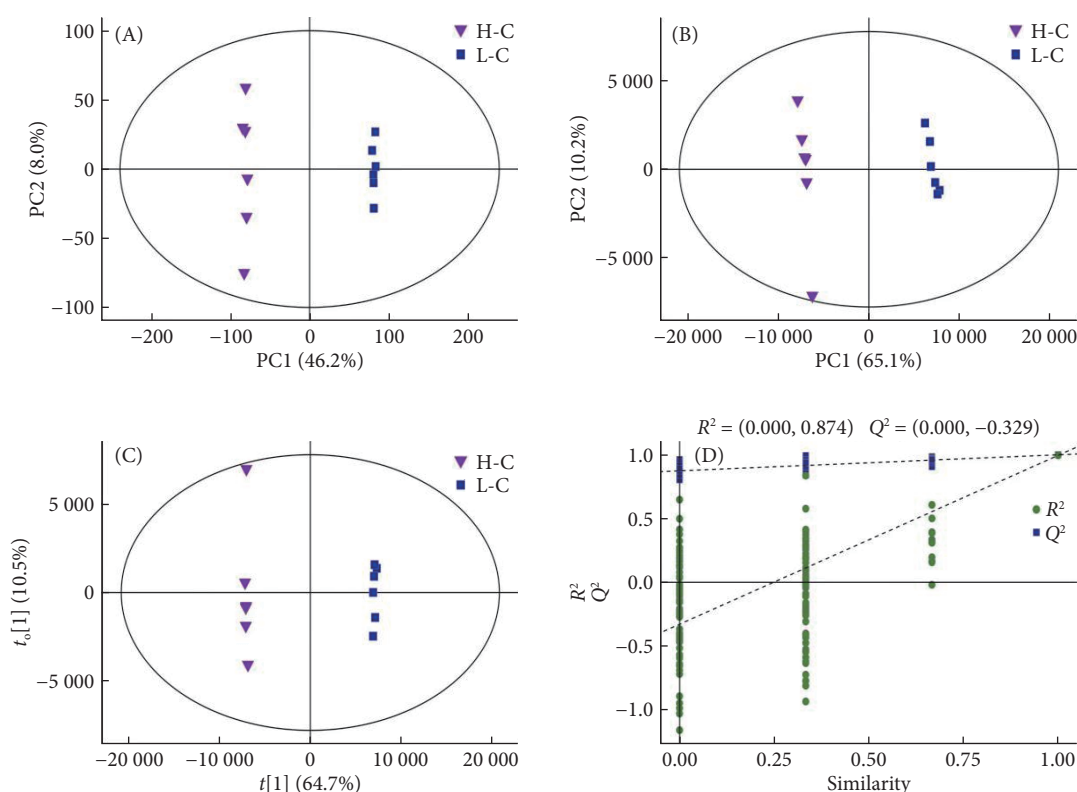


Figure 1 Multivariate statistical analysis of colostrum samples from high-yield and low yield Guanzhong dairy goats. (A) Principal component analysis score chart; (B) PLS-DA score chart; (C) OPLS-DA score chart; (D) Permutation test chart. Each point in the graph represents a sample. H-C represents high yielding Guanzhong dairy goat colostrum; L-C represents low yielding Guanzhong dairy goat colostrum.

Table 1 Results of multivariate statistical analysis for H-C and L-C groups.

Group	Model	R^2_X (cum)	R^2_Y (cum)	Q^2 (cum)	Q^2
H-C/L-C	PCA	0.614			
H-C/L-C	PLS	0.809	1	0.997	
H-C/L-C	OPLS	0.809	1	0.995	-0.329

Note: H-C represents high yielding Guanzhong dairy goat colostrum; L-C represents low yielding Guanzhong dairy goat colostrum. R^2_X represents the cumulative explanatory rate of the model in the X-axis direction when modeling the multiple statistical analysis; R^2_Y represents the cumulative explanatory rate of the model in the Y-axis direction; Q^2 represents the cumulative predictive rate of the model; Q^2 was used to measure whether the model was overfitting. When R^2_X and R^2_Y are higher, it indicates that the model can better explain the relationship between X and Y, indicating that the model has stronger explanatory power.

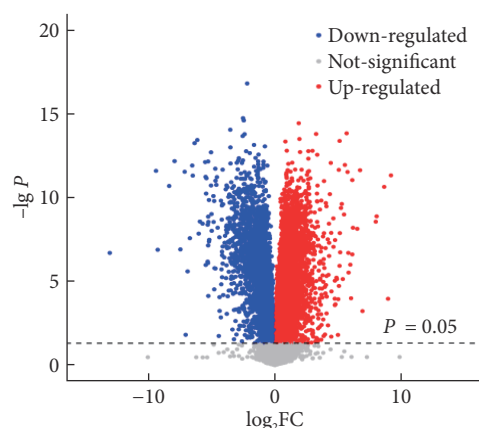


Figure 2 Volcano map of all metabolites.

3.3 Identification and screening of differential metabolites in colostrum of Guanzhong dairy goats

Differential metabolites were screened by VIP values > 1 and $P < 0.05$. The top 95 differential metabolites were selected from the colostrum of Guanzhong dairy goats in the H and L groups for analysis. These differential metabolites belong to 17 organic oxides,

17 lipids and lipid molecules, 1 propane and polyketone, 3 benzene rings, 32 nucleosides, nucleotides and analogues, 6 organic heterocyclic compounds, and 17 organic acids and derivatives, and 2 organic nitrogen compounds were identified (Table 2). Through clustering heatmap analysis of the top 50 differential metabolites (Figure 3), it was found that there was a significant clustering and stratification effect between the H and L groups of metabolites, with 9 metabolites having the highest relative content, namely taurocholic acid, L-carnitine, hygromycin A, D-alanine-D-serine, α -lactose, isovitellin 2'-(6'-coumaroyl glucoside), D-maltose, pravastatin, diketogulonic acid. The metabolite most expressed in Group H was D-alanine-D-serine, α -lactose, 2'-(6'-coumaroyl glucoside), D-maltose, and diketogulonic acid, while taurocholic acid, L-carnitine, and hygromycin A were more abundant metabolites in the group L (as shown in Figure 3).

3.4 Enrichment analysis of colostrum metabolic pathways in Guanzhong dairy goats

MetaboAnalyst 5.0 analysis platform was used to analyze the metabolic pathways of differential metabolites in the colostrum of two groups of Guanzhong dairy goats. The results showed that 96 identified differential metabolites were involved in 38 metabolic pathways. KEGG pathway enrichment analysis was performed on the top 20 significant pathways (Figure 4). The enriched differential

Table 2 Differential metabolites of colostrum between H and L groups of Guanzhong dairy goats.

No.	Superclasses	Metabolites name	P-value	VIP	log ₂ FC	Trends	Retention time (min)
1	Organic oxygen compounds	D-Maltose	< 0.001	28.63	0.67	Up	0.76
2		3'-CMP	< 0.001	1.10	-0.80	Down	0.78
3		α -Lactose	< 0.001	4.95	0.12	Up	0.78
4		D-Galactose	0.040	1.13	0.04	Up	0.78
5		D-Fructose	< 0.001	4.50	-0.27	Down	0.77
6		(R)-5-Pentanoic acid diphosphate	< 0.001	1.03	1.08	Up	0.91
7		D-Mannose 6-phosphate	< 0.001	3.60	-0.63	Down	0.73
8		Diketogulonic acid	< 0.001	6.41	1.03	Up	0.92
9		Gluconic acid	< 0.001	5.48	0.33	Up	0.77
10		Glyceraldehyde 3-phosphate	< 0.001	1.37	1.12	Up	0.53
11		Inulobiose	< 0.001	19.47	0.22	Up	0.76
12		Myo-inositol 1-phosphate	< 0.001	1.54	0.56	Up	0.71
13		N-Acetylmannosamine	0.002	1.46	-0.07	Down	0.78
14		Pantothenic acid	< 0.001	1.98	0.29	Up	3.00
15		Pseudouridine 5'-phosphate	< 0.001	3.41	-1.41	Down	0.83
16		Raffinose	0.020	1.62	0.18	Up	0.76
17		Threonic acid		1.34	0.91	Up	1.33
18	Lipids and lipid-like molecules	12-Keto-10,11,14,15-tetrahydro-LTB4	< 0.001	4.13	1.69	Up	0.90
19		24,25-Dihydrolanosterol	< 0.001	1.79	-0.80	Down	13.75
20		3 β -Hydroxypregn-5-en-20-one sulfate	< 0.001	4.54	1.04	Up	8.56
21		7-Oxo-8-amino-nonanoic acid	0.040	1.24	0.07	Up	4.68
22		Chenodeoxycholic acid glycine conjugate	< 0.001	1.09	-2.22	Down	9.40
23		Cholic acid	< 0.001	1.23	1.78	Up	9.05
24		Dehydroepiandrosterone sulfate	< 0.001	1.09	0.68	Up	7.18
25		DL-Acetylcarnitine	< 0.001	1.09	0.75	Up	7.18
26		L-Acetylcarnitine	< 0.001	17.38	0.65	Up	1.18
27		Estrone 3-sulfate	< 0.001	1.50	0.77	Up	6.88
28		Glycerophosphoric acid	< 0.001	1.20	-0.62	Down	0.75
29		Glycerolphosphorylethanolamine	0.020	1.11	-0.24	Down	0.76
30		Lithocholate 3-O-glucuronide	< 0.001	1.17	1.05	Up	4.27
31		Taurochenodesoxycholic acid	< 0.001	11.36	-0.68	Down	8.34
32		Taurochenodeoxycholic acid	< 0.001	6.99	-0.63	Down	8.01
33		Taurocholic acid	< 0.001	3.83	-0.26	Down	6.94
34		TG(16:1(9Z)/16:1(9Z)/18:2(9Z,12Z))	< 0.001	1.06	2.15	Up	4.29
35	Phenylprope and polyketones	2-Hydroxycinnamic acid	< 0.001	1.73	-0.29	Down	1.22

Table 2 (Continued)

No.	Superclasses	Metabolites name	P-value	VIP	log ₂ FC	Trends	Retention time (min)
36	Benzenoids	2-Phenyl-1,3-propanediol monocarbamate	< 0.001	1.19	-1.28	Down	1.20
37		4-Hydroxy-5-phenyltetrahydro-1,3-oxazin-2-one	0.030	1.36	0.09	Up	4.68
38		Hippuric acid	< 0.001	3.61	0.64	Up	4.47
39		AMP	< 0.001	1.79	0.75	Up	1.33
40		6-Mercaptopurine ribonucleoside 5'-diphosphate	< 0.001	1.13	-1.83	Down	4.12
41		Adenosine	< 0.001	1.96	-0.41	Down	1.35
42		Adenosine 3'-monophosphate	< 0.001	2.86	-0.17	Down	0.81
43		Adenosine 3',5'-diphosphate	< 0.001	5.30	0.97	Up	1.11
44		Adenosine monophosphate (AMP)	< 0.001	2.26	-0.54	Down	0.83
45		Adenosine phosphosulfate (ADP)	< 0.001	4.93	0.23	Up	0.78
46		ADP	< 0.001	4.83	1.09	Up	0.83
47		Cyclic AMP (cAMP)	< 0.001	1.51	0.74	Up	1.28
48		Cytidine	< 0.001	2.16	-1.04	Down	0.83
49		Cytidine monophosphate	< 0.001	1.57	-0.82	Down	0.81
50		Guanosine diphosphate (GDP)-D-rhamnose	< 0.001	1.53	0.51	Up	0.94
51		GDP-L-fucose	< 0.001	7.13	0.45	Up	0.93
52		Glucose-uridine-C1,5'-diphosphate	< 0.001	2.24	-0.25	Down	0.90
53		Guanosine	< 0.001	1.65	-0.89	Down	1.42
54		GDP	< 0.001	1.04	0.55	Up	0.94
55	Nucleosides, nucleotides, and analogues	GDP mannose	< 0.001	1.59	1.26	Up	0.83
56		Guanosine monophosphate	< 0.001	1.08	-0.73	Down	0.85
57		Inosine	< 0.001	3.18	-0.46	Down	1.44
58		Nicotinamide adenine dinucleotide	< 0.001	1.32	5.29	Up	1.09
59		Orotidine	< 0.001	1.26	-0.64	Down	0.78
60		Phosphoribosyl-AMP	< 0.001	2.96	0.81	Up	0.81
61		Pseudouridine	< 0.001	2.25	-0.98	Down	1.18
62		Uridine diphosphate-D-galactose	< 0.001	15.56	-0.40	Down	0.69
63		Uridine diphosphate-D-xylose	< 0.001	1.77	-0.29	Down	0.81
64		Uridine diphosphate-N-acetyl-D-mannosamine	< 0.001	3.00	-0.63	Down	0.85
65		Uridine	< 0.001	7.55	-0.95	Down	1.17
66		Uridine 3'-monophosphate	< 0.001	2.10	-1.62	Down	0.85
67		Uridine 5'-diphosphate	< 0.001	2.80	-0.28	Down	0.80
68		Uridine diphosphate-N-acetylglucosamine	< 0.001	19.64	-0.78	Down	0.78
69	Organoheterocyclic compounds	2-Methyl-1-hydroxypropyl-ThPP	< 0.001	1.62	-0.50	Down	0.78
70		5-Methoxyindoleacetate	< 0.001	1.62	0.95	Up	5.43
71		Adenine	0.001	1.17	0.34	Up	13.94
72		Hypoxanthine	< 0.001	1.98	-0.58	Down	1.44
73		Orotic acid	0.005	1.36	0.12	Up	0.85
74		Riboflavin	0.002	1.70	0.40	Up	4.43
75		Uracil	< 0.001	1.70	-0.92	Down	1.18
76		Uric acid	< 0.001	1.75	-1.98	Down	1.15
77		5-Aminopentanoic acid	< 0.001	2.07	0.70	Up	1.00
78		Aconitic acid	< 0.001	3.84	1.19	Up	1.15
79		Citric acid	< 0.001	15.50	0.96	Up	1.135
80		Creatine	< 0.001	5.71	-0.25	Down	0.80
81		Gamma-glutamyl-L-putrescine	< 0.001	1.04	5.67	Up	2.74
82		Isocitrate	< 0.001	13.52	1.33	Up	0.91
83	Organic acids and derivatives	L-Arginine	< 0.001	1.12	0.54	Up	0.70
84		L-Isoleucine	< 0.001	2.36	-0.46	Down	1.55
85		L-Pipecolic acid	< 0.001	1.26	0.63	Up	0.83
86		L-Valine	< 0.001	8.60	0.53	Up	0.80
87		Malic acid	< 0.001	3.08	0.87	Up	0.87
88		Oxoglutaric acid	< 0.001	1.81	0.59	Up	0.87
89		Phenylacetyl glycine	< 0.001	1.69	0.23	Up	4.75
90		Phosphocreatine	< 0.001	2.01	0.24	Up	0.80
91		Pyroglutamic acid	< 0.001	2.01	0.75	Up	0.76
92		Thyroxine	< 0.001	1.21	1.33	Up	4.27
93		Ureidosuccinic acid	< 0.001	1.83	0.76	Up	0.87
94	Organic nitrogen compounds	Acetylcholine	< 0.001	2.54	0.71	Up	0.81
95		L-carnitine	0.002	4.80	-0.13	Down	0.783

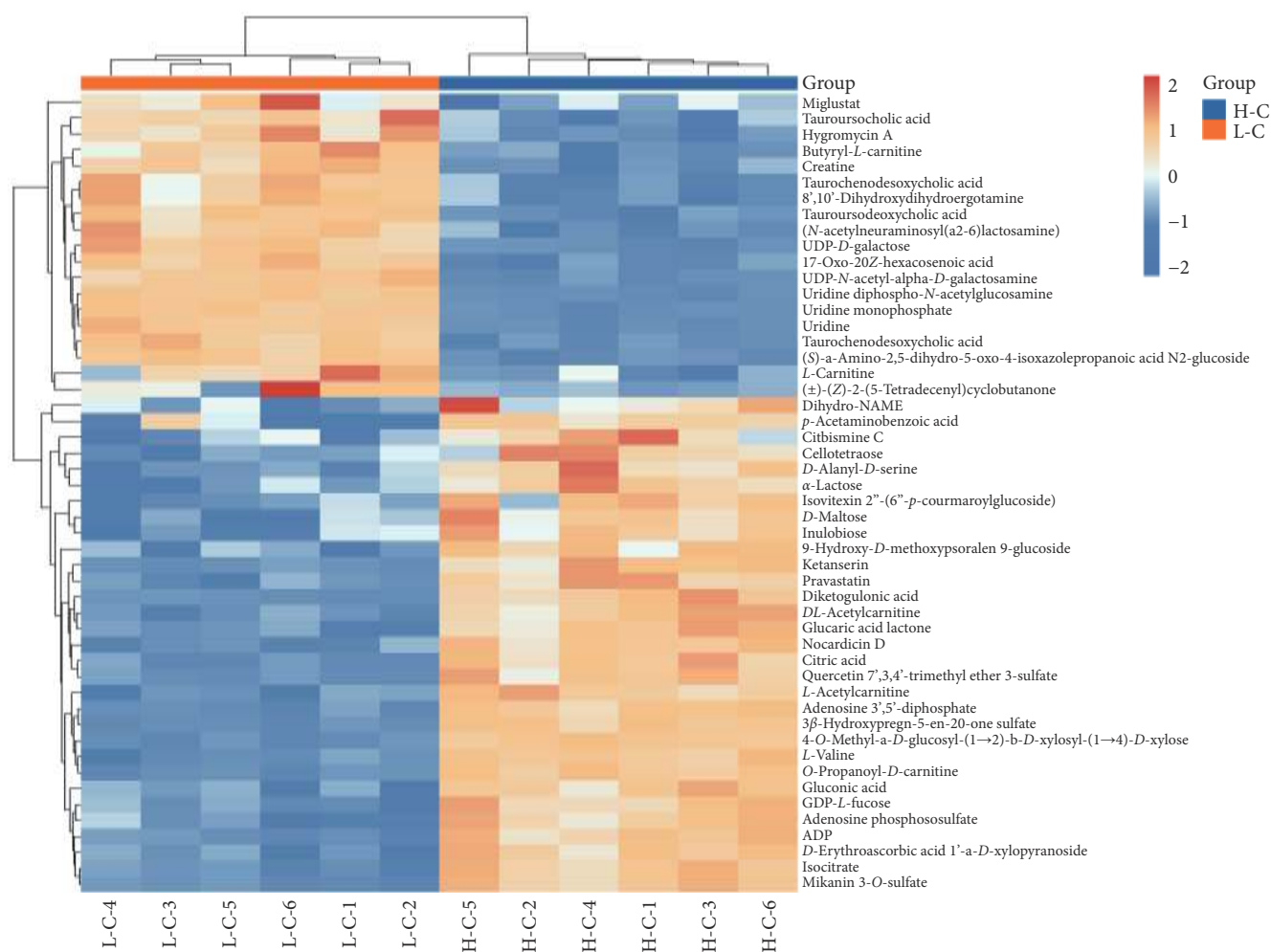


Figure 3 Hierarchical analysis clustering heatmap of the top 50 differential metabolites. The horizontal axis represents 6 replicates of H-C-1 to H-C-6 groups; L-C-1 to L-C-6 represent 6 replicates of the L-C group. The color from blue to red indicates a low to high expression abundance of metabolites, meaning that the redder the color, the higher the expression abundance of differential metabolites.

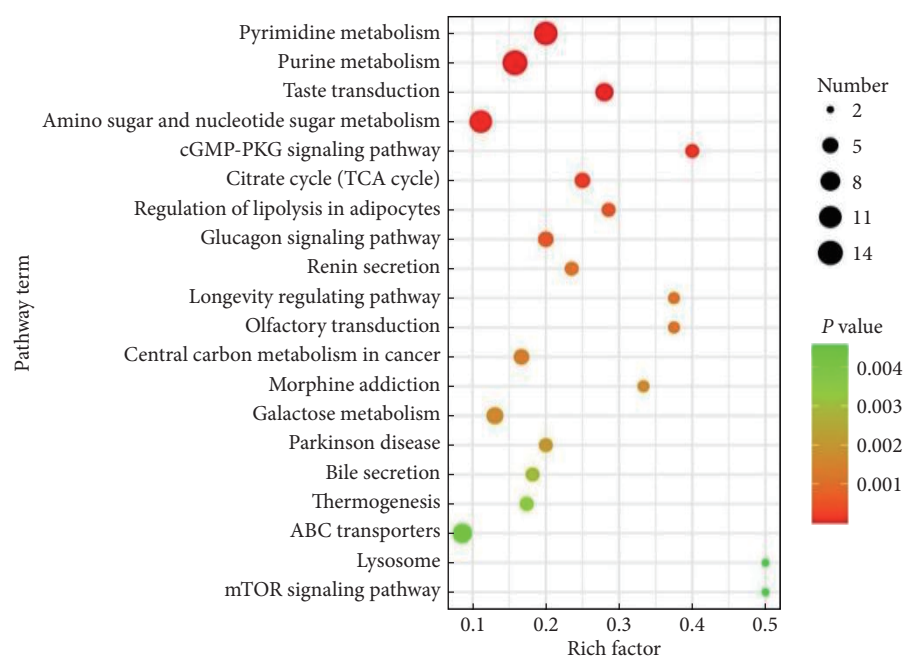


Figure 4 Bubble plots of the top 20 metabolic pathways in colostrum samples from H and L groups. The larger the bubble, the more metabolites are enriched on the pathway.

Table 3 Enrichment table of metabolic pathways in colostrum samples of Guanzhong dairy goats in groups H and L.

Metabolic pathways name	Related metabolites	Rich factor	−lgP
Pyrimidine metabolism	Uridine 5'-diphosphate; Cytidine; Monophosphate; Orotic acid; Uridine 3'-monophosphate; Pseudouridine; 3'-CMP	0.20	7.81
Purine metabolism	ADP; AMP; Adenosine; ADP; Hypoxanthine; cAMP;	0.15	7.48
Gustatory conduction	ADP; Adenosine monophosphate; Guanosine monophosphate; Alicacid; Citric acid; Acetylcholine	0.28	5.44
Amino acid and nucleotide sugar metabolism	Uridinediphosphate- <i>N</i> -acetylglucosamine; UDP- <i>D</i> -galactose; UDP- <i>D</i> -xylose; Mannose-6-phosphate; UDP- <i>N</i> -acetyl- <i>D</i> -mannosamine; <i>D</i> -fructose; GDP- <i>D</i> -rhamnose; Guanosine monophosphate	0.11	4.42
CGMP-PKG signaling pathway	Guanosine monophosphate; AMP	0.4	3.96
Citric acid cycle (TCA cycle)	Citric acid; Socitrate; Aconitic acid	0.25	3.74
Regulation of fat breakdown by adipocytes	Adenosine; AMP	0.28	3.31
Glucagon signaling pathway	TG(16:1(9Z)/16:1(9Z)/18:2(9Z,12Z)); cAMP	0.20	3.26

metabolites are mainly concentrated in metabolic pathways such as pyrimidine metabolism, purine metabolism, taste transduction, amino sugar, and nucleotide sugar metabolism. The most significantly enriched pathway is the cGMP-PKG signaling pathway. The metabolic pathway with $-\lg P > 3$ is considered the most critical in this experiment, and 8 important metabolic pathways related to the regulation of milk production in Guanzhong dairy goats were further screened, including purine metabolism, taste transduction, amino and nucleotide sugar metabolism, cGMP-PKG signaling pathway, citric acid cycle (TCA cycle), regulation of adipocyte lipolysis, and glucagon signaling pathway (as shown in Table 3).

4 Discussion

In recent years, metabolomics has matured in the detection of endogenous low molecular weight compounds, mainly involving fields such as food, biology, and medicine^[15–16]. Jia et al.^[17] used metabolomics methods to study and found that 108 metabolites and 174 lipids related to sensory quality were identified in fermented brown goat milk. After fermentation, the content of organic acids, peptides, and medium and long chain fatty acids in brown goat milk significantly increased. Tan et al.^[18] used liquid chromatography electrospray ionization tandem mass spectrometry and multivariate statistical methods to evaluate the effects of pasteurization and spray drying on goat milk lipids. A total of 1 061 lipids were identified, and 185 different lipids ($VIP \geq 1$ and $|\log_2 FC| \geq 1.0$) were screened out. Among them, glycerol phospholipid had the greatest impact on spray drying.

This study was based on LC-MS/MS detection for qualitative and quantitative analysis. It was found that a total of 452 differential metabolites were identified in the colostrum metabolites of Guanzhong dairy goats in the high-yield and low yield groups. Under certain conditions, the top 95 differential metabolites were screened for analysis. Among them, the content of 55 metabolites was significantly upregulated, while the content of 40 metabolites was significantly downregulated. These differential metabolites belong to 8 major categories of substances, namely organic oxygen compounds, organic heterocyclic compounds, lipids and lipid molecules, nucleosides, nucleotides and analogues, benzene rings, polyketones, organic nitrogen compounds, organic acids, and their derivatives. Cluster analysis of the top 50 differential metabolites revealed that 9 of them were relatively abundant in content, namely α -lactose, 2'-(6'-coumaryl glucoside), *D*-maltose, pravastatin

(reductase inhibitor), diketogulonic acid, taurocholic acid, hygromycin A, *L*-carnitine, *D*-alanine-*D*-serine. Among them, there were six differentially expressed metabolites with the highest relative content in the colostrum of high-yielding Guanzhong dairy goats, namely *D*-alanine-*D*-serine, α -lactose, 2'-(6'-coumaroyl glucoside), *D*-maltose, pravastatin (reductase inhibitor), and diketogulonic acid are the three most abundant differential metabolites in the colostrum of low yielding Guanzhong dairy goats, namely taurocholic acid, hygromycin A, and *L*-carnitine. *D*-maltose is the main component and energy supply substance of cellular structure, and has important functions in regulating cellular activity^[19]; *D*-alanine is another potent *N*-methyl-*D*-aspartate receptor co-agonist produced by the natural breakdown of host gut microbiota, which has the ability to affect neuroendocrine stress responses and subsequently affect human emotions^[20]; Statins have the effects of reducing lipid, antioxidation, and anti-inflammatory^[21]; the pravastatin has the effect of reducing the decrease of cytochrome c and mitochondrial oxygen consumption^[22]. Lactose is a disaccharide secreted by the mammary glands of mammals and is the main carbohydrate in milk. Some scholars used liquid chromatography high resolution mass spectrometry to analyze 200 serum samples of atherosclerotic patients and normal people and found that taurocholic acid, cholic acid, cortisol, and other metabolites were significantly up-regulated in patients' serum. the significant changes in the levels of these metabolites may be related to the occurrence of diseases^[23]. Hysteromycin is an aminoglycoside antibiotic that has certain effects on leptospira and mycoplasma^[24]. *L*-carnitine, also known as carnitine or *L*-carnitine, is present in plants with low levels and mainly in animal liver and skeletal muscle^[25]. Studies have shown that *L*-carnitine, as a carrier for transporting long-chain fatty acids into mitochondria, can promote the synthesis of fatty acids β -oxidation reduces the excess fat content in the body^[26], and there is a certain correlation between the amount of fat content and the milk production of Mora buffalo^[27].

The top 95 significantly different metabolites screened were projected onto the pathway database in the KEGG and matched with them^[28], resulting in a total of 38 metabolic pathways. A bubble plot analysis was performed on the top 20 significant metabolic pathways, and differential metabolites were enriched onto the KEGG metabolic pathway under the condition of $-\lg P > 3$. Eight key pathways with the strongest correlation with the regulation of milk production performance by Guanzhong dairy goat colostrum metabolism were identified, including pyrimidine metabolism,

purine metabolism, taste transduction, metabolism of amino sugars and nucleotide sugars, cGMP PKG signaling pathway, TCA cycle, regulation of fat breakdown in adipocytes, and glucagon signaling pathway. Bubble plot analysis revealed that these differential metabolites were mainly concentrated in three pathways: purine metabolism, pyrimidine metabolism, and amino and nucleotide sugar metabolism; The enriched differential metabolites are mainly concentrated in metabolic pathways such as pyrimidine metabolism, purine metabolism, taste transduction, amino sugar and nucleotide sugar metabolism. The most significantly enriched pathway is the cGMP-PKG signaling pathway. Inserte et al.^[29] found that the cGMP/PKG pathway can regulate the recovery rate of intracellular pH and directly affect Ca^{2+} oscillation and mitochondrial permeability transition. However, the specific mechanism of action of the key metabolic pathways enriched and screened in this experiment needs further research.

5 Conclusion

This experiment is based on LC-MS/MS non-targeted metabolomics technology, and 452 metabolites were identified from the colostrum of high-yield and low yield Guanzhong dairy goats. Under relevant conditions, the top 95 significant differential metabolites were screened, of which 55 were significantly upregulated and 40 were downregulated. Moreover, identification revealed that there were 9 metabolites with the highest expression levels in the two groups, namely *D*-alanine-*D*-serine, α -lactose, 2'-(6'-coumaryl glucoside), *D*-maltose, pravastatin, diketogulonic acid, taurocholic acid, hygromycin A, *L*-carnitine. Eight potential metabolic pathways related to the regulation of milk production performance in Guanzhong dairy goats were identified through KEGG metabolic pathway enrichment. The results of this experiment provide certain reference materials for the breeding and selection of high-yield dairy goat populations.

Authorship contribution statement

Akang Shari: Investigation, data curation, formal analysis, visualization, writing (original draft). Xingyang Ren: Writing (review & editing). Yingxin Qu: Conceptualization, writing (review & editing). Guang Wang: Resources, investigation, writing (review & editing). Mengqi Yu: Resources, Writing (review & editing). Lu Chen: Investigation, writing (review & editing). Yuxin Yuan: Conceptualization, methodology, formal analysis. Guang Li: Supervision, writing (review & editing, Funding acquisition).

Conflict of 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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