SHIMADZU

TP043

Targeted Metabolomics Profile Sow Milk Components using ultra performance liquid chromatograph-tandem mass spectrometry

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1. Introduction

In animal husbandry, pigs are rare animals that produce many offspring. Usually a sow gives birth to 10-16 piglets. Therefore, in order to maintain the growth of piglets, sows must secrete large amounts of milk every day for the growth of piglets. The quality and quantity of pig milk directly affect the growth potential of piglets. With the help of targeted metabonomics, this paper analyzed the small and medium molecular metabolites (such as protein, amino acid, carbohydrate and lipid metabolism) in colostrum and normal milk of sows, and then determined what differentiated metabolites existed in the milk of sows of high and low lactation performance groups.The results of this study will help to better understand the composition differences of high and low quality milk and the physiological status of sows, and provide useful information for human and animal nutrition.

2. Methods and Materials

Sample extraction and purification :

Take 300 μL milk sample, add internal standard (2-Morpholinoethane sulfonic acid , MES) into sample, then mix with 900 μL acetonitrile. Sample are mixed for for 10 minutes at -20℃, and then centrifugated for 10 min at 12 000 r/min. Take 200 μL supernatant for testing by LCMS-8050.

Samples were divided into three groups:

1 > The sow milk in different time periods were taken: 0 days (D0), 3 days (D3), 5 days (D5), 7 days (D7), 14 days (D14), 21 days (D21), 6 groups, and QC1 samples; a total of 52 samples. 2> Colostrum samples: sows were sampled within 24 h after delivery; there were 32 samples for high lactating performance group, low lactating performance group and QC2 sample. 3> Mature milk samples: sows were sampled on the eighteenth day after delivery; there were 32 samples for high lactating performance group, the low lactating performance group and the QC3 sample.

Analysis conditions :

LC condition: LC-30A

Column: PFPP (2.0 mm I.D. × 150 mm L., 3 μm) ;

Mobile phase: A-water / 0.1% formaic acid ; B-Acetonitrile/0.1% formaic acid ;

Injection volume: 1 μL.

Reference to "Primary Metabolites Method Package" for other conditions

MS condition: LCMS-8050

Ion type : ESI; Scan mode :MRM; Interface temp.: 300℃ , DL temp.: 250℃; Heating block temp.: 400℃ , Nebulizing gas :3 L/min , Drying gas :10 L/min , Heating gas :10 L/min.

Figure 1. Shimadzu LCMS-8050 and Primary Metabolites MP

3. Result

58 compounds were screened from QC samples by the Primary Metabolites MP.

Table 1. List of 58 compounds and IS in QC sample

No.	Compand	No.	Compand	No.	Compand
1	Cystine	21	2-Aminobutyric acid	41	Tyrosine
2	Asparagine	22	Histidine	42	Adenosine
3	Aspartic acid	23	Arginine	43	Isoleucine
4	Serine	24	Creatine	44	Leucine
5	Alanine	25	Cytosine	45	Phenylalanine
6	4-Hydroxyproline	26	Choline	46	S-Adenosylhomocysteine
7	Cystathionine	27	Valine	47	Kynurenine
8	Glycine	28	Creatinine	48	Acetylcarnitine
9	Citicoline	29	Norepinephrine	49	Tryptophan
10	Glutamine	30	Carnitine	50	Serotonin
11	Threonine	31	Methionine	51	Allantoin
12	Cysteine	32	Niacinamide	52	Malic acid
13	Dimethylglycine	33	Thymine	53	Isocitric acid
14	Methionine sulfoxide	34	Histamine	54	Lactic acid
15	Glutamic acid	35	Guanosine	55	Guanine
16	Cytidine monophosphate	36	Inosine	56	FMN
17	Citrulline	37	FAD	57	Taurocholic acid
18	Guanosine monophosphate	38	Pantothenic acid	58	Cholic acid
19	Proline	39	Cytidine	59	2- Morpholinoethanesulfonic acid (IS)
20	Ornitine	40	Adenine	60	Methionine sulfone Morpholinoethanesulfonic acid (IS)

1> The Stability of Instruments

QCs were used to examine the stability of the instrument. QCs data are processed by Traverse MS software. The quality control results of QCs of three groups samples are shown in Fig. 3. RSD of most compounds in QCs of is within 15%.

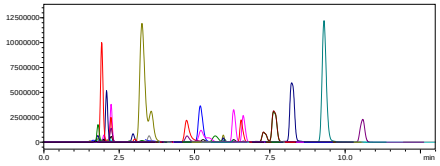

Figure 2. MRM chromatogram of QC1 in Group1 Milk in Different Time

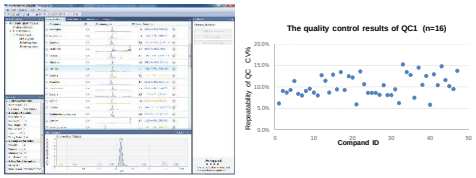

Figure 3. Traverse MS software and the quality control results of QC1 in Group1

2> Sample Analysis at Different Time Points During Lactation

By analyzing sow milk samples of 6 time points (D0, D3, D5, D7, D14, D21), the metabolites of pigs at different time points during lactation were studied. As shown in Fig. 4 (left), D0, D14 and D21 can be separated obviously; D3-D7 clustering has no obvious separation trend, but D3-D7 has a transitional trend. If only D3 is used as the mid-lactation representative in D3-D7 , the trend will be clearer, as shown in Fig. 4 (right). Studies have shown that the secretion and energy of sow milk tend to be stable on the 6-7 day after delivery.

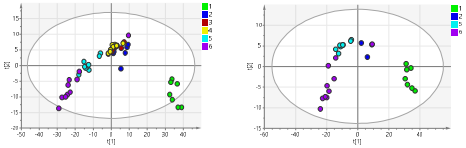

Figure 4. PLS-DA diagrams of lactation samples at different time points (D0-D21 expressed by 1-6, respectively)

3> Analysis of Colostrum Samples and Normal Milk Samples

According to the above trends, samples from colostrum (D0) and normal milk (D18) periods were selected to study the difference between the milk of high lactation performance sows and low lactation performance sows in colostrum and normal milk periods. Figure 5 shows that during colostrum period, the high lactation performance group and the low lactation performance group tend to separate gradually, but there is no significant difference between the two groups during normal breast period. The difference compounds are shown in Table 2-3.

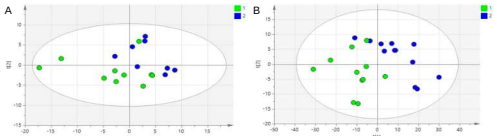

Figure 5. PLS-DA diagrams of colostrum (A, R^2X=0.606) and mature milk (B, R^2X=0.427) (■ High lactation performance group, ■ Low lactation performance group)

Table 2. Differential compounds screened from colostrum (p<0.05)

No	Compounds	HL（Area ratio）	LL（Area ratio）	FC	P-Value	VIP
1	Creatinine	0.008	0.036	0.215	0.044	5.100
2	Glutamine	1.389	2.598	0.535	0.019	0.458
3	Tyrosine	0.165	0.274	0.602	0.038	0.185
4	Histamine	23.384	37.194	0.629	0.019	0.041

Attachment: HL: high lactation performance group; LL: low lactation performance group; Area ratio: area ratio of target to internal standard; FC = HL / LL, if FC > 1, HL is higher than LL.

Table 3. Differential compounds screened from mature milk (p<0.05)

No	Compounds	HL（Area ratio）	LL（Area ratio）	FC	P-Value	VIP
1	Creatine	502.78	374.06	1.34	0 003	4.47
2	Glutamine	253.17	181.55	1.39	0.01	2.44
3	Creatinine	100.85	53.16	1.90	0.03	2.08
4	Glutamic acid	171.92	128.21	1.34	0.04	1.58
5	4-Hydroxyproline	57.34	32.76	1.75	0.002	0.87
6	Proline	111.16	88.76	1.25	0.03	0.78
7	Alanine	39.7	27.67	1.43	0.01	0.54
8	Citrulline	11	6.63	1.66	0.05	0.24
9	Asparagine	19.81	13.95	1.42	0.002	0.22
10	Allantoin	0.16	0.08	1.94	0.004	0.07
11	Glycine	5.59	3.86	1.45	0.01	0.06
12	Phenylalanine	20.63	15.01	1.37	0.05	0.04
13	Isocitric acid	5.93	10.55	0.56	0.01	0.69
14	Pantothenic acid	38.34	19.89	1.93	0.002	2.58
15	Acetylcarnitine	49.22	30.14	1.63	0.003	2.49
16	Carnitine	50.95	34.34	1.48	0.05	2.4
17	Adenosine	10.55	4.71	2.24	0.003	0.78
18	Citicoline	0.17	0.28	0.59	0.03	0.02
19	Thymine	0.06	0.02	2.43	0.01	0.004

Pathway	ID（KEGG database）	Compound
Biosynthesis of amino acids	map01230	Glycine, Threonine, Citrulline, Tyrosine, S-Adenosylhomocysteine, Isocitric acid
Protein digestion and absorption	map04974	Glycine, Threonine, Histamine, Tyrosine
Alanine, aspartate and glutamate metabolism	map00250	Alanine, Aspartate, Glutamate, Arginine
Arginine and proline metabolism	map00330	Arginine, Citrulline, Creatinine, Creatine
Arginine biosynthesis	map00220	Aspartate, Glutamate,

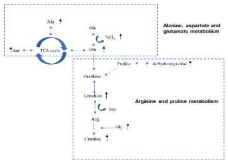

Figure 6. Metabolic Pathway of Differential Metabolites

These differential compounds in Tables 3-4 are involved in amino acid metabolism. Combined with the tracing analysis of metabolic pathways (Fig.6), it was found that arginine, proline, alanine, aspartic acid and glutamate metabolites may be biomarkers for nutritional needs of piglets. High concentrations of glutamate and glutamine may play an important role in intestinal barrier structure, intestinal cell proliferation and immune regulation. They are not only functional amino acids that maintain and promote intestinal development, but also participate in the metabolism of arginine and proline. The higher the concentration of proline, glycine, citrulline and creatine, the better the development of muscle and nervous system and the regulation of immune function.

4. Conclusions

In this study, the lactation performance of sows was studied from the perspective of metabolomics by using Shimadzu LCMS-8050 and primary metabolites MP. The results showed that the secretion of arginine, proline, alanine, aspartic acid and glutamic acid metabolites in mammary gland of sows with high lactation performance might be more active. These metabolites could be used as nutritional factors to provide higher growth performance for piglets. The differentially identified metabolites can be used as potential biomarkers to characterize lactation performance and help to understand the physiological status of sows during lactation.