Apocynum venetum Feed Affects the Serum Metabolism of Tan-sheep through the Amino Acid and Glycolipid Metabolic Pathways

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Abstract **[Objective]** The paper was to explore the effects of *Apocynum venetum* diet on nutritional metabolism of Tan sheep. **[Method]** Forty Ningxia Tan sheep were randomly divided into 4 groups (A, B, C and D), with 10 sheep in each group. The sheep were fed with different contents of *A. venetum* granule feedstuff (0%, 5%, 10% and 15%). Blood samples were collected from jugular vein on the 20th, 40th and 60th day of the experimental period. Serum samples were prepared and analyzed for differential metabolites by UPLC-Q-TOF/MS technique and annotated to the KEGG pathway. **[Result]** (1) The differential metabolites increased with the extension of feeding time. The up-regulated metabolites in Tan sheep serum were more than the down-regulated ones, and reached the maximum on the 40th day. The up-regulated differential metabolites included 2 amino acids (valine and alanine), 7 organic acids (β-hydroxybutyric acid, fumarate, ethylmalonic acid, hydroxyphenylacetic acid, gentiolic acid, protocatechuic acid, and oxycholic acid), pyrocatechol and taurochenocholate. The down-regulated differential metabolite was only p-chlorophenol. (2) With the increase of *A. venetum* content in pelleted diet, the differential metabolites in the serum of Tan sheep also increased, and the serum metabolic level gradually stabilized after the 10% concentration level. Similarly, the up-regulated metabolites were far more than the down-regulated metabolites. The up-regulated differential metabolites included 3 organic acids (β-hydroxybutyric acid, oxycholic acid and gentiolic acid), 2 amino acids (alanine and valine) and catechol, and the down-regulated differential metabolite was only taurochenocholate. (3) The metabolic pathways involving in differential metabolites were mainly tricarboxylic acid cycle, tyrosine metabolism, taurine and bile acid pathway. **[Conclusion]** The results will provide a scientific basis for the green breeding of Tan sheep.

Keywords *Apocynum venetum*; Granulated feed; Tan-sheep; Serum; Differential metabolites; Metabolic pathways

Sheep is one of the earliest domesticated animals, and the evolution of its feeding pattern has witnessed the civilization process of human domestication of livestock. Tan sheep breeding is a traditional high-quality industry in Ningxia. Tan sheep has the characteristics of strong survival, cold resistance, rough feeding resistance, fresh meat, uniform fat distribution, and no odor[1], and has become an important breed resource in the development of halal beef and mutton industry in Ningxia. Since 2000, with the implementation of grassland ecological construction project, grazing has been completely banned in Ningxia, and the sheep husbandry has gradually changed from traditional extensive grazing to intensive house feeding. In recent years, the rapid development of grass husbandry has laid the foundation for the breeding of energy industry.

Although large-scale intensive farming, high energy feed and the utilization of additives can promote the livestock production and shorten the production cycle, it causes many problems such as large deposition of subcutaneous and abdominal fat in livestock[2–3]. Excessive fat reduces not only the quality, but also the nutritional value of mutton.

As a perennial herb, *Apocynum venetum* has strong salt resistance and drought tolerance, and is widely distributed in arid and semi-arid areas of northwest China. The medicinal ingredients of *A. venetum* are mainly flavonoids which can regulate immunity, promote digestion and reduce blood lipids [4–5]. In addition to ecological value[6] and medicinal value[7], it can also be used as high-quality feed for livestock and poultry, regulate the fermentation process of herbage[8], and promote the quality and production performance of sheep livestock[9]. Studies have found that the addition of appropriate amount of *A. venetum* leaves in the feed can reduce serum triglyceride and total cholesterol of copper foot chicken[10]. At present, Chinese herbal medicine instead of antibiotics as feed additives to regulate animal nutrition metabolism has become a research hotspot in the field of animal nutrition and feed, but there are few studies on *A. venetum* as a functional feed, and the mechanism of its action is even less reported. Our research group has carried out a feeding experiment to study the effects of *A. venetum*

Received: 2022–10–15 Accepted: 2022–11–10
Supported by Ningxia Natural Science Foundation (2020AAC03076).
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pellet on the intestinal flora[11], body weight, serum and meat quality of Tan sheep[12]. On this basis, through strict feeding experiment, metabolomics technology was used to analyze the effects of different feeding time and various feeding dose on different groups of Tan sheep, as well as the changes of related metabolic pathways, which will lay the foundation for further utilization of the feed, and provide a scientific basis for the green breeding of Tan sheep.

1 Materials and Methods

1.1 Animal management The experiment was conducted from October to December 2019 at the Helan County Xiangxintai Breeding Professional Cooperative in Ningxia. Forty Ningxia Tan-sheep were selected, and they were all about six months old and weighed (21.5 ±0.5) kg. After weighing in the morning, they were randomly divided into four groups and each group got three. The animals were dewormed and were identified using ear tags. The test period was 60 d and the pre-feeding period was 5 d, and in two periods, the sheep all grew healthily. *A. venetum* was derived from our experimental field. After tested, the nutrient compositions of *A. venetum* were 7.59% crude protein, 32.42% crude fiber, 12.68% lignin, 0.61% calcium, and 0.14% phosphorus.

To conform to the recommended nutrient level from the NY/T816-2004 *Feeding Standard of Meat-Producing Sheep and Goats*, some rice husk powder and corn flour were substituted with *A. venetum* to keep nutrition balance. The 4 groups of Tan-sheep fed with 0%, 5%, 10% and 15% *A. venetum* were assigned as A, B, C and D, respectively. We disinfected the pens and feeding utensils, adopted the house-fed mode, isolated the pens, and fed them individually. We fed Tan-sheep at 8:00 and 18:00 every day to ensure they have the same conditions. The pellet feed composition and nutrient levels are shown in Tab.1.

1.2 Sample collection On the 20th, 40th, and 60th day of the experimental period, blood samples were collected from the jugular vein of all experimental sheep before morning feeding. Vacuum blood collection vessel ($EDTANa_2$ anticoagulant tube) was used to collect 5 mL of each blood. After standing for 30 min, the blood samples were centrifuged at 3 000 r/min for 15 min, and the serum was separated and placed in a frozen storage tube and stored at −80 ℃.

Tab.1 Composition of pellet feed and nutrient level (DM basis)

Ingredient	Content//%			
	A	B	C	D
Corn flour	37.00	29.00	22.00	15.00
Cottonseed meal	3.00	3.00	3.00	3.00
Soybean meal	3.00	3.00	3.00	3.00
Inorganic salt	1.00	1.00	1.00	1.00
1% Premix	1.00	1.00	1.00	1.00
Rice bran	10.00	10.00	10.00	10.00
A. venetum	0.00	5.00	10.00	15.00
Alfalfa	10.00	10.00	10.00	10.00
Powdered rice hulls	35.00	38.00	40.00	42.00
Total	100.00	100.00	100.00	100.00
Nutritional level				
DE//MJ/kg	14.88	14.81	14.88	14.93
CP	16.02	15.77	15.43	15.24
EE	7.47	7.17	6.98	7.23
Ca	0.45	0.49	0.53	0.58
TP	0.55	0.51	0.49	0.52

Note: Additive premix is supplied per kg of feed: 8 g Fe, 1.5 g Cu, 9 g Zn, 5 g Mn, 90 mg I, 45 mg Co, 700,000 IU vitamin A, 3.4 million IU vitamin D, 2 200 IU vitamin E, 1 500 mg monensin; DE. Digestive energy; CP. Crude protein; EE. ether extract; TP. Total phosphorus.

1.3 Serum metabolomics test

1.3.1 Instruments and reagents. High performance liquid (1290 UHPLC, Agilent), high resolution mass spectrometry (Triple TOF 5600, AB Sciex), centrifuge (Heraeus Fresco17, Thermo Fisher Scientific), balance (BSA124S-CW, Sartorius), pure water instrument (Clear D24 UV, Merck Millipore), ultrasonic instrument (PS-60AL, Shenzhen Redbond Electronics Co., Ltd.), chromatographic column (ACQUITY UPLC BEH Amide, Waters); Methanol (LC-MS grade), acetonitrile (LC-MS grade), ammonium acetate (LC-MS grade), Ammonia (LC-MS grade), L-2-chlorophenylalanine (analytical pure, Shanghai Hengbai Biotechnology Co., Ltd.).

1.3.2 Sample pretreatment. Accurately 100 μL of serum was added with 400 μL of methanol and 5 μL of internal standard (2.8 mg/mL 2-chlorophenylalanine). The mixture was shaken, and then centrifuged at 12 000 r/min at 4 ℃ for 15 min. The 200 μL supernatant was transferred into the injection vial, and 5 μL was injected for detection and analysis.

1.3.3 Sample analysis. (1) Chromatographic conditions: Agilent 1290 UHPLC system, column ACQUITY UPLC BEH Amide (1.7 μm 2.1 mm×100 mm), column temperature 25℃, flow rate 0.5 mL/min, injection volume 5 μL. Mobile phase composition: liquid A (25 mmol/L ammonium acetate, 25 mmol/L ammonia), liquid B (acetonitrile).

(2) Mass spectrometry conditions: AB Sciex 5600 Triple TOF mass spectrometer. Atomization pressure (GS1): 60 Psi; auxiliary pressure: 60 Psi; curtain pressure: 35 Psi; temperature: 650 ℃; spray voltage:

–4 000 V (negative ion mode).

1.4 Data processing After ProteoWizard software was used to convert the original data into mzML format, XCMS program was used for peak alignment, retention time correction and peak area extraction; minfrac was set as 0.5, cutoff was set as 0.6, and R program package and self-built secondary mass spectrometry database were used for peak material identification. Ion peaks with more than 50% missing value in the group were deleted from the extracted data. Principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were performed by SIMCA-P14.1. The volcano map was drawn by R software. The variable importance for the projection (VIP) value obtained by OPLS-DA model was greater than 1. Meanwhile, the *P* value of *t*-test (<0.05) was used to screen differentially expressed metabolites and annotate within KEGG pathway.

2 Results and Analysis

2.1 Comparison of sample total ion chromatogram (TIC) As shown in Fig. 1, the total ion current graph of QC sample UPLC-Q-TOF/MS was compared by atlas overlap, and the response intensity and retention time of each chromatographic peak were basically overlapped. The results showed that the instrumental analysis system was stable and the experimental data were stable and reliable, and the differences in metabolic profiles obtained could reflect the biological differences between the treatments of the samples.

2.2 Effects of different feeding time on serum metabolites of Tan sheep In terms of time series, serum samples of A (0%) and C (10%) groups were selected for analysis. In the negative ion mode, 832 metabolites were detected in 60 samples. Orthogonal partial least squares discriminant analysis (OPLS-DA) was used to establish the relationship model between metabolite expression levels and sample categories to predict sample categories. The closer R^2X, R^2Y and Q^2Y are to 1, the more stable and reliable the model is. Among the three models, $Q^2Y>0.5$ is valid. As shown in Fig.2, the OPLS-DA model can well divide the data into two groups. In terms of time, the samples in group C became more and more concentrated. The results showed that the difference of serum samples in group C was reduced, which verified that the metabolites of the two groups were different in terms of type or quantity.

As shown in Fig.3, compared with the control group, the total numbers of different metabolites in the experimental groups were 155, 203 and 180, accounting for 18.6%, 24.4% and 21.6% of the total metabolites detected. The numbers of up-regulated metabolites were 127, 86 and 144, and those of down-regulated metabolites were 28, 117 and 36. With the increase of time, the differential metabolites increased, and the up-regulated metabolites were more than the down-regulated ones. On the 40th day of the experimental period, the metabolism of Tan sheep was significantly different, with the most differential metabolites, and the number of down-regulated metabolites exceeded the up-regulated ones. The results indicated that the overall differential metabolites had a rise trend in spite of someway fluctuation during the experimental period.

As shown in Tab.2, the significant differential metabolites screened in three periods were d-beta-hydroxy butyric acid, pyrocatechol, parachlorophenol, mesaconic acid, ethylmalonic acid, 2-hydroxyphenylacetic acid, gentisic acid and protocatechuic acid. Among them, parachlorophenol was down-regulated, and others were up-regulated.

2.3 Effects of different feeding doses on serum metabolites of Tan sheep We chose serum samples from A (0%), B (5%), C (10%) and D (15%) groups of Tan-sheep fed with different contents of *A. venetum* for 60 d to analyze the changes of different metabolites in Tan sheep serum samples. A total of 821 metabolites were detected in 40 serum samples at the concentration level. The OPLS-DA model can filter irrelevant signals, such as sample noise, time factors and individual differences, and explain the differences between groups more scientifically. As can be seen from Fig.4, group A and groups B, C, and D can all be significantly clustered together, and the sample points of groups B, C, and D were concentrated in the oval area (95% confidence interval) of the score chart, showing an obvious clustering trend. Individual No.8 in group A was not in the confidence interval, and other sample points were relatively concentrated. The aggregation degree of the three percentages of *A. venetum* addition groups was significantly higher than that of the control group.

As shown in Fig.5, compared with group A, the numbers of up-regulated

Tab.2 Differential metabolites

Number	Name	Fold change	*P* value	*VIP*	Regulated
1	D-beta-hydroxy butyric acid	1.459 8	0.007 9	1.700 2	up
2	Pyrocatechol	1.609 0	0.009 0	1.687 0	up
3	Parachlorophenol	0.746 9	0.044 7	1.327 3	down
4	Mesaconic acid	1.275 0	0.044 2	1.292 3	up
5	Ethylmalonic acid	1.494 5	0.011 5	1.684 5	up
6	2-Hydroxyphenylacetic acid	1.446 8	0.018 9	1.590 1	up
7	Gentisic acid	2.473 6	0.000 1	2.375 5	up
8	Protocatechuic acid	1.567 4	0.014 7	1.639 2	up

Note: Fold change represents the difference multiple; *VIP* is the *VIP* value of OPLS-DA model, and *P* value is the *P* value of *t* test.

metabolites in groups B, C and D were 247, 144 and 157, and those of down-regulated metabolites were 16, 36 and 33, respectively. The results indicated that different contents of *A. venetum* had an effect on serum metabolism of Tan sheep, and the up-regulated metabolites were far more than the down-regulated ones. With the increase of the addition amount of *A. venetum*, the down-regulated metabolites increased, while the up-regulated metabolites decreased, and the serum metabolism level had a gradual trend of stability after 10% concentration level.

As shown in Tab.3, the metabolites with significant differences in each group were screened, including alanine, oxycholic acid and catechol in group B, β-hydroxybutyric acid, taurocholate and gentian acid in group C, and p-oxycholic acid, catechol and valine in group D. Taurogoocholate was down-regulated, while alanine, oxycholic acid, catechol, β-hydroxybutyric acid, gencholic acid and valine were up-regulated.

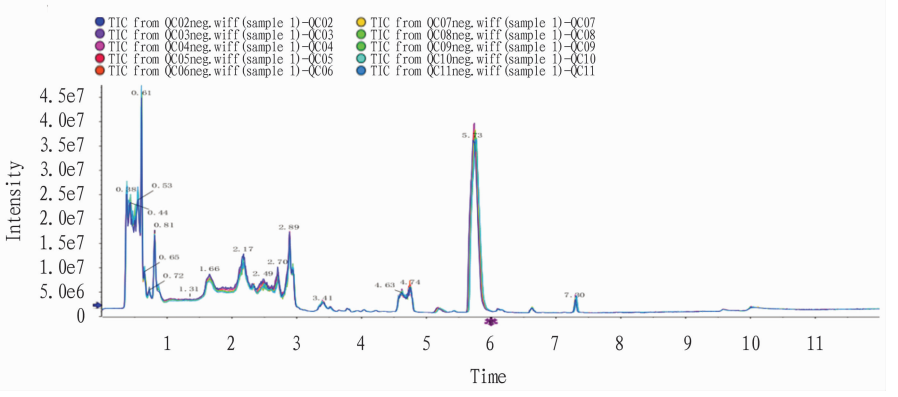


Fig.1 Total ion current UPLC-Q-TOF/MS diagram in serum of Tan–sheep

2.4 KEGG path enrichment analysis

Differential metabolites interact *in vivo* to form distinct pathways. The serum differential metabolites of Tan sheep fed with 10% *A. venetum* feed for 20, 40, and 60 d

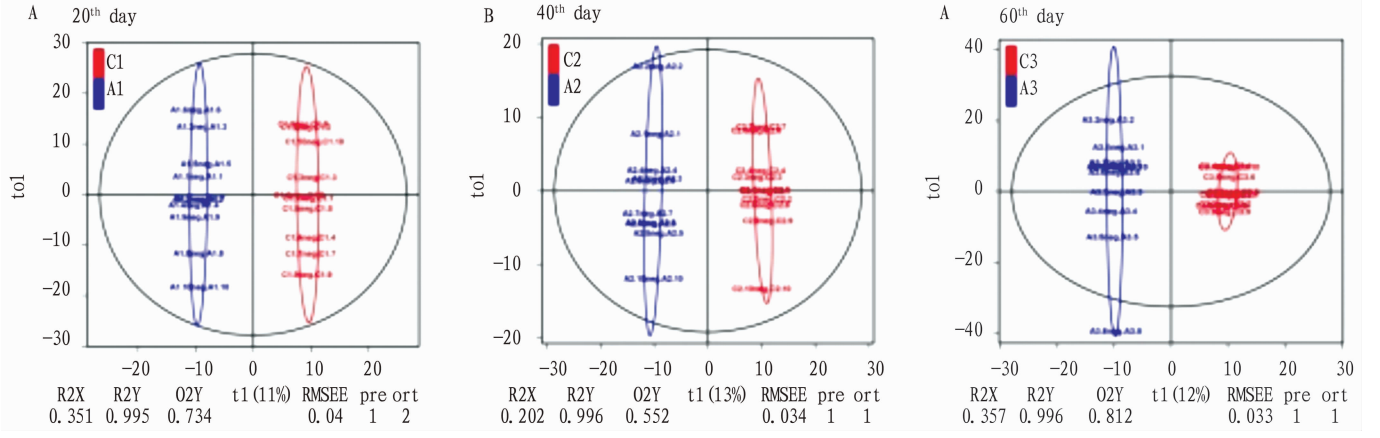
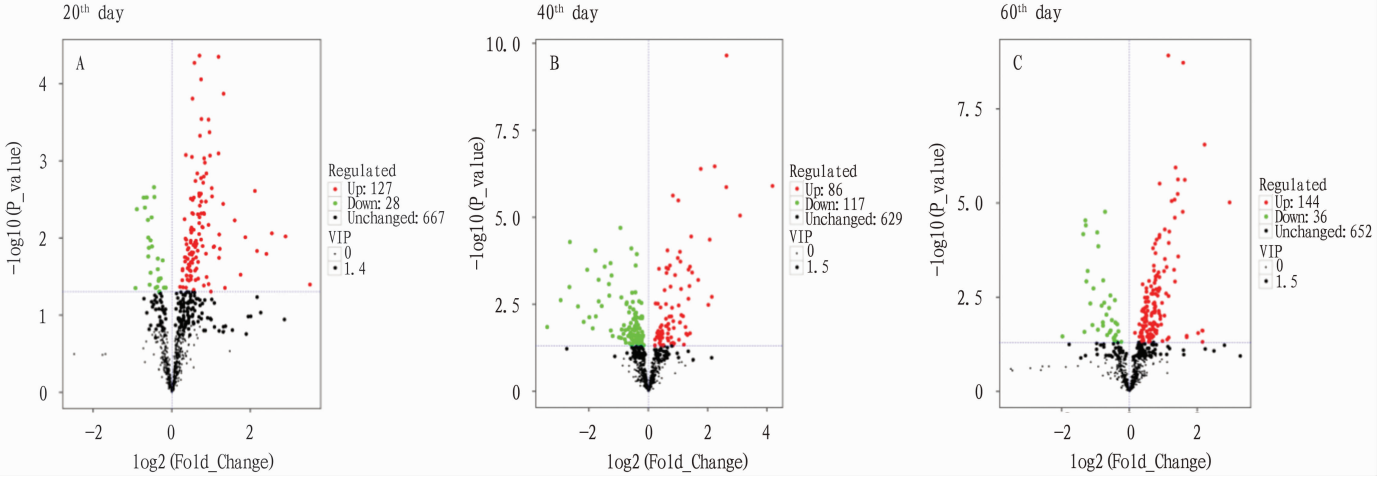


Fig.2 Analysis diagram of serum OPLS-DA of Tan sheep in three periods



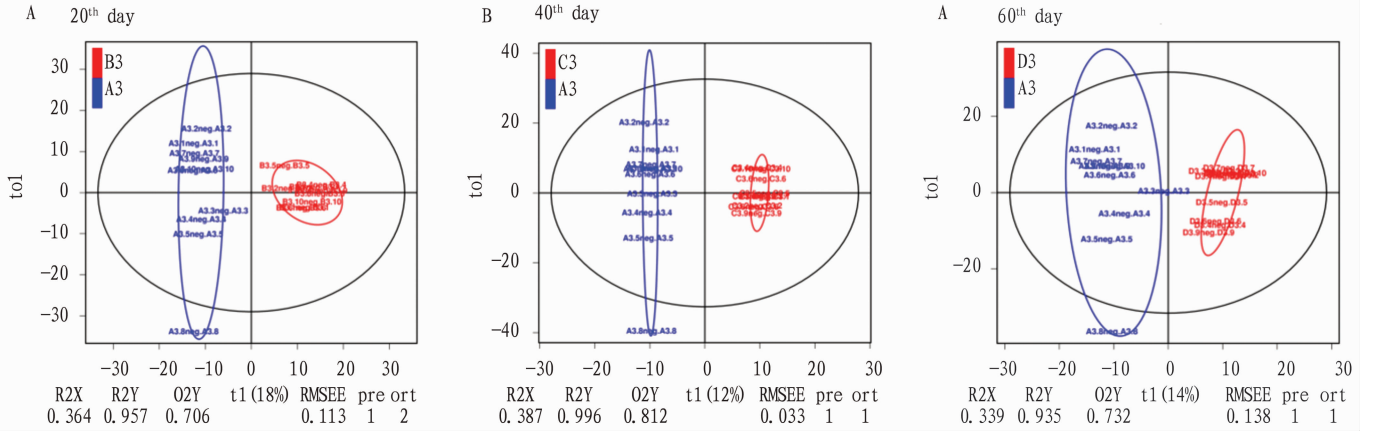
Note: A, B and C are volcanic maps of different metabolites of Tan-sheep fed with 10% *A. venetum* feed for 20, 40 and 60 d, respectively. Each point in the figure represents a metabolite; the horizontal coordinate represents the multiple change of each material, the vertical coordinate represents the *P* value, and the scatter point size represents the *VIP* value of the OPLS-DA model; the green point represents the metabolite of down-regulated differential expression, the red point represents up-regulated differential expression, and the black point represents the detected but insignificant metabolite.

Fig.3 Volcanic diagram of differential metabolites in three periods

were annotated through the KEGG database. Metabolites up-regulated pathways mainly include carbohydrate metabolism, involving in the tricarboxylic acid cycle, glycolysis, fructose, mannose metabolism and galactose metabolism; organic acid metabolism involves ascorbate, vitamin metabolism and bile acid metabolism; amino acid metabolism involves fine acid, serine, threonine, tyrosine, and taurine metabolism; pentose phosphate pathway and glutathione metabolism, and glycosylphosphatidylinositol synthesis pathway are down-regulated. The annotation results of the differential metabolite KEGG were classified according to the pathway type. As shown in Fig.6, in addition to autophagy, the metabolic pathways were roughly classified into five categories: metabolism, pathology, environmental factors, genes, and organ development. In the three periods, the metabolic class accounted for the largest proportion, and concentrated on carbohydrate metabolism and amino acid metabolism. The pathways for digestion and absorption of protein and vitamin increased, and the pathological metabolic pathway decreased with time. *A. venetum* may have a positive impact on the body immunity and energy metabolism of Tan-sheep.

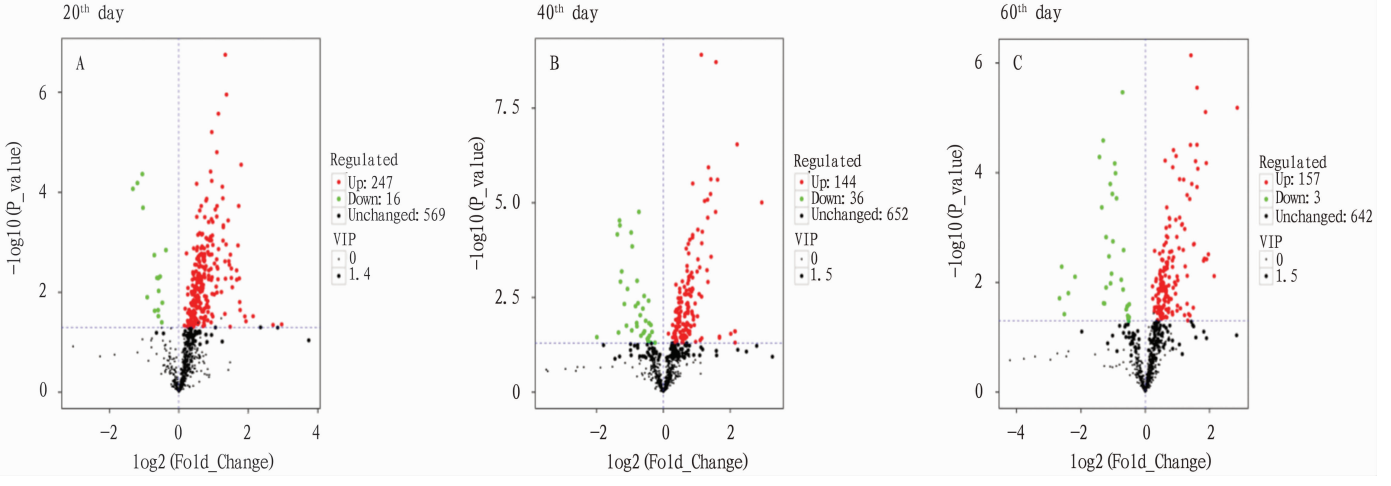
3 Discussion

Metabolomics mainly studies the changes of small molecule metabolites produced by organisms after disturbance of internal and external environment. It has been widely used to study animal pathogenesis and drug metabolism process. The research method mainly combines high-throughput technology platform and data analysis to explore the differential expression of metabolites in body fluids (blood, semen, milk, urine, *etc.*) as well as liver, muscle, fat and other samples of animals (cattle, sheep and pigs), so as to find key markers and analyze metabolic pathways[13]. Metabonomics analysis of Tan sheep serum samples from time series and concentration levels showed that some organic acids, amino acid, phenol and taurochenocholate involve in the metabolic pathways of carbohydrates, amino acids and bile acids in Tan sheep. These differential metabolites are organic acids (β-hydroxy butyric acid, fumaric acid, ethyl malonic acid, hydroxy benzene acetic acid, gentian



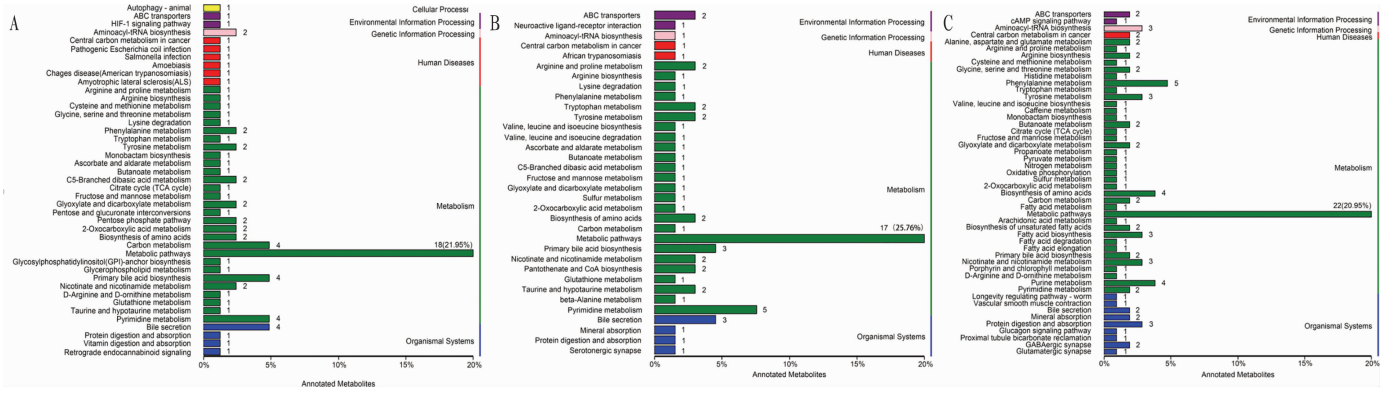
Note: A, B and C are serum OPLS-DA scores of Tan sheep fed with 5%, 10% and 15% *A. venetum* for 60 d, respectively.

Fig.4 Serum OPLS-DA scores of different groups of Tan-sheep



Note: A, B, and C are volcanic maps of differential metabolites of Tan sheep fed with 5%, 10% and 15% *A. venetum* for 60 d, respectively.

Fig.5 Volcanic map of different metabolites in Tan sheep serum with different dosages



Note: A, B and C are the KEGG annotation results of different metabolites fed with 10% *A. venetum* feed for 20, 40 and 60 d, respectively.

Fig.6 Classification of different metabolic pathways in different periods

Tab.3 Metabolite difference at concentration levels

Term	Name	Fold change	*P* value	*VIP*	Trend
AvsB	L-alanine	1.196	0.025	1.190	up
	Glycochenodeoxycholate	1.185	0.001	1.994	up
	Pyrocatechol	2.201	0.006	2.193	up
AvsC	D-beta-hydroxy butyric acid	1.322	0.035	1.262	up
	Taurochenodeoxycholate	0.690	0.007	1.745	down
	Gentisic acid	2.473	0.001	2.376	up
AvsD	Glycochenodeoxycholate	1.623	0.025	1.408	up
	Pyrocatechol	1.428	0.003	1.689	up
	L-valine	1.362	0.013	1.576	up

acid, protocatechuic acid, and oxygen cholic acid), amino acid (valine and alanine), phenol (pyrocatechol and parachlorophenol) and taurochenocholate. β-hydroxybutyrate is an important intermediate metabolite of amino acid and fatty acid metabolism [14]. β-hydroxybutyrate was up-regulated in the study, indicating that fatty acid metabolism was enhanced. Pyrocatechol, parachlorophenol and protocatechuic acid opened their loops under the catalysis of dioxygenase, then changed into small molecules through a series of catalytic reaction, and ultimately entered the glycolysis pathway and tricarboxylic acid cycle[15]. Protocatechuic acid has the effects of anti platelet aggregation, reducing myocardial oxygen consumption, increasing myocardial oxygen resistance ability, slowing heart rate, and antibacterial and analgesic pharmacological activities, as well as antioxidant, anti-tumor and neuroprotective effects [16]. Both oxycholic acid and tauroaminocholic acid can promote fatty acid metabolism[17–18].

Metabolomics is the identification and quantification of all metabolites in a system under a given set of conditions[19]. As an important branch of systems biology, metabolomics mainly studies the changes of small molecule metabolites produced by biological systems after internal and external environmental disturbances (gene changes or environmental changes), and has been widely used to study animal pathogenesis and drugs. The main research idea is to combine high-throughput technology platform and data analysis to discover the differential expression of metabolites in blood, semen, milk, urine, cerebrospinal fluid, bile, excretion and liver, muscle and fat samples of cattle, sheep and pigs[20], and then look for key markers to analyze metabolic pathways. This experiment performed metabolomics analysis on the serum samples of Tan-sheep from time series and concentration levels, and found differential metabolites such as β-hydroxybutyric acid, pyrocatechol, p-chlorophenol, fumaric acid, ethylmalonic acid, hydroxyphenylacetic acid, gentisic acid, protocatechuic acid, oxycholic acid, taurocholate, proline and alanine. Differential metabolites mainly involve in carbohydrate, amino acid and bile acid metabolism pathways. β-hydroxybutyrate acid is an important intermediate metabolite of amino acid and fatty acid metabolism[21]. In the study, β-hydroxybutyrate showed up-regulation, indicating enhanced fatty acid metabolism. Protocatechuic acid, pyrocatechol, p-chlorophenol and other phenolic acids have certain antioxidant and hepatoprotective effects [22]. Protocatechuic acid has the effects of antiplatelet aggregation, reducing myocardial oxygen consumption, improving myocardial oxygen tolerance, slowing down heart rate, antibacterial, analgesic and other pharmacological activities, playing an antioxidant, anti-tumor and neuroprotective role[23].

The tricarboxylic acid cycle is a common pathway for complete oxidative decomposition of sugars, fats and proteins, as well as a metabolic hub for the mutual transformation of carbohydrates, fatty acids and certain amino acids[20]. The carbohydrates circulating in animals are mainly glucose and are metabolized in a phosphorylated form (such as glycolysis, tricarboxylic acid cycle), in which fumaric acid is hydrated to malic acid in the tricarboxylic acid cycle to form oxaloacetic acid[24]. Phosphatidinositol-3-kinase (PI-3K)

regulates blood glucose by mediating insulin signaling pathways[25], and PI-3K accelerates glucose transporter 4 (GLUT4) and GLUT1. On the one hand, membrane transport regulates glucose uptake by muscle cells, adipocytes and hepatocytes[26]; on the other hand, gluconeogenesis is inhibited by inhibition of enolpyruvate carboxykinase, ultimately producing a variety of biological effects, such as glucose transport, glycogen synthesis, protein synthesis, anti-lipid breakdown and inhibition of apoptosis[27]. In this study, the tricarboxylic acid cycle, metabolism of fructose, mannose metabolism and galactose metabolism were up-regulated, and the phosphatidylinositol synthesis pathway was inhibited, indicating that the glucose metabolism of Tan-sheep was enhanced.

Amino acids are the precursors of the body's proteins and the basic substances of cell signaling in the body's metabolism [28–29]. The results of the study indicate that the amino acids in the metabolic pathway are mainly alanine, valine, tyrosine, arginine, proline, glycine, cysteine, methionine and taurine. The essential amino acids and medicinal amino acids in the total amino acids of *A. venetum* are similar[30], and affect the synthesis and metabolism of amino acids and sugars and lipids. Tyrosine produces 3,4-dihydroxyphenylalanine under the action of tyrosine hydroxylase, which is catalyzed by dopa decarboxylase to form dopamine. They are all synthetic precursors of neurotransmitter catecholamines, and play an important role in regulating blood glucose[31]. Hydroxyphenylacetic acid has an inhibitory effect on tyrosinase monophenolase[32], affecting tyrosine metabolism. The important free amino acid, synthesized by methionine and cysteine in hepatocytes, suggests that taurine can promote cholesterol and bile acid catabolism by increasing CYP7A1 enzyme expression activity[33–34]. Arginine participates in nutritional and physiological processes such as NO and polyamine synthesis and immune response[35], and is also an intermediate product of urea cycle[36]. Primary bile acids bind to glycine or taurine under the guidance of bile-acid CoA synthase (BACS) and bile acid-amino acid transferase (BAT) to form bound bile acid, regulate intestinal and hepatic circulation[37], and contribute to fat emulsification and prevention of gallstone formation. Related studies have shown that bile acids can also mediate processes related to intestinal bacterial community structure, dysbiosis and disease status[38–39].

In addition, pathway enrichment results indicate that *A. venetum* feed may also have an effect on immune function, vitamin metabolism, and gastrointestinal flora. *A. venetum* contains flavonoids such as hyperoside, quercetin, isoquercetin, astragalin, and kaempferol[40–41], and combines various components to affect various biochemical metabolism in Tan-sheep. The results of this study indicated that *A. venetum* can induce energy metabolism pathways such as glucose metabolism, amino acid metabolism and lipid utilization in beach sheep, which provide a reference for elucidating the mechanism of *A. venetum* feed affecting the nutritional metabolism of Tan-sheep. Therefore, Chinese herbal medicine additives need more precise and in-depth research to regulate the energy metabolism of ruminants.

4 Conclusions

In this study, UPLC-Q-TOF/MS method was used to compare and analyze the differences in serum metabolomics between the control group and the experimental group from two aspects of feeding time and feed content, and it was found that a total of 13 differential metabolites (10 of which were up-regulated and 3 were down-regulated) involved in glucose metabolism, amino acid metabolism, bile acid metabolism and other pathways. This study initially explained the nutritional metabolism of Tan sheep in the material basis, and provided a scientific basis for further research on the nutritional value of *A. venetum* functional diet.

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