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Study of metabolomics in selenium deprived Przewalski's Gazelle (*Procapra przewalskii*)

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Abstract

To understand why *Procapra przewalskii* does not show the same white myopathy as sheep in se-deficient regions and to provide reference for feeding nutrition level of artificial population and selection of wild reintroduction areas in the later period, a se-deficient model was established. The mineral elements content, physiological and biochemical parameters in blood and serum metabonomics were determined. In the se-deficient group compared with the control group, the se content was highly significantly lower ($P < 0{\cdot}01$), and the Cu content was significantly higher ($P < 0{\cdot}05$). The activity of glutathione peroxidase was significantly lower ($P < 0{\cdot}05$), but total superoxide dismutase was significantly higher ($P < 0{\cdot}05$). By matching the mass spectrum data of compounds with the Kyoto Encyclopedia of Genes and Genomes (KEGG database), eighty-six types of differential metabolites in the serum were identified. The main metabolic pathways included secondary bile acid biosynthesis, biosynthesis of unsaturated fatty acids and pyrimidine metabolism. Further analysis showed that there were seven different metabolites in pyrimidine metabolism pathway between the two groups. And there was no significant difference in erythrocyte, Hb and total antioxidant capacity between the two groups ($P > 0{\cdot}05$). The above results showed that the differential metabolism of substances exhibited complementary functions, thus alleviating some adverse effects and resulting normal activities of *P. przewalskii* can be carried out under the condition of dietary se content lower than 0·05 mg/kg.

Key words: **Metabolism: Metabolomics: Procapra *przewalskii*: Pyrimidine metabolism: Selenium deprivation**

Przewalski's gazelle (*Procapra przewalskii*), a rare, endangered and endemic animal in China, was once widely distributed in Inner Mongolia, Ningxia, Gansu, Qinghai, Xinjiang and Tibet[1,2]. However, owing to population growth, grassland degradation and habitat fragmentation, the range of distribution has been shrinking, and populations have been declining. Now, *P. przewalskii* is found only in the Qinghai Lake Basin[2]. According to the results of a survey conducted around Qinghai Lake from 1994 to 1998, only five distribution points of *P. przewalskii* were found, approximately 300 gazelles[3]. The endangered status of *P. przewalskii* has attracted worldwide attention, and this species was listed as endangered in the Nature Red List by the International Union for Conservation in 2016[4,5]. In the study of *P. przewalskii* habitat, researchers have found that se in soil and forage in the *P. przewalskii* active area are highly unevenly distributed, and the Hudong and Yuanzhe areas are se-deprived pastures[6–8]. se plays an important biological role in organisms[9]. se deprivation can lead to growth retardation, decline in fecundity, emaciation, skeletal muscle degeneration and necrosis, cell membrane degeneration, protein damage and DNA mutation[10]. However, investigations have found that *P. przewalskii* in the Hudong and Yuanzhe areas does not show obvious symptoms of se deprivation. Two possibilities were suggested to explain this finding: (1) se deficiency has no significant influence on *P. przewalskii*, because an adaptive mechanism may exist in the body, and (2) *P. przewalskii* has been affected and may be in a subclinical state, and the minimum se requirement may be lower than that in other animals. Previous studies reported the mechanisms of species coexistence[11–13], reproductive behaviour, genetic diversity[14,15], digestive system anatomy and feeding habits[16,17], the environmental characteristics of living[1,4,18], feeding strategies under se stress[19,20] and

Abbreviations: GSH-Px, glutathione peroxidase; KEGG, Kyoto Encyclopedia of Genes and Genomes; OPLS-DA, orthogonal partial least squares discriminant analysis; TOF, time of flight.

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proteomics of *P. przewalskii*[21]; however, to our knowledge, no metabolomics study in *P. przewalskii* has been reported. Metabolomics is used to study the endogenous metabolites in an organism[16,22], which directly reflect the real-time changes of functions and activity of substances synthesised and decomposed by intracellular biochemical reactions. Therefore, metabolomics is more closely associated with phenotype than genomics and transcriptomics[23,24]. Metabolomics involves quantitative measurements of the dynamic multiparameter metabolic responses of biological systems induced by pathophysiological or genetic changes. It can be used to conduct an overall evaluation of biological effects across time and space. Since Nicholson *et al.* proposed the concept of metabolomics, it has been widely used in the fields of disease diagnosis, treatment and prognostics, chemistry and toxicology[25–27]. By studying the overall dynamic changes in terminal metabolites of organisms found in serum, urine or exhaled gas, metabolomics can be used to analyse the changes in biological processes in animals[24,28]. Compared with other chromatography-MS techniques, ultra-liquid chromatography quadrupole time-of-flight MS (HILIC UHPLC-Q-TOF/MS) has advantages of high throughput, separation capacity, sensitivity and specificity, which can decrease matrix interference and improve the separation of extremely complex samples[29].

In this work, not only basic indexes were analysed, such as mineral element content and blood physiological and biochemical indexes, but also the serum metabolome of se-deficient *P. przewalskii*, with a HILIC UHPLC-Q-TOF/MS technique. Through one-dimensional and multidimensional statistical analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG database) matching, the effects of se-deficient feed on metabolome of *P. przewalskii* were studied to reveal why *P. przewalskii* does not show symptoms of se deficiency on the basis of metabonomics, and to provide a reference for further studies of the molecular mechanism underlying the adaptation of *P. przewalskii* to se stress in the environment, and to ascertain the dietary se level fed during the expansion of the artificial population as well as the selection of wild release places at a later stage.

Experimental design and method

Study area

The Wildlife Conservation Station is located in the Bird island area, Qinghai, P. R. China. The Bird island area is located in the Western part of the Qinghai Lake National Nature Reserve, the altitude range is 3194–3226 m, with large temperature difference between day and night, the annual average temperature of 0·3~1·1°C, rain and heat in the same season, sufficient light and the frost free period is short.

Model of Se-deprived animals

Fourteen healthy *P. przewalskii* reared in captivity were selected from the Wildlife Conservation Station in the Bird island area, Qinghai, P. R. China and were randomly divided into se-deficient and control groups, with seven animals in each group. The se-deficient group was fed se-deficient feed (dietary composition in Table 1), the control group was fed a complete feed (0·3 mg/kg Na_2SeO_3 was added to the se-deficient feed), the concentration of se was 0·161 mg/kg and the feeds of both groups were purchased from Trophic Animal Feed High-Tech Co. Ltd. The diets were formulated to meet or exceed all nutrient concentrations recommended by the National Research Council (2012), except the se content in se-deficient feed. The entire experiment lasted for 50 d; during the period, food and water were made available *ad libitum*. Ethical standards: all protocols used in the study were approved by the School of Life Science and Engineering, Southwest University of Science and Technology Animal Care and Use Committee.

Sample collection and preparation

At the end of the feeding trial, the animals were fasted start at 10 p.m. until 6 a.m. the following day fasting venous blood was collected. Five millilitre blood from the jugular vein of each animal was collected into a vacutainer for mineral element analysis, 5 ml blood was collected into a vacutainer containing heparin Na for blood index analyses and 5 ml blood was collected for serum metabolomics analyses. The blood samples for the metabolomics analyses were centrifuged at 3000 r/min for 10 min at 4°C to obtain serum. Serum samples were cryopreserved in aliquots of 150 μl at −80°C for the determination of serum metabolites. The serum samples were thawed at 4°C and ground in liquid N_2, and then 100 mg and 400 μl of cold methanol/acetonitrile/aqueous solution (2:2:1, v/v) were added. Samples were then mixed by vortexing, allowed to rest at −20°C for 60 min, and centrifuged at 14 000 × g for 4 min at 4°C. The supernatant was removed, and the sample was vacuum dried. For UPLC-Q-TOF/MS analysis, the samples were re-dissolved in 100 μl acetonitrile/water (1:1, v/v) solvent, mixed by vortexing and centrifuged for 15 min (14 000 × g, 4°C); 2 μl of the supernatant was then collected for injection analysis.

Sample analysis

Mineral contents in blood. Blood samples were digested with the microwave digestion method[30]. The content of elements such as Cu, Mn, Fe, Zn and se was measured with XDY-2A atomic absorption spectrometry (Perkin-Elmer).

Physiological and biochemical indexes. A fully automatic blood analyser for animals (SF-3000, Sysmex-Toa Medical Electronics) was used to collect measurements of leucocyte, erythrocyte, Hb, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular haemoglobin concentration.

A UV1900 spectrophotometer (Shimadzu) was used to measure blood biochemical indicators. Glutathione peroxidase (GSH-Px) and total antioxidant capacity were determined with a colorimetric method[31]. Malondialdehyde, catalase and total superoxide dismutase were determined with the thiobarbituric acid method, visible light photometric method[32] and hydroxylamine method, respectively. The kits used in the experiment were purchased from the Nanjing Jiancheng Bioengineering Institute.

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Table 1. Composition of se-deficient diet

Ingredient	Content (%)	Nutrients (air dry weight)	Level
Maize starch	31	DM (%)	84·41
Sugar	21	Crude protein, CP (%)	18·13
Urea	4	Ether extract, EE (%)	1·97
Cellulose	32	Ash (%)	5·36
Vegetable oil	1	Crude fibre, CF (%)	2·61
Vitamins	1	Ca (%)	0·94
Mineral elements	10	P (%)	1·19
		se (mg/kg)	0·029
Total	100	Gross energy, GE (MJ/kg)	15·78

Metabolomics analyses. A non-targeted metabolomic method was used in this study.

Chromatographic conditions. Metabolic profiling of serum samples was performed on an Agilent 1290 Infinity LC system (Agilent Technologies) coupled with an AB SCIEX Triple TOF 6600 System (AB SCIEX). Chromatographic separation was performed on ACQUITY HSS T3 1·7 μm (1 × 150 mm) columns for both positive and negative modes. The column temperature was set at 25°C, and the injection volume was 3 μl. The mobile phase composition was A: water + 25 mm ammonium acetate + 25 mm ammonium hydroxide, and B: acetonitrile. The gradient elution procedure was as follows: 0–1 min, 95 % B; 1–14 min, B linearly changed from 95 % to 65 %; 14–16 min, B linearly changed from 65 % to 40 %; 16–18 min, B maintained at 40 %; 18–18·1 min, B from 40 % linearly changed to 95 %; 18·1–23 min, and B maintained at 95 %. TOF/MS was performed in positive ion mode and negative ion mode. During the entire analysis process, the sample was placed in an automatic injector at 4°C. To avoid the influence of signal fluctuation in instrumental detection, the samples were analysed in random order. Quality control samples were inserted into the sample queue to monitor and evaluate the stability of the system and the reliability of the experimental data.

Q-time-of-flight MS conditions. MS analysis was performed with a Triple TOF 6600 Mass Spectrometer (AB SCIEX) operated in positive and negative ion modes. The electrospray ionisation source conditions on the Triple TOF were set as follows: ion source gas 1: 60 psi, ion source gas 2: 60 psi, curtain gas: 30 psi, source temperature: 600°C, ion spray voltage floating ± 5500 V (positive and negative modes); TOF MS scan m/z range: 60–1000 Da, product ion scan m/z range: 25–1000 Da, TOF MS scan accumulation time 0·20 s/spectra, product ion scan accumulation time 0·05 s/spectra. Secondary MS was performed with information-dependent acquisition in high sensitivity mode, with a declustering potential of ± 60 V (positive and negative modes), collision energy of 35 ± 15 eV and information-dependent acquisition settings to exclude isotopes within 4 Da and monitor six candidates per cycle.

To process the data from the serum metabolic analysis, the original data were converted into mzXML format in ProteoWizard 3.0.4472 and then performed peak alignment, retention time correction and peak area extraction using the XCMS programme. The metabolite structure was identified by accurate mass matching (< 25 ppm) and MS/MS spectrum alignment to the standard model compounds. For data extraction by XCMS, ion peaks with more than 50 % missing values in the group were deleted. SIMCA-P 14.1 (Umetrics) was used for pattern recognition. Principal component analysis and orthogonal partial least squares discriminant analysis (OPLS-DA) were used to analyse the differences between groups. The variable importance in projection obtained from the OPLS-DA model was used to evaluate the effects of the expression patterns of the metabolites in the different groups of samples, and variable importance in projection > 1 was used as the screening criterion to preliminary screen the differential substance between the two groups. Univariate statistical analysis was used to verify the significance of differentially present metabolites. The expression amount of qualitative significant difference metabolites was used to conduct hierarchical clustering for each group of samples, so as to help us to accurately screen marker metabolites and to analyse the related metabolic process the changes.

Data processing

The results for the mineral contents and the physiological and biochemical indexes in blood were evaluated with Student's '*t*-test' in SPSS 20.0 (version 20.0 for windows). Data are expressed as 'means with their standard error of mean'. After pre-processed the data of metabonomics by Pareto-scaling, multidimensional statistical analysis was carried out, including unsupervised principal component analysis and OPLS-DA. One-dimensional statistical analysis included Student's '*t*-test' and variation multiple analysis.

Results

Mineral elements in the blood

The content of mineral element in blood samples is given in Table 2. It is found that the se content in blood in the se-deficient group was highly significantly lower than that in the control group ($P < 0·01$), but the Cu concentrations were significantly higher than that in the control group ($P < 0·05$). The concentration of Mn in se-deficient group had a rising trend ($0·05 < P < 0·1$). The content of Fe and Zn in the se-deficient group was higher than those in the control group, but the results were not significantly different between the two groups ($P > 0·05$).

Blood physiological and biochemistry indexes

The blood physiological and biochemistry indexes in *P. przewalskii* were measured on the 50th d of the feeding test, and the results are given in Tables 3 and 4, respectively. Comparison of blood parameters between the two groups, the results showed that the physiological indexes of leucocyte, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration in the se-deficient group decreased, but no statistical difference was observed ($P > 0·05$). The values of erythrocyte and Hb in the se-deficient group increased, but there was no significant difference between the two groups ($P > 0·05$). The activity of GSH-Px in the se-deficient group was significantly lower than that in the

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Table 2. The content of mineral elements in the blood of *P. przewalskii* (μg/g)
(Mean values and standard deviations, *n* 7)

	se-deficient group		Control group		
Element	Mean	SD	Mean	SD	*P*
Cu	2·387[a]	0·100	1·670[b]	0·041	0·035
Mn	0·661	0·040	0·525	0·009	0·096
Fe	37·149	1·646	26·013	0·523	0·113
Zn	1·933	0·063	1·463	0·057	0·952
se	0·24[B]	0·046	1·392[A]	0·021	0·009

In the same row, different little letters show significant difference (*P*-value < 0 05), different capital letters show extremely significant difference (*P*-value < 0 01).

Table 3. Blood physiological index in *P. przewalskii*
(Mean values and standard deviations, *n* 7)

	se-deficient group		Control group		
Item	Mean	SD	Mean	SD	*P*
Leucocyte ($\times 10^9$/l)	24·461	0·805	27·087	1·060	0·387
Erythrocyte ($\times 10^{12}$/l)	16·306	0·581	11·821	0·358	0·170
Hb (g/l)	183·051	5·602	160·012	3·730	0·141
MCV (fl)	42·716	1·446	52·440	0·706	0·377
MCH (pg)	12·362	0·104	13·053	0·173	0·119
MCHC (g/l)	236·354	1·657	251·776	2·264	0·455

MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

Table 4. Blood biochemical index in *P. przewalskii*
(Mean values and standard deviations, *n* 7)

Item	se-deficient group		Control group		*P*
	Mean	SD	Mean	SD	
T-SOD (U/ml)	92·107[a]	1·737	62·820[b]	3·692	0·033
T-AOC (/ml)	2·354	0·217	2·969	0·403	0·235
MDA (nmol/l)	2·2988	0·202	1·613	0·108	0·064
CAT (U/ml)	9·821	0·883	10·688	0·815	0·999
GSH-Px (U/ml)	317·706[b]	2·321	365·540[a]	13·059	0·019

T-SOD, total superoxide dismutase; T-AOC, total antioxidative capacity; MDA, malondialdehyde; CAT, catalase; GSH-Px, glutathione peroxidase.
In the same row, different little letters show significant difference (*P*-value < 0 05).

control group ($P < 0{\cdot}05$), and the total superoxide dismutase activity was significantly higher ($P < 0{\cdot}05$). In addition, compared with the values in the control group, the total antioxidant capacity and catalase values were lower, and the concentration of malondialdehyde in se-deficient group had a rising trend ($0{\cdot}05 < P < 0{\cdot}1$).

Inter-group principal component analysis

The ion peaks of metabolites were recorded with XCMS. There were 6060 ion peaks of metabolites in positive ion mode and 7897 ion peaks of metabolites in negative ion mode. After Pareto-scaling, principal component analysis was performed, which can be used to observe the overall trend in the distribution of all samples and determine differences in metabolism between se-deficient group and control group. As shown in Fig. 1, on a PC1 and PC2 dimension chart with positive and negative ion mode data, a trend of separation was visible between the se-deficient and control group, thus indicating that the spectrum of serum metabolism in the two groups had changed.

Inter-group orthogonal partial least squares discriminant analysis

A supervised discriminant analysis statistical method, OPLS-DA, was used to analyse samples. OPLS-DA is modified on the basis of PLS-DA, due to filter out the noises irrelevant to the classification information, which is more powerful and effective for model analysis[24]. The OPLS-DA model is shown in Fig. 2. According to the model evaluation parameters, particularly under anionic mode, $R2Y = 0{\cdot}998 > 0{\cdot}5$ and $Q2 = 0{\cdot}953 > 0{\cdot}5$, thus indicating that the OPLS-DA anionic mode was stable and reliable and showed distinct metabolomic plasma profiles for the se-deficient and control groups (Fig. 2(a)). Under cationic mode, $R2Y = 0{\cdot}849 > 0{\cdot}5$ and $Q2 = 0{\cdot}423 < 0{\cdot}5$; therefore, the stability and reliability of the OPLS-DA cationic mode were poor (Fig. 2(b)). To prove the reliability and accuracy of the model, used a 200 permutation one component permutation test to evaluate the model, the OPLS-DA model established by the positive and negative ione mode data in this experiment has not been fitted (Fig. 3).

Metabolites with significant differences between groups

Using *P*-value < 0·05 and variable importance in projection > 1 as screening criteria, forty and fifty-three types of differential metabolites were identified in the serum of *P. przewalskii* in positive and negative ion modes, respectively (online Supplementary Table S1). Compared with the control group, twenty-three substances were up-regulated, and seventeen were down-regulated in positive ion mode in the se-deficient group. In negative ion mode, twenty-three metabolites were up-regulated, and thirty were down-regulated in the se-deficient group. The hierarchical clustering results of significant differences between positive and negative ion modes are shown in Fig. 4(a) and (b), respectively.

Kyoto Encyclopedia of Genes and Genomes metabolic pathway analysis of metabolites

Metabolites were searched and screened in the KEGG database to determine chemical and metabolic pathways that might be involved in se deprivation in animals. Given that a substance is not necessarily involved in only one metabolic pathway in the KEGG database, fifty-three specific metabolites involved in ninety-six possible pathways were discovered (online Supplementary Table S2). The main metabolic pathways included secondary bile acid biosynthesis, biosynthesis of unsaturated fatty acids, pyrimidine metabolism, bile secretion,

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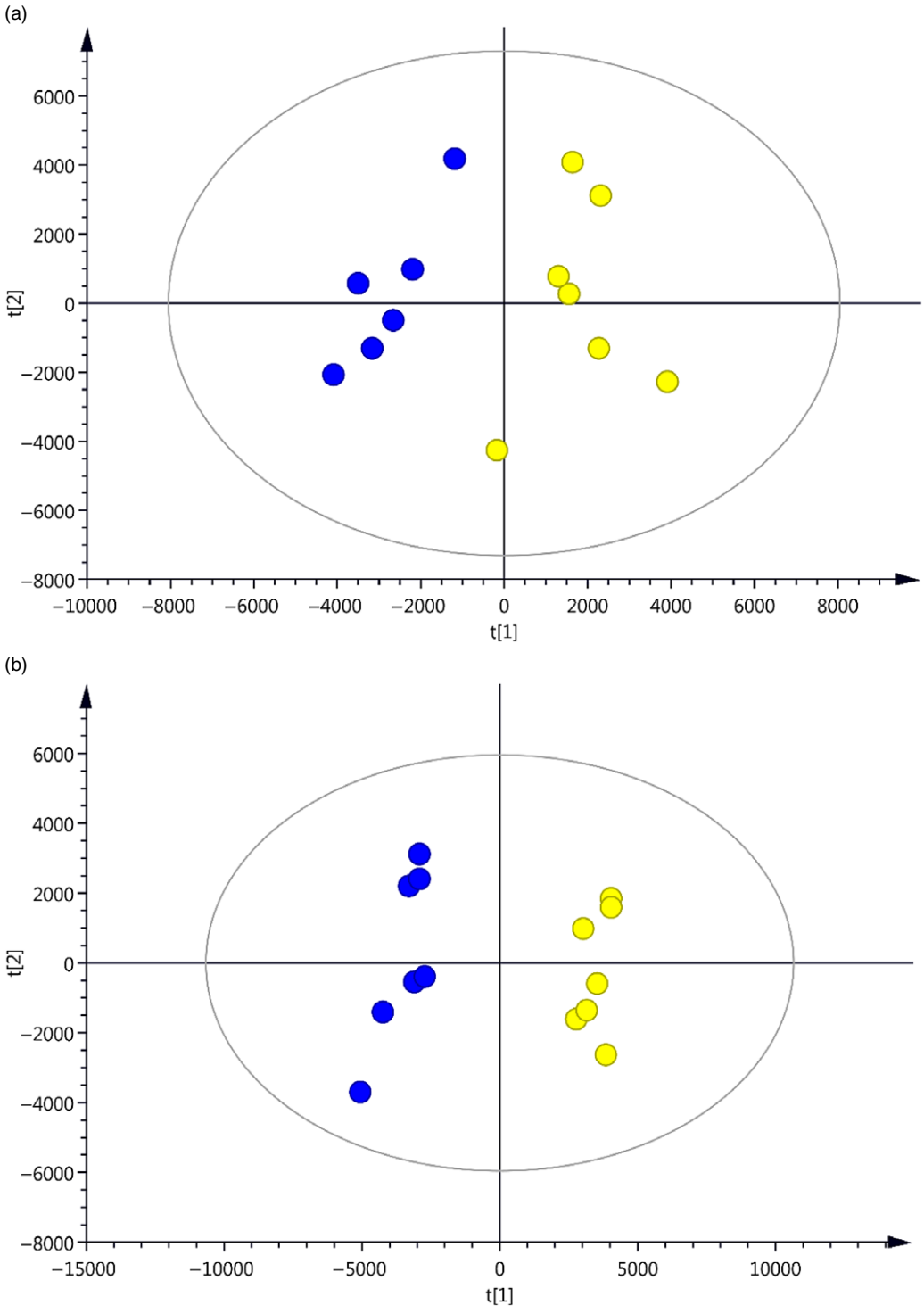

Fig. 1. Principal component analysis scores for se-deficient and control groups under the cationic and anionic mode. ■, control; ■, Se deficiency.

primary bile acid biosynthesis, biosynthesis of plant secondary metabolite, arginine and proline metabolism, metabolic pathway, protein digestion and absorption, and aminoacyl tRNA biosynthesis; the results of KEGG enrichment analysis are shown in Fig. 5. The pyrimidine metabolism pathway included seven different metabolites, 2′-deoxyuridine, 5,6-dihydrothymine, L-glutamine, pseudouridine, thymidine, uracil and uridine.

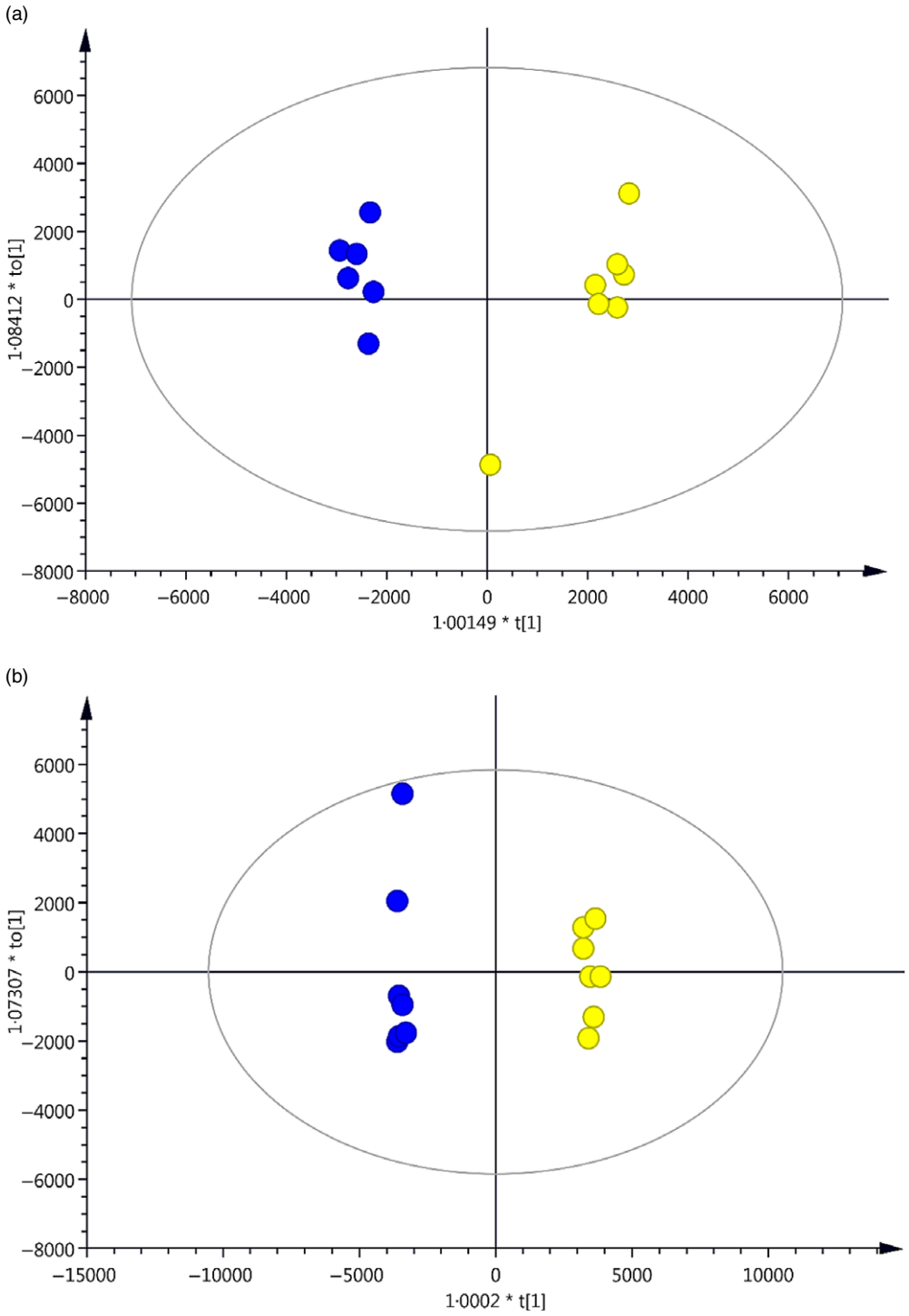

Fig. 2. Orthogonal partial least squares discriminant analysis scores for se-deficient and control groups under the cationic and anionic mode. ■, control; ■, Se deficiency.

Discussion

se plays a key role in animals by acting as a component of some proteins and enzymes, participating in basic metabolism and effectively improving the immunity of the organism[33,34]. se deprivation can cause disorders in metabolic processes and biochemical reactions, thereby leading to many injuries and diseases, for example, causing white muscle disease in calves, sheep and goat, exudative diathesis in poultry and mulberry heart disease in pigs[35]. Metabolic changes reflect the effects of genetic mutations and environmental changes[36]. At

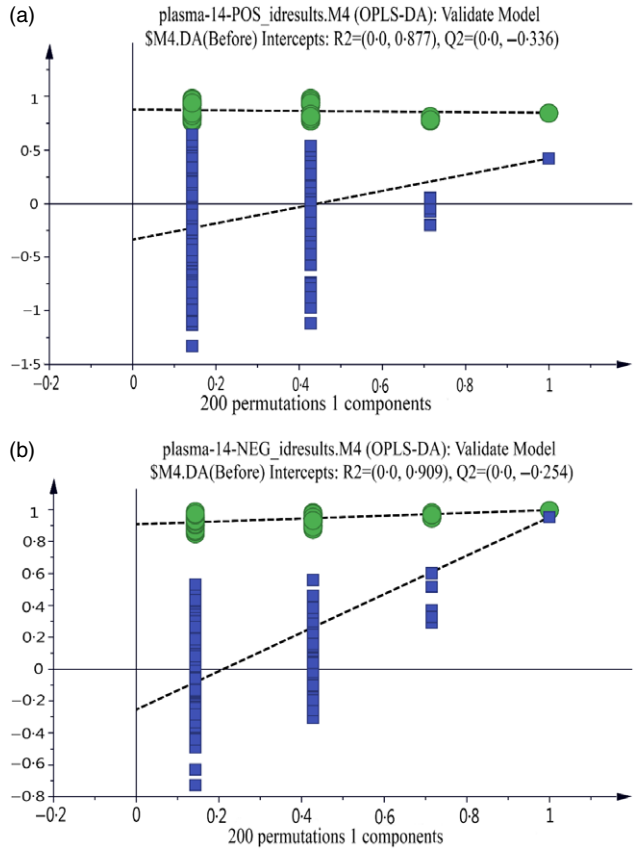

Fig. 3. Orthogonal partial least squares discriminant analysis displacement test under the cationic and anionic mode. ●, R2; ■, Q2.

present, the biological markers of se can be roughly divided into two types[37]. One type can provide information based on indirect inference of the se level in food, tissue, urine or faeces, which can indicate the possibility of an insufficient or adverse reaction but cannot provide direct evidence. The other type of marker can directly provide functional information. This information enables identification of se-deprived disorders and tracking the responses of se-deficient individuals to se therapy, such as selenoproteins, especially glutathione peroxidase 3 and selenoprotein P[38]. Therefore, health risk can be feasibly assessed through testing specific substances. Many studies have shown that the physiological and pathological changes in many animals and micro-organisms are usually accompanied by abnormal changes in metabolic processes[39,40]. It follows that differences in metabolite levels between the se-deprived group and control group can be detected even in early stages of the disease, and the analysis of the variations in metabolites can be used to evaluate the health status of *P. przewalskii*.

The serum metabolomics of the Se-deprived group and the control group were analyzed by non-targeted metabolomics. On the basis of data from ultra-high voltage programmable logic controller Q-TOF/MS, the serum metabolites differed between the se-deficient and control animals. Principal component analysis and OPLS-DA analysis indicated significant differences in endogenous metabolites between the se-deficient and control groups. The KEGG database was searched to identify the possible different metabolites and metabolic pathways associated with se deprivation. Forty differential metabolites were found in positive ion mode, and fifty-three differential metabolites were found in negative ion mode, including amino acids, fatty acids, pyrimidines and other major substances (online Supplementary Table S1). As substrates or intermediate products, these metabolites affect bile acid synthesis, amino acid metabolism, pyrimidine metabolism, protein digestion and metabolic absorption pathways to varying degrees (Fig. 5). Compared with the control group levels, the levels of 2′-deoxyuridine (fold change (FC) = 1·422458925), 5,6-dihydrothymine (FC = 1·49709639), L-glutamine (FC = 1·372975589), pseudouridine (FC = 1·174172203), thymidine (FC = 1·570781589), uracil (FC = 1·398440554) and uridine (FC = 1·543435143) in the serum were higher. The content of metabolites in the pyrimidine metabolism pathway increased to varying degrees, thus leading to pyrimidine metabolism pathway disorder; this result is consistent with the results of the study on the metabolic pathway of se deficiency in wild *P. przewalskii*[21,41]. Severe pyrimidine metabolism disorder can lead to orotic aciduria, which is characterised by hypopigmentation giant cell anaemia and physical development disorders[42,43]; it is also related to chronic kidney disease[44].

Studies on the differentially present metabolites in the pyrimidine metabolic pathway have demonstrated that 5,6-dihydrothymine is an important product of DNA base damage under hypoxia[45]. The experimental results indicated that 5,6-dihydrothymine was elevated in the serum in se-deficient *P. przewalskii*, and the erythrocyte count and Hb concentration increased. Erythrocyte and Hb in the blood are substances that transport oxygen, but erythrocyte, as the most important medium for transporting oxygen in the blood of vertebrates, transports oxygen through glucose synthesis energy. When the se content in the body decreases, the content of selenoprotein involved in electron transfer subsequently decreases, and the synthesis of ATP decreases[46]. The energy to transport oxygen decreases, thus ultimately diminishing the efficiency of oxygen transport, and that may be the reason for the increased content of 5,6-dihydrothymine without a decrease in erythrocyte and Hb[46].

L-Glutamine, despite being considered a non-essential amino acid, is essential for cell proliferation under appropriate conditions. It can be used as a breathing fuel, which can enhance lymphocyte proliferation, cytokine production, macrophage phagocytosis, secretory activity and neutrophil bacterial lethality[47]. High utilisation of glutamine can enhance proliferation, phagocytosis and secretion of the immune cells, thereby improving the body's immune ability. However, in our serum metabolomics studies, the content of L-glutamine in the serum of se-deficient *P. przewalskii* was higher than that in the control group, thus indicating that the utilisation of L-glutamine in the body in se-deficient *P. przewalskii* was decreased. Studies on the

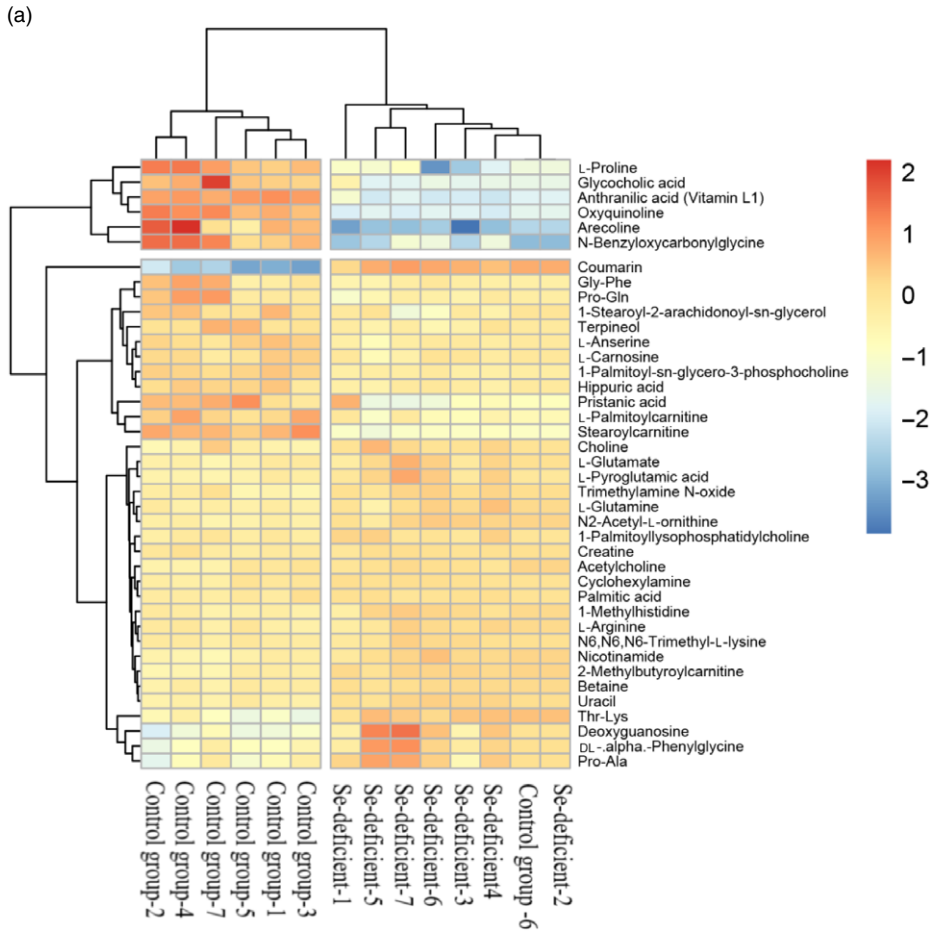

Fig. 4. (Continued)

physiological role of se in immunity have shown that se modulates the presence of compounds in cells, regulates the proliferation and differentiation of immune cells and participates in and affects non-specific immune processes including chemotaxis, phagocytosis and killing of antigens[48]. A lack of se in the body can lead to decreased immunity. L-Glutamine has been speculated to participate in the process through which se affects the body's immunity; consequently, the immune ability of se-deficient *P. przewalskii* might be affected. L-Glutamine participates in the synthesis of glutathione, some of them are hydrolysed by glutathione hydrolase and a portion of them be catalysed by GSH-Px to produce oxidised glutathione GSSG and exert an antioxidant effect. However, the protein expression of glutathione hydrolase 6 in the serum in se-deficient *P. przewalskii* was down-regulated[41] and the activity of GSH-Px decreased (Table 4), which resulted in restriction of the pathway of glutathione metabolism in organism. Based on the feedback regulation mechanism *in vivo*[49,50], the synthesis of glutathione was limited and the consumption of L-glutamine was reduced, which is one of the reasons why the content of L-glutamine in se-deficient *P. przewalskii* was higher than that in the control group. This suggests that se deficiency affects the antioxidant system of *P. przewalskii*.

Pseudouridine is a t-RNA catabolite that has been found to be associated with tissue destruction. Pseudouridine excretion in the urine is also more generally related to growth rate or cell turnover and has been thought to be a tumour marker[28]. In chronic diseases, in which cell turnover is assumed to be higher than normal, pseudouridine excretion is elevated[51,52]. Hocher & Adamski have used metabolomics to identify chronic kidney disease and have found that pseudouridine performs better as a biomarker for identifying chronic kidney disease than creatinine[53]. A study by Gerrits *et al.* has shown that the plasma concentration of pseudouridine increases to varying degrees, reaching a maximal value of more than ten times the upper normal limit[41]. This finding has also been described in a report of kidney damage biomarkers and chronic kidney disease incidence by Zhang *et al.*[54]. These results suggest that pseudouridine might be used as a biomarker to evaluate the kidney health of *P. przewalskii*. In this study, the FC of pseudouridine content in serum in se-deficient *P. przewalskii* was 1·174 172. This

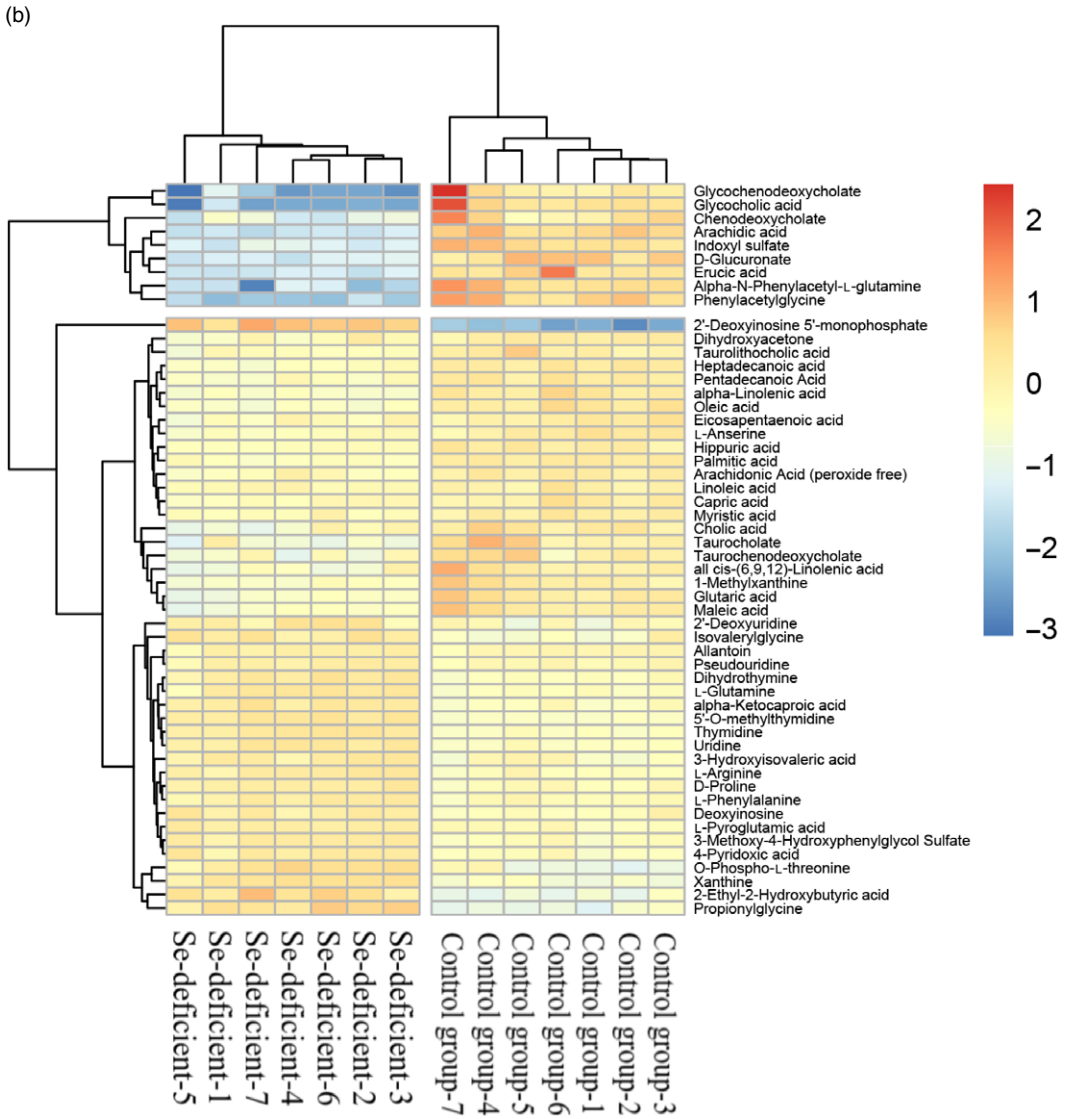

Fig. 4. Hierarchical clustering results of metabolites with significant difference.

showed that se deficiency had an effect on kidney function of *P. przewalskii*, but does not cause kidney disease.

In addition, in the analysis of blood biochemical indexes, it is found that the activity of GSH-Px and total superoxide dismutase decreased and increased, respectively ($P < 0{\cdot}05$), but the total antioxidant capacity value in blood was not significantly different from that in the control group ($P > 0{\cdot}05$) (Table 4). This finding may be related to the increased activity of Cu/Zn-SOD caused by the increased Cu and Zn concentrations in the blood in se-deficient *P. przewalskii*, because when Cu and Zn meet the demand of animals, se-deficient promotes the absorption of Cu and Zn[55] (Table 2).

The basis of a comprehensive analysis based on the results of mineral element content, physiological and biochemical indexes and metabonomics shows that *P. przewalskii* does not show se deficiency symptoms when the dietary se content is lower than 0·05 mg/kg. The reasons for this are some supplement functions between the changed metabolites and metabolic pathways, thus alleviating the harm of se deficiency on the body.

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W. T., H. J. and S. X. Y. conceived and designed the research; W. T. performed experiments, analysed data, interpreted the experimental results; prepared figures; and drafted, edited and revised the manuscript. W. T., H. J. and S. X. Y. approved the final version of the manuscript.

There are no conflicts of interest.

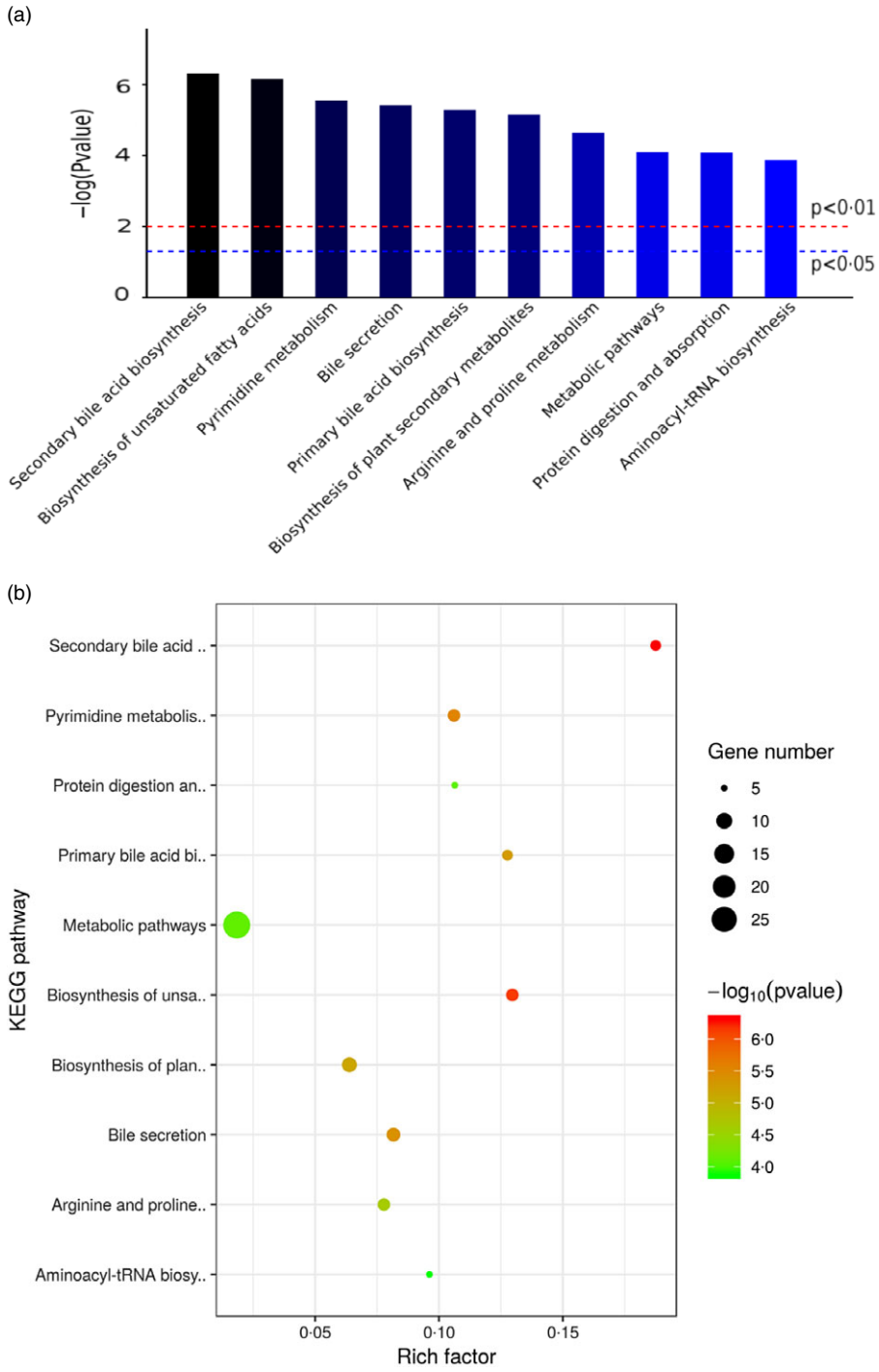

Fig. 5. Enrichment analysis of Kyoto Encyclopedia of Genes and Genomes pathway.

Supplementary material

For supplementary materials referred to in this article, please visit https://doi.org/10.1017/S000711452100355X

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