Effects of Guava (*Psidium Guajava L.*) Leaf Extract on Growth Performance and Metabolomics in Weaned Piglets Challenged by *Escherichia Coli*

Dingfa Wang
Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences
Luli Zhou
Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences
Hanlin Zhou (zhouhanlin8@163.com)
Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences
https://orcid.org/0000-0002-6990-8792
Guanyu Hou
Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agriculyural Sciences

Research

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Abstract

Background: The effects of dietary supplementation with guava leaf extracts (GE) on growth performance, diarrhea and intestinal barrier function, as well as associated with its modulation of serum and fecal metabolic changes in weaned piglets challenged by enterotoxigenic *Escherichia coli* (ETEC) were investigated.

Method: Fifty weaned piglets (Duroc × Yorkshire × Landrace) from 5 pens (2 piglets per pen) were randomly divided into five groups: blank control group (BC), negative control group (NC), or those supplemented with 50 mg kg^{-1} (S50), 100 mg kg^{-1} (S100), or 200 (S200) mg kg^{-1} diet GE, respectively. On day 4, all piglets (except for BC) were orally challenged with about 1.0×10^9 colony-forming units (CFU) enterotoxigenic ETEC. After 28-day trial, growth performance, diarrhea incidence, intestinal barrier function and metabolomics of serum and fecal were investigated.

Results: We demonstrated that dietary supplementation with GE (50-200 mg kg^{-1}) reduced diarrhea incidence of piglets and increased expression of intestinal tight junction proteins (ZO-1, Occludin, Claudin-1) ($P < 0.05$) and sodium hydrogen exchanger 3 (NHE3) ($P < 0.05$). Moreover, dietary supplementation with GE (50-200 mg kg^{-1}) upregulated level of tetrahydrofolic acid (THF) and reversed higher level of nicotinamide-adenine dinucleotide phosphate (NADP) caused by ETEC in serum compared with NC group ($P < 0.05$), and enhanced antioxidant ability of piglets. In addition, dietary addition with GE (100 mg kg^{-1}) reversed the lower level of *L*-pipecolic acid caused by ETEC in feces compared with NC group ($P < 0.05$), and decreased oxidative stress response of piglets. Further, there were no differences ($P > 0.05$) in the final weight, average daily feed intake (ADFI) and F/G among dietary groups during the overall period, and piglets in S50 group has the higher average daily gain (ADG).

Conclusion: Dietary supplementation with 50-200 mg kg^{-1} GE reduced diarrhea incidence of weaned piglets challenged by ETEC and exhibited positive effect on improving intestinal barrier function. Meanwhile, dietary addition with GE organized and redistributed energy resources through similar or dissimilar metabolic pathways, and finally enhanced antioxidant ability of piglets challenged by ETEC.

Background

Weaned piglets challenged by enterotoxigenic *Escherichia coli* (ETEC) could have caused diarrhea, thus in turn leading to stunted growth performance and disrupted innate and adaptive immunity systems of piglets. These negative factors increased morbidity and mortality of piglets, and caused large economic losses in the swine industry worldwide [1, 2]. Regarding the mechanisms by which ETEC induces diarrhea, it has been demonstrated that ETEC can produce colonization factors (CFs) and enterotoxins that adhere to the intestinal mucosa of piglets, and this action inhibited intestinal immune function and perturbed hydro-electrolytic secretions in the intestine and finally resulting in the occurrence of diarrhea [3].

As a consequence of this, veterinary antibiotics have been commonly used to treat intestinal infections for improving animal growth performances and health in several decades, but concerns about antimicrobial

resistance, accumulations of residues in animal production and pollutions in the environment, led to the limited application for antibiotics as growth promoters [4, 5]. Due to these factors, the search for alternatives to antibiotic growth promoters, such as pro- and prebiotics, organic acids, enzymes, plant extracts, have been attracted more and more interest [5–7]. Among the candidates for replacement, plant extracts appear to have one of the most widespread acceptance at this time [8, 9].

Guava (*Psidium guajava L.*) is an economically fruit crop and medicinal plant that has been mainly distributed in tropical and subtropical areas. Guava leaf extracts (GE) has been widely used as a kind of herbal medicine for respiratory and gastrointestinal disorder diseases [10], and is reported to contain phenolics, triterpenoids and other compounds that have antibacterial, antioxidant and anti-inflammatory activities [11–13]. Pruning usually can be used to stimulate growth and influence fruiting in guava [14], thus, residual guava leaves from pruned processing are promising sources of natural feed additives which may be utilized as potential alternative for in-feed antibiotics.

In recent years, a series of studies have demonstrated that GE possess antidiarrheal effect in various diarrhea models [15, 16]. However, the link between antidiarrheal effect of GE and related metabolic regulation has been rarely researched. Today, metabolomics is considered as an emerging profiling method, which focuses on the dynamic changes of small molecules in response to the disturbance of the organism [17]. As a matter of fact, advances in metabolomic approaches will result in a better appreciation of how the complex mechanisms of between the intestinal diarrhea disease and metabolism events is occurred and can be manipulated for therapeutic benefit [18]. Here we analyzed the intervention of GE on metabolic profiling and related endogenous differential metabolites by metabolomic approaches in weaned piglets. Meanwhile, we also evaluated the effects of GE on diarrhea incidence, growth performance and expression of tight junction-related proteins in weaned piglets, aiming to provide a potential window through which to explore the crosstalk between GE-mediate metabolic changes and its antidiarrheal processes during the progression of weaned piglets challenged by ETEC.

Material And Methods

Preparation of guava leaf extract (GE)

Fresh guava leaf was collected from the guava plantations in Qionghai city of Hainan Province, China, during the pruning period in July 2018, and fresh leaves were dried (60 °C, 24 h) and powdered. The powder (50 kg) was exhaustively extracted with 95% ethanol three times at room temperature and filtered. The solvent was evaporated under reduced pressure using a rotary vacuum evaporator to afford GE (3.47 kg), which was stored at 4 °C for animal experiment.

Feeding trial and experimental design

All procedures involving animal handling were conducted in accordance with the China official guidelines for domestic animal care [19], and the study was approved by the Institutional Animal Care and Use Committee of the Chinese Academy of Tropical Agricultural Sciences (Haikou, China).

Fifty 21 ± 3 d-old crossbred weaned piglets (Duroc ×Yorkshire ×Landrace, 7.35 ± 0.18 kg) were selected and transported from the piggery to the barn, where they were randomly allotted to five groups of five replicate pens per group (2 piglets per pen). The five groups were as follows: (1) blank control group (BC), piglets were fed diet without supplements and ETEC challenge; (2) negative control group (NC), piglets were fed diet without supplements and challenged by ETEC; (3) S50 group (S50), piglets were fed diet supplemented with 50 mg kg^{-1} GE and challenged by ETEC; (4) S100 group (S100), piglets were fed diet supplemented with 100 mg kg^{-1} GE and challenged by ETEC; (5) S200 group (S200), piglets were fed diet supplemented with 200 mg kg^{-1} GE and challenged by ETEC. The diet was formulated to meet the nutrient recommendations of National Research Council (2012). The ingredient and nutrient composition of basal diet were presented in Table 1S.

Feed and water were available ad libitum during the 28-d experimental period. All piglets were housed in a weaner facility temperature-maintained at 25 ± 0.5 ℃, with 12 - h periods of light and dark. Individual pig body weight (BW) was recorded on day 1 and day 29 to calculate average daily gain (ADG) for each replicate. Average daily feed intake (ADFI) was determined per replicate from the difference between the sums of feed additions and feed remaining at the end of trial. Feed efficiency (F/G) was calculated by dividing ADFI by ADG. On day 4, all piglets (except for BC) were orally challenged with about 1.0×10^9 colony-forming units (CFU) enterotoxigenic ETEC according to the method developed by Wu et al [20]. ETEC was obtained from China Veterinary Culture Collection Center (Beijing, China). The occurrence of diarrhea for each group was assessed as described by Wang et al [21], and calculated based on each group.

Sample collection

On day 29, one pig was randomly selected from each pen and the blood samples were collected from the jugular vein, and serum was separated from whole blood by centrifugation at 700 × *g* for 15 min at 4 °C and stored at -80 °C until serum metabolomics analysis. After blood sampling, all piglets were anesthetized by an intraperitoneal injection of 50 mg kg^{-1} pentobarbital sodium and were killed by exsanguination. Fecal samples were collected directly in 10 mL sterile plastic tubes from the rectum of piglets and stored at -80 °C until feces metabolomics analysis. The small intestine was removed and a piece (4 cm-length) of the middle jejunum was collected, gently rinsed with 0.1 M phosphate buffered saline (PBS) at pH 7.2, and then fixed in 10% formaldehyde-phosphate buffer for subsequent immunohistochemical analysis.

Immunohistochemistry

Immunohistochemical assay was used to detect the Claudin-1, Occludin, zonula occludens 1 (ZO-1) and sodium hydrogen exchanger 3 (NHE3) proteins expression in the jejunal mucosa with densitometric analysis as described previously [22]. Polyclonal primary antibodies against Claudin-1, Occludin, ZO-1 and NHE3 (1: 200 dilution, Abcam) were employed. The average integrated optical density of the positive products was detected by using the Image-pro plus software (version 6.0 for Windows) at 200 × magnification.

Serum and feces sample preparation and analysis by UPLC-QTOF-MS

Each 100 μL of serum sample and 100 mg of feces sample were extracted to be used in ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS) for positive ionization mode analysis, respectively. The serum samples were diluted with 400 μL of methanol (TEDIA, Fairfield, USA). The extraction procedure was performed at − 80 ℃ for 6 h after 2 min vortex. After that, the samples were centrifuged at 20,000 × *g* for 10 min at 4 ℃. Then 400 μL of supernatant was transferred into an eppendorf tube, and the supernatant was evaporated to dryness using SPD121P SpeedVac Concentrator (Thermo Fisher, Germany) and 100 μL of acetonitrile (ACN) (MERCK, Darmstadt, Germany) were added to the dried residue for UPLC-QTOF-MS analysis as described previously [23]. Additionally, for extraction of the feces samples, 500 μL methanol was added. Then the samples were vigorously vortexed for 5 min, and centrifuged at 20,000 × *g* at 4 ℃ for 10 min. After the supernatant was collected, the residue again was extracted according to the above extraction procedure and combined with the previous supernatant. At last, 200 μL of supernatant was transferred into the sampling vial for UPLC-QTOF-MS analysis as described previously [24].

Briefly, the prepared sample (5 μL) was injected into a XBridge HILIC (2.1 × 100 mm, 3.5 μm particle size) column (Waters, USA) at 30 °C in a LC-30AD series ultra-performance liquid chromatography system (Nexera™, Shimadzu, Japan), and the analytes in the eluent were introduced into and detected in a TOF plus mass spectrometer (Sciex TripleTOF5600, USA, Concord, ON.) equipped with Turbo V sources and a Turbolon Spray interface. Mass spectrum was scanned and collected (70 to 1,000 *m/z*) with electrospray ionization (ESI) positive mode at a flow rate of 0.25 mL min^{-1}. The chromatographic gradient condition for samples analysis was 80% − 20% B over 0–24 min, 20% − 80% B over 24–24.5 min and the composition was held at 80 % B for 8.5 min, where A = a mixture of 50 % ACN and 50 % water contains 0.1 % formic acid (SIGMA, Deisenhofen, Germany) and B = a mixture of 95 % ACN and 5 % water contains 0.1 % formic acid.

Drying gas temperature and the ion spray voltage were set at 550 °C and 5,000 V, respectively. Atomization gas pressure, auxiliary heating gas pressure, and curtain gas pressure were set at 45, 45, and 35 psi, respectively. The instrument was mass calibrated by automatic calibration infusing the Sciex Positive Calibration Solution (part no. 4460131, Sciex, Foster City, CA, USA) after every six-sample injections. One quality control sample and one blank vial were run after each cycle of 10 samples.

Automatic peak extraction, peak matching, peak alignment, and normalization preprocessing on the acquired data were performed using MarkerView software (Sciex, USA). The retention time and *m/z* tolerances were 0.1 min and 10 ppm, respectively; the response threshold was 100 counts and the isotope peak were removed. After Pareto-scaling, principal components analysis (PCA), and partial least-squares discrimination analysis (PLS-DA) models were carried out to visualize the metabolic difference among BC, NC, S50, S100 and S200 groups. The quality of the models was described using the R^2X (PCA) or R^2Y (PLS-DA) values. To avoid model over-fitting, 999 cross-validations in SIMCA-P 13.0 were performed

throughout to determine the optimal number of principal components. R^2X, R^2Y and Q^2Y values of models were nearly 1.0, indicating that these models retain the ability to explain and predict variations in the X and Y matrix.

Furthermore, the value of fold change (FC) was calculated as the average normalized peak intensity ratio between two groups. Differences between data sets with FC > 1.10 or < 0.90, and $p < 0.05$ (student *t*-test) were considered statistically significant. The structural identification of differential metabolites was performed by matching the mass spectra with an in-house standard library, including accuracy mass, retention time, MS/MS spectra, and online databases Metlin (http://www.metlin.scripps.edu) and HMDB (http://www.hmdb.ca).

The impact of ETEC and GE on metabolic pathways was evaluated based on metaboanalyst, a tool for metabolomics data analysis platform, which is available online (https://www.metaboanalyst.ca). The pathway analysis module combines results from powerful pathway enrichment analysis with pathway topology analysis to help researchers identify the most relevant pathways involved in the conditions being investigated. A results report was then presented graphically as well as in a detailed table. Potential biomarkers for GE efficacy were identified based on the identified metabolic pathways and statistics analysis.

Statistical analysis

Statistical analysis of growth performance and integral optical density among the groups were evaluated by using the one-way analysis of variance (ANOVA), performed using SPSS 23.0 (IBM-SPSS Inc., Chicago, USA). The results were presented as mean ± standard error of mean (SEM). Orthogonal polynomial contrasts were used to test for linear and quadratic effects of GE by comparing with the negative control group. Significant differences were evaluated by Tukey multiple comparisons test at $P < 0.05$.

Results

Growth performance and diarrhea incidence of piglets

Results for the effects of diet type and ETEC challenge on the growth performance in piglets from day 0 to 28 are summarized in Table 1. There were no differences ($P > 0.05$) in the final weight, ADFI and F/G among dietary groups during the overall period. However, S50 group had higher ADG compared with S200 group ($P < 0.05$). Additionally, the supplementation of 50–200 mg kg^{-1} of GE in piglet diets reduced diarrhea incidence compared with the NC group (Fig. 1). In the present study, we observed that diarrhea mainly occurred in the first 5 days after ETEC challenge, and there was no obvious diarrhea on the 10th day after ETEC challenge among NC, S50, S100 and S200 groups, which indicating piglets challenged by ETEC were recovered gradually during 14–28 days.

Table 1
Effect of GE on the growth performance of piglets

Items	Groups					SEM	*P*	Contrast [a]	
	BC	NC	S50	S100	S200			*L*	*Q*
Initial weight, kg	7.31	7.34	7.17	7.31	7.49	0.20	0.889	0.946	0.852
Final weight, kg	14.12	13.88	15.20	13.87	13.95	0.29	0.595	0.711	0.606
ADFI, g	456.94	456.85	456.48	456.73	445.49	2.23	0.513	0.161	0.458
ADG, g	244.38^{ab}	236.03^{ab}	300.86^{a}	238.26^{ab}	230.60^{b}	8.63	0.030	0.242	0.175
F/G	1.87	1.94	1.55	1.92	1.93	0.05	0.059	0.404	0.242

In the same row, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$), while with different letter superscripts mean significant difference ($P < 0.05$). n = 5. ADFI: average daily feed intake; ADG: average daily gain; F/G: ADFI/ADG. BC: blank control group, piglets were fed diet without supplements and ETEC challenge; NC: negative control group, piglets were fed diet without supplements and challenged by ETEC; S50, piglets were fed diet supplemented with 50 mg kg^{-1} GE and challenged by ETEC; S100, piglets were fed diet supplemented with 100 mg kg^{-1} GE and challenged by ETEC; S200, piglets were fed diet supplemented with 200 mg kg^{-1} GE and challenged by ETEC.

[a] L = Linear, Q = Quadratic; Linear and quadratic effect of adding GE compared to the NC.

Immunohistochemistry

As seen to Fig. 2 and Table 2, the results of immunohistochemistry indicated that the color signals and the integral optical density of Occludin and Claudin-1 in S100 and S200 groups were significantly higher than the NC group ($P < 0.05$), and NHE3 in the GE groups (GEs) were significantly higher than the NC group ($P < 0.05$). Supplementation of GE in the diet linearly increased the integral optical density of Claudin-1 ($P < 0.001$), Occludin ($P < 0.001$) and NHE3 ($P = 0.002$) compared with the NC group.

Table 2
Effect of GE on the integral optical density of ZO-1, Claudin-1, Occludin and NHE3 in jejunal mucosa of piglets

Items	Groups					SEM	*P*	Contrast [a]	
	BC	NC	S50	S100	S200			*L*	*Q*
Claudin-1	22.59^{a}	5.98^{d}	10.09 ^{cd}	15.05^{b}	14.03^{bc}	1.53	< 0.001	< 0.001	0.003
Occludin	52.08^{b}	29.41^{c}	55.93^{b}	53.62^{b}	74.29^{a}	3.83	< 0.001	< 0.001	< 0.001
ZO-1	34.49^{a}	24.95^{b}	29.47^{ab}	26.47^{b}	25.51^{b}	1.03	0.001	0.574	0.059
NHE3	12.93^{a}	3.24^{c}	6.60^{b}	6.86^{b}	6.21^{b}	0.86	< 0.001	0.002	< 0.001

In the same row, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$), while with different letter superscripts mean significant difference ($P < 0.05$), n = 5. BC: blank control group, piglets were fed diet without supplements and ETEC challenge; NC: negative control group, piglets were fed diet without supplements and challenged by ETEC; S50, piglets were fed diet supplemented with 50 mg kg^{-1} GE and challenged by ETEC; S100, piglets were fed diet supplemented with 100 mg kg^{-1} GE and challenged by ETEC; S200, piglets were fed diet supplemented with 200 mg kg^{-1} GE and challenged by ETEC.

[a] L = Linear, Q = Quadratic; Linear and quadratic effect of adding GE compared to the NC.

Analysis of feces metabolomics

Using the optimal UPLC- QTOF- MS condition described above, the representative total ion chromatograms (TICs) for feces samples of five groups were presented in Fig. 1S a. In order to better visualize the subtle similarities and differences among the data sets, PCA and PLS-DA were used to investigate the metabolic alterations and identify the differential metabolites (Fig. 3). The score plots of PCA overlapped partly both in the direction of PC1 and PC2 based on the feces samples from NC *vs* BC group (Fig. 3a1), S50 *vs* NC group (Fig. 3b1), S100 *vs* NC group (Fig. 3c1) and S200 *vs* NC group (Fig. 3d1). Further supervised PLS-DA suggested that there were significant differences between two groups, indicating that NC *vs* BC group (Fig. 3a2), S50 *vs* NC group (Fig. 3b2), S100 *vs* NC group (Fig. 3c2) and S200 *vs* NC group (Fig. 3d2), all have distinctly different metabolites. Many metabolites in the feces were significantly changed based on the results of NC *vs* BC group, S50 *vs* NC group, S100 *vs* NC group and S200 *vs* NC group, respectively. (Table 2S). *L*-pipecolic acid is a single differential metabolite detected between NC group and BC group. Different metabolic pathways were enriched from the groups of S50 *vs* NC, S100 *vs* NC and S200 *vs* NC, respectively (Fig. 2S). The details of top 4 ranked metabolic pathways and relevant differential metabolites affected by the BC, NC and GEs groups were presented in Table 3. It suggested that the S50 group significantly increased the production of 3-methoxytyramine ($P < 0.05$) and decreased the production of epinephrine, normetanephrine, *N*-acetylserotonin, melatonin and caffeine ($P < 0.05$) compared with the NC group. Further, the S100 group significantly upregulated the levels of biliverdin and *L*-pipecolic acid ($P < 0.05$) and downregulated the levels of 5-aminolevulinic acid and

phosphorylcholine ($P < 0.05$) compared with the NC group. Moreover, the S200 group significantly downregulated the levels of uridine 5'-monophosphate (UMP), deoxycytidine monophosphate (dCMP), deoxyguanosine and *L*-phenylalanine ($P < 0.05$) compared with the NC group.

Table 3
Top 4 ranked metabolic pathways and relevant differential metabolites in feces affected by S50 *vs* NC group, S100 *vs* NC group and S200 *vs* NC group, respectively, as well as a single differential metabolite affected by NC *vs* BC group

Metabolic pathways	**Differential metabolites**	
	Upregulation (P<0.05, FC>1.10)	**Downregulation (P<0.05, FC<0.90)**
NC vs **BC group**		
/	/	*L*-pipecolic acid
S50 vs **NC group**		
tyrosine Metabolism	3-methoxytyramine	epinephrine; normetanephrine
tryptophan Metabolism	/	*N*-acetylserotonin; melatonin
catecholamine Biosynthesis	/	epinephrine
caffeine Metabolism	/	caffeine
S100 vs **NC group**		
porphyrin metabolism	biliverdin	5-aminolevulinic acid
phosphatidylcholine biosynthesis	/	phosphorylcholine
phospholipid biosynthesis	/	phosphorylcholine
lysine degradation	*L*-pipecolic acid	/
S200 vs **NC group**		
pyrimidine metabolism	/	UMP; dCMP
lactose synthesis	/	UMP
phenylalanine and tyrosine metabolism	/	*L*-phenylalanine
purine metabolism	/	deoxyguanosine

Analysis of serum metabolomics

The UPLC-QTOF-MS system can obtain metabolic profiling of five groups with total ion chromatograms (TICs) (Fig. 1S b). To investigate the global metabolic alterations in the serum among NC, BC, S50, S100 and S200 groups, all observations were integrated and analyzed using PCA (Fig. 4). The score plots of PCA overlapped partly both in the direction of PC1 and PC2 based on the serum samples from NC *vs* BC

group (Fig. 4a1) and S50 *vs* NC group (Fig. 4b1). The score plots of PCA from S100 *vs* NC group (Fig. 4c1) and S200 *vs* NC group (Fig. 4d1) were separated from each other. To further explore the differences between two groups, supervised PLS-DA was applied for chemometrics analysis (Fig. 4). The score plots of PLS-DA suggested that NC *vs* BC group (Fig. 4a2), S50 *vs* NC group (Fig. 4b2), S100 *vs* NC group (Fig. 4c2) and S200 *vs* NC group (Fig. 4d2) could be clearly separated, which reflected the remarkably distinct metabolic status of the serum samples among BC, NC, S50, S100 and S200 groups. Many metabolites in the serum were significantly changed based on the results of NC *vs* BC group, S50 *vs* NC group, S100 *vs* NC group and S200 *vs* NC group, respectively (Table 3S). Finally, different metabolic pathways were identified and enriched from the groups of NC *vs* BC, S50 *vs* NC, S100 *vs* NC and S200 *vs* NC, respectively (Fig. 3S).

As shown in Table 4, top 4 ranked metabolic pathways affected by the BC, NC and GEs groups displayed characteristic differences in serum of piglets, respectively. It is interesting that, nicotinamide-adenine dinucleotide phosphate (NADP), as a node molecule, was observed, and significant features ($P < 0.05$) in the NC group being upregulated compared to the BC group but that in the GE groups being downregulated compared to the NC group (Fig. 5a). Notably, tetrahydrofolic acid (THF) is a key node molecule affected by GE and supplementation of 50–200 mg kg^{-1} GE significantly upregulated level of THF ($P < 0.05$) compared with the NC group (Fig. 5b). Additionally, considering the addition of different dosages of GE in the GE groups, important top 4 ranked metabolic pathways and relevant metabolites affected also yielded dissimilar results. Especially, it suggested that GE in the S100 group significantly increased the production of adenosine triphosphate (ATP), *L*-glutamic acid (*L*-glu) and *L*-glutamine (Gln) ($P < 0.05$) compared with the NC group. On the other hand, the NC group significantly reduced the production of thiamine pyrophosphate (TPP) ($P < 0.05$) compared with the BC group. However, GE in the S200 group significantly increased the production of TPP and *L*-aspartic acid ($P < 0.05$) compared with the NC group.

Table 4
Top 4 ranked metabolic pathways and relevant differential metabolites in serum affected by NC *vs* BC group, S50 *vs* NC group, S100 *vs* NC group and S200 *vs* NC group, respectively.

Metabolic pathways	**Differential metabolites**	
	Upregulation (P<0.05, FC>1.10)	**Downregulation (P<0.05, FC<0.90)**
NC vs **BC group**		
glutamate metabolism	NADP	glucosamine 6-phosphate; oxidized glutathione
glutathione metabolism	NADP	oxidized glutathione
transfer of acetyl groups into mitochondria	NADP; thiamine pyrophosphate	/
pyrimidine metabolism	NADP	CMP; dUTP
S50 vs **NC group**		
glycerolipid metabolism	glycerol	NADP
pterine biosynthesis	tetrahydrofolic acid	NADP
folate metabolism	tetrahydrofolic acid	NADP
histidine metabolism	tetrahydrofolic acid	NADP
S100 vs **NC group**		
amino sugar metabolism	*L*-glutamic acid; ATP; *L*-glutamine; glucosamine 6-phosphate	uridine diphosphate-*N*-acetylglucosamine
betaine metabolism	*L*-methionine; tetrahydrofolic acid; ATP; *S*-adenosylhomocysteine	/
nicotinate and nicotinamide metabolism	*L*-glutamic acid; ATP; *L*-glutamine; *S*-adenosylhomocysteine	NADP
glycine and serine metabolism	*S*-adenosylhomocysteine; *L*-glutamic acid; 5-aminolevulinic acid; ATP; *L*-methionine; tetrahydrofolic acid	/
S200 vs **NC group**		
betaine metabolism	*L*-methionine; tetrahydrofolic acid	/

Metabolic pathways	Differential metabolites	
	Upregulation (P<0.05, FC>1.10)	Downregulation (P<0.05, FC<0.90)
transfer of acetyl groups into mitochondria	/	NADP; thiamine pyrophosphate
phytanic acid peroxisomal oxidation	/	NADP; thiamine pyrophosphate
pterine biosynthesis	tetrahydrofolic acid	NADP

Discussion

Growth performance and diarrhea incidence

Due to the post-weaning piglets are usually exposed to environmental, nutritional, and psychological stress responses, the combined and additive effects of these factors directly or indirectly contributed to the results of increased diarrhea which can deteriorate gut health of weaned piglets. In the current study, compared with the NC group, piglets fed the diet with supplementation of 50–200 mg kg^{-1} GE had lower diarrhea incidences. In a previous study, Ezekwesili *et al*. investigated the underlying mechanism of antidiarrheal effect of GE, and they suggested that phenolics and alkaloids in GE present antimicrobial activity as well as reduce gastrointestinal motility of piglets [25]. Similarly, Morales *et al*. and Jaisinghani also demonstrated that quercetin, as a main active phenolics of GE, not only have a good antibacterial activity, but also could reduce intracellular calcium release of the sarcoplasmic reticulum to relaxes smooth muscles and inhibit bowel contraction [26, 27]. Collectively, due to GE was rich in bioactive composition, such as phenolics, alkaloids, etc., which display a remarkable array of antimicrobial and antioxidant activities [28], dietary addition of GE might be advantageous in preventing from ETEC infection, and improving gut health in weaned piglets.

Intestinal mucosal barrier

In general, diarrhea disease cause by enterotoxigenic ETEC infections is a major risk factor for impaired intestinal structure and barrier function of piglets. It has been reported that claudins and occludins are considered to the tight junction protein components of that primarily regulated permeability of uncharged and charged molecules, respectively. Further, ZO-1 is the adaptor protein of that modulated the actin cytoskeleton [29, 30], and NHE3 is a primary mediator of the absorptive route for Na^+ entering the intestinal epithelium from the lumen [31]. Thus, all of them play important roles in mediating functional integrity of the junction in epithelial and endothelial cells of intestine [29–31]. Our results showed that ETEC decreased the expression of epithelial tight junctions such as claudin-1, occludin, ZO-1 and NHE3, thereby in turn increased cellular permeability and disturbed intestinal mucosal barrier. Subsequently,

luminal antigens rather than bacteria may enter the lamina propria, resulting in inflammation [32]. However, weaned piglets fed a diet supplementation with GE (50–200 mg kg^{-1} in the diet) increased the proteins expression of claudin-1, occludin, ZO-1 and NHE3, which are crucial for the formation of a semi-permeable mucosal barrier and the recovery of the barrier function of intestinal tight junctions compared with the NC group. Furthermore, previous studies also suggested that GE was rich in phenolics [33], and phenolics has a positive effect on gut health [34]. Specifically, with respect to quercetin and myricetin, as the main phenolic constituents in GE, which have been demonstrated to enhance the intestinal barrier function [35]. Consequently, it is assumed the abundant phenolics in GE exerted anti-inflammatory effect [36] to improve the intestinal barrier function and increase gut mucosal integrity of piglets.

Feces metabolomics

In mammals, *L*-pipecolic acid has long been recognized as a metabolite of lysine degradation pathway [37]. In this pathway, peroxisomal sarcosine oxidase (PSO) can catalyze *L*-pipecolic acid and oxygen to give (*S*)-2,3,4,5-tetrahydropiperidine-2-carboxylate and hydrogen peroxide (H_2O_2). As seen in Table 3 and Fig. 6, the findings of our study indicated that *L*-pipecolic acid was significantly lower in NC compared with BC group and suggested that ETEC might activated the reactions mentioned above, which resulting in the consumption of *L*-pipecolic acid and the production of H_2O_2. Whereas the H_2O_2 accumulated can induced disruption of intestinal epithelial barrier function by a mechanism involving phosphatidylinositol 3-kinase and c-Src kinase [38, 39]. Here, consisted with the results of immunohistochemistry in the present study, and it suggested that H_2O_2-induced oxidative stress in gut might has been considered to be one of the important pathogenic mechanisms in NC compared with BC group, which disrupted intestinal epithelial tight junctions and barrier functions.

As was well known that the catecholamines are generally associated with stressful events that result in high levels of gram-negative pathogens, such as *Escherichia coli* [40]. Based on S50 *vs* NC group, the results suggested that dietary addition with 50 mg kg^{-1} GE can decrease the production of stress hormones, such as catecholamines (epinephrine and normetanephrine), and increase the production of 3-methoxytyramine (an in-active metabolite of dopamine) through tyrosine metabolism and catecholamine biosynthesis pathways and finally to inhibit the growth of *Escherichia coli* and resist oxidative stress in gut. In addition, it has been reported that caffeine can increase the intracellular calcium levels through directly effects on metabolic phosphorylase-like enzymes (PHOS) regulation and calcium mobilization from the sarcoplasmic reticulum [41]. Our results revealed the caffeine in feces was downregulated in S50 compared with NC group and suggested it may be beneficial to a decrease in cytosolic calcium to inhibit pathophysiologic processes which resulting in diarrhea [42]. Moreover, previous studies have suggested that nuclear translocation of nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) can induce melatonin synthesis in macrophages [43]. Whereas GE in S50 group can attenuate inflammatory response, achieve its antioxidant and antimicrobial actions and protect the intestinal epithelial barrier, resulting in decrease of intestinal melatonin synthesis in macrophages compared with the NC group. Meanwhile, small doses of melatonin in S50 group may be propitious to increase intestinal motility [44] to

improve digestion and absorption of nutrients in gut. Thus, it suggested that the downregulation of melatonin of piglets in S50 group probably play an important role in increase of ADG.

Based on the fecal metabolomic data of S100 *vs* NC group, it showed that 100 mg kg^{-1} GE upregulated the level of biliverdin and downregulated the level of 5-aminolevulinic acid in gut via porphyrin metabolism pathway. This process started as the condensation of glycine and succinyl-CoA by 5-aminolevulinate synthase (ALAS), and generated 5-aminolevulinic acid. Presumably, the resulting 5-aminolevulinic acid has two fates. On one hand, it may be finally converted into biliverdin via a series of steps, leading to the accumulation of biliverdin in gut (Fig. 6). The biliverdin generated in this process can protect intestine from oxidant and inflammatory injury [45, 46]. On the other hand, 5-aminolevulinic acid was also potentially absorbed into the blood, resulting in the high level of 5-aminolevulinic acid in serum via glycine and serine metabolism pathway (Fig. 7). Here, 5-aminolevulinic acid reduced intracellular carbon monoxide concentration and inhibited oxidative stress and inflammation response [47]. Moreover, phosphorylcholine was downregulated in S100 compared with NC group, which associated with the phosphatidylcholine and phospholipid biosynthesis pathways. It means that most of choline might not be break down in gut but was absorbed into the blood in S100 group, and then choline went to the betaine metabolism pathway and was probably used in betaine biosynthesis (Fig. 7). Notably, it was showed that *L*-pipecolic acid in feces was significantly higher in S100 group than NC group. Conversely, *L*-pipecolic acid in feces was significantly lower in NC group than BC group. Our findings suggested that 100 mg kg^{-1} GE might inhibit the activity of PSO and reversed the lower levels of *L*-pipecolic acid caused by ETEC, which in turn prevented the production of H_2O_2 and decreased oxidative stress level.

Based on S200 *vs* NC group, *L*-phenylalanine was downregulated, which is a double-edged sword. On one hand, due to *L*-phenylalanine is not only an essential amino acid and the precursor of the amino acid tyrosine as well as also a precursor for catecholamines including tyramine, dopamine, epinephrine, and norepinephrine, the lower level of *L*-phenylalanine might decrease oxidative stress in gut [40]. On the other hand, the lower level of *L*-phenylalanine also might lead to a decrease of gut hormone secretion, including glucose-dependent insulinotropic peptide (GIP) and cholecystokinin (CCK) [48]. While GIP and CCK are important hormonal regulators of the ingestion, digestion, and absorption of intestinal nutrients [49, 50]. Hence, it suggested that the lower ADG in S200 group probably as a result of the downregulation of *L*-phenylalanine in gut. Additionally, our results indicated that UMP and dCMP were downregulated in S200 group compared with NC group, which were involved in the pyrimidine metabolism and lactose synthesis pathways and suggested that UMP and dCMP finally may be degraded to β-alanine through pyrimidine metabolism pathway and then β-alanine synthesized probably went to the alanine metabolism pathway. In this pathway, alanine and glyoxylic acid can be converted into glycine and pyruvic acid via serine-pyruvate aminotransferase. Meanwhile, *D*-glucose probably participated in the biosynthesis of pyruvic acid, leading to the lower level of UMP in lactose synthesis pathway. Then the pyruvic acid generated via two pathways may be absorbed into the blood and was involved in the transfer of acetyl groups into mitochondria pathway (Fig. 7). Furthermore, our present data discovered the lower level of deoxyguanosine in feces was associated with the higher level of inosine-5'-monophosphate (IMP) in serum in S200 group compared with NC group (Table 3S). While the higher level of IMP, as a nucleotide,

may be propitious to the growth and maturation of intestinal epithelial cells and plays an important role in intestinal immunity and health [51].

Serum metabolomics

As seen in Table 4 and Fig. 7, based on NC vs BC group, it indicated that ETEC exposure decreases glucosamine 6-phosphate (G6P) and oxidized glutathione (GSSG) levels and increases NADP levels in serum by affecting glutamate and glutathione metabolism pathways, which resulted in accumulation of H_2O_2 in serum. On one hand, the producing H_2O_2 is not reduced to water (H_2O) which resulted in peroxide interference and cell damage through oxidation of lipids, proteins, and nucleic acids [52]. On the other hand, H_2O_2 is not a radical but is considered a reactive oxygen species and it can induce a cascade of radical reactions and inactivate pyruvate dehydrogenase (PDH) [53, 54], leading to accumulation of TPP in serum and meaning that pyruvic acid cannot be create acetyl-CoA. While the latter was closely associated with fatty acid biosynthesis. In addition, the lower levels of cytidine monophosphate (CMP) and deoxyuridine triphosphate (dUTP) in NC group compared with BC group revealed that ETEC perturbed pyrimidine metabolism, then might inhibit the process of pyrimidine-related nucleotide biosynthesis.

Interestingly, based on S50 *vs* NC group, it was found that caffeine was significantly downregulated in feces (Table 3), while it was significantly upregulated in serum (Table 3S) and suggested most caffeine allowed for absorption into blood through intestinal mucosa. Furthermore, the high level of caffeine in serum could lead to an increase of lipolysis and it is usually accompanied by accumulation of glycerol in serum [55]. Then the high level of glycerol could raise blood osmolality, and it in turn probably played a favorable role in an increase of intestinal water absorption and a decrease of sodium efflux into the intestinal lumen, and finally resulted in attenuation of secretory diarrhea caused by ETEC [56]. It is of note that, based on S50 *vs* NC group, indoleamine 2, 3-dioxygenase 1 (IDO1) or tryptophan 2, 3-dioxygenase 2 (TDO2) drives tryptophan down the kynurenine pathways that produce tryptophan catabolites, such as the high level of kynurenic acid in serum (Table 3S). In this process, it is usually accompanied by the production of folic acid and *L*-glu, meaning that the generated folic acid and *L*-glutamate can be synthesized to THF through folate metabolism pathway. Meanwhile, THF also was biosynthesized in serum via two pathways, including pterine biosynthesis pathway and histidine metabolism.

Based on S100 *vs* NC group, the higher level of *L*-glu, as a note molecule, was known to be relevant in three pathways, including amino sugar metabolism, nicotinate and nicotinamide metabolism, and glycine and serine metabolism. It revealed that after glutamine synthetase or glutaminase liver isoform (GLS2) converting *L*-glu into Gln, and glutamine-fructose-6-phosphate aminotransferase (GFPT1) subsequently converted Gln and fructose 6-phosphate (F6P) into *L*-glu and G6P, which suggested that 100 mg kg^{-1}GE reversed the ETEC-induced downregulation of G6P. The higher level of G6P in S100 group, in turn, can be converted into *N*-acetyl-*D*-glucosamine-6-phosphate (*N*-AG6P) (via glucosamine 6-phosphate *N*-acetyltransferase) compared with NC group. Here, downregulation of uridine diphosphate-*N*-acetylglucosamine and upregulation of ATP in serum indicated that most *N*-AG6P generated likely can be converted into *N*-acetyl-*D*-glucosamine (*N*-AG) (a polysacchatide) and ATP, via *N*-acetyl-*D*-glucosamine kinase (NAGK). The resulting *N*-AG has been confirmed its anti-inflammatory efficacy for inflammatory

bowel disease [57]. It is worth mentioning that betaine, which might be synthesized from choline, can be degraded via two pathways. The first pathway involves the betaine metabolism pathway. Compared with NC group, the higher levels of *S*-adenosyl-*L*-homocysteine (SAH), *L*-methionine, THF and ATP in S100 group indicated that THF cofactors was probably used to carry and activate one-carbon units via the folate-mediated one-carbon transfer pathway, resulting in the remethylation of homocysteine to *L*-methionine and the synthesis of purine nucleotides and thymidylate [58]. In the second pathway, betaine can be synthesized to dimethylglycine in methionine cycle, then the generated dimethylglycine can be converted into sarcosine and went to the glycine and serine metabolism. Here, the sarcosine synthesized via two routes to create 5-aminolevulinic acid and serine, respectively. Of them, the formation pathway of serine was accompanied by the production of THF. Whereas the producing *L*-methionine, purine nucleotides and 5-aminolevulinic acid may participate in the processes of attenuated inflammatory responses and inhibited oxidative stress [59–61].

Furthermore, it showed that 200 mg kg^{-1} of GE reversed the ETEC-induced upregulation of NADP and TPP in serum via the transfer of acetyl groups into mitochondria and phytanic acid peroxisomal oxidation pathways and thereby participated in the production of acetyl-CoA, and the latter was related to the synthesis of fatty acids and sterols and the metabolism of many amino acids [62]. Meanwhile, similar to the S50 or S100 groups, S200 group also upregulated the levels of THF and *L*-methionine via the betaine metabolism and pterine biosynthesis pathway compared with the NC group.

It is worth noting that dietary addition of 50, 100 and 200 mg kg^{-1} GE all can upregulate the level of THF and reversed the high level of NADP caused by ETEC compared with the NC group (Fig. 5). It suggested that THF is probably a main antioxidative force for GE indirectly [63, 64]. Meanwhile, GE downregulated level of NADP also meaning that the NADP pool is probably maintained in a highly reduced state, which improved antioxidant ability in response to oxidative damage [65].

Conclusions

Results of our studies collectively demonstrate that dietary supplementation with 50–200 mg kg^{-1} of GE reduced the occurrence of diarrhea incidence of weaned piglets challenged by ETEC and exhibited positive effect on the recovery of intestinal tight junctions and barrier function. Meanwhile, serum and fecal metabonomic analysis indicated that dietary GE (50, 100 and 200 mg kg^{-1}) addition could organize and redistribute energy resources through similar or dissimilar metabolic pathways and finally enhance antioxidant ability of weaned piglets challenged by ETEC. There were closely link between GE-mediate metabolic changes and its antidiarrheal effect.

Abbreviations

GE: Guava leaf extracts; ETEC: Enterotoxigenic *Escherichia coli*; NHE3: Sodium hydrogen exchanger 3; ZO-1: Zonula occludens protein 1; THF: tetrahydrofolic acid; NADP: nicotinamide-adenine dinucleotide phosphate; ADFI: Average daily feed intake; ADG: Average daily gain; BW: Body weight; F/G: Feed to gain

ratio; PBS: phosphate buffered saline; UPLC-QTOF-MS: Ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry; CAN: Acetonitrile; ESI: Electrospray ionization; PCA: Principal components analysis; PLS-DA: Partial least-squares discrimination analysis; TICs: Total ion chromatograms; UMP: Uridine 5'-monophosphate; dCMP: Deoxycytidine monophosphate; ATP: Adenosine triphosphate; *L*-glu: *L*-glutamic acid; Gln: *L*-glutamine; TPP: Thiamine pyrophosphate; PSO: Peroxisomal sarcosine oxidase; H_2O_2: Hydrogen peroxide; PHOS: Phosphorylase-like enzymes; ALAS: 5-aminolevulinate synthase; GIP: Glucose-dependent insulinotropic peptide; CCK: cholecystokinin; IMP: Inosine-5'-monophosphate; G6P: Glucosamine 6-phosphate; GSSG: Oxidized glutathione; PDH: Ppyruvate dehydrogenase; CMP: Cytidine monophosphate; dUTP: Deoxyuridine triphosphate; IDO1: Indoleamine 2, 3-dioxygenase 1; TDO2: Tryptophan 2, 3-dioxygenase 2; GLS2: Glutaminase liver isoform; GFPT1: Glutamine-fructose-6-phosphate aminotransferase; F6P: Fructose 6-phosphate; *N*-AG6P; *N*-acetyl-*D*-glucosamine-6-phosphate; SAH: *S*-adenosyl-*L*-homocysteine.

Declarations

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Authors' contributions

The authors' contributions are as follows: D. W. and H. Z. contributes to the study design; L. Z analyzed the data and wrote the manuscript; D. W. and H. G. finished the animal experiments and determination. All authors reviewed and approved the final version of the manuscript.

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Availability of data and materials

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

Ethics approval

All animal experiments were conducted in accordance with the Laboratory Animal Requirements of Environment and Housing Facilities and approved by the Institutional Animal Care and Use Committee of the Chinese Academy of Tropical Agricultural Sciences (Haikou, China).

Consent for publication

Not applicable.

Competing interests

The authors declared that there were no competing interests.

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Figures

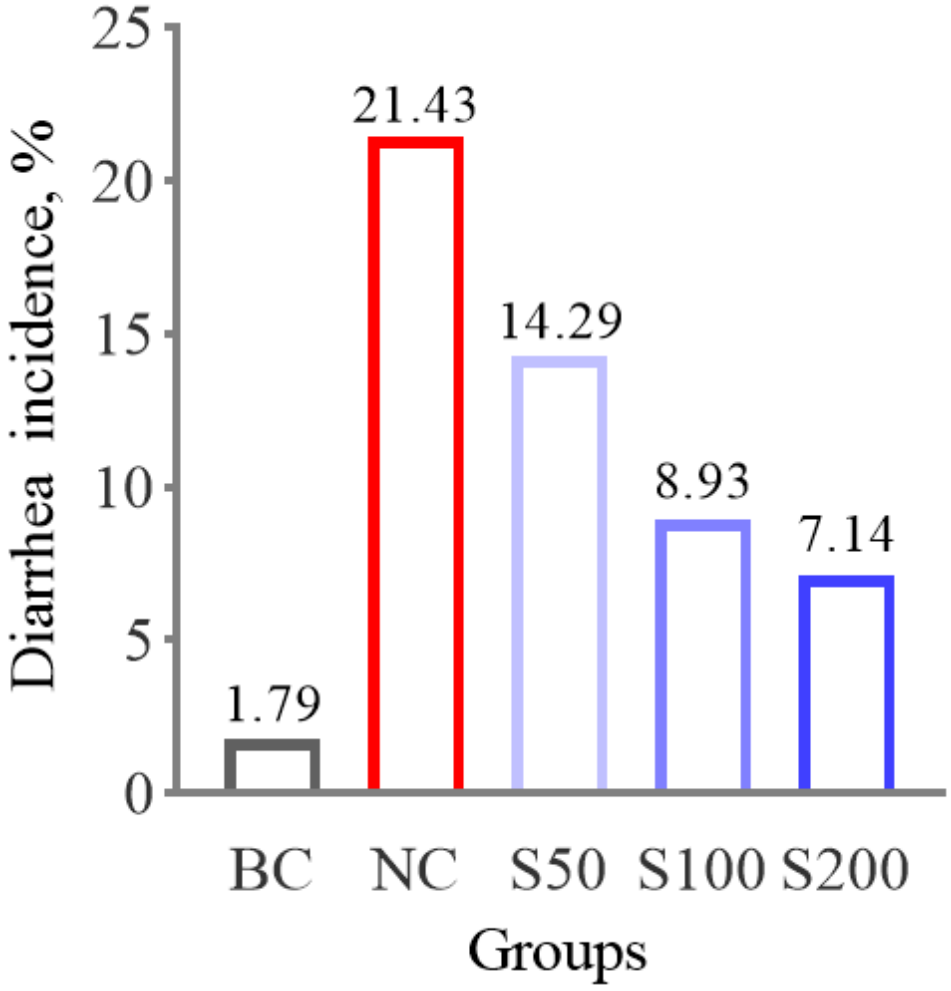

Figure 1

Effect of GE on the diarrhea incidence of piglets

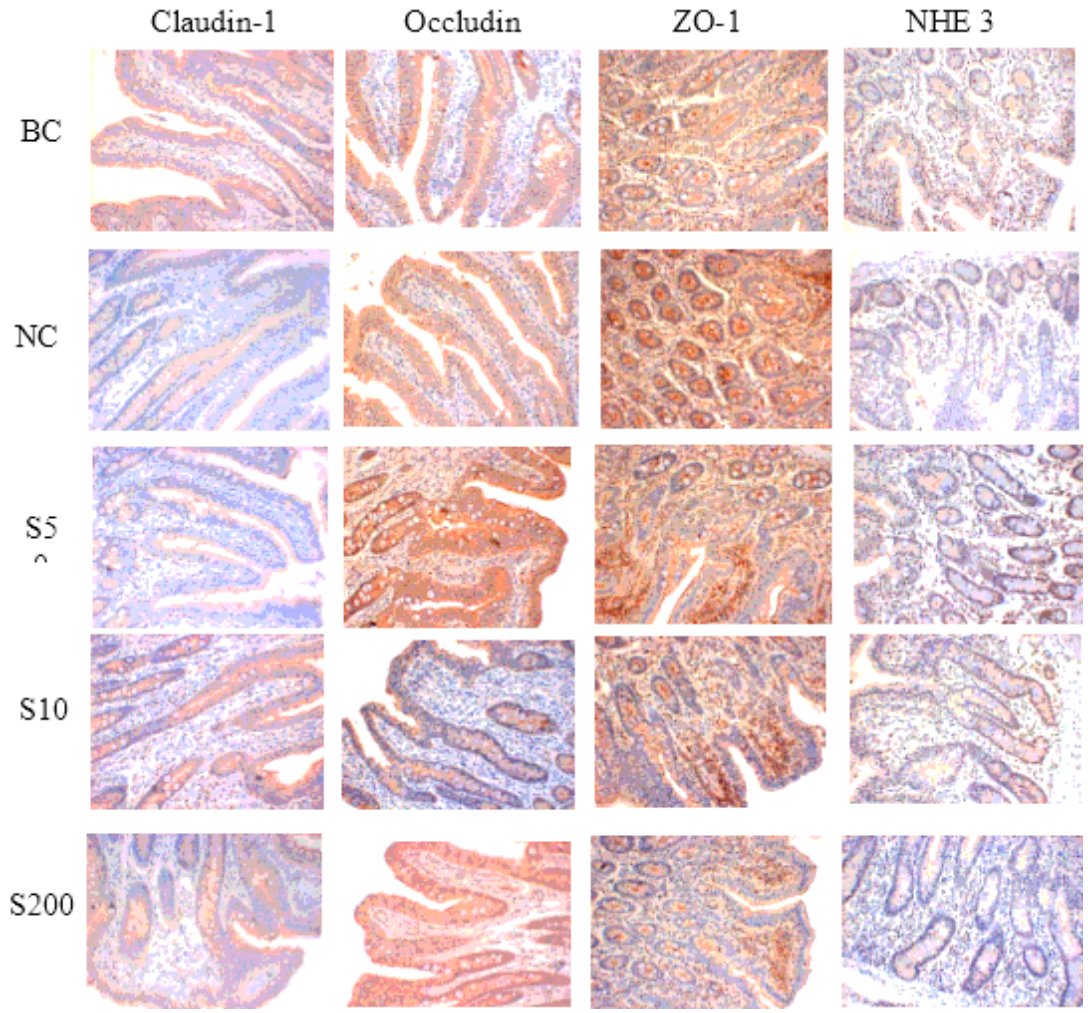

Figure 2

The representative figure of Claudin-1, Occludin, ZO-1 and NHE 3 protein expression in different groups by immunohistochemical staining (×200). The staining was visualized using DAB (brown), and slides were counterstained with hematoxylin (n=5). BC: blank control group, piglets were fed diet without supplements and ETEC challenge; NC: negative control group, piglets were fed diet without supplements and challenged by ETEC; S50, piglets were fed diet supplemented with 50 mg kg-1 GE and challenged by ETEC; S100, piglets were fed diet supplemented with 100 mg kg-1 GE and challenged by ETEC; S200, piglets were fed diet supplemented with 200 mg kg-1 GE and challenged by ETEC.

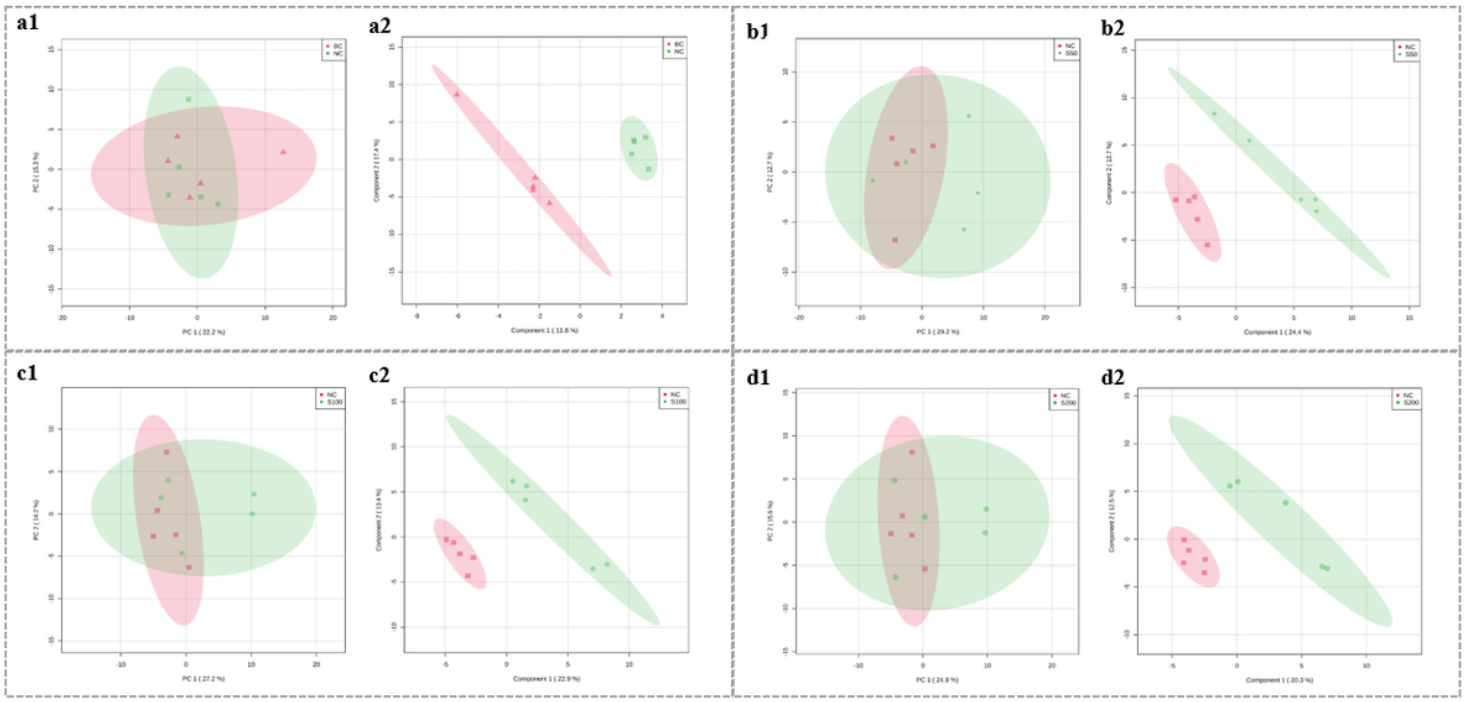

Figure 3

Score plots of metabolites in feces from piglets. (a1), (b1), (c1) and (d1) represent the score plots of the PCA models (NC vs BC group, S50 vs NC group, S100 vs NC group and S200 vs NC group, respectively.); and (a2), (b2), (c2) and (d2) represent the score plots of the PLS-DA models (NC vs BC group, S50 vs NC group, S100 vs NC group and S200 vs NC group, respectively).

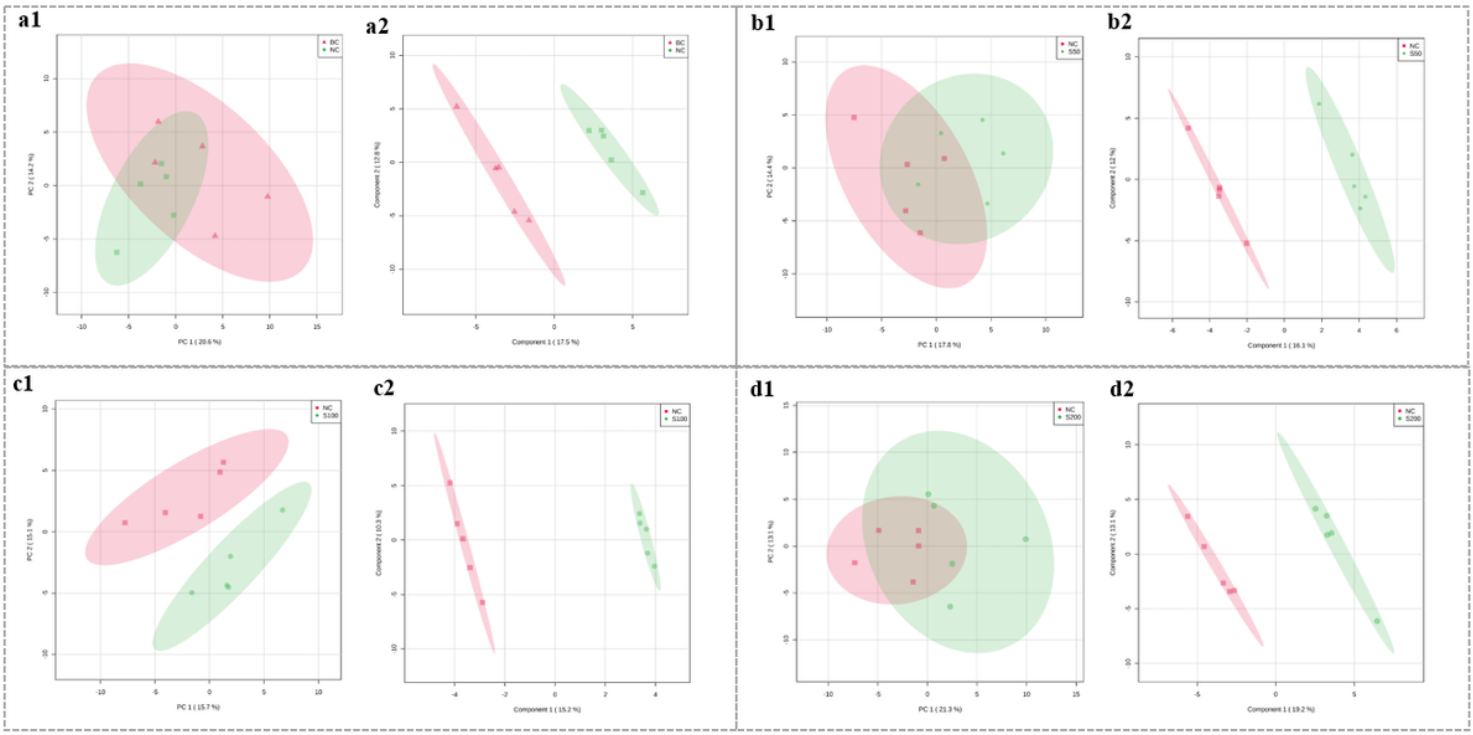

Figure 4

Score plots of metabolites in serum from piglets. (a1), (b1), (c1) and (d1) represent the score plots of the PCA models (NC vs BC group, S50 vs NC group, S100 vs NC group and S200 vs NC group, respectively.); and (a2), (b2), (c2) and (d2) represent the score plots of the PLS-DA models (NC vs BC group, S50 vs NC group, S100 vs NC group and S200 vs NC group, respectively).

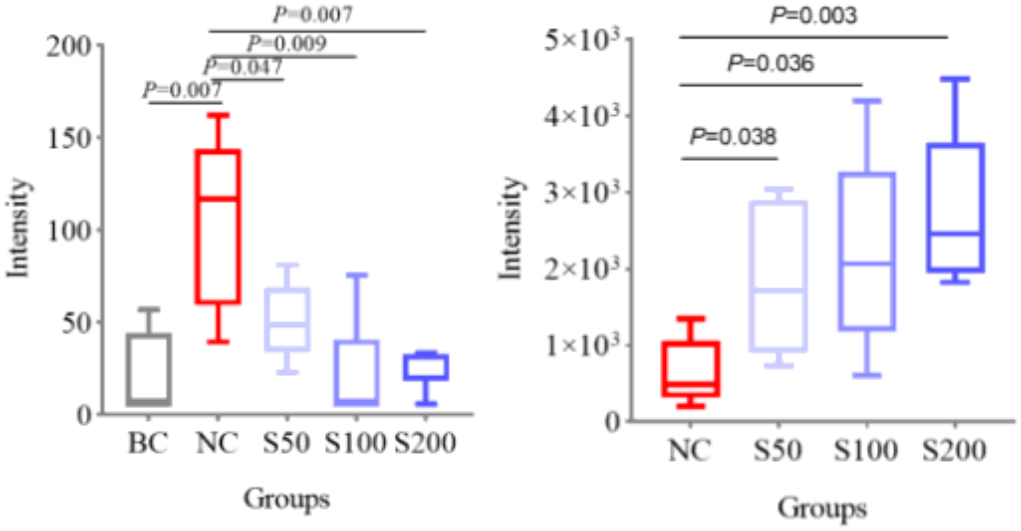

Figure 5

Key node molecules detected in serum of piglets: a) NADP; b) THF.

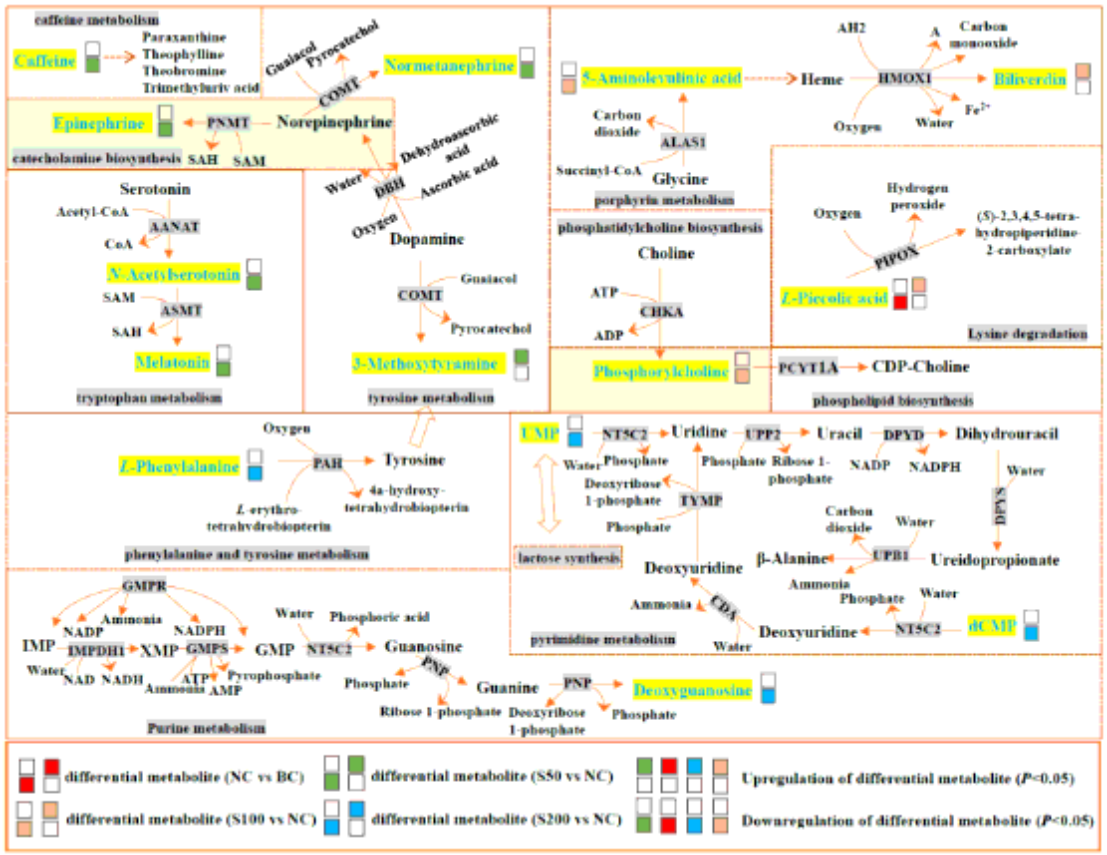

Figure 6

Top 4 ranked metabolic pathways and related differential metabolites in feces of piglets affected by S50 vs NC group, S100 vs NC group and S200 vs NC group, respectively. The details of abbreviated name: AANAT: serotonin N-acetyltransferase; ADP: adenosine diphosphate; AMP: adenosine monophosphate; ASMT: acetylserotonin O-methyltransferase; ATP: adenosine triphosphate; ALAS1: 5-aminolevulinate synthase; CDA: calcium-transporting ATPase; CHKA: choiline kinase alpha; COMT: catechol O-methyltransferase; DBH: dopamine beta-hydroxylase; DPYD: dihydropyrimidine dehydrogenase [NADP(+)]; DPYS: dihydropyrimidinase; GMP: guanosine 5'-monophosphate; GMPR: guanosine 5'-monophosphate oxidoreductase 1; GMPS: GMP synthase [glutamine-hydrolyzing]; HMOX1: heme oxygenase; IMP: inosine-5'-monophosphate; IMPDH1: inosine-5'-monophosphate dehydrogenase 1; NAD: nicotinamide adenine dinucleotide; NADH: reduced nicotinamide adenine dinucleotide; NADP: nicotinamide adenine dinucleotide phosphate; NADPH: reduced nicotinamide adenine dinucleotide phosphate; NT5C2: cytosolic purine 5`-nucleotidase; PAH: phenylalanine-4-hydroxylase; PCYT1A: choline phosphate cytidylyltransferase A; PNMT: phenylethanolamine N-methyltransferase; PNP: polyribonucleotide nucleotidyltransferase; PIPOX: peroxisomal sarcosine oxidase; SAH: S-adenosyl-L-homocysteine; SAM: S-adenosylmethionine; TYMP: thymidine phosphorylase; UPB1: beta-ureidopropionase; UPP2: uridine phosphorylase 2; XMP: xanthosine monophosphate; AH2 and A are two generic compounds.

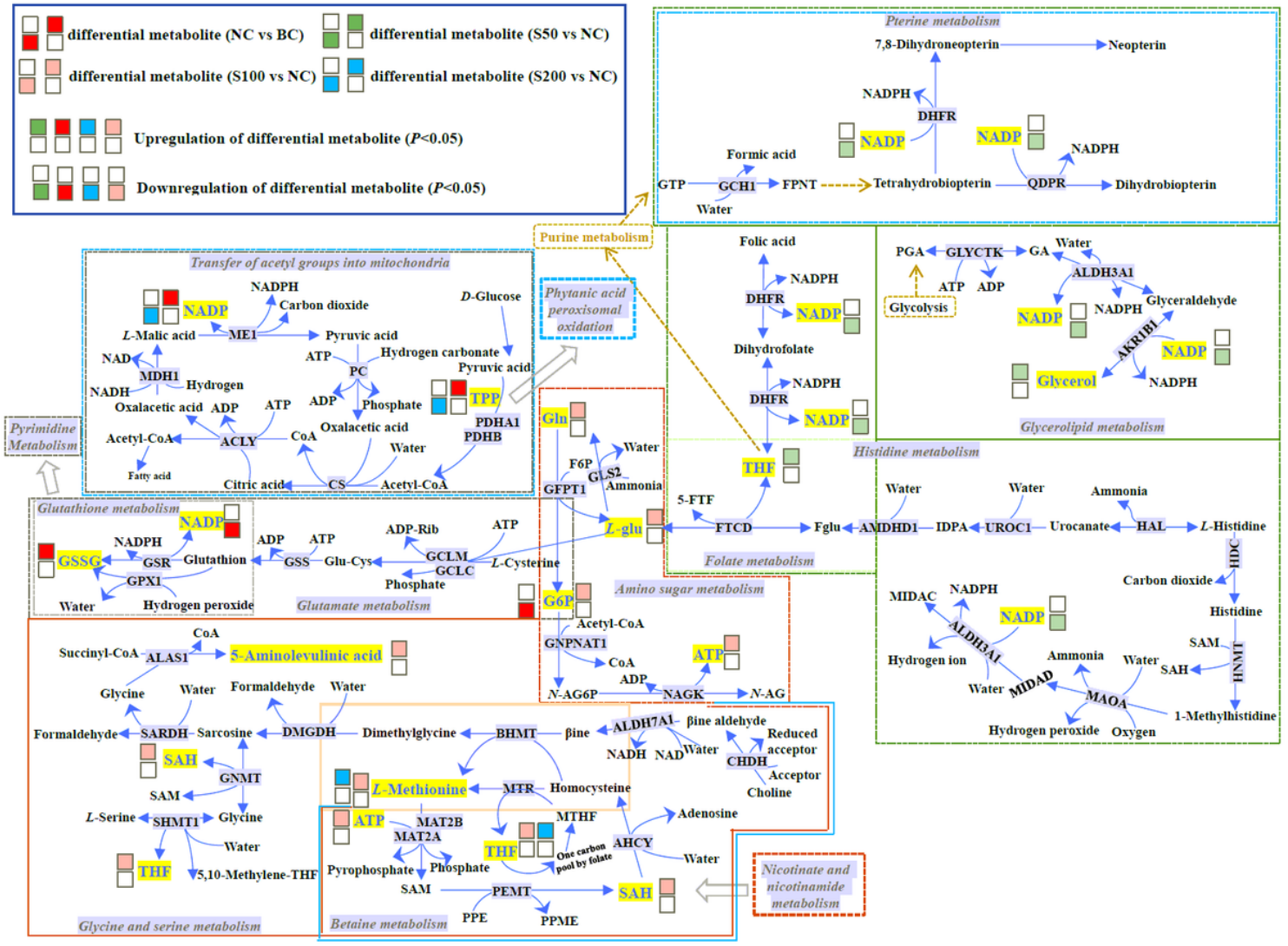

Figure 7

Top 4 ranked metabolic pathways and related differential metabolites in serum of piglets affected by NC vs BC group, S50 vs NC group, S100 vs NC group and S200 vs NC group, respectively. The details of abbreviated name: ACLY: ATP-citrate synthase; ADP-Rib: adenosine diphosphate ribose; AHCY: adenosylhomocysteinase; AKR1B1: aldose reductase; ALAS1: 5-aminolevulinate synthase; ALDH3A1: aldehyde dehydrogenase dimeric NADP-preferring; ALDH7A1: alpha-aminoadipic semialdehyde dehydrogenase; AMDHD1: probable imidazolonepropionase; BHMT: betaine–homocysteine S-methyltransferase 1; βine: betaine; CHDH: choline dehydrogenase; CS: citrate synthase; DHFR: dihydrofolate reductase; DMGDH: dimethylglycine dehydrogenase, mitochondrial; F6P: fructose 6-phosphate; Fglu: N-formyl-L-glutamic acid; FPNT: formamidopyrimidine nucleoside triphosphate; 5-FTF: N5-formyl-THF; FTCD: formimidoyltransferase-cyclodeaminase; G6P：glucosamine 6-phosphate; GA：glyceric acid; GCH1：GTP cyclohydrolase 1; GCLC：glutamate–cysteine ligase catalytic subunit; GCLM：glutamate–cysteine ligase regulatory subunit; GFPT1：glutamine-fructose-6-phosphate aminotransferase; Gln：L-glutamine; GLS2: glutaminase liver isoform; Glu-Cys：glutamylcycteine; GLYCTK：glycerate kinase; GNMT：glycine N-methyltransferase; GNPNAT1：glucosamine 6-phosphate N-acetyltransferase; GPX1：glutathione peroxidase 1; GSSG：oxidized glutathione; GSR：glutathione reductase; GSS：glutathione synthetase; GTP：guanosine triphosphate; HAL：histidine ammonia-lyase; HDC：histidine decarboxylase; HNMT：histamine N-methyltransferase; IDPA：4-imidazolone-5-propionic acid; L-glu：L-glutamic acid; MAOA：amine oxidase [flavin-containing] A; MAT2A：S-adenosylmethionine synthase isoform type-2; MAT2B：methionine adenosyltransferase 2 subnit beta; MDH1：malate dehydrogenase; ME1：NADP-dependent malic enzyme; MIDAC：methylimidazoleacetic acid; MIDAD：methylimidazole acetaldehyde; MTHF：5-methyltetrahydrofolic acid; MTR：methionine synthase; N-AG6P：N-acetyl-D-glucosamine-6-phosphate; N-AG：N-acetyl-D-glucosamine; NAGK：N-acetyl-D-glucosamine kinase; PC: pyruvate carboxylase; PDHA1: pyruvate dehydrogenase E1-alpha; PDHB: pyruvate dehydrogenase E1-beta; PEMT: phosphatidylethanolamine N-methyltransferase; PGA: 3-phosphoglyceric acid; PPE: phosphatidyl-ethanolamide; PPME: phosphatidyl-N-methylethanolamide; QDPR: dihydropteridine reductase; SARDH: sarcosine dehydrogenase; SHMT1: serinr hydroxymethyltransferase cytosolic; THF: tetrahydrofolic acid; TPP: thiamine pyrophosphate; UROC1: urocanate hydratase.

Supplementary Files

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- SupplementaryTablesandFigures.docx