Applied Microbiology and Biotechnology (2020) 104:1227–1242
https://doi.org/10.1007/s00253-019-10302-6

APPLIED MICROBIAL AND CELL PHYSIOLOGY

Sulfur-containing amino acid supplementation to gilts from late pregnancy to lactation altered offspring's intestinal microbiota and plasma metabolites

Md. Abul Kalam Azad[1,2] · Gang Liu[1,3] · Peng Bin[1,4] · Sujuan Ding[3] · Xiangfeng Kong[1] · Guiping Guan[3] · Yulong Yin[1,5]

Received: 20 October 2019 / Revised: 14 November 2019 / Accepted: 8 December 2019 / Published online: 18 December 2019

Abstract

Maternal nutrition during late pregnancy and lactation is highly involved with the offspring's health status. The study was carried out to evaluate the effects of different ratios of methionine and cysteine (Met/Cys: 46% Met, 51% Met, 56% Met, and 62% Met; maintained with 0.78% of total sulfur-containing amino acids; details in "Materials and methods") supplements in the sows' diet from late pregnancy to lactation on offspring's plasma metabolomics and intestinal microbiota. The results revealed that the level of serum albumin, calcium, iron, and magnesium was increased in the 51% Met group compared with the 46% Met, 56% Met, and 62% Met groups. Plasma metabolomics results indicated that the higher ratios of methionine and cysteine (0.51% Met, 0.56% Met, and 0.62% Met)–supplemented groups enriched the level of hippuric acid, retinoic acid, riboflavin, and δ-tocopherol than in the 46% Met group. Furthermore, the 51% Met–supplemented group had a higher relative abundance of *Firmicutes* compared with the other three groups ($P < 0.05$), while the 62% Met–supplemented group increased the abundance of *Proteobacteria* compared with the other three groups ($P < 0.05$) in piglets' intestine. These results indicated that a diet consisting with 51% Met is the optimum Met/Cys ratio from late pregnancy to lactation can maintain the offspring's health by improving the serum biochemical indicators and altering the plasma metabolomics profile and intestinal gut microbiota composition, but higher proportion of Met/Cys may increase the possible risk to offspring's health.

Keywords Sulfur-containing amino acids · Pregnancy · Lactation · Intestinal microbiota · Plasma metabolomics

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00253-019-10302-6) contains supplementary material, which is available to authorized users.

✉ Gang Liu
gangle.liu@gmail.com

[1] Laboratory of Animal Nutritional Physiology and Metabolic Process, Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, National Engineering Laboratory for Pollution Control and Waste Utilization in Livestock and Poultry Production, Changsha 410125, Hunan, China

[2] University of Chinese Academy of Sciences, Beijing 100049, China

[3] College of Bioscience and Biotechnology, Hunan Agricultural University, Changsha 410128, Hunan, China

[4] Jiangsu Co-Innovation Center for Important Animal Infectious Diseases and Zoonoses, Joint International Research Laboratory of Agriculture and Agri-Product, Safety of Ministry of Education of China, College of Veterinary Medicine, Yangzhou University, Yangzhou 225009, Jiangsu, China

[5] Yunnan Yin Yulong Academician Workstation at Yunnan Xinan Tianyou Animal Husbandry Technology Co., Ltd., Kunming 650032, Yunnan, China

Introduction

Deficiency or excess of maternal nutrition, especially maternal dietary protein intake during pregnancy or lactation can significantly influence the growth and development of offspring piglets. Adequate maternal nutrition supplementation during gestation enhances placental growth, vascular development, and placental nutrient transport (Herring et al. 2018; Zhang et al. 2019). Insufficient protein intake during pregnancy can cause fetal loss, intra-uterine growth restriction (IUGR), and reduced neonatal or postnatal growth due to a deficiency in specific amino acids, which are essential for cell metabolism and function. Excess dietary protein intake during pregnancy can also cause IUGR and fetal loss due to the toxicity of ammonia, homocysteine, and H_2S, which are induced from amino acid catabolism (Herring et al. 2018; MacKay et al. 2012; Wu 2016). Furthermore, sufficient maternal

nutrition during lactation is necessary for mammary development, milk volume, and milk quality, and that continues to regulate the piglets' growth and development through maternal milk, which contains carbohydrates, proteins, and oligosaccharides. In addition, the transmission of colostrum or milk nutrition to neonatal piglets can enhance immunity, gastrointestinal development, digestive absorption, barrier function, and stimulate visceral organ and protein synthesis (Martin Agnoux et al. 2015; Uruakpa et al. 2002; Zhang et al. 2018).

Sulfur-containing amino acids (SAAs), particularly methionine (Met) and cysteine (Cys), are important for maintaining the cell integrity functions by altering the redox state of cells. Furthermore, Met and Cys can reduce the toxicity of toxic compounds, free radicals, and ROS (reactive oxygen species; Townsend et al. 2004). Met is one of the most important essential amino acids in animal nutrition that is obtained from diets, whereas Cys is a semi/non-essential amino acid obtained from Met metabolism necessary for the synthesis of glutathione. These SAAs provide the cellular pool of sulfur homeostasis and play a crucial role in the regulation of one-carbon metabolism during pregnancy (Kalhan 2016; Shoveller et al. 2005). Furthermore, SAAs are also shown to be indispensable for neonatal piglets' normal growth, nutrients metabolism, normal mucosal growth, and gut barrier function (Fang et al. 2010; Liu et al. 2019). Moreover, recent studies revealed that maternal essential amino acid supplementation during pregnancy and lactation affects fetal growth, reproductive performance, IUGR, offspring health, and diseases later in life (Dallanora et al. 2017; Wei et al. 2019; Xu et al. 2019; Zhong et al. 2016).

Intestinal microbiota in the gastrointestinal ecosystem plays a crucial role in host health. The pig gut microbiota demonstrates dynamic composition and diversification, which alters over time and on the entire gastrointestinal tract. The colonization of piglets' gut microbiota started at birth and shaped by the consumption of the sow's colostrum and milk, building a milk-oriented microbiome. Thus, the suckling period is a crucial window of gut microbiota modification (Frese et al. 2015; Isaacson and Kim 2012; Li et al. 2018). Furthermore, plasma biomarkers and metabolomics are also effective tools for the detection of early health complications (Azad et al. 2018a; Verheyen et al. 2007). However, the collation of SAAs in maternal diet from late pregnancy to lactation on offspring piglets' gut microbiota alteration and metabolites in serum plasma remained unknown. Therefore, the present study was aimed to evaluate the effects of different ratios of maternal Met and Cys supplementation from late pregnancy to lactation on offspring piglets' plasma metabolomics and alteration of intestinal gut microbiota composition.

Materials and methods

Animals and experimental treatment

A total of 40 pregnant (Landrace × Large White) gilts (approximately at day 90 of gestation), with similar parity (2–3 fetuses) were randomly allotted into four dietary treatment groups: (a) 46% Met group: a basal diet with additional 0.12% Cys (diet contained 0.36% Met and 0.42% Cys, Met/SAAs = 46%); (b) 51% Met group: a basal diet with additional 0.04% Met and 0.08% Cys (diet contained 0.40% Met and 0.38% Cys, Met/SAAs = 51%); (c) 56% Met group: a basal diet with additional 0.08% Met and 0.04% Cys (diet contained 0.44% Met and 0.34% Cys, Met/SAAs = 56%); and (d) 62% Met group: a basal diet with additional 0.12% Met (diet contained 0.48% Met and 0.30% Cys, Met/SAAs = 62%), were fed from late gestation (85–90 days of gestation) to days 21 of lactation. The basal diet (Supplemental Table S1) was formulated with 0.36% Met and 0.30% Cys, Met/Cys ratio of the four groups were same 0.78% of total SAAs, and met the NRC-(2012) requirements.

Experimental gilts were separately kept in gestation crates (2.0 m × 0.6 m) during late pregnancy (days 90 to 110 of gestation) and were transferred to the farrowing house approximately on day 110 of gestation (2.2 m × 1.5 5 m), where they remained until weaning. In the farrowing house, crates had a piglet creep area provided with a heat lamp. The sows were fed at 6:00 am and 2:00 pm with approximately 3.2 kg of food daily. Experimental animal had free access to drink water at all times.

Sample collection

At day 21 of post-farrowing, one piglet from each sow (similar body weight), a total of ten piglets from each supplemented group were taken for sample collection. 5 mL of blood from each piglet was collected from the jugular vein, and then centrifuged at 3000 rpm at 4 °C for 10 min to obtain plasma and immediately shifted into a new centrifuge tube and kept at − 80 °C for future analyses. Then the piglets were sacrificed as described in our previous study (Azad et al. 2018a), and the sample of intestinal content (jejunum, ileum, colon, and cecum) were collected and then immediately frozen in liquid nitrogen and stored at − 80 °C for future analyses.

Analysis of plasma biochemical indicators

The plasma samples obtained from piglet serum (stored at − 80 °C) were thawed naturally. Then the samples were centrifuged at 3000 rpm for 10 min, and the supernatants (approximately 360 μL) were transferred into a 1.5-mL

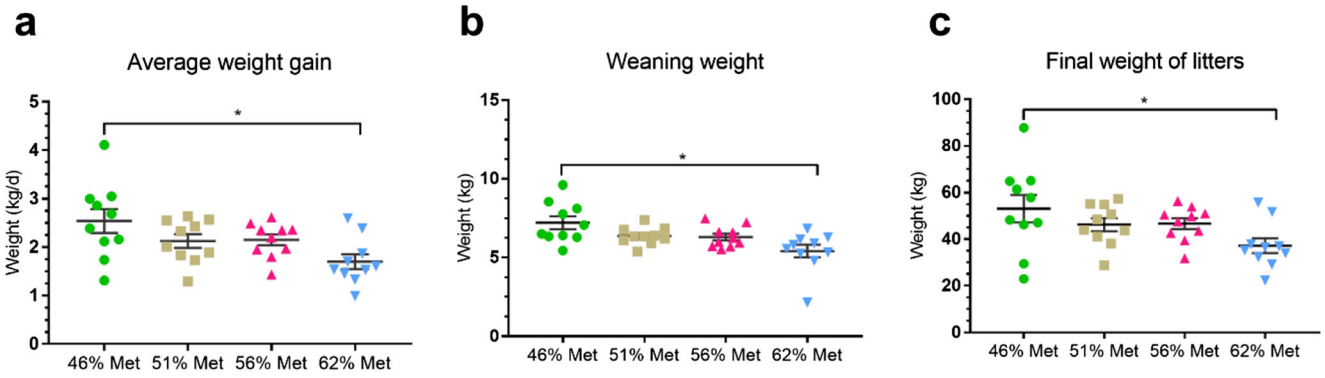


Fig. 1 Effects of different maternal Met and Cys ratios in sow diet from late pregnancy to lactation on piglets (**a**) average daily weight gain from day 1 to day 21 of lactation, (**b**) weaning weight, and (**c**) final body weight of litters on day 21 of lactation. Values are expressed as means ($n = 10$) with their SEM indicated by vertical bars. Asterisks represent significantly different means ($P < 0.05$). 46% Met, sows fed a basal diet with additional 0.12% Cys; 51% Met, sows fed a basal diet with additional 0.04% Met and 0.08% Cys; 56% Met, sows fed a basal diet with additional 0.08% Met and 0.04% Cys; and 62% Met, sows fed a basal diet with additional 0.12% Met from late pregnancy to day 21 of lactation

centrifuge tube. The plasma biochemical indicators including TP (total protein), ALB (albumin), LDH (lactate dehydrogenase), AST (glutamic oxaloacetic aminotransferase), ALT (alanine aminotransferase), GGT (gamma-glutamyl transpeptidase), HDL (high-density lipoprotein), LDL (low-density lipoprotein), BILT (total bilirubin), CREA (creatinine), GLU (glucose), LACT (lactic acid), BUN (blood urea nitrogen), NH_3 (serum ammonia), Ca (calcium), I (iron), Mg (magnesium), and P (phosphate) were determined using commercially available kits (F. Hoffmann-La Roche Ltd., Basel, Switzerland) in line with the manufacturers' guidelines.

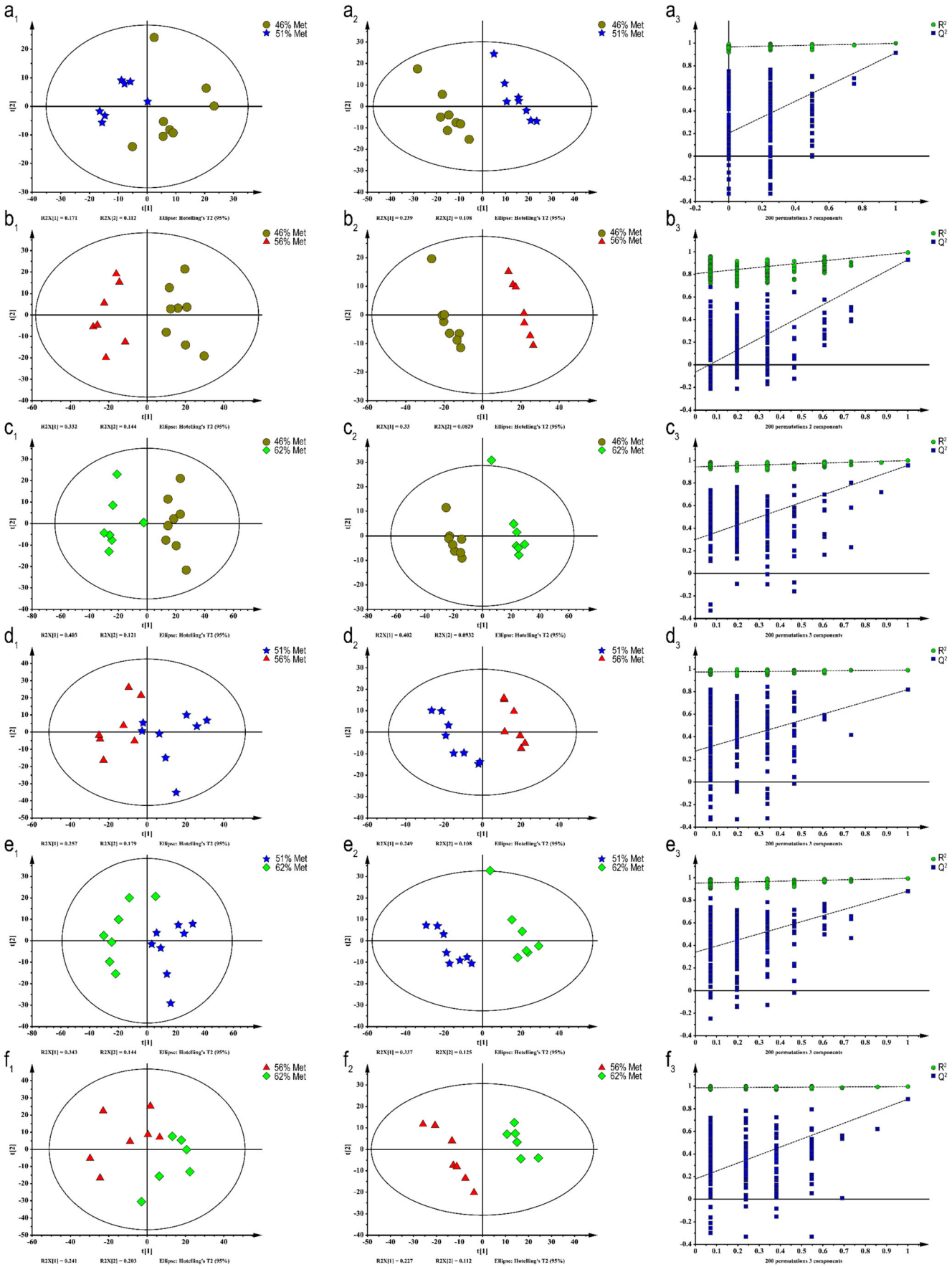
Determination of plasma metabolite changes using LC-MS analysis

Preserved plasma samples (− 80 °C) were thawed at room temperature, then 100 μL of plasma aliquots ($n = 8$) were transferred to 300 μL methanol (Merck, Darmstadt, Germany) and added 10 μL internal standard (2-chloro-L-phenylalanine, 3.1 mg/mL) (Sigma-Aldrich, St. Louis, MA, USA); after that, the samples were vortexed for 30 s and centrifuged at 12,000 rpm for 15 min at 4 °C. 200 μL of the supernatant was transferred to sample vial for LC-MS analysis.

Table 1 Effects of different maternal Met/Cys ratio on piglet's plasma biochemical indicators

Parameters	46% Met	51% Met	56% Met	62% Met
TP (g/L)	71.60 ± 1.58b	73.28 ± 0.95ab	75.93 ± 1.37a	75.60 ± 1.41ab
ALB (g/L)	41.18 ± 0.87	43.42 ± 0.69	41.50 ± 0.94	40.92 ± 1.58
AST (U/L)	33.6 ± 3.81b	32.50 ± 3.93b	32.17 ± 2.38b	48.17 ± 2.38a
LDH (U/L)	377.20 ± 9.5b	387.00 ± 10.86b	382.50 ± 24.09b	452.17 ± 13.66a
BUN (mmol/L)	5.22 ± 0.50	5.68 ± 0.35	5.30 ± 0.44	5.55 ± 0.49
CREA (μmol/L)	187.00 ± 12.55	178.83 ± 13.04	173.17 ± 15.26	184.17 ± 11.60
GLU (mmol/L)	4.70 ± 0.51	4.92 ± 0.10	5.38 ± 0.34	5.05 ± 0.55
Ca (mmol/L)	2.67 ± 0.02	2.74 ± 0.02	2.71 ± 0.05	2.67 ± 0.04
P (mmol/L)	2.55 ± 0.16	2.55 ± 0.05	2.67 ± 0.11	2.63 ± 0.11
HDL (mmol/L)	0.76 ± 0.03	0.75 ± 0.06	0.67 ± 0.03	0.71 ± 0.06
LDL (mmol/L)	0.67 ± 0.05	0.74 ± 0.03	0.66 ± 0.03	0.80 ± 0.05
I (μmol/L)	24.49 ± 2.38	29.29 ± 1.96	25.67 ± 1.76	24.92 ± 2.90
NH_3 (μmol/L)	133.32 ± 20.39	117.17 ± 6.43	121.22 ± 16.94	121.50 ± 13.28
Mg (mmol/L)	1.99 ± 0.02	2.03 ± 0.02	1.99 ± 0.03	2.01 ± 0.03

Values are expressed as means ± SEM, $n = 10$. Means in the same row with different lowercase letters were significantly different among groups ($P < 0.05$). 46% Met, sows fed a basal diet with additional 0.12% Cys; 51% Met, sows fed a basal diet with additional 0.04% Met and 0.08% Cys; 56% Met, sows fed a basal diet with additional 0.08% Met and 0.04% Cys; and 62% Met, sows fed a basal diet with additional 0.12% Met from late pregnancy to day 21 of lactation


a1
46% Met
51% Met
t[1]
t[2]
R2X[1] = 0.171 R2X[2] = 0.112 Ellipse: Hotelling's T2 (95%)
a2
46% Met
51% Met
R2X[1] = 0.239 R2X[2] = 0.108 Ellipse: Hotelling's T2 (95%)
a3
R2
Q2
200 permutations 3 components
b1
46% Met
56% Met
R2X[1] = 0.332 R2X[2] = 0.144 Ellipse: Hotelling's T2 (95%)
b2
46% Met
56% Met
R2X[1] = 0.33 R2X[2] = 0.0829 Ellipse: Hotelling's T2 (95%)
b3
R2
Q2
200 permutations 2 components
c1
46% Met
62% Met
R2X[1] = 0.403 R2X[2] = 0.121 Ellipse: Hotelling's T2 (95%)
c2
46% Met
62% Met
R2X[1] = 0.402 R2X[2] = 0.0932 Ellipse: Hotelling's T2 (95%)
c3
R2
Q2
200 permutations 3 components
d1
51% Met
56% Met
R2X[1] = 0.257 R2X[2] = 0.179 Ellipse: Hotelling's T2 (95%)
d2
51% Met
56% Met
R2X[1] = 0.249 R2X[2] = 0.108 Ellipse: Hotelling's T2 (95%)
d3
R2
Q2
200 permutations 3 components
e1
51% Met
62% Met
R2X[1] = 0.343 R2X[2] = 0.144 Ellipse: Hotelling's T2 (95%)
e2
51% Met
62% Met
R2X[1] = 0.337 R2X[2] = 0.125 Ellipse: Hotelling's T2 (95%)
e3
R2
Q2
200 permutations 3 components
f1
56% Met
62% Met
R2X[1] = 0.241 R2X[2] = 0.203 Ellipse: Hotelling's T2 (95%)
f2
56% Met
62% Met
R2X[1] = 0.227 R2X[2] = 0.112 Ellipse: Hotelling's T2 (95%)
f3
R2
Q2
200 permutations 3 components

◀ **Fig. 2** Score plots of PCA, PLS-DA models, and permutation test results of PLS-DA ($n = 8$). The PCA score plots between the (a_1) 46% Met group (brown dots) and 51% Met group (blue stars), (b_1) 46% Met group (brown dots) and 56% Met group (red triangles), (c_1) 46% Met group (brown dots) and 62% Met group (green rhombuses), (d_1) 51% Met group (blue stars) and 56% Met group (red triangles), (e_1) 51% Met group (blue stars) and 62% Met group (green rhombuses), and (f_1) 56% Met group (red triangles) and 62% Met group (green rhombuses); the PLS-DA score plots between the (a_2) 46% Met group (brown dots) and 51% Met group (blue stars), (b_2) 46% Met group (brown dots) and 56% Met group (red triangles), (c_2) 46% Met group (brown dots) and 62% Met group (green rhombuses), (d_2) 51% Met group (blue stars) and 56% Met group (red triangles), (e_2) 51% Met group (blue stars) and 62% Met group (green rhombuses), and (f_2) 56% Met group (red triangles) and 62% Met group (green rhombuses); and the permutation test results of PLS-DA (a_3–f_3), respectively. 46% Met, sows fed a basal diet with additional 0.12% Cys; 51% Met, sows fed a basal diet with additional 0.04% Met and 0.08% Cys; 56% Met, sows fed a basal diet with additional 0.08% Met and 0.04% Cys; and 62% Met, sows fed a basal diet with additional 0.12% Met from late pregnancy to day 21 of lactation

Plasma metabolic analysis was carried out using an ACQUITYTM UPLC-QTOF system (Waters, Manchester, England). A Waters ACQUITY UPLC HSS T3 column (2.1 mm × 100 mm, 1.8 μm) was used for chromatographic separation of all samples and the temperature of the column was maintained at 40 °C. The mobile phase (A) contained with water with 0.1% formic acid (Sigma-Aldrich, St. Louis, MO, USA) and the mobile phase (B) contained with acetonitrile (Merck, Darmstadt, Germany) with 0.1% formic acid. The column flow rate was 0.30 mL/min, and the sample injection volume was 6 μL. The gradient conditions for the metabolic separation process of the chromatograph are shown in supplemental Table S2. Electrospray ionization (ESI) was performed in both positive and negative modes. The positive mode conditions are presented in supplemental Table S3 and the negative mode conditions are presented in supplemental Table S4. A Massxlynx 4.1 software (Waters, Dublin, Ireland) was used for data processing. Finally, the processed data were standardized using Excel 2007 to obtain Rt (retention time), Mz (mass/charge ratio), observation (samples), and peak intensity. Furthermore, the SMICA-P 11.0 (Umetrics, Umea, Sweden) was used for group data standardization, and principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA) were carried out to obtain the metabolic information and significant differences of group data.

16S rDNA sequencing of intestinal microbiota

Piglet intestinal samples ($n = 8$) from four different supplemented groups were sequenced 16S rDNA as previously described (Azad et al. 2018a). Briefly, the intestinal DNA was extracted using the QIAamp DNA stool Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. A NanoDrop ND-1000 instrument (NanoDrop Technologies Inc., Wilmington, DE, USA) was used to determine the DNA concentration and purity. The PCR primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3′) and 926R (5'-CCGTACAATTCMTTTGAGTTT-3′) were used for the amplification of 16S rDNA. The amplified PCR products were then extracted by agarose gel electrophoresis (2% agarose; Qiagen, Hilden, Germany).

According to the manufacturer's guidelines, a TruSeq® DNA PCR-free Sample Preparation kit (Illumina, San Diego, CA, USA) was used to construct sequencing libraries. The constructed gene-sequencing library was quantified using Qubit@ 2.0 Fluorimeter and Q-PCR. Finally, an Illumina HiSeq2500 (Illumina, San Diego, CA, USA) was used to sequence each library. Raw sequences are available in the NCBI Sequence Read Archive with accession number PRJNA579317.

Statistical analysis

All experimental results were expressed as mean ± SEMs (standard error of mean). Statistical data were analyzed using the SPSS 23.0 (SPSS Inc., Chicago, IL, USA) software for Windows. The growth performance and bacterial community compositions were tested using a one-way analysis of variance (ANOVA) program, and significant differences between the groups were analyzed using Duncan's multiple range test. To determine the correlation between various levels of plasma metabolite and gut microbial genera abundance, the Pearson correlation test was performed using GraphPad Prism v.7.0 (GraphPad Software, San Diego, CA, USA). P values of <0.05 were taken to indicate statistical significance.

Results

Effects of different maternal Met/Cys ratio on offspring's growth

We observed the effects of different ratios of Met and Cys supplements in the sow diet from late pregnancy to lactation on offspring's growth performance. The results for the average weight gain of piglets, weaning weight, and final weight of piglets on day 21 of lactation are depicted in Fig. 1. The results show that the 46% Met group significantly improved the average weight gain, weaning weight, and final body weight of litters compared with the 62% Met group, while 51% Met group and 56% Met group also improved these indices compared with the 62% Met group but not significantly.

Effects of different maternal Met/Cys ratio on piglet's plasma biochemical indicators

At day 21 of lactation, plasma biochemical indicators of piglets are presented in Table 1. Compared with the 56%

Table 2 Effects of different Met/Cys ratio on piglet plasma metabolomics

Metabolites	46% Met vs. 51% Met			46% Met vs. 56% Met			46% Met vs. 62% Met			51% Met vs. 56% Met			51% Met vs. 62% Met			56% Met vs. 62% Met		
	VIP	FC	*P*	VIP	FC	*P*	VIP	FC	*P*	VIP	FC	*P*	VIP	FC	*P*	VIP	FC	*P*
Hypotaurine	1.87	0.280	0.000	1.71	0.544	0.000	1.56	0.695	0.000	1.85	0.264	0.000	1.65	0.415	0.000	1.81	0.20	0.001
Lactic acid	–	–	–	1.03	0.110	0.026	1.02	0.184	0.014	–	–	–	–	–	–	1.55	0.135	0.013
Phenylacetic acid	1.19	0.374	0.037	1.34	0.832	0.001	1.38	1.338	0.000	1.50	0.460	0.007	1.63	0.960	0.000	–	–	–
Acetyl phosphate	–	–	–	–	–	–	–	–	–	–	–	–	1.01	0.240	0.041	1.58	0.194	0.010
Thymine	–	–	–	1.24	0.223	0.005	1.24	0.375	0.001	1.44	0.164	0.011	1.38	0.317	0.002	1.83	0.227	0.001
Pyroglutamic acid	–	–	–	1.22	0.271	0.005	1.39	0.460	0.000	–	–	–	1.07	0.301	0.029	1.46	0.239	0.021
Creatine	1.76	0.373	0.000	1.68	0.683	0.000	1.40	0.770	0.000	1.65	0.310	0.002	1.15	0.397	0.017	–	–	–
L-Asparagine	1.82	0.400	0.000	1.69	0.707	0.000	1.44	0.814	0.000	1.61	0.306	0.003	1.20	0.414	0.011	–	–	–
Hypoxanthine	1.31	0.533	0.019	1.43	1.006	0.000	1.42	1.467	0.000	–	–	–	1.35	0.933	0.003	–	–	–
L-Histidinol	–	–	–	1.37	0.932	0.001	1.38	1.376	0.000	1.44	0.584	0.010	1.53	1.029	0.000	1.64	0.549	0.007
m-Coumaric acid	–	–	–	–	–	–	–	–	–	1.34	0.599	0.019	1.38	0.864	0.002	–	–	–
Caprylic acid	–	–	–	1.24	0.694	0.005	1.21	0.808	0.001	1.32	0.396	0.023	1.30	0.510	0.005	–	–	–
3-Methoxytyramine	–	–	–	1.37	0.765	0.001	1.25	0.778	0.001	1.52	0.515	0.006	1.27	0.528	0.007	–	–	–
L-Glutamate	–	–	–	1.43	0.793	0.000	1.39	1.236	0.000	1.59	0.501	0.004	1.52	0.943	0.000	1.74	0.614	0.003
Phosphohydroxypyruvic acid	1.55	0.185	0.003	1.41	0.277	0.001	1.13	0.278	0.005	1.17	0.109	0.048	1.10	0.180	0.024	1.44	0.135	0.023
Nonanedioic acid	–	–	–	1.06	0.603	0.022	1.20	0.926	0.002	–	–	–	1.11	0.563	0.021	–	–	–
Hippuric acid	1.77	0.439	0.000	1.62	0.720	0.000	1.46	0.968	0.000	–	–	–	1.20	0.529	0.011	–	–	–
Sebacic acid	1.32	0.448	0.018	1.40	0.854	0.001	1.33	1.206	0.000	1.67	0.406	0.001	1.47	0.759	0.001	–	–	–
Myo-Inositol	1.87	0.489	0.000	1.65	0.765	0.000	1.49	1.067	0.000	1.26	0.276	0.031	1.31	0.578	0.004	1.36	0.416	0.036
L-Tyrosine	1.71	0.353	0.001	1.69	0.786	0.000	1.51	1.036	0.000	1.83	0.433	0.000	1.53	0.683	0.000	1.41	0.344	0.027
L-Tryptophan	1.54	0.191	0.004	1.67	0.395	0.000	1.44	0.457	0.000	1.58	0.204	0.004	1.27	0.266	0.007	1.32	0.124	0.042
Indolelactic acid	1.61	0.217	0.002	1.61	0.391	0.000	1.37	0.468	0.000	1.51	0.174	0.006	1.17	0.251	0.014	1.45	0.156	0.023
Gluconic acid	1.48	0.451	0.006	1.50	0.878	0.000	1.47	1.301	0.000	1.45	0.427	0.010	1.61	0.851	0.000	1.53	0.485	0.014
Pantothenic acid	1.25	0.371	0.027	1.45	0.868	0.000	1.42	1.281	0.000	1.73	0.497	0.001	1.65	0.910	0.000	1.43	0.426	0.025
Xanthurenic acid	1.29	0.401	0.021	1.48	0.860	0.000	1.45	1.123	0.000	1.24	0.460	0.034	1.36	0.723	0.003	–	–	–
Traumatic acid	–	–	–	–	–	–	1.05	1.367	0.011	1.47	0.729	0.009	1.53	1.241	0.000	–	–	–
L-Cystine	1.20	0.360	0.035	1.59	0.995	0.000	1.48	1.321	0.000	1.51	0.635	0.007	1.43	0.961	0.001	1.60	0.514	0.009
Cytidine	1.33	0.393	0.017	1.42	0.733	0.001	1.32	1.001	0.000	1.48	0.340	0.008	1.32	0.608	0.004	1.40	0.402	0.029
γ-Glu-Cys	–	–	–	1.36	0.810	0.001	1.40	1.164	0.000	1.44	0.597	0.011	1.51	0.951	0.000	–	–	–
Cis-9-plmitoleic acid	–	–	–	–	–	–	–	–	–	1.31	0.556	0.024	1.13	0.613	0.019	–	–	–
D-Glucose-6-phosphate	1.21	0.549	0.033	1.36	1.084	0.001	1.33	1.422	0.000	1.32	0.536	0.022	1.40	0.874	0.002	–	–	–
Alpha-CEHC	1.73	0.228	0.001	1.66	0.429	0.000	1.40	0.491	0.000	1.62	0.201	0.003	1.20	0.263	0.012	–	–	–
Tetradecanedioic acid	1.65	0.275	0.001	1.61	0.534	0.000	1.34	0.471	0.000	1.45	0.259	0.010	–	–	–	–	–	–
Oleamide	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.38	−1.43	0.031

Table 2 (continued)

Metabolites	46% Met vs. 51% Met			46% Met vs. 56% Met			46% Met vs. 62% Met			51% Met vs. 56% Met			51% Met vs. 62% Met			56% Met vs. 62% Met		
	VIP	FC	*P*	VIP	FC	*P*	VIP	FC	*P*	VIP	FC	*P*	VIP	FC	*P*	VIP	FC	*P*
Oleic acid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.42	−1.45	0.026
Sedohetulose-7-phosphate	–	–	–	–	–	–	1.09	0.801	0.007	–	–	–	1.07	0.633	0.028	–	–	–
Retinoic acid	1.58	0.552	0.003	1.48	0.914	0.000	1.44	1.294	0.000	–	–	–	1.36	0.742	0.003	–	–	–
Sphinganine	1.13	0.161	0.049	1.17	0.308	0.009	1.41	0.580	0.000	–	–	–	1.48	0.419	0.000	1.36	0.321	0.035
Xanthosine	1.84	0.580	0.000	1.59	0.938	0.000	1.51	1.250	0.000	1.23	0.359	0.036	1.41	0.670	0.002	1.34	0.456	0.038
Phytosphingosine	–	–	–	1.07	0.298	0.020	1.42	0.629	0.000	–	–	–	1.48	0.454	0.001	1.51	0.365	0.016
Progsterone	–	–	–	–	–	–	–	–	–	1.32	0.497	0.022	–	–	–	–	–	–
cAMP	1.18	0.257	0.040	1.37	0.557	0.001	1.27	0.682	0.001	1.55	0.300	0.005	1.32	0.425	0.004	–	–	–
Adrenic acid	–	–	–	–	–	–	–	–	–	1.45	2.107	0.010	–	–	–	–	–	–
PGF1α	–	–	–	–	–	–	–	–	–	–	–	–	1.05	1.498	0.033	–	–	–
Cortisone	–	–	–	1.38	0.401	0.001	1.05	1.795	0.010	1.37	0.227	0.016	–	–	–	1.46	0.946	0.022
Cortisol	–	–	–	–	–	–	–	–	–	–	–	–	1.07	−0.81	0.029	–	–	–
Doccosanoic acid	–	–	–	–	–	–	1.07	0.651	0.008	–	–	–	1.16	0.434	0.015	1.40	0.282	0.030
XMP	–	–	–	1.23	0.769	0.005	1.37	1.267	0.000	1.23	0.447	0.037	1.58	0.945	0.000	1.33	0.536	0.040
Riboflavin	1.64	0.640	0.002	1.22	0.633	0.005	1.33	1.040	0.000	–	–	–	–	–	–	–	–	–
Campesterol	–	–	–	–	–	–	–	–	–	1.24	0.161	0.035	–	–	–	–	–	–
δ-Tocopherol	1.86	0.224	0.000	1.33	0.285	0.002	1.35	0.429	0.000	–	–	–	1.05	0.205	0.033	–	–	–
UDP	–	–	–	1.46	0.865	0.000	1.45	1.241	0.000	–	–	–	1.13	0.857	0.020	–	–	–
Cholic acid	–	–	–	–	–	–	–	–	–	1.41	2.327	0.013	1.01	1.495	0.041	–	–	–
α-Tocopherol	1.19	−0.26	0.036	1.43	−0.581	0.000	–	–	–	1.36	−0.33	0.018	–	–	–	–	–	–
CPA (18:1(9Z)/0:0)	–	–	–	1.26	0.598	0.004	–	–	–	1.79	0.762	0.000	–	–	–	1.37	−0.32	0.034
Deoxycholic acid glycine conjugate	–	–	–	–	–	–	–	–	–	1.61	1.736	0.003	1.16	1.188	0.016	–	–	–
LysoPE(0:0/16:1(9Z))	–	–	–	1.40	0.818	0.000	1.45	1.063	0.000	1.65	0.692	0.002	1.66	0.937	0.000	–	–	–
LysoPE(0:0/16:0)	–	–	–	1.06	0.659	0.008	1.02	0.752	0.013	–	–	–	–	–	–	–	–	–
Glycocholic acid	–	–	–	1.58	0.602	0.000	1.53	0.797	0.000	1.78	2.265	0.001	1.61	0.618	0.000	1.39	0.242	0.030
LysoPE (0:0/18:3(6Z,9Z,12Z))	–	–	–	1.18	0.581	0.008	–	–	–	1.78	0.668	0.001	1.03	0.409	0.038	–	–	–

VIP, variable importance in the projection; FC, fold change. 46% Met, sows fed a basal diet with additional 0.12% Cys; 51% Met, sows fed a basal diet with additional 0.04% Met and 0.08% Cys; 56% Met, sows fed a basal diet with additional 0.08% Met and 0.04% Cys; and 62% Met, sows fed a basal diet with additional 0.12% Met from late pregnancy to day 21 of lactation

Table 3 Effects of different ratio of Met/Cys on Alpha diversity indices of intestinal communities of piglets

Item	46% Met	51% Met	56% Met	62% Met
Cecum				
Effective tags	68,794 ± 2788b	77,608 ± 2030.43a	81,202 ± 1000.46a	75,378 ± 2269.09a
OTU	522 ± 25.65a	561 ± 20.87a	380 ± 34.12b	475 ± 1636a
Shannon	5.43 ± 0.27a	5.10 ± 0.13a	4.12 ± 0.42b	5.17 ± 0.21a
Chao	523.55 ± 25.32a	486.78 ± 17.62a	388.46 ± 37.11b	481.54 ± 15.66a
Colon				
Effective tags	71,262 ± 3364.62b	79,544 ± 2308.86ab	83,166 ± 2493.60a	74,014 ± 3419.03b
OTU	545 ± 33.58	552 ± 18.04	469 ± 40.02	538 ± 21.85
Shannon	5.65 ± 0.17a	5.05 ± 0.10a	4.40 ± 0.34b	5.38 ± 0.10a
Chao	535.51 ± 39.85	568.77 ± 21.95	474.58 ± 39.76	549.49 ± 17.73
Ileum				
Effective tags	80,175 ± 27.74	80,168 ± 42.18	78,227 ± 3032.40	80,508 ± 13.9.57
OTU	841.25 ± 43.93	762.51 ± 75.46	924.72 ± 50.56	829.36 ± 70.99
Shannon	6.15 ± 0.36	6.00 ± 0.42	6.31 ± 0.25	6.62 ± 0.15
Chao	836.56 ± 41.30	766.43 ± 75.81	927.10 ± 43.97	835.57 ± 68.14

Values are expressed as means ± SEM, $n = 6$. Means in the same row with different lowercase letters were significantly different among groups ($P < 0.05$). 46% Met, sows fed a basal diet with additional 0.12% Cys; 51% Met, sows fed a basal diet with additional 0.04% Met and 0.08% Cys; 56% Met, sows fed a basal diet with additional 0.08% Met and 0.04% Cys; and 62% Met, sows fed a basal diet with additional 0.12% Met from late pregnancy to day 21 of lactation

Met groups, the 46% Met group significantly ($P < 0.05$) decreased the level of TP, and the concentration of ALB was also lower than in the 51% Met and 56% Met groups. In addition, the 46% Met group showed a lower BUN and iron concentration compared with the other three groups. The 62% Met group significantly increased ($P < 0.05$) plasma AST and LDH contents compared with the 46% Met, 51% Met, and 56% Met groups. Furthermore, the 62% Met group had also increased GLU and NH_3 contents compared with other supplemented groups but not significantly. Moreover, compared with the 46% Met, 56% Met, and 62% Met groups, the level of serum Ca, I, and Mg was improved in the 51% Met group.

Effects of different maternal Met/Cys ratio on piglet's plasma metabolomics

The results of PCA showed the clear separation between inter-groups on the score plots (Fig. 2a_1–f_1). SMICA-P 11.00 was used for this process. Later, the supervised multidimensional statistical method and PLS-DA was used to obtain the metabolic information with significant differences. The PLS-DA models (Fig. 2a_2–f_2) showed that the 46% Met group (brown dots) and 51% Met group (blue stars) ($R^2X = 0.461$, $R^2Y = 0.997$, $Q^2 = 0.913$), the 46% Met group (brown dots) and 56% Met group (red triangles) ($R^2X = 0.413$, $R^2Y = 0.994$, $Q^2 = 0.929$), the 46% Met group (brown dots) and the 62% Met group (green rhombuses) ($R^2X = 0.56$, $R^2Y = 0.998$, $Q^2 = 0.956$), the 51% Met group (blue stars) and the 56% Met group (red triangles) ($R^2X = 0.476$, $R^2Y = 0.99$, $Q^2 = 0.818$), the 51% Met group (blue stars) and the 62% Met group (green rhombuses) ($R^2X = 0.534$, $R^2Y = 0.994$, $Q^2 = 0.881$), and the 56% Met group (red triangles) and the 62% Met group (green rhombuses) ($R^2X = 0.506$, $R^2Y = 0.995$, $Q^2 = 0.886$) were clearly separated. Thereafter, the models were sort out to determine whether the model was "over-fitting" (Fig. 2a_3–f_3).

The results presented in Table 2 indicated the different metabolite changes in piglets' plasma after addition of different Met/Cys ratio in maternal sow diets (46% Met vs. 51% Met, 46% Met vs. 56% Met, 46% Met vs. 62% Met, 51% Met vs. 56% Met, 51% Met vs. 62% Met, and 56% Met vs. 62% Met), with VIP > 1 and $t < 0.045$. Sixty metabolites were obtained; 46% Met vs. 51% Met, 46% Met vs. 56% Met, 46% Met vs. 62% Met, 51% Met vs. 56% Met, 51% Met vs. 62% Met, 56% Met vs. 62% Met, containing 27, 45, 45, 41, 49, and 26 metabolites, respectively (Table 2). Twenty-six metabolites (hypotaurine, phenylacetic acid, creatine, L-asparagine, hypoxanthine, phosphohydroxypyruvic acid, hippuric acid, sebacic acid, myo-inositol, L-tyrosine, L-tryptophan, indolelactic acid, gluconic acid, pantothenic acid, xanthurenic acid, L-cysteine, cytidine, D-glucose-6-phosphate, alpha-CEHC, tetradecanedioic acid, retinoic acid, sphinganine, xanthosine, cAMP, riboflavin, and δ-tocopherol) were found only in the lower Met/Cys ratio groups comparison (46% Met vs. 51% Met, 46% Met vs. 56% Met, and 46%

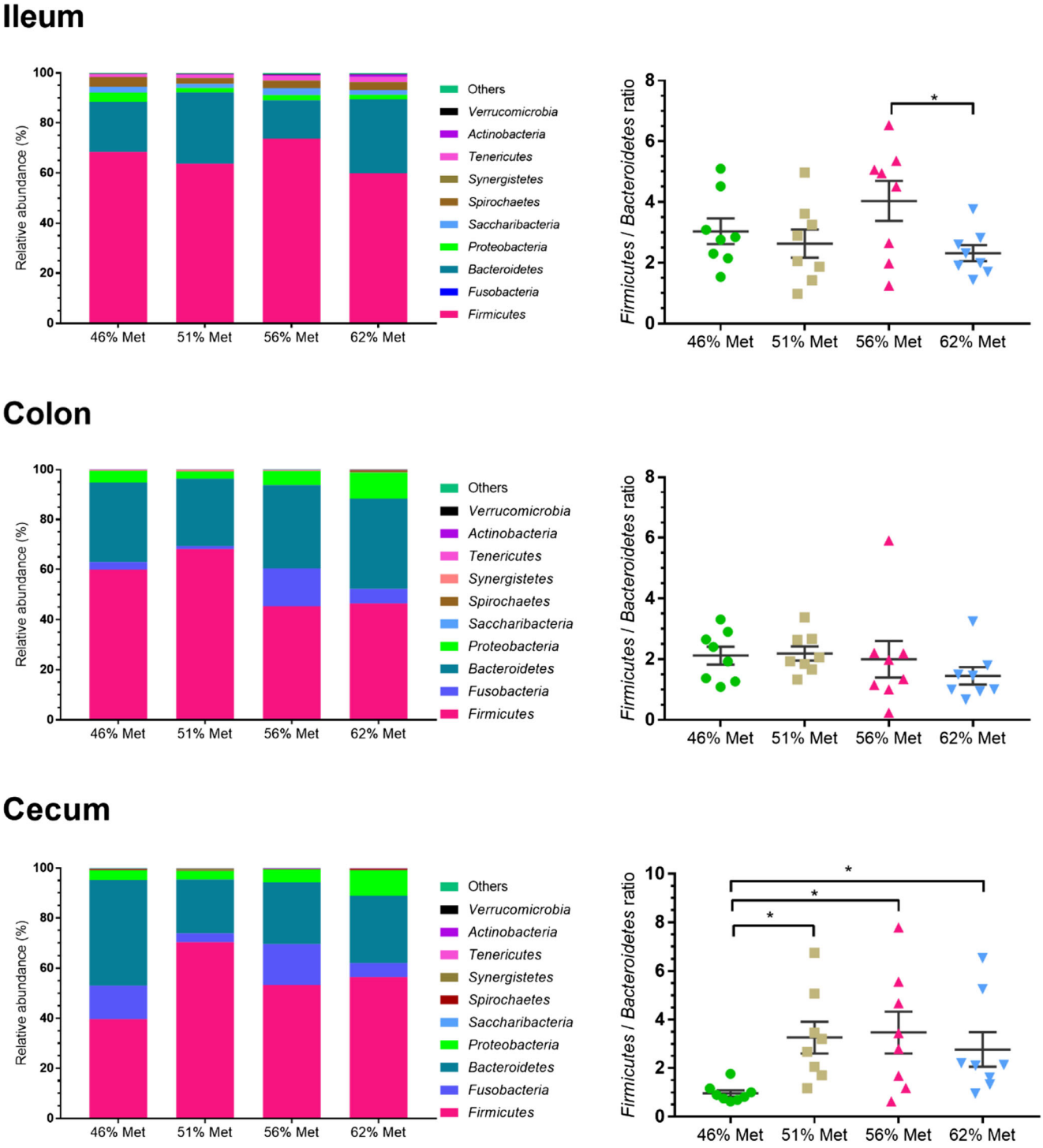


Fig. 3 The relative abundance of piglets' intestinal microbiota (ileum, colon, and cecum) and the ratio of *Firmicutes* to *Bacteroidetes* at the phylum level at day 21 of lactation. Values are expressed as means ($n = 8$) with their SEMs indicated by vertical bars. Asterisks represent significantly different means ($P < 0.05$). 46% Met, sows fed a basal diet with additional 0.12% Cys; 51% Met, sows fed a basal diet with additional 0.04% Met and 0.08% Cys; 56% Met, sows fed a basal diet with additional 0.08% Met and 0.04% Cys; and 62% Met, sows fed a basal diet with additional 0.12% Met from late pregnancy to day 21 of lactation

Met vs. 62% Met), while sixteen differential metabolite including hypotaurine, thymine, L-histidine, L-glutamate, phosphohydroxypyruvic acid, myo-nositol, L-tyrosine, L-tryptophan, indolelactic acid, gluconic acid, pantothenic acid, L-cystine, cytidine, xanthosine, XMP (xanthosine monophosphate), and glycocholic acid were found in the higher Met/Cys ratio groups (51% Met vs. 56% Met, 51% Met vs. 62% Met, and 56% Met vs. 62% Met).

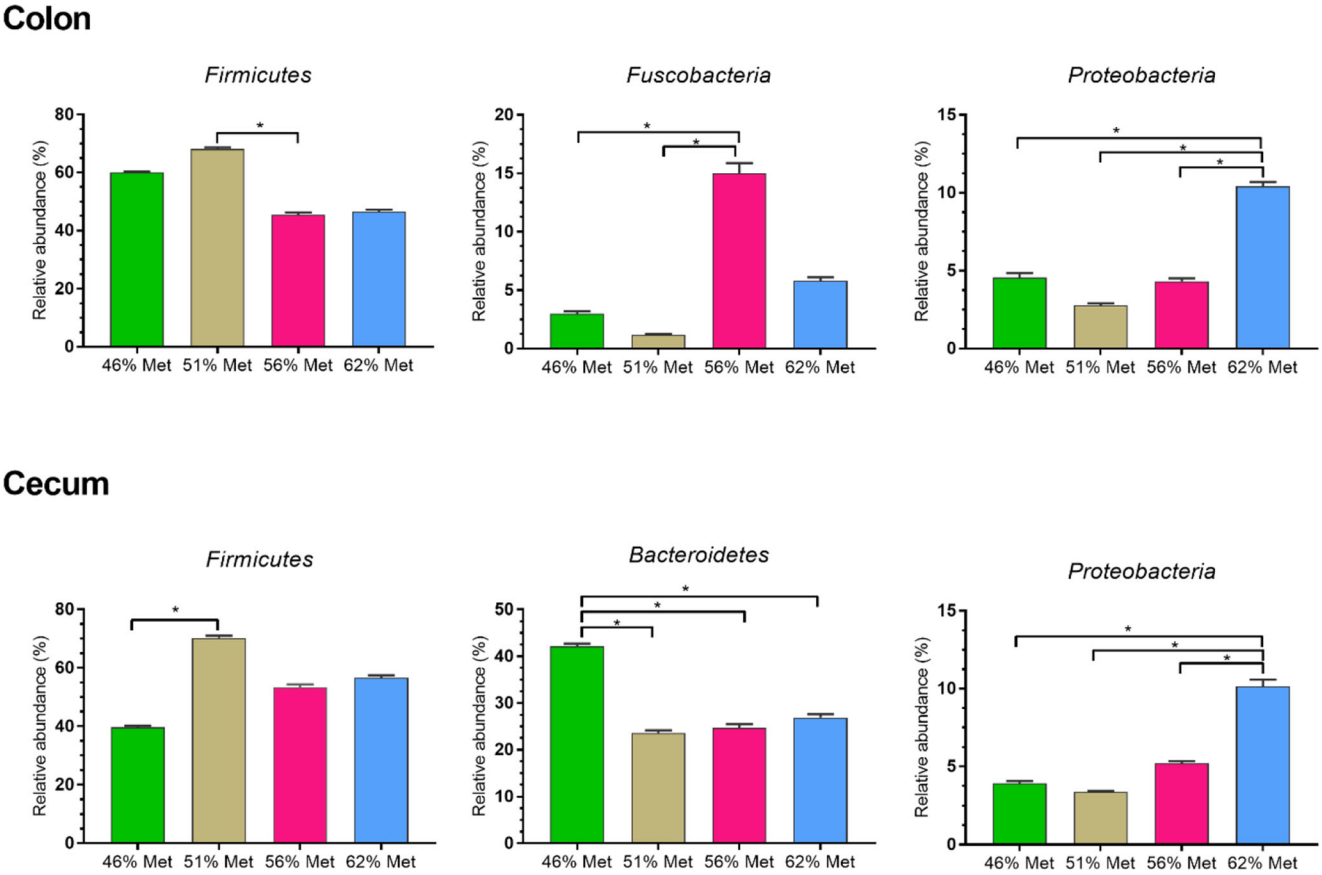


Fig. 4 Taxonomic differences in piglets' intestinal microbiota (colon and cecum) in the various groups at the phylum level at day 21 of lactation. Values are expressed as means ($n = 8$) with their SEMs indicated by vertical bars. Asterisks represent significantly different means ($P < 0.05$). 46% Met, sows fed a basal diet with additional 0.12% Cys; 51% Met, sows fed a basal diet with additional 0.04% Met and 0.08% Cys; 56% Met, sows fed a basal diet with additional 0.08% Met and 0.04% Cys; and 62% Met, sows fed a basal diet with additional 0.12% Met from late pregnancy to day 21 of lactation

Effects of different maternal Met/Cys ratio on piglet's intestinal microbiota

We sequenced the V4-V5 region of the 16S rDNA of intestinal samples obtained from four different Met/Cys supplementation group. The alpha diversity data were obtained through the process of trimming, assembly, and quality filtering. The effective tags, OTUs (operational taxonomic unit), Shannon, and Chao indices of piglets intestinal are presented in Table 3. In the cecum, the results showed that the OTU, Shannon, and Chao indices in the 56% Met group were significantly ($P < 0.05$) lower than in the other three groups. Furthermore, 56% Met group also had a significantly lower Shannon index in the colon compared with the other three groups. There was no significant difference observed of these indices in the ileum.

The intestinal microbial taxonomy was determined using a taxon-dependent analysis. Figure 3 shows the piglets' intestinal microbial composition at the phylum level. *Firmicutes*, *Fuscobacteria*, and *Bacteroidetes* were the most dominant phyla in the ileum, colon, and cecum and were > 90% of the total composition. The most dominant taxa in the ileum were *Firmicutes* and *Bacteroidetes* (59% to 74% and 15% to 30%, respectively). In the colon and cecum, *Firmicutes* and *Bacteroidetes* were also most abundant, (45% to 60% and 27% to 37% in the colon, and 39% to 71% and 23% to 43% in the cecum, respectively). In addition, *Firmicutes* to *Bacteroidetes* ratio in the 56% Met group was significantly ($P < 0.05$) higher compared with the other three groups (Fig. 3). Furthermore, taxonomic differences at the phylum level revealed that the abundance of *Firmicutes* in the colon and cecum was significantly ($P < 0.05$) higher in the 51% Met group compared with the other three groups, while the abundance of *Proteobacteria* in the colon and cecum was higher in the 62% Met group than in the other three groups (Fig. 4). In the ileum, there was no taxonomic difference observed among the various groups.

The most dominant bacterial genera in the piglets' ileum, colon, and cecum at the genus level are shown in Fig. 5. *Lactobacillus, Fuscobacterium, Bacteroides,* and *Ruminococcaceae_UCG-002* were the most abundant genera in the piglets' ileum, colon, and cecum. The abundance of

Lactobacillus in the colon and cecum was significantly ($P < 0.05$) higher in the 51% Met group compared with the other three groups (Fig. 6). In the ileum, the abundance of *Clostridium_sensu_stricto_1* was significantly ($P < 0.05$) higher in the 56% Met group than in the other groups. In the colon, the relative abundance of *Fuscobacterium* was significantly higher in the 56% Met group compared with the other groups, while 51% Met group had a significantly higher abundance of *Ruminococcaceae_UCG-002* compared with the other groups (Fig. 6). The 46% Met group had significantly ($P < 0.05$) higher abundance of *Alloprevotella* in the cecum compared with the other three groups.

Correlation between the plasma metabolites levels and abundance of piglet's intestinal microbial genera

Pearson correlation (r) analysis results are shown in Fig. 7. The correlation between the levels of different plasma metabolite and the piglets' intestinal gut microbiota abundance at the genus level ($P < 0.05$) is as follows: positive correlation between L-glutamate levels and *Ruminococcaceae_UCG-002* abundance ($r = 0.384$, $P = 0.0642$), between L-tryptophan levels and *Ruminococcaceae_UCG-002* abundance ($r = 0.369$, $P = 0.0763$) in the colon; and between hypotaurine levels and *Alloprevotella* abundance ($r = 0.477$, $P = 0.0082$),

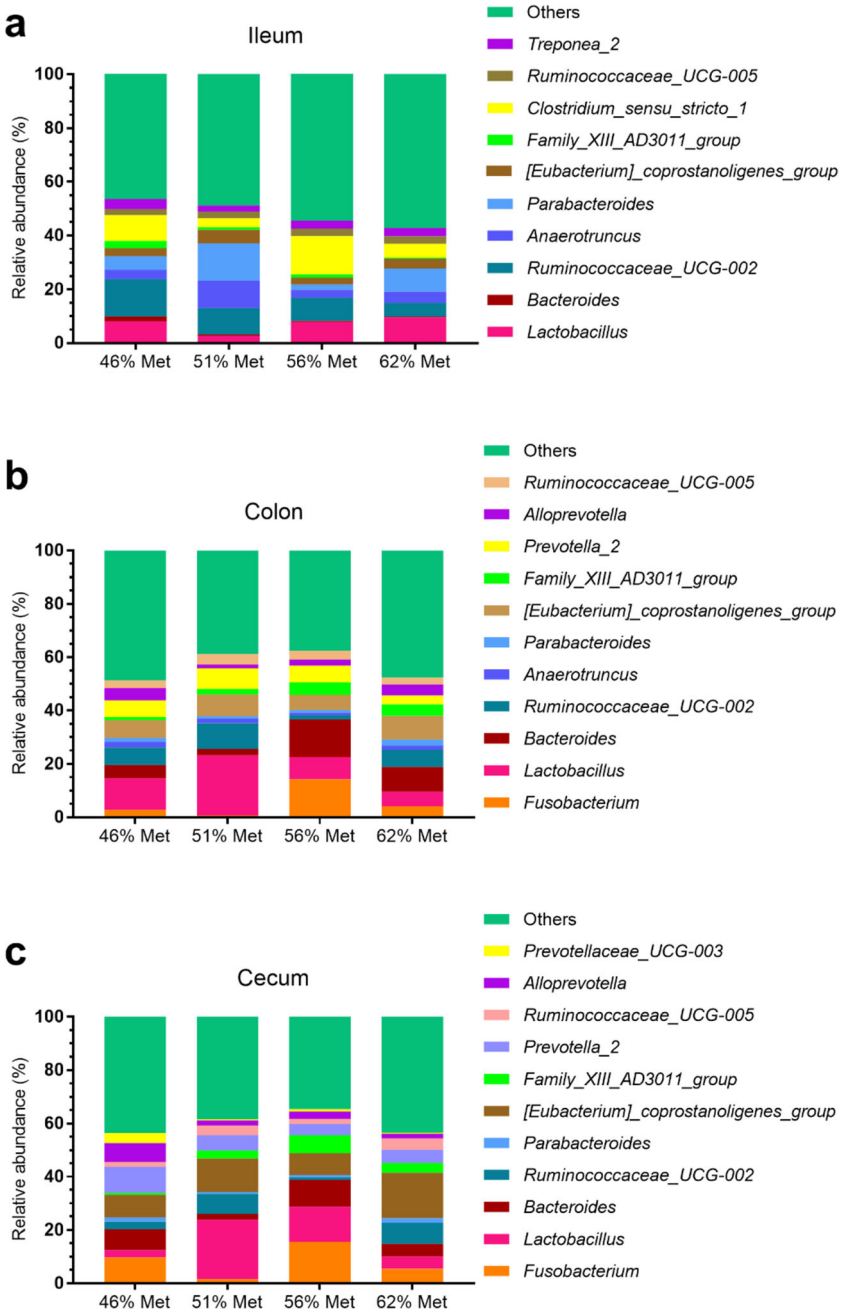


Fig. 5 The relative abundance of piglets' intestinal microbiota in various groups at the genus level at day 21 of lactation ($n = 8$). (**a**) Ileum, (**b**) colon, and (**c**) cecum. 46% Met, sows fed a basal diet with additional 0.12% Cys; 51% Met, sows fed a basal diet with additional 0.04% Met and 0.08% Cys; 56% Met, sows fed a basal diet with additional 0.08% Met and 0.04% Cys; and 62% Met, sows fed a basal diet with additional 0.12% Met from late pregnancy to day 21 of lactation

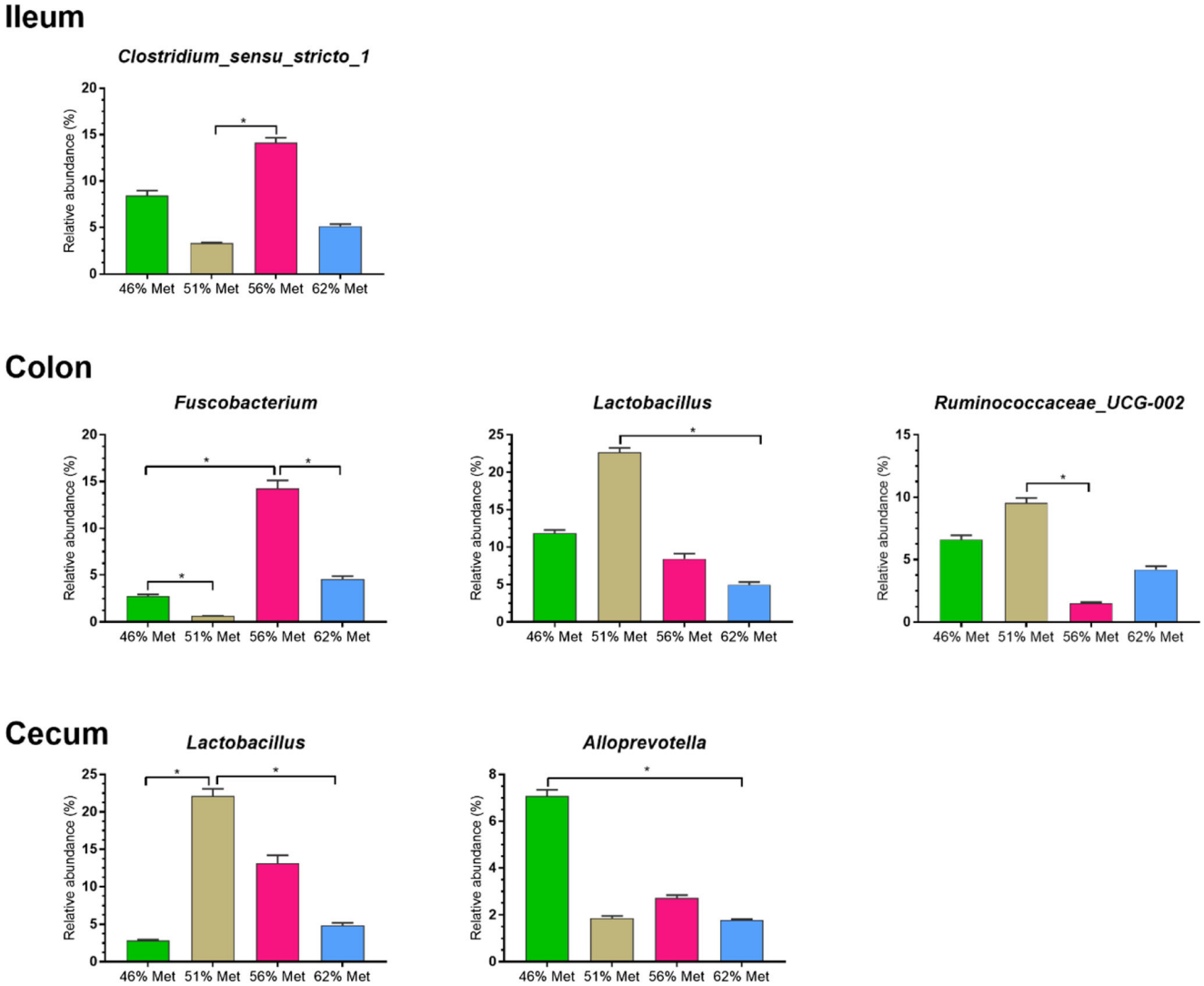


Fig. 6 Taxonomic differences in piglets' intestinal microbiota (ileum, colon, and cecum) in various groups at the genus level at day 21 of lactation. Values are expressed as means ($n = 8$) with their SEMs indicated by vertical bars. Asterisks represent significantly different means ($P < 0.05$). 46% Met, sows fed a basal diet with additional 0.12% Cys; 51% Met, sows fed a basal diet with additional 0.04% Met and 0.08% Cys; 56% Met, sows fed a basal diet with additional 0.08% Met and 0.04% Cys; and 62% Met, sows fed a basal diet with additional 0.12% Met from late pregnancy to day 21 of lactation

between gluconic acid levels and the abundance of *Alloprevotella* ($r = 0.433$, $P = 0.0344$), between L-glutamate levels and the abundance of *Alloprevotella* ($r = 0.480$, $P = 0.0176$), and between δ-tocopherol and the abundance of *Alloprevotella* ($r = 0.504$, $P = 0.0119$) in the cecum.

Discussion

Maternal nutrition is essential for fetal growth and development. Research evidence has proven that adverse nutritional conditions during late gestation may permanently change the structure and function of specific organs in the offspring and lead to many diseases later in life (Hsu and Tain 2019; Mennitti et al. 2015). SAAs are involved in numerous crucial roles in the gut to maintain its function such as digestion, absorption, and metabolism of nutrients, the immune functions of the intestinal layer, and maintenance of mucosal barrier against foreign antigens (Fang et al. 2010). Met and Cys are known to affect protein metabolism. In addition to their role in protein synthesis, methionine and cysteine are also important in special conditions, like stress and inflammation. The demand for SAAs is essential for pregnant sows' health and as well as their offspring piglets' growth, but inadequate intake or an excessive SAAs in the maternal diet may cause negative effects (Garlick 2006; Stipanuk et al. 2006; van de Poll et al. 2006). In the present study, the 46% Met group significantly increased the average weight gain, weaning weight, and the final weight of piglets compared with the other three groups. The 51% Met and 56% Met groups had an

increased tendency on the average weight, weaning weight, and the final weight of piglets, but the higher proportion of SAAs supplementation (62% Met group) decreased these parameters. One of the possible explanations might be the excess proportion of SAAs supplementation from late pregnancy to lactation reduced average weight, weaning weight, and final body weight of offspring piglets.

Dietary supplements from gestation to lactation not only are effective for the pregnant sows but also have a collateral effect on the offspring piglet's growth and health. Met and Cys are the constituents' tissue proteins, and, when insufficient, they lead to reduced protein synthesis (Tesseraud et al. 2009). In general, serum total protein and serum albumin are the indicators of sufficient supply of dietary protein, whereas AST, BUN, and CREA are the identifying biomarkers of kidney and liver damage or failure (Aiello 2016; Huang et al. 2019; Remus et al. 2019). The increases in total protein and albumin levels in serum reflect improved nutrient utilization. In addition, increased serum albumin can prevent irreversible oxidative losses by absorbing excess SAAs and then transfer them to peripheral tissues to assist local protein synthesis (De Feo et al. 1992; Remus et al. 2019). The current study showed that the serum protein was increased according to the increase of SAAs proportion. Furthermore, maternal supplemented 51% Met group showed an improved serum ALB than in the other groups but not significantly. The 51% Met group also increased the concentration of Ca, I, and Mg in piglets' serum than in the other groups. These increases indicate that

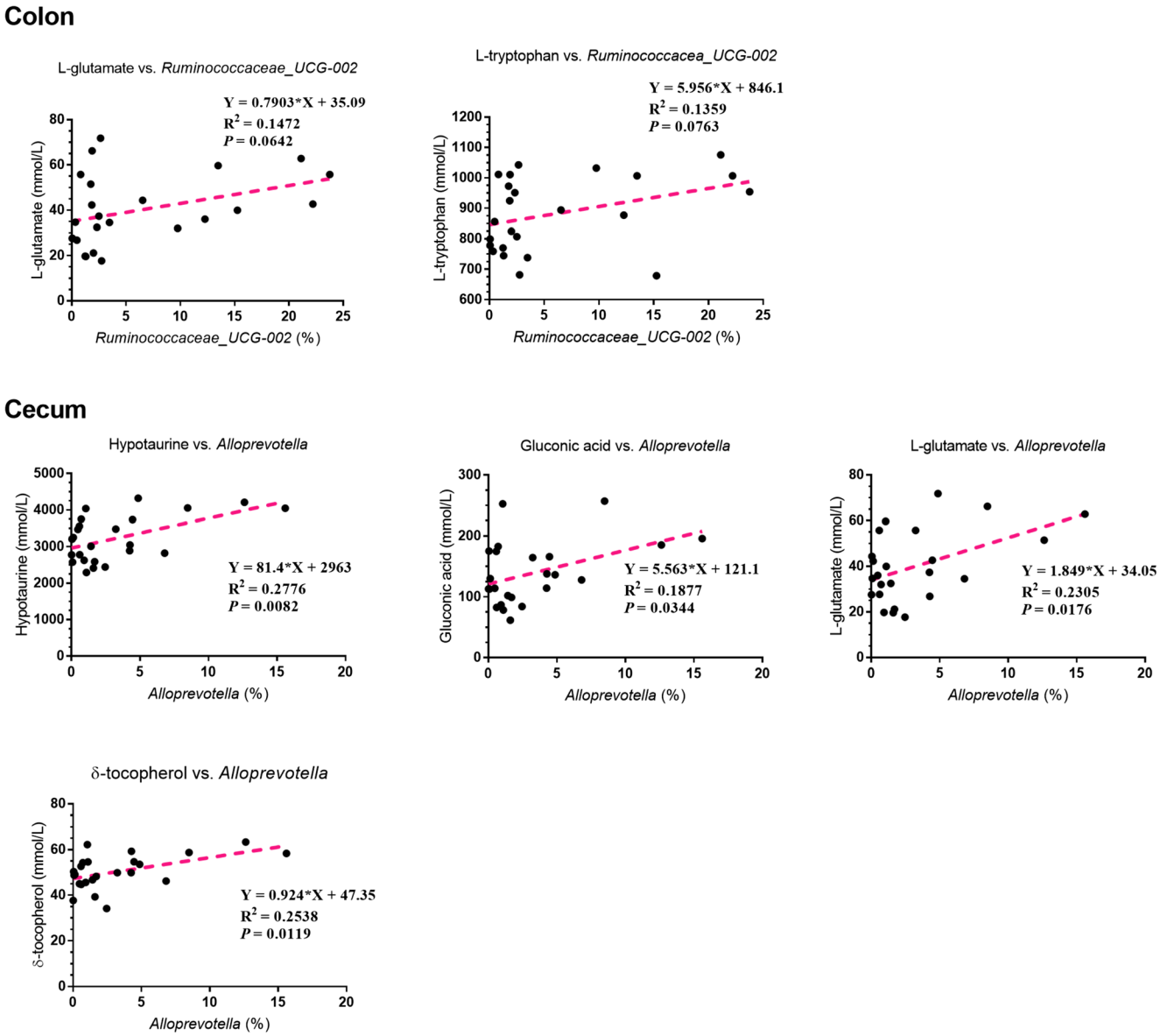


Fig. 7 Pearson's correlation analysis between piglets' plasma metabolite levels and the abundance of piglets' intestinal gut microbiota at the genus level ($n = 6$)

maternal dietary SAAs supplementation increases the mineral absorption during late pregnancy and lactation, which are most needed during the late gestation and lactation for maternal health and neonatal growth and development (da Silva et al. 2019; Kovacs 2016).

Metabolism is one of the most important expressions of the body's abrupt response to environmental disturbance that plays a crucial role in the biological ecosystem (Ding et al. 2019b). Plasma metabolite alteration has been shown to be possible biomarkers for different diseases (Gao et al. 2013). In the present study, the different proportion of maternal SAAs supplementation during late gestation to lactation was altered 233 metabolites in piglet's plasma. In comparison with the 46% Met group, the 51% Met and 56% Met groups increased the levels of hypotaurine, pyroglutamic acid, hippuric acid, retinoic acid, riboflavin, and δ-tocopherol. These improved levels of plasma metabolites benefit the offspring piglet's health. Studies have shown that a lower concentration of pyroglutamic acid may increase the risk of traumatic brain injury, mild cognitive impairment, and Alzheimer's disease (Trushina et al. 2013; Yi et al. 2016). In addition, pyroglutamic acid can protect protein degradation by CD26/dipeptidyl peptidase IV (Ding et al. 2019b). An intermediate product in the biosynthesis and antioxidant activity, hypotaurine, plays a crucial role in oxidative stress and acts as a protective agent under different physiological conditions (Fontana et al. 2004). An increased concentration of serum hypotaurine and taurine have also been found in inflammatory diseases, oxidative stress, and chronic kidney disease (Suliman et al. 2002; Zhang et al. 2015). Weaning is the most critical health-challenging stage for piglets. Several studies have been reported that the levels of vitamin E (tocopherol) and vitamin A (retinoic acid) plasma concentration dramatically reduced at/after weaning (Barbalho et al. 2019; Buchet et al. 2017; Lauridsen and Jensen 2005). Like other mammals, piglets cannot synthesize vitamin E and vitamin A and must be taken in from supplemented diet. Vitamin A and vitamin E play potential roles in embryonic growth and development, regulation of cell growth, antioxidant activity, immune regulation, and maintenance of the epithelial surface (Barbalho et al. 2019; Traber 2012). The present study showed that maternal SAAs supplementation during late gestation to lactation improved vitamin E and vitamin A concentrations in piglets' serum. Furthermore, the concentration of plasma riboflavin was also improved in the 51% Met, 56% Met, and 62% Met groups in comparison with the 46% Met group. Riboflavin (vitamin B_2) is one of the B vitamins that act as coenzymes for a number of enzymatic reactions and plays key metabolic functions for biological oxidation-reduction associated with energy production, antioxidant protection, and homocysteine metabolism (Thakur et al. 2017; Xin et al. 2017). Moreover, the addition of a different proportion of SAAs in the maternal diet also improved gluconic acid, L-tryptophan, pantothenic acid, indolelactic acid, and L-glutamate levels in piglet plasma, which have been found in related with improved growth, intestinal functions, and mucosal barrier functions in weaning piglets (Hou and Wu 2018; Liang et al. 2018; Sabui et al. 2018).

Gut microbiota colonization plays a crucial role in normal intestinal development, the establishment of gut homeostasis, and mucosal function (Ding et al. 2019a; Hooper and Macpherson 2010). Accumulating evidence indicates that maternal diet supplementation affects the newborn's gut microbiota structure (Azad et al. 2018a; Fukumori et al. 2019; Robertson et al. 2018). Bacteria *Firmicutes* and *Bacteroidetes* are the most abundant phyla in piglets' gut microbiota during the weaning transition (Chen et al. 2017). In our present study, *Firmicutes* and *Bacteroidetes* were the most abundant phyla in piglet's cecum, colon, and ileum. The endogenous infection may occur when the immune function of the body becomes imbalanced or the gut microbiota composition alters (Azad et al. 2018b). In the present study, a higher proportion of maternal SAAs supplementation (62% Met group) significantly increased the concentrations of *Proteobacteria* in piglet's colon and cecum. *Proteobacteria*, which consists of a variety of pathogens, such as *Escherichia, Salmonella, Vibrio,* and *Helicobacter*. In addition, 56% Met group increased the abundance of *Clostridium_sensu_stricto_1* in ileum, which may contribute to inflammation (Janowski et al. 2013).

In conclusion, the health of young animals is highly dependent upon maternal nutrition. An adequate maternal SAAs supplementation from late gestation to lactation may affect the offspring piglet's health. In our present study, the 51% Met group increased the piglets' serum ALB, Ca, and Mg, but the higher proportion of SAAs supplementation (62% Met group) decreased the level of ALB and Ca and associated with increased *Proteobacteria* abundance in colon and cecum. Furthermore, the young animal's growth and health may improve through the upregulation of plasma metabolites such as hypotaurine, pyroglutamic acid, and vitamins (A, B, and E). Therefore, the present study output provides a dose reference for future research into the different ratios of SAAs supplementation during late gestation and lactation.

Acknowledgments The authors acknowledge the CAS-TWAS President's Fellowship for international PhD students.

Funding information This study was supported by National Natural Science Foundation of China (No. 31772642, 31672457), International Partnership Program of Chinese Academy of Sciences (161343KYSB20160008), Hunan Provincial Science and Technology Department (2017NK2322, 2018CT5002, 2018WK4025), Local Science and Technology Development Project guided by The Central Government (YDZX20184300002303), Double first-class construction project of Hunan Agricultural University (SYL201802003, YB2018007, CX20190497), Project for Yunnan Yin Yulong Academician Workstation from Yunan Province (2018IC087), and China Postdoctoral Science Foundation (2018 M632963, 2019 T120705).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study was carried out in accordance with the guidelines of the Laboratory Animal Ethical Commission of the Chinese Academy of Science and approved by the Animal Care Committee of the Institute of Subtropical Agriculture (201703-64C), Chinese Academy of Science, Changsha, China.

References

Aiello SE (2016) Serum proteins and the dysproteinmias. The Merk veterinary manual. Merk & Co. Inc, Kenilworth, pp 3173–3174

Azad MAK, Bin P, Liu G, Fang J, Li T, Yin Y (2018a) Effects of different methionine levels on offspring piglets during late gestation and lactation. Food Funct 9(11):5843–5854. https://doi.org/10.1039/c8fo01343h

Azad MAK, Sarker M, Li T, Yin J (2018b) Probiotic species in the modulation of gut microbiota: an overview. Biomed Res Int 2018: 9478630. https://doi.org/10.1155/2018/9478630

Barbalho SM, Goulart RA, Batista G (2019) Vitamin A and inflammatory bowel diseases: from cellular studies and animal models to human disease. Expert Rev Gastroenterol Hepatol 13(1):25–35. https://doi.org/10.1080/17474124.2019.1543588

Buchet A, Belloc C, Leblanc-Maridor M, Merlot E (2017) Effects of age and weaning conditions on blood indicators of oxidative status in pigs. PLoS One 12(5):e0178487. https://doi.org/10.1371/journal.pone.0178487

Chen L, Xu Y, Chen X, Fang C, Zhao L, Chen F (2017) The maturing development of gut microbiota in commercial piglets during the weaning transition. Front Microbiol 8:1688. https://doi.org/10.3389/fmicb.2017.01688

da Silva BP, Toledo RCL, Grancieri M, Moreira MEC, Medina NR, Silva RR, Costa NMB, Martino HSD (2019) Effects of chia (*Salvia hispanica* L.) on calcium bioavailability and inflammation in Wistar rats. Food Res Int 116:592–599. https://doi.org/10.1016/j.foodres.2018.08.078

Dallanora D, Marcon J, Walter MP, Biondo N, Bernardi ML, Wentz I, Bortolozzo FP (2017) Effect of dietary amino acid supplementation during gestation on placental efficiency and litter birth weight in gestating gilts. Livest Sci 197:30–35. https://doi.org/10.1016/j.livsci.2017.01.005

De Feo P, Horber FF, Haymnd MW (1992) Meal stimulation of albumin synthesis: a significant contributor to whole body protein synthesis in humans. Am J Physiol Endocrinol Metab 263:E794–E799. https://doi.org/10.1152/ajpendo.1992.263.4.E794

Ding S, Azad MAK, Fang J, Zhou X, Xu K, Yin Y, Liu G (2019a) Impact of sulfur-containing amino acids on the plasma metabolomics and intestinal microflora of the sow in late pregnancy. Food Funct 10: 5910. https://doi.org/10.1039/c9fo01456j

Ding S, Fang J, Liu G, Veeramuthu D, Naif Abdullah AD, Yin Y (2019b) The impact of different levels of cysteine on the plasma metabolomics and intestinal microflora of sows from late pregnancy to lactation. Food Funct 10(2):691–702. https://doi.org/10.1039/c8fo01838c

Fang Z, Yao K, Zhang X, Zhao S, Sun Z, Tian G, Yu B, Lin Y, Zhu B, Jia G, Zhang K, Chen D, Wu D (2010) Nutrition and health relevant regulation of intestinal sulfur amino acid metabolism. Amino Acids 39(3):633–640. https://doi.org/10.1007/s00726-010-0502-x

Fontana M, Pecci L, Duprè S, Cavallini DJNR (2004) Antioxidant properties of sulfinates: protective effect of hypotaurine on peroxynitrite-dependent damage. Neurochem Res 29(1):111–116. https://doi.org/10.1023/B:NERE.0000010439.99991.cf

Frese SA, Parker K, Calvert CC, Mills DA (2015) Diet shapes the gut microbiome of pigs during nursing and weaning. Microbiome 3:28. https://doi.org/10.1186/s40168-015-0091-8

Fukumori C, Casaro MB, Thomas AM, Mendes E, Ribeiro WR, Crisma AR, Murata GM, Bizzarro B, Dias-Neto E, Setubal JC, Oliveira MA, Tavares-de-Lima W, Curi R, Bordin S, Sartorelli P, Ferreira CM (2019) Maternal supplementation with a synbiotic has distinct outcomes on offspring gut microbiota formation in A/J and C57BL/6 mice, differentially affecting airway inflammatory cell infiltration and mucus production. J Funct Foods 61. https://doi.org/10.1016/j.jff.2019.103496

Gao J, Yang H, Chen J, Fang J, Chen C, Liang R, Yang G, Wu H, Wu C, Li S (2013) Analysis of serum metabolites for the discovery of amino acid biomarkers and the effect of galangin on cerebral ischemia. Mol BioSyst 9(9):2311–2321. https://doi.org/10.1039/c3mb70040b

Garlick PJ (2006) Toxicity of methionine in humans. Nutr J 136(6): 1722S–1725S. https://doi.org/10.1093/jn/136.6.1722S

Herring CM, Bazer FW, Johnson GA, Wu G (2018) Impacts of maternal dietary protein intake on fetal survival, growth, and development. Exp Biol Med (Maywood) 243(6):525–533. https://doi.org/10.1177/1535370218758275

Hooper LV, Macpherson AJ (2010) Immune adaptations that maintain homeostasis with the intestinal microbiota. Nat Rev Immunol 10: 159–169. https://doi.org/10.1038/nri2710

Hou Y, Wu G (2018) L-glutamate nutrition and metabolism in swine. Amino Acids 50(11):1497–1510. https://doi.org/10.1007/s00726-018-2634-3

Hsu CN, Tain YL (2019) The good, the bad, and the ugly of pregnancy nutrients and developmental programming of adult disease. Nutrients 11(4). https://doi.org/10.3390/nu11040894

Huang C, Chiba LI, Magee WE, Wang Y, Griffing DA, Torres IM, Rodning SP, Bratcher CL, Bergen WG, Spangler EA (2019) Effect of flaxseed oil, animal fat, and vitamin E supplementation on growth performance, serum metabolites, and carcass characteristics of finisher pigs, and physical characteristics of pork. Livest Sci 220:143–151. https://doi.org/10.1016/j.livsci.2018.11.011

Isaacson R, Kim HB (2012) The intestinal microbiome of the pig. Anim Health Res Rev 13(1):100–109. https://doi.org/10.1017/S1466252312000084

Janowski A, Kolb R, Zhang W, Sutterwala F (2013) Beneficial and detrimental roles of NLRs in carcinogenesis. Front Immunol 4(370). https://doi.org/10.3389/fimmu.2013.00370

Kalhan SC (2016) One carbon metabolism in pregnancy: impact on maternal, fetal and neonatal health. Mol Cell Endocrinol 435:48–60. https://doi.org/10.1016/j.mce.2016.06.006

Kovacs CS (2016) Maternal mineral and bone metabolism during pregnancy, lactation, and post-weaning recovery. Physiol Rev 96(2): 449–547. https://doi.org/10.1152/physrev.00027.2015

Lauridsen C, Jensen SK (2005) Influence of supplementation of all-rac-α-tocopheryl acetate preweaning and vitamin C postweaning on α-tocopherol and immune responses of piglets. Anim Sci J 83(6): 1274–1286. https://doi.org/10.2527/2005.8361274x

Li Y, Guo Y, Wen Z, Jiang X, Ma X, Han X (2018) Weaning stress perturbs gut microbiome and its metabolic profile in piglets. Sci Rep 8(1):18068. https://doi.org/10.1038/s41598-018-33649-8

Liang H, Dai Z, Kou J, Sun K, Chen J, Yang Y, Wu G, Wu Z (2018) Dietary L-tryptophan supplementation enhances the intestinal mucosal barrier function in weaned piglets: implication of tryptophan-metabolizing microbiota. Int J Mol Sci 20(1). https://doi.org/10.3390/ijms20010020

Liu D, Zong EY, Huang PF, Yang HS, Yan SL, Li JZ, Li YL, Ding XQ, He SP, Xiong X, Yin YL (2019) The effects of dietary sulfur amino acids on serum biochemical variables, mucosal amino acid profiles,

and intestinal inflammation in weaning piglets. Livest Sci 220:32–36. https://doi.org/10.1016/j.livsci.2018.12.013

MacKay DS, Brophy JD, McBreairty LE, McGowan RA, Bertolo RF (2012) Intrauterine growth restriction leads to changes in sulfur amino acid metabolism, but not global DNA methylation, in Yucatan miniature piglets. J Nutr Biochem 23(9):1121–1127. https://doi.org/10.1016/j.jnutbio.2011.06.005

Martin Agnoux A, Antignac JP, Boquien CY, David A, Desnots E, Ferchaud-Roucher V, Darmaun D, Parnet P, Alexandre-Gouabau MC (2015) Perinatal protein restriction affects milk free amino acid and fatty acid profile in lactating rats: potential role on pup growth and metabolic status. J Nutr Biochem 26(7):784–795. https://doi.org/10.1016/j.jnutbio.2015.02.012

Mennitti LV, Oliveira JL, Morais CA, Estadella D, Oyama LM, Oller do Nascimento CM, Pisani LP (2015) Type of fatty acids in maternal diets during pregnancy and/or lactation and metabolic consequences of the offspring. J Nutr Biochem 26(2):99–111. https://doi.org/10.1016/j.jnutbio.2014.10.001

NRC (2012) Nutrient requirements of swine: eleventh revised edition. The National Academies Press, Washington, DC. https://doi.org/10.17226/13298

Remus A, Hauschild L, Corrent E, Letourneau-Montminy MP, Pomar C (2019) Pigs receiving daily tailored diets using precision-feeding techniques have different threonine requirements than pigs fed in conventional phase-feeding systems. J Anim Sci Biotechnol 10:16. https://doi.org/10.1186/s40104-019-0328-7

Robertson RC, Kaliannan K, Strain CR, Ross RP, Stanton C, Kang JX (2018) Maternal omega-3 fatty acids regulate offspring obesity through persistent modulation of gut microbiota. Microbiome 6(1):95. https://doi.org/10.1186/s40168-018-0476-6

Sabui S, Kapadia R, Ghosal A, Schneider M, Lambrecht NWG, Said HM (2018) Biotin and pantothenic acid oversupplementation to conditional SLC5A6 KO mice prevents the development of intestinal mucosal abnormalities and growth defects. Am J Physiol Cell Physiol 315(1):C73–C79. https://doi.org/10.1152/ajpcell.00319.2017

Shoveller AK, Stoll B, Ball RO, Burrin DG (2005) Nutritional and functional importance of intestinal sulfur amino acid metabolism. Nutr J 135(7):1609–1612. https://doi.org/10.1093/jn/135.7.1609

Stipanuk MH, Dominy JE Jr, Lee J-I, Coloso RM (2006) Mammalian cysteine metabolism: new insights into regulation of cysteine metabolism. Nutr J 136(6):1652S–1659S. https://doi.org/10.1093/jn/136.6.1652S

Suliman ME, Bárány P, Filho JCD, Lindholm B, Bergström J (2002) Accumulation of taurine in patients with renal failure. Nephrol Dial Transplant 17(3):528–529. https://doi.org/10.1093/ndt/17.3.528

Tesseraud S, Metayer Coustard S, Collin A, Seiliez I (2009) Role of sulfur amino acids in controlling nutrient metabolism and cell functions: implications for nutrition. Br J Nutr 101(8):1132–1139. https://doi.org/10.1017/S0007114508159025

Thakur K, Tomar SK, Singh AK, Mandal S, Arora S (2017) Riboflavin and health: a review of recent human research. Crit Rev Food Sci Nutr 57(17):3650–3660. https://doi.org/10.1080/10408398.2016.1145104

Townsend DM, Tew KD, Tapiero H (2004) Sulfur containing amino acids and human disease. Biomed Pharmacother 58(1):47–55. https://doi.org/10.1016/j.biopha.2003.11.005

Traber MG (2012) Vitamin E, vol 11th Ed. Lippincott Williams & Wilkins, Baltimore

Trushina E, Dutta T, Persson XM, Mielke MM, Petersen RC (2013) Identification of altered metabolic pathways in plasma and CSF in mild cognitive impairment and Alzheimer's disease using metabolomics. PLoS One 8(5):e63644. https://doi.org/10.1371/journal.pone.0063644

Uruakpa FO, Ismond MAH, Akobundu ENT (2002) Colostrum and its benefits: a review. Nutr Res 22(6):755–767. https://doi.org/10.1016/S0271-5317(02)00373-1

van de Poll MCG, Dejong CHC, Soeters PB (2006) Adequate range for sulfur-containing amino acids and biomarkers for their excess: lessons from enteral and parenteral nutrition. Nutr J 136(6):1694S–1700S. https://doi.org/10.1093/jn/136.6.1694S

Verheyen AJM, Maes DGD, Mateusen B, Deprez P, Janssens GPJ, Ld L, Counotte G (2007) Serum biochemical reference values for gestating and lactating sows. Vet J 174(1):92–98. https://doi.org/10.1016/j.tvjl.2006.04.001

Wei H, Zhao X, Xia M, Tan C, Gao J, Htoo JK, Xu C, Peng J (2019) Different dietary methionine to lysine ratios in the lactation diet: effects on the performance of sows and their offspring and methionine metabolism in lactating sows. J Anim Sci Biotechnol 10:76–11. https://doi.org/10.1186/s40104-019-0373-2

Wu G (2016) Dietary protein intake and human health. Food Funct 7:1251–1265

Xin Z, Pu L, Gao W, Wang Y, Wei J, Shi T, Yao Z, Guo C (2017) Riboflavin deficiency induces a significant change in proteomic profiles in HepG2 cells. Sci Rep 7:45861. https://doi.org/10.1038/srep45861

Xu M, Che L, Gao K, Wang L, Yang X, Wen X, Jiang Z, Wu D (2019) Effects of dietary taurine supplementation to gilts during late gestation and lactation on offspring growth and oxidative stress. Animals (Basel) 9(5). https://doi.org/10.3390/ani9050220

Yi L, Shi S, Wang Y, Huang W, Xia ZA, Xing Z, Peng W, Wang Z (2016) Serum metabolic profiling reveals altered metabolic pathways in patients with post-traumatic cognitive impairments. Sci Rep 6:21320. https://doi.org/10.1038/srep21320

Zhang ZH, Wei F, Vaziri ND, Cheng XL, Bai X, Lin RC, Zhao YY (2015) Metabolomics insights into chronic kidney disease and modulatory effect of rhubarb against tubulointerstitial fibrosis. Sci Rep 5:14472. https://doi.org/10.1038/srep14472

Zhang S, Chen F, Zhang Y, Lv Y, Heng J, Min T, Li L, Guan W (2018) Recent progress of porcine milk components and mammary gland function. J Anim Sci Biotechnol 9:77–13. https://doi.org/10.1186/s40104-018-0291-8

Zhang S, Heng J, Song H, Zhang Y, Lin X, Tian M, Chen F, Guan W (2019) Role of maternal dietary protein and amino acids on fetal programming, early neonatal development, and lactation in swine. Animals (Basel) 9(1). https://doi.org/10.3390/ani9010019

Zhong H, Li H, Liu G, Wan H, Mercier Y, Zhang X, Lin Y, Che L, Xu S, Tang L, Tian G, Chen D, Wu FZ (2016) Increased maternal consumption of methionine as its hydroxyl analog promoted neonatal intestinal growth without compromising maternal energy homeostasis. J Anim Sci Biotechnol 7:46. https://doi.org/10.1186/s40104-016-0103-y