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The improvement of parturition duration by high intake of dietary fibre in late gestation is associated with gut microbiota and metabolome in sows

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Abstract

Prolonged parturition duration has been widely demonstrated to be a risk factor for incidence of stillbirth. This study evaluated the supply of dietary fibre on the parturition duration, gut microbiota and metabolome using sows as a model. A total of 40 Yorkshire sows were randomly given diet containing normal level of dietary fibre (NDF, 17·5 % dietary fibre) or high level of dietary fibre (HDF, 33·5 % dietary fibre). Faecal microbiota profiled with 16S rRNA amplicon sequencing, SCFA and metabolome in the faeces and plasma around parturition were compared between the dietary groups. Correlation analysis was conducted to further explore the potential associations between specific bacterial taxa and metabolites. Results showed that HDF diet significantly improved the parturition process as presented by the shorter parturition duration. HDF diet increased the abundance of the phyla Bacteroidetes and Synergistetes and multiple genera. Except for butyrate, SCFA levels in the faeces and plasma of sows at parturition were elevated in HDF group. The abundances of fifteen and twelve metabolites in the faeces and plasma, respectively, markedly differ between HDF and NDF sows. These metabolites are involved in energy metabolism and bacterial metabolism. Correlation analysis also showed associations between specific bacteria taxa and metabolites. Collectively, our study indicates that the improvement of parturition duration by high fibre intake in late gestation is associated with gut microbiota, production of SCFA and other metabolites, potentially serving for energy metabolism.

Key words: **Dietary fibre: Parturition duration: SCFA: The gut microbiota: Metabolomics**

Intensive genetic selection resulted in gradually increased litter size, thereby prolonged parturition[1]. It has been shown that stillbirth rate increases along with longer farrowing duration[2,3]. Of note, about 75 % of the stillbirths occurred during parturition process due to asphyxia[4]. For litter-bearing animals like sows, parturition duration can be rather long. Our previous study has reported that diet enriched with inulin, a fermentable fibre from roots, shortened parturition duration in a sow model[5]. Although several hypotheses have been proposed, such as reducing constipation or body fat deposition by high fibre intake[6], the underlying mechanisms of high fibre intake during gestation on improving parturition duration remain obscure.

The microbial residents of the gut is associated with various physiological aspects of host and largely affected by diet[7]. The intake of dietary fibre had been largely related to the change of composition and function of gut microbiota[8,9]. The symbiotic metabolites produced by gut microbiota have been proposed to play roles in gut microbiota affecting host physiology[10]. Dietary fibres, including NSP, oligosaccharides and resistant starches, are microbiota-accessible carbohydrates and fermented by gut microbiota to produce various symbiotic metabolites[10,11]. SCFA are one major type of symbiotic metabolites, which decrease the pH in the gut lumen to suppress the pathogenic bacterial, and serving as an energy substrate of the host[12,13]. To our knowledge, however, it is unknown that the gut microbiota and symbiotic metabolites, such as SCFA, are involved in the effect of dietary fibre on parturition duration.

In this study, therefore, we aimed to assess whether the improved parturition duration by high intake of dietary fibre is associated with the gut microbiota and related symbiotic metabolites, using sows as a model. The gut microbiota of sows given different level of dietary fibre was profiled by 16S rRNA amplicon

Abbreviations: HDF, high dietary fibre diet; NDF, normal dietary fibre diet.

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sequencing. Levels of SCFA in both faeces and plasma were quantified, and metabolome was profiled by untargeted metabolomics. Abundance of these metabolites and bacterial taxa were correlated to explore potential association.

Materials and methods

All institutional and national guidelines for the care and use of laboratory animals were followed. The animal experiment was approved by the Animal Care and Use Committee of the Sichuan Agricultural University (DKY-B20121602) and was performed in accordance with the National Research Council's Guidelines for Care and Use of Laboratory Animals.

Animals and dietary intervention

A total of forty Yorkshire sows with similar parity and body weight were randomly allocated into two dietary treatments (twenty sows per treatment) from day 90 of gestation (G90) to parturition. The diets were formulated based on maize–soyabean meal to meet or exceed the nutrient requirements recommended by NRC (2012), as shown in Table S1: normal dietary fibre diet (NDF, 17·5 % total dietary fibre) and high dietary fibre diet (HDF, 33·5% total dietary fibre). The daily gross energy and crude protein intake were comparable between dietary treatments by adjusting daily feed intake (3·0 kg/d in NDF group and 3·2 kg/d in HDF group). Sows in the NDF group consumed dietary fibre at 485 g/d, while sows in the HDF group consumed 964 g/d. All sows were housed in gestation stalls until moved to farrowing room on G110. The body weight and backfat thickness of sows were recorded on G110. The temperature in the farrowing room was kept between 20°C and 22°C. Three sows from NDF group and four sows from HDF group were eliminated due to illness and abnormal litter size (total piglets born is less than 4) and were excluded from analysis. Therefore the final count for sows was seventeen in NDF group and sixteen in HDF group. The litter size was recorded at parturition and did not markedly differ between two groups (data not shown). Parturition duration was defined as the time elapsed from birth of the first-born to the last-born piglet in the litter[14] and was recorded accurately during parturition process by timer.

Sample collection

Blood samples (n 9) of sows were collected from the ear vein into Na-heparinised tubes at 1200 h on G107 and at the onset of parturition. Blood samples were centrifuged at 3000 rpm for 15 min, and plasma was harvested and stored immediately at −20°C for later analysis. Fresh faecal samples of sows were collected into sterile cryopreservation tube on G110 and stored immediately in liquid N_2 for later analysis.

Faecal microbiota

Genomic DNA was extracted from faecal samples using Mo Bio Power Faecal DNA Isolation Kit (Mo BIO). The v4 hypervariable regions of 16S rRNA was amplified using primers 515F and 806R, and the amplicon pyrosequencing was carried out on an Illumina HiSeq PE250 platform (Illumina). Raw data were processed with RRR pipeline-based UPARSE (version 7.0.1001). Ribosomal Database Project classifier (version 2.2) was used to assign taxonomic rank. Operational taxonomic units (OTUs) were clustered at 97 % sequence identity (sequences with ≥ 97 % similarity were assigned to the same OTU). OTU and sample information were imported into R software interfaced with R Studio for further analysis using the Phyloseq and VEGAN packages. Bray–Curtis and unifrac dissimilarities were calculated to present the β-diversity and displayed by Non-metric Multi-dimensional Scaling (NMDS), and difference between the dietary groups was tested by Permutational Multivariate Analysis of Variance (PERMANOVA) test with permutation 1000 times.

Quantification of SCFA

Concentrations of SCFA (acetate, propionate, butyrate, isobutyrate, valerate and isovalerate) and sum of them (total SCFA) in faecal and plasma samples were determined as previously described[15], using GC (Varian CP-3800, manual injection, flame ionisation detector, 10-μl micro-injector). Briefly, supernatant obtained from faecal suspension (0·7 g of faecal matter in 1·5 ml of water) or plasma was mixed with 25 % metaphosphoric acid and crotonic acid solution (210 mmol/l for faecal samples and 21 mmol/l for plasma samples) and centrifuged at 12 000 rpm for 10 min. Then, the supernatant was mixed with methanol (1:3 dilution), centrifuged at 12 000 rpm for 15 min and filtered by 0·22-μm filter (Millipore) before being manually applied onto a gas chromatographer with flame ionisation detector for quantification.

Faecal and plasma metabolomics

Faecal and plasma samples were thawed and mixed with cold methanol/acetonitrile (1:1, v/v) to remove the protein. The supernatant was dried and re-dissolved in 50 % acetonitrile before being applied onto a ultra high performance liquid chromatography (Infinity 1290, Agilent) coupled to a quadrupole time-of-flight mass spectrometer (qTOF MS, 6550, Agilent) for analysis in random order within the same type of samples. ACQUITY BEH amide column (2·1 mm × 100 mm, 1·7 μm, Waters) was used as the separation column and the mobile phase was mixture of solvent A (25 mmol/l $CH._3COONH_4$ and 25 mmol/l NH_4OH) and B (acetonitrile). The 12-min elution gradient was starting with 95 % solvent B (acetonitrile) for 0·5 min, decreasing to 60 % of B in 6·5 min in linear fashion, further to 40 % of B in 1 min, holding for 1 min, changing back to 95 % of B in 0·1 min, then holding for 2·9 min for re-equilibration. To assist the chemical assignment, pooled sample was analysed on a TripleTOF MS (AB Sciex 6600) to obtain MS and MS/MS data in an information-dependent acquisition mode. Obtained data were used for compound assignment against an in-house database established with available authentic standards.

Statistical analysis

The variance homogeneity and normality of data for body weight, backfat thickness, parity, gestation length, parturition duration and SCFA concentrations were evaluated by the

British Journal of Nutrition

Shapiro–Wilk test and Levene's test procedures of SAS 9.4 (SAS Institute, Inc.), respectively. Then, these data were analysed using linear model against the dietary groups. Difference with $P < 0{\cdot}05$ was regarded as significant.

For gut microbiota, difference in α-diversity was presented by Shannon index, and differential abundance between the dietary groups of specific genus or phylum was tested by the non-parametric Wilcoxon rank-sum test. *P*-values indicating significance of difference at genus level were further adjusted by a two-stage Benjamini and Hochberg (TSBH) step-up false discovery rate (FDR)-controlling procedure with type I error rate (α) set to 0·20 using the mt.rawp2adjp function in multcomp package. *P*-values of phyla were not adjusted as there were less than twenty tests conducted. Effect size was also calculated using equation, effect size $= \frac{Z}{\sqrt{N}}$, in which Z is the Wilcoxon Z and N is the number of samples.

Raw LC-MS data for untargeted metabolomics were converted into MzXML format using MSconvert (version 3.0.6458, ProteoWizard, Palo Alto), then imported into XCMS (version, Scripps Center) for data pre-processing. Processed data were annotated with chemical compound ID as aforementioned, then imported into R software integrated with R studio for abundance analysis. All the identified ions in the faeces or plasma were combined together and applied to principal component analysis. Each MS feature was fitted to a linear model using *lm* function with treatment (NDF *v.* HDF) as the predictor. *P*-value for the significance of diet was generated by comparing these two models with *anova* function. *P*-values were further adjusted within each data set (faeces or plasma) by a two-staged TSBH FDR procedure as described above. Adjusted *P*-value less than 0·05 was the threshold of significance.

To explore the associations between composition of gut microbiota and abundance of metabolites in faeces or plasma, Spearman's correlation was applied to the microbiota data and individual SCFA data, whilst unsupervised regularised canonical correlation analysis with the shrinkage method was performed on the microbiota and metabolomic data across the dietary groups using the mixOmics package. Correlations were shown as heatmap with either Spearman's r or the regularised canonical correlation analysis similarity scores. Pearson's correlation analyses were applied to further explore potential associations between SCFA concentration and plasma glucose concentration and parturition duration of sows. The data for plasma glucose concentration have been published in our recent paper[16] and were used for this correlation analysis.

Results

Body condition, parity and gestation length

As shown in Table 1, the body weight and backfat thickness on G110, parity and gestation length did not markedly differ between NDF and HDF sows ($P > 0{\cdot}05$).

Parturition duration and mean birth interval

As shown in Fig. 1(a), sows fed NDF diet had longer parturition duration than sows fed HDF diet (303 min *v.* 229 min in average, $P < 0{\cdot}05$). Additionally, the newborn piglets from HDF sows had significantly lower mean birth interval ($P < 0{\cdot}05$).

Table 1. Body condition, parity and gestation length between NDF and HDF sows
(Mean values with the standard errors of the mean)

	NDF		HDF		
	Mean	SEM	Mean	SEM	*P*
Body weight on G110 (kg)	285·37	4·61	287·00	4·07	0·80
Backfat thickness on G110 (mm)	15·00	0·45	15·25	0·37	0·67
Parity	4·65	0·21	4·81	0·21	0·58
Gestation length (day)	115·2	1·01	114·7	0·96	0·22

NDF, normal dietary fibre diet; HDF, high dietary fibre diet.
n 17 for NDF group and *n* 16 for HDF group.

Faecal microbiota

The microbiota data cover 16 phyla and were further divided into 114 genera. No significant difference was found between HDF and NDF sows in α-diversity shown as Shannon index (Fig. 2(a)) or in β-diversity shown by Bray–Curtis and unifrac dissimilarity at genus level (Fig. 2(b) and (c)). Firmicutes was the most abundant phylum, followed by Bacteroidetes, Proteobacteria, Spirochaetes and Euryarchaeota. Relative abundance of phylum Firmicutes ($P < 0{\cdot}05$) and Actinobacteria ($P = 0{\cdot}09$) decreased in HDF sows, relative to NDF sows, while that of Bacteroidetes, Synergistetes (both *P*s $< 0{\cdot}05$) and Elusimicrobia ($P = 0{\cdot}07$) increased (online Supplementary Table S2). *Lactobacillus* was the most abundant genus with a known ID, followed by *Streptococcus*, *Methanobrevibacter*, *Parabacteroides* and *Oscillospira*. Multiple genera were found with higher abundance in HDF sows (12/18) (Fig. 2(d)). Information of these genera, including genus name, *P*-value, adjusted *P* and effect size, is listed in Table S3. However, none of these significance retained after the FDR correction, but most of them had relatively large effect size (all $> 0{\cdot}5$, online Supplementary Table S3).

Faecal and plasma SCFA concentration

In both faecal samples of sows on G110 and plasma samples of sows at parturition, levels of total SCFA, acetate, propionate, isobutyrate ($P = 0{\cdot}06$ in plasma), v.alerate and isovalerate ($P = 0{\cdot}07$ in faeces) were higher in HDF sows than those in NDF ones (all *P*s $< 0{\cdot}05$ unless otherwise stated). No significant difference in level of butyrate was found between HDF and NDF sows in either faecal or plasma samples ($P > 0{\cdot}05$). In plasma samples of sows on G107, levels of isobutyric acid ($P = 0{\cdot}09$) and valeric acid ($P < 0{\cdot}05$) were higher in HDF sows than those in NDF ones, whereas no significant difference in level of total SCFA, acetic acid, propionic acid, butyric acid and isovaleric acid were found between dietary treatments (Table 2).

Faecal and plasma metabolomics

In total, 186 metabolites in positive mode and 170 in negative mode were annotated in faeces, while 217 (positive) and 179 (negative) metabolites were found in plasma, respectively. Among these metabolites, fifteen metabolites in faeces and twelve in plasma were found with significant difference (adjusted

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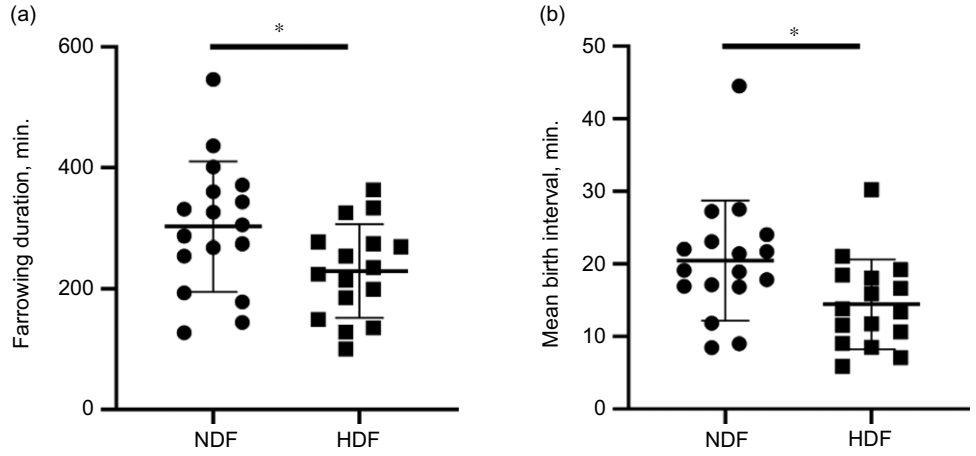

Fig. 1. Parturition duration (a) and mean birth interval (b) between NDF and HDF sows. *$P < 0{\cdot}05$. *n* 17 for NDF group and *n* 16 for HDF group. NDF, normal dietary fibre diet; HDF, high dietary fibre diet.

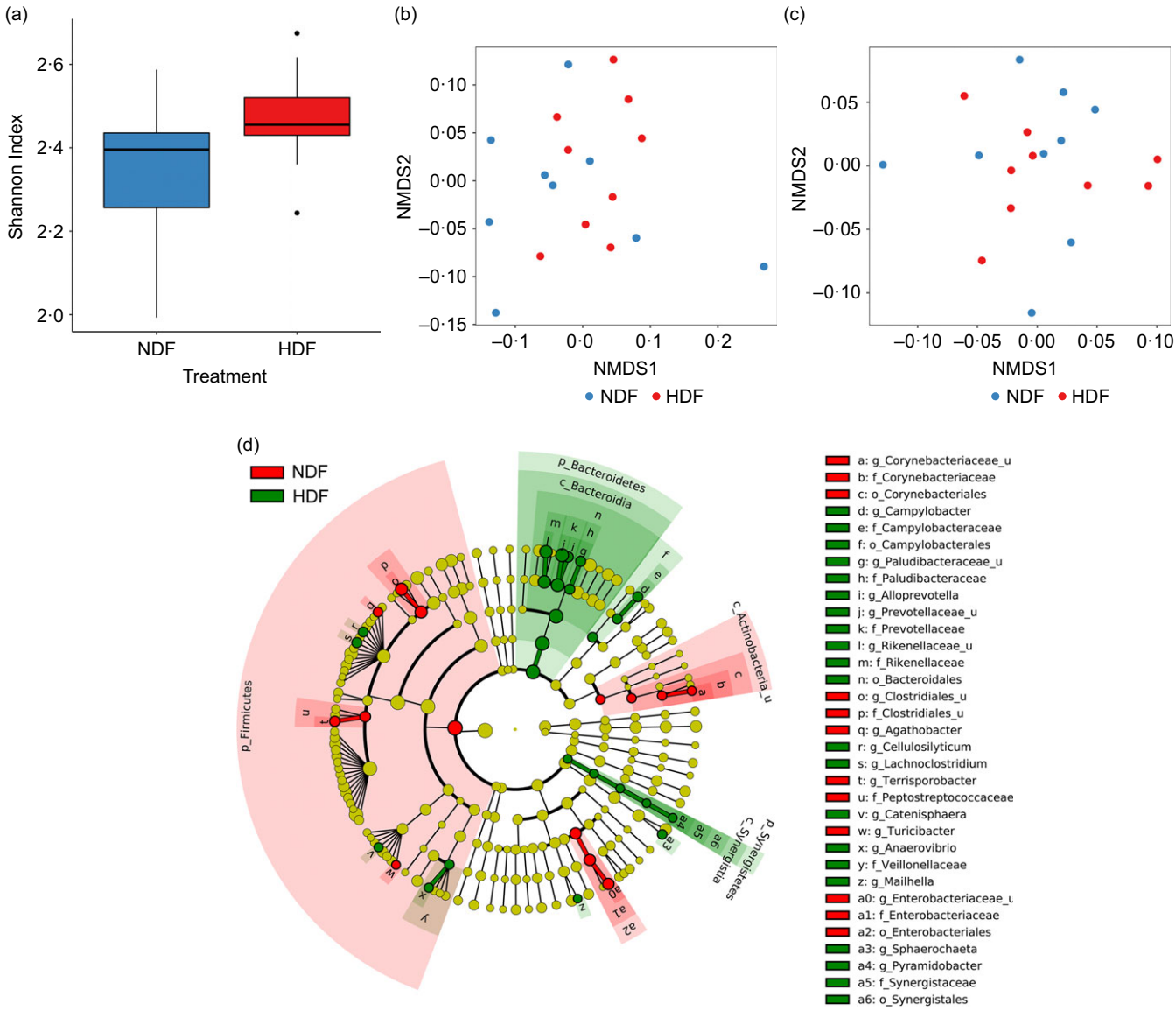

Fig. 2. α-diversity (a), β-diversity using Bray–Curtis distance (b) and unifrac distance (c) of the gut microbiota, and taxa with differential abundance between HDF and NDF sows (d). NDF, normal dietary fibre diet; HDF, high dietary fibre diet.

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Table 2. Faecal and plasma SCFA concentrations between NDF and HDF sows
(Mean values with the standard errors of the mean, *n* 9 for each group)

	Faeces					Plasma on G107					Plasma at parturition				
	NDF		HDF			NDF		HDF			NDF		HDF		
	Mean	SEM	Mean	SEM	*P*	Mean	SEM	Mean	SEM	*P*	Mean	SEM	Mean	SEM	*P*
Acetate	42·6	3·4	53·0	1·2	0·03	323·0	16·5	367·9	21·8	0·42	204·1	12·1	267·9	16·3	0·01
Propionate	14·9	1·3	18·5	0·8	0·04	11·1	1·0	14·3	1·9	0·16	18·9	1·4	29·2	3·8	0·02
Butyrate	7·1	0·6	7·9	0·3	0·20	11·9	1·4	11·7	1·6	0·92	8·2	1·3	10·1	1·1	0·29
Isobutyrate	2·1	0·1	2·5	0·1	0·02	1·4	0·1	1·8	0·2	0·09	1·8	0·2	2·5	0·3	0·06
Valerate	1·3	0·1	1·5	0·1	0·04	9·6	0·5	12·1	0·8	0·02	25·0	2·2	34·9	2·8	0·01
Isovalerate	2·4	0·1	2·7	0·1	0·07	5·2	0·4	5·4	0·4	0·73	4·8	0·3	7·4	0·9	0·01
Total SCFA	70·4	5·3	86·1	2·0	0·03	365·0	17·6	414·2	20·9	0·38	262·7	13·0	354·2	24·5	< 0·01

NDF, normal dietary fibre diet; HDF, high dietary fibre diet.
Faecal data in μmol/g, plasma data in μmol/l. Total SCFA = the sum of acetate, propionate, butyrate, isobutyrate, valerate and isovalerate.

Table 3. Faecal and plasma metabolites with differential abundance between NDF and HDF sows
(Mean values with the standard errors of the mean)

				Abundance				
				NDF		HDF		
	Ion	RT (min)	M/Z	Mean	SEM	Mean	SEM	Fold change
Faecal metabolites								
Saccharin	[M-H]-	0·53	181·9931	851·5	155·2	230·3	85·8	0·27
Arachidic acid	[M-H]-	0·65	311·2948	6·7	1·1	19·3	2·0	2·90
Norharmane	[M + H]+	0·74	169·0744	6·2	0·6	9·7	0·6	1·57
Sphinganine	[M + H]+	1·96	302·3046	10·6	0·9	6·7	0·8	0·63
3b-Hydroxy-5-cholenoic acid	[M + CH3COO]-	2·28	433·2956	39·1	2·9	24·3	3·3	0·62
Ile-Leu	[M + H]+	2·81	245·1849	15·1	2·1	8·5	0·7	0·56
LysoPC (14:0)	[M + H]+	2·96	468·3104	27·2	2·6	38·4	2·7	1·41
LysoPE (16:0)	[M + H]+	3·00	454·2950	125·9	14·2	178·7	9·9	1·42
Jasmonic acid	[M + H]+	3·13	211·1325	27·7	1·5	22·4	0·5	0·81
Perillyl alcohol	[M + H-H2O]+	3·87	135·1153	20·1	0·6	14·5	0·4	0·72
Pro-Ala	[M + NH4]+	3·88	204·1333	0·6	0·1	2·6	0·4	4·10
Hydroxyproline	[M + H-2H2O]+	4·51	96·0427	1·9	0·2	3·4	0·1	1·76
Pelletierine	[M + CH3COO + 2H]+	5·69	202·1427	8·5	0·6	5·3	0·2	0·63
3,3-Dimethylacrylic acid	[2M + NH4]+	6·46	218·1382	12·6	1·1	25·4	0·7	2·01
Vincamine	[M + H]+	6·50	355·1980	67·1	6·2	38·8	2·0	0·58
Plasma metabolites								
Quinone	[M + H]+	0·50	109·0271	1·4	0·1	3·5	0·5	2·49
2,3-Dihydroxy-3-methylbutyric acid	[M-H]-	1·82	133·0489	35·4	3·9	146·2	16·4	4·13
2'-Deoxy-ribose	[2M-H]-	2·48	267·1065	1·6	0·2	4·0	0·4	2·56
LysoPC (18:0)	[M-H + 2Na]+	2·58	568·3359	18·3	1·1	29·3	2·2	1·60
Anthranilic acid	[M + H]+	3·50	138·0535	4·1	0·3	19·4	1·7	4·79
His-Gln	[M-H + 2Na]+	4·03	328·1005	1·5	0·1	3·9	0·5	2·56
Propionate	[M + CH3COO]-	4·29	133·0489	8·6	0·4	14·1	0·7	1·64
Betaine	[M + H]+	4·35	118·0856	12 690·4	306·3	16 365·2	607·2	1·29
Dimethylglycine	[M + H]+	4·81	104·0696	8·9	0·5	15·6	0·7	1·76
Lys-Pro	[M + H]+	8·13	244·1641	0·8	0·0	1·9	0·1	2·28
Pro-Asn	[M + NH4]+	8·22	247·1386	1·7	0·0	2·2	0·1	1·28
Arg	[M + H-H2O]+	8·49	157·1068	4·1	0·2	5·6	0·3	1·36

NDF, normal dietary fibre diet; HDF, high dietary fibre diet; RT, retention time; M/Z, mass-to-charge ratio.
Results of abundance are shown as mean ± SEM.
Fold change refers to abundance (HDF)/abundance (NDF).

$P < 0·05$) between NDF and HDF sows (Table 3). Principal component analysis score plots of faecal and plasma metabolome are shown in Fig. 3. Tendency of separation in both score plots indicates difference between the two groups in both faecal and plasma metabolome. While only nearly half of the faecal metabolites (7/15) showed higher abundance in HDF sows, levels of all identified plasma metabolites were higher in the HDF sows.

Correlation

Associations between abundance of specific taxa and SCFA in faeces and plasma by Spearman's correlation analysis, and that metabolites in faeces and plasma by regularised canonical correlation analysis are shown as heatmaps in Figs. 4, 5 and 6, respectively. Across the diet groups, multiple taxa were correlated with SCFA in abundance in faeces

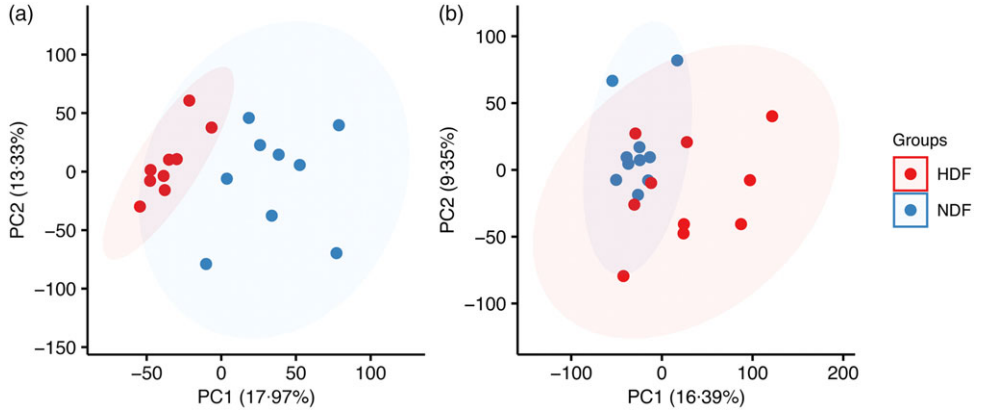

Fig. 3. PCA score plots of faecal (a) and plasma (b) metabolome. PCA, principal component analysis; NDF, normal dietary fibre diet; HDF, high dietary fibre diet.

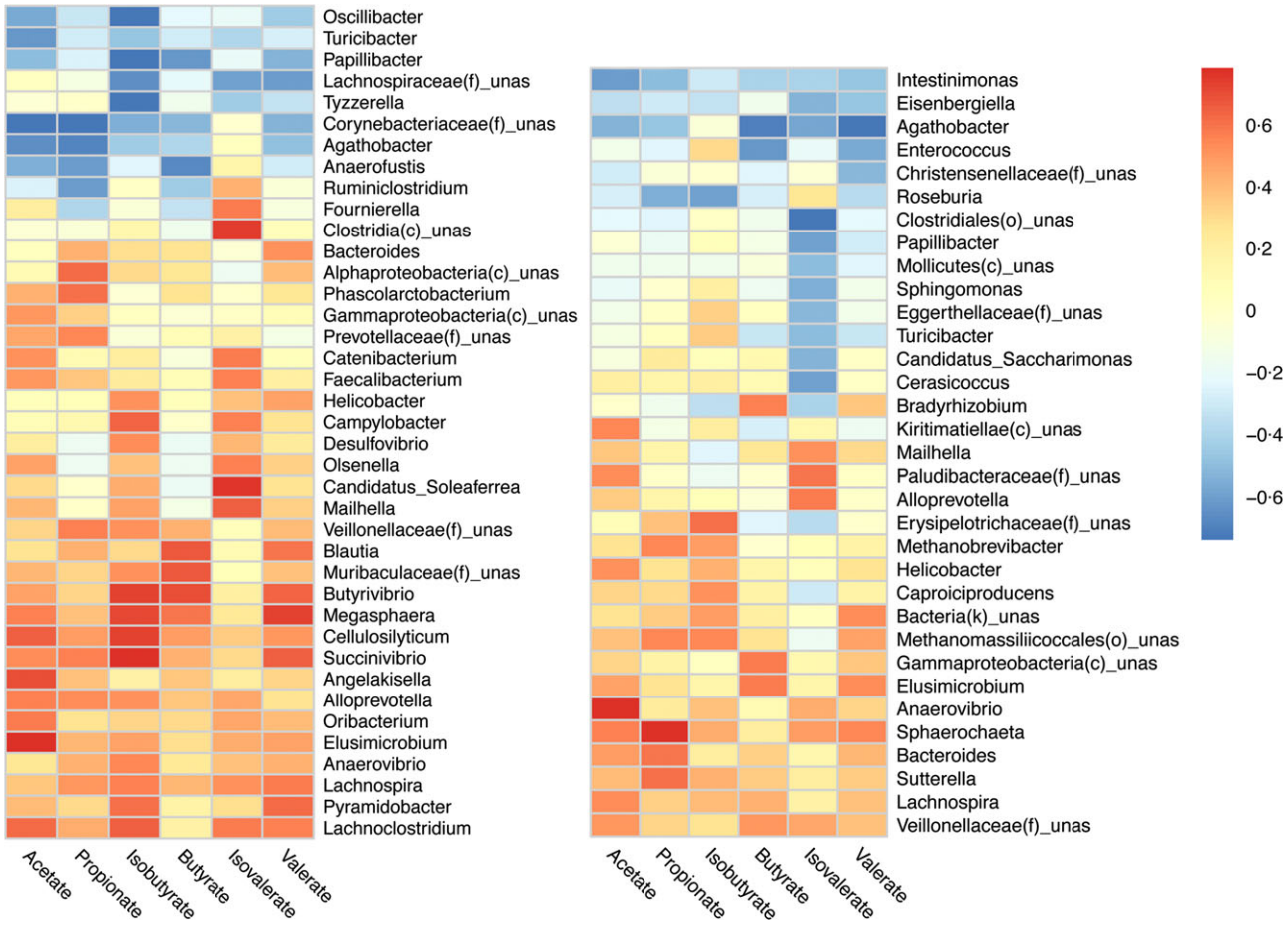

Fig. 4. Heatmap of correlation of the gut bacterial taxa and SCFA in faeces (left) and plasma (right). Correlation coefficients were coloured according to the scale listed on the right.

and plasma. Specifically, *Cellulosilyticum*, *Angelakisella*, *Elusimicrobium* and *Lachnoclostridium* were positively correlated with faecal acetate, *Alphaproteobacteria(c)_unas* and *Phascolarctobacterium* were positively correlated with faecal propionate, and *Blautia*, *Muribaculaceae(f)_unas* and *Butyrivibrio* were positively correlated with faecal butyrate (Fig. 4(a)). Moreover, *Anaerovibrio* was positively correlated with plasma acetate, while *Sphaerochaeta*, *Sutterella* and *Bacteroides* were positively correlated with plasma propionate (Fig. 4(b)). Indole metabolites, such as norharmane in faeces (Fig. 5) and imidazoleacetic acid in plasma (Fig. 6), were also correlated with bacterial taxa, including *Anaerovibrio*, which was also correlated with abundance of betaine. The gut bacterial taxa, such as *Cellulosilyticum* and *Lachnoclostridium*, were found associated with intermediate metabolites related to energy metabolism, such as

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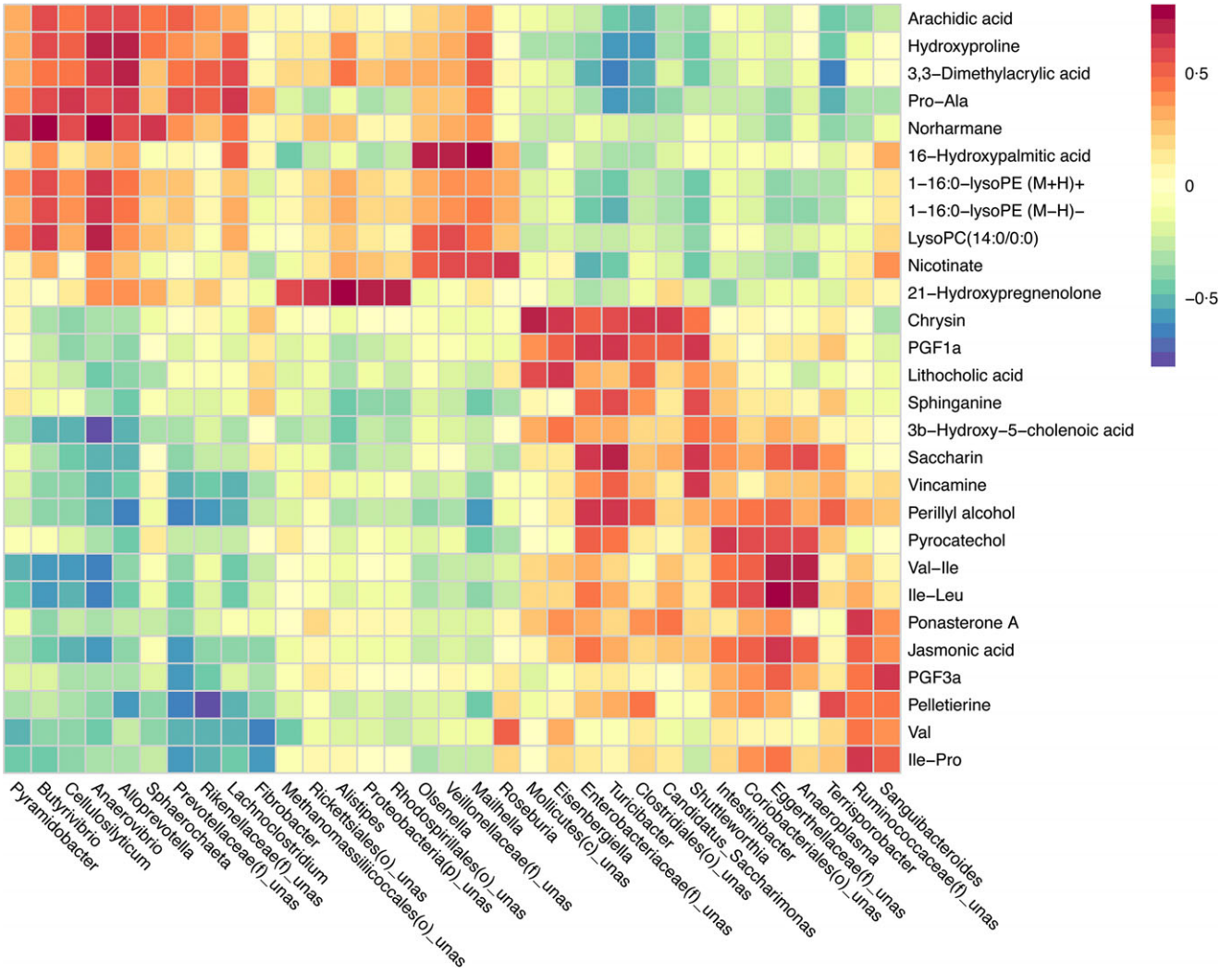

Fig. 5. rCCA of the gut bacterial taxa and metabolites in faeces. rCCA Similarity Scores are coloured according to the scale listed on the right. rCCA, regularised canonical correlation analysis.

malic acid and 2-keto-gluconic acid as well as acetylcarnitine in plasma (Fig. 6). As shown in Fig. 7, plasma acetate ($r = 0{\cdot}59$, $P < 0{\cdot}05$) and total SCFA ($r = 0{\cdot}59$, $P < 0{\cdot}05$) concentrations on G107 were positively correlated with plasma glucose concentration on G107. Moreover, plasma propionate concentration on G107 was positively correlated with parturition duration ($r = 0{\cdot}55$, $P < 0{\cdot}05$).

Discussion

During birth, the blood and oxygen supply to the brain of piglets are affected by parturition duration[17], and piglets with a long parturition duration have higher risk of death. Consistent with previous report[18], in this study, sows fed HDF diet had a substantial reduction (24 %) in the parturition duration and mean birth interval as compared with NDF ones.

For mammals, energy is of importance for the maternal uterine contractility during parturition. In humans, ATP production of myometrium increases two- to threefold during uterine contraction[19]. Likewise, a recent retrospective study emphasised the difference of uterine energy expenditure in sows with short or long farrowing duration[20]. The increasing energy intake of sows before the onset of parturition is an effective strategy to improve parturition process[21,22]. However, it should be noted that the feed intake is decreased when sows approach farrowing, and the intense physical activity due to nest-building behaviour increases the energy expenditure of sows prior to parturition[23]. Previous study had pointed out that sows fed high-fibre diet have a longer postprandial energy uptake from the gastrointestinal tract[24] and a more stable level of blood glucose[13], indicating a more sustainable energy supply during parturition.

In addition to glucose, SCFA can also be utilised as energy source contributing to nearly 30 % of the energy requirements of pigs[13,25], whereas 10 % in humans[26]. In this study, we found that concentrations of plasma acetate and total SCFA on G107 were positively correlated with plasma glucose on G107, indicating the role of SCFA as energy substrates. Consistent with recent study[14], moreover, the plasma concentrations of acetate, butyrate and total SCFA at parturition were lower than that on

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Fig. 6. rCCA of the gut bacterial taxa and metabolites in plasma. rCCA Similarity Scores are coloured according to the scale listed on the right. rCCA, regularised canonical correlation analysis.

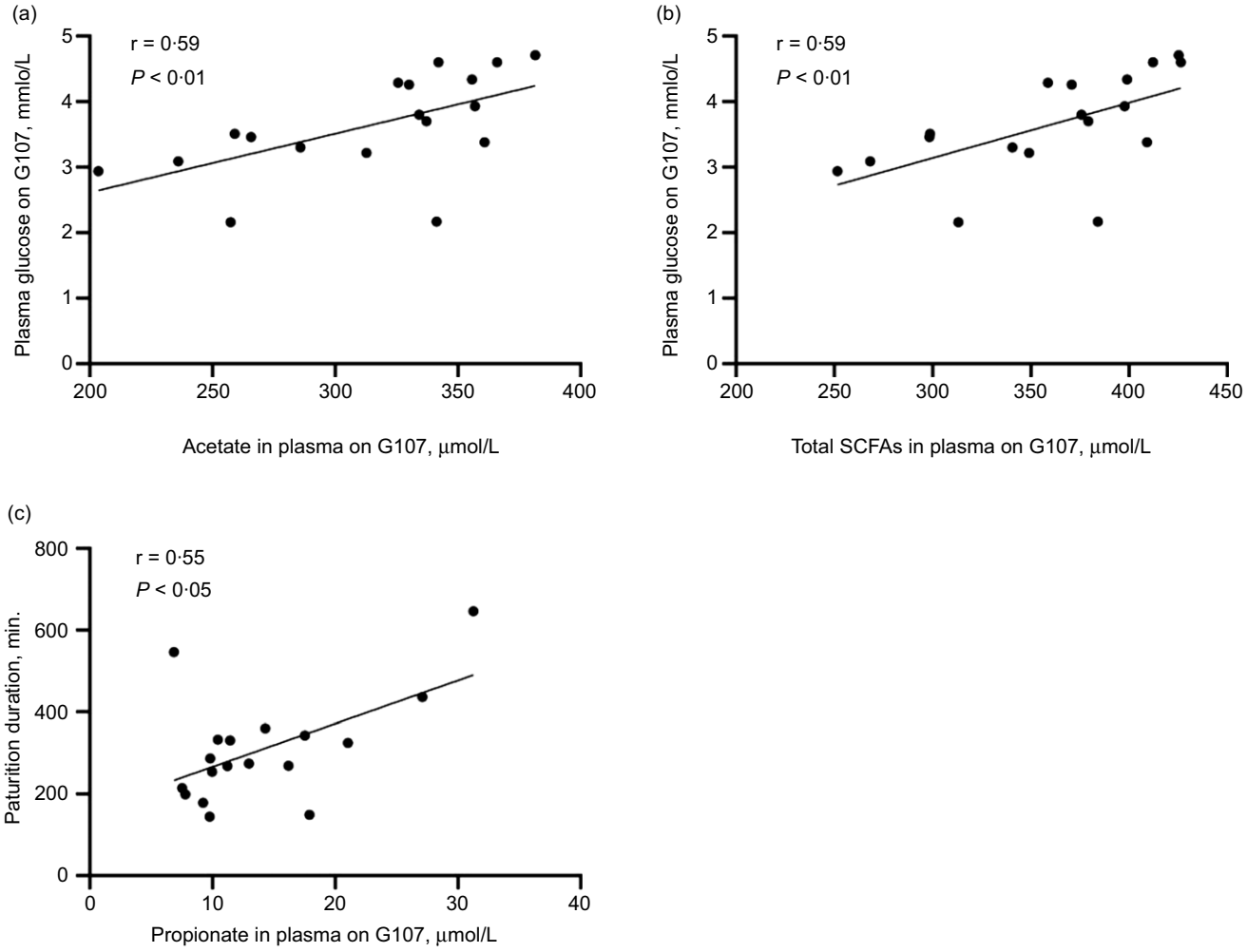

Fig. 7. Correlation between SCFA and plasma glucose (a) and (b) and parturition duration (c) of sows.

G107, further suggesting the expenditure of SCFA during parturition.

In contrast to acetate, butyrate and total SCFA, the level of plasma propionate increased at parturition as compared with G107. The propionate acts as a precursor for gluconeogenesis in the liver[26], and about 50 % of propionate were utilised as the substrate of hepatic gluconeogenesis in humans[26], and 69 % of total glucose were synthesised from propionate in a mice model[27]. Parturition is a highly energy-demanding process[28]; thus, the elevated plasma propionate may largely support the high energy (glucose) requirement during parturition process. In the present study, the level of plasma propionate was comparable between NDF and HDF sows on G107. When it comes to parturition, however, the HDF sows had significantly higher level of plasma propionate as compared with NDF ones. The higher concentration of plasma propionate in HDF sows (91 % increase) indicated the gluconeogenesis process. Intriguingly, the plasma propionate concentration on G107 was positively correlated with parturition duration, which is also in line with the recent retrospective study[20].

The molar ratio of acetate, propionate and butyrate in faeces of sows is 66:23:11 in this study, a little different with that in human faeces, 60:20:20[10]. Of note, most of SCFA, except for butyrate, showed higher levels in both faeces and plasma of HDF sows at parturition. In fact, findings concerning the effect of dietary fibre intake on butyrate level are inconsistent across different studies. The intake of inulin-type fructans by patients with type 2 diabetes showed unchanged butyrate level[29], whereas inulin intake by mice showed higher level of butyrate in faeces, but not in plasma[30]. Alfalfa-containing diet also increased butyrate level in the caecal digesta of pigs[31] and caecal levels of mucosal genes involved in SCFA sensing and absorption[31]. In our previous study, sows supplemented with guar gum and cellulose during the whole gestation had increased faecal level of butyrate[32]. Therefore, it is speculated that this inconsistency could be due to the heterogeneity in fibre types, species and pathophysiological status of the subjects. A previous study pointed out that butyrate and valerate have competitive metabolic pathways[33], which may explain the reasons that valerate increased but butyrate did not increase in this study.

In addition to SCFA, we observed some metabolites were markedly altered by dietary treatment, using non-targeted metabolomics analysis. Particularly, some metabolites are related to energy metabolism, such as betaine and

dimethylglycine (DMG). Previous study has reported that betaine improves energy utilisation, especially when energy intake is insufficient[34]. It has been reported that betaine can be catalysed by betaine homocysteine methyltransferase to produce DMG[35]. In this study, the increased DMG in the plasma of sows fed HDF diet coincided with the higher betaine. In parallel, the result of plasma metabolomics showed a higher concentration of propionic acid in HDF sows, which is in consistent with the SCFA results in this study. Furthermore, dietary fibre can serve as the metabolism substrate for specific bacteria producing secondary bile acids[36]. Our untargeted metabolomic analysis showed there was decreased faecal level of 3b-hydroxy-5-cholenoic acid, which is a monohydroxy bile acid, an intermediate of synthesis of lithocholate, chenodeoxycholate and cholate involving in gut microbiota[37]. However, there were inconsistent reports that wheat bran fibre decreased the faecal levels of lithocholate and deoxycholate in humans[38,39], whilst soluble β-glucans increased faecal levels of primary and secondary bile acids[40].

Given the crucial role of gut microbiota on fermentation of dietary fibre and production of SCFA and other metabolites, the 16S rRNA sequencing was applied to determine the composition of gut microbiota. We found HDF intake in late gestation altered gut microbiota, with lower abundance of Firmicutes, but higher abundance of Bacteroidetes, which are in accordance with previous study[41]. The phylum Synergistetes, digesting fibre to produce acetic acid[33], showed increased abundance in HDF sows. Genera *Turicibacter* and *Terrisporobacter* showed decreased abundance in HDF sows, as reported in pigs and children consuming inulin-enriched diet[31,42,43]. The changes of multiple genera involving in fibre degradation are positively correlated with the levels of specific SCFA in faeces and plasma, suggesting their roles in the production of SCFA. The family *Rikenellaceae* and genus *Cellulosilyticum* are reported to degrade carbohydrates[44,45]. Accordingly, our results showed that *Cellulosilyticum* was positively correlated with acetate and isobutyrate in faeces. *Lachnoclostridium* degrades complex polysaccharides to produce SCFA[46], and its positive correlation with acetate and isobutyrate was found in the current study. *Alloprevotella* and *Pyramidobacter* from the Synergistetes phylum have been reported to produce acetate[46,47], but only a positive correlation between *Pyramidobacter* and faecal valerate was found in this study. *Anaerovibrio* was reported with lipolytic activity in producing glycerol for propionate synthesis[33], but instead a strong correlation between the *Anaerovibrio* abundance and plasma acetate was observed. Nevertheless, these newly observed correlations suggest potential roles of these bacterial taxa in the production of specific SCFA.

The correlations analyses showed the positive correlations between phyla *Succinivibrio*, *Butyrivibrio* and isobutyrate, valuerate, genus *butyrivibrio* and butyrate in the faeces. Also we found the positive correlations between *Succinivibrio*, *Cellulosilyticum* and pseudouridine, which is a metabolite reflecting cell turn-over of the host. The family *Prevotellaceae*, genus *Anaerovibrio* and imidazoleacetic acid involved in bacterial metabolism of tryptophan. Positive correlation between *Lachnoclostridium* and 2-keto-gluconic acid, an intermediate of energy metabolism, was also observed by regularised canonical correlation analysis. Moreover, the strong positive correlation found between *Anaerovibrio* and betaine suggested the role of gut microbiota in betaine metabolism. These correlations suggest new associations between bacterial taxa and host metabolites, revealing the potential mechanism of dietary fibre intake on host physiology.

Conclusion

Our study showed that high dietary fibre intake in late gestation improved parturition duration, which could be associated with altered gut microbiota, production of SCFA and other metabolites involving in energy metabolism. However, the further investigations are needed whether other mechanism remains about the improvement of parturition duration by dietary fibre, also fibre types and gut microbiota differences across species need to be concerned.

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In this work, L. C. and Y. Liu. designed the study. Y. Liu. and N. C. carried out the animal and laboratory experiments. Y. J., R. Z., Z. F., Y. Lin., S. X., B. F., Y. Z., D. W., P. K. T. and L. C. analysed the data. Y. Liu and P. J. wrote the manuscript and L. C. helped to revise the manuscript.

The authors declare that they have no conflicts of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114522000502

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