The Improvement of Parturition Progress by High Intake of Dietary Fibre in Late Gestation is Associated with the Altered Gut Microbiome and Metabolome in Sows

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Research
Abstract

Background: Gestational intake of dietary fibre improves the parturition progress, which largely affects developmental outcomes of the offspring. Dietary fibre can alter the gut microbiome and production of symbiotic metabolites, e.g. short chain fatty acids (SCFAs). We hypothesized that the improvement of parturition progress by dietary fibre is associated with the symbiotic metabolites generated by the gut microbiome.

Methods: Yorkshire sows were randomly given diet containing normal level of fibre (NDF, 16.2% dietary fibre, n = 20) or high level (HDF, 30.1%, n = 20) with other nutrients identical from days 90 of gestation to parturition. Faecal microbiome profiled with 16S amplicon sequencing, SCFAs 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: HDF significantly improved the parturition progress, indicated by the shorter parturition duration. HDF increased abundance of the phyla Bacteroidetes and Synergistetes and multiple genera. Except for butyrate, SCFAs levels in the faeces and plasma of sows at parturition were increased in HDF group. The abundances of 15 and 12 metabolites in the faeces and plasma, respectively, markedly differ between HDF and NDF sows. These metabolites are involved in the bacterial metabolism of amino acids, bile acids, SCFAs and dietary fibre. Correlation analysis also showed associations between specific taxa (genera Cellulosilytica and Lachnoclostridia) and metabolites (acetate and isobutyrate).

Conclusions: The improvement of parturition process by high fibre intake in late gestation is associated with altered gut microbiome, production of SCFAs and other metabolites, potentially serving for energy metabolism.

Background

Parturition progress is associated with birth and developmental outcomes of the offspring [1]. In polytocous animals, such as pigs, parturition process can be rather long. Prolonged parturition duration increases the number of stillbirth and adversely affects sow health postpartum. Around 75% of the stillbirth can be attributed to asphyxia, which affects last-born pigs even worse [1]. Dietary factors, besides length of gestation, can also affect parturition progress [2]. Fibrous diet during gestation has been shown to reduce the parturition length of sows with a range of 9–29% [3]. Our own study also demonstrated that diet enriched with inulin, a fermentable fibre from roots, shortened parturition duration in sows [4]. Although several hypotheses have been proposed, including high fibre intake reducing constipation or/and removing excess body fat, the underlying mechanisms of high fibre intake during gestation on improving parturition process remains exclusive.

The gut microbiome is associated with various physiological aspects of host and largely affected by diet [5]. A plethora of studies have associated intake of dietary fibre with the change of composition and
function of gut microbiome [6–8]. The production of symbiotic metabolites by gut microbiome had been proposed to play roles in gut microbiome affecting host physiology [9]. Dietary fibres, including non-starch polysaccharides, oligosaccharides and resistant starches, are microbiota-accessible carbohydrates and fermented by gut microbiome to produce various symbiotic metabolites [9]. Short-chain fatty acids (SCFAs) are one major type of symbiotic metabolites [10], which decrease the pH in the gut lumen to suppress the bacterial growth, and serving as an energy substrate of the host [11]. SCFAs can also regulate metabolism and immunity not only based on the gut, but also the liver and peripheral tissues via the peripheral circulation [5]. To the best of our knowledge, whether the gut microbiome and symbiotic metabolites, such as SCFAs, are involved in the high fibre diet-improved parturition progress is not known yet.

Against this background, we aimed to test whether the altered gut microbiome and -related metabolites are associated with the improved parturition progress by high intake of dietary fibre, using sows as a model. The gut microbiome of sows given high level of dietary fibre was profiled by 16S rRNA amplicon sequencing and compared with that of sows given normal level of dietary fibre from days 90 (d 90) of gestation to parturition. Levels of SCFAs in faeces and plasma were quantified and metabolome were profiled by untargeted metabolomics. Abundance of these metabolites and bacterial taxa were correlated to further explore any potential association.

**Materials And Methods**

The animal experiment was approved by the Animal Care and Use Committee of the Sichuan Agricultural University (Permit No. DKY-B20121602), and was conducted at Tianfu Pig Farm, Giastar Group, Chengdu, China in accordance with the National Research Council's Guidelines for Care and Use of Laboratory Animals.

**Animals and dietary intervention**

Based on parity (4~6) and body weight (267.4 ± 16.8 kg), 40 Yorkshire sows were randomly allocated into 2 groups (20 per treatment) receiving two feedings different in fibre content. The two feedings were formulated based on corn-soybean meal to meet or exceed the recommendation of NRC (2012) as shown in Table S1: Normal dietary fibre diet (NDF, 16.2 % total dietary fibre) and high dietary fibre diet (HDF, 30.1% total dietary fibre). In addition to dietary fibre, other nutrients such as digestible energy and crude protein intake were similar via adjusting feed intake (3.0 kg/d in CON and 3.2 kg/d in HDF) from d 90 of gestation to parturition. The daily intake of dietary fibre in HDF group was about twice as much as CON group (485 g/d in CON and 964 g/d in HDF). During d 90 to 110 of gestation, the diet was supplied once a day (08:00). On d 111 of gestation, sows were moved to the farrowing room and feeding frequency turned to twice a day (08:00 and 15:00) until parturition. Parturition duration was recorded accurately during delivery of the sows. The health care and immunization procedures of the sows and newborn piglets followed the regulation of the farm.

**Sample Collection**
Blood samples (10 mL, n=10) from ear vein were collected into sodium heparinized tubes on d 107 of gestation and within 1 h of parturition. Plasma was obtained by centrifuging at 3,000 × g for 15 min, and stored immediately at -20 °C for later analysis. Fresh faecal samples of sows were collected on d 110 of gestation and stored immediately in liquid nitrogen for later analysis.

**Faecal Microbiomics**

Genomic DNA was extracted from faecal samples using Mo Bio Power Faecal DNA Isolation Kit (Mo BIO, Carlsbad, CA, USA). The v4 hypervariable regions of 16S rRNA was amplified using primers 515F and 806, and the amplicon pyrosequencing was carried out on an Illumina HiSeq PE250 platform (Illumina, San Diego, CA, USA). Raw data was processed with RRR pipeline based UPARSE (Version 7.0.1001). Ribosomal Database Project classifier (Version 2.2) was used to assign taxonomic rank. OTUs were clustered at 97% sequence identity (Sequences with ≥ 97% similarity were assigned to the same OTUs). OTU and sample information were imported into R [12] interfaced with R Studio for further analysis using the Phyloseq and VEGAN [13] 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 1,000 times. Difference in α-diversity presented by Shannon index and differential abundance between the dietary groups of specific genus or phylum were tested by the nonparametric Wilcoxon sum rank test. P values indicating significance of difference at genus level were further adjusted by a two-stage Benjamini and Hochberg (TSBH) step-up FDR-controlling procedure with type I error rate (α) set to 0.20 using the mt.rawp2adjp function in multcomp package [14]. P values of phyla were not adjusted as there were less than 20 tests conducted. Effect size was also calculated using equation, Effect size = , in which Z is the Wilcoxon Z and N is the number of samples.

**Quantification of SCFAs in faeces and plasma**

Concentrations of SCFAs (Acetate, Propionate, Butyrate, Isobutyrate, Valerate and Isovalerate) and sum of them (Total SCFAs) in faecal and plasma samples were determined as previously described [15] with minor modifications. Briefly, supernatant obtained from faecal suspension (0.7 g faecal matter in 1.5 mL water) or plasma was mixed with 25% metaphosphoric acid and crotonic acid solution (210 mmol/L). The extract was further mixed with methanol (1: 3 dilution), filtered by 0.22-µm filter (Millipore, Bedford, MA, USA) before being manually applied onto a gas chromatographer with flame ionization detector (Varian CP-3800, manual injection, flame ionization detector, FID, 10 µL micro-injector) for quantification. Plasma was directly mixed with metaphosphoric acid and crotonic acid for SCFAs extraction. Parturition duration, SCFA concentrations were analysed in R using linear modelling against the dietary groups. Difference with $P < 0.05$ was regarded as significant.

**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
UHPLC (Infinity 1290, Agilent, Santa Clara, CA, USA) 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, Milford, MA, USA) was used as the separation column and the mobile phase was mixture of solvent A (25 mmol/L CH$_3$COONH$_4$ and 25 mmol/L NH$_4$OH) 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, Framingham, MA, USA) to obtain MS and MS/MS data in an information dependent acquisition (IDA) mode. Obtained data was used for compound assignment against an in-house database established with available authentic standards.

Raw LC-MS data was converted into MzXML format using MSconvert (Version 3.0.6458, ProteoWizard, Palo Alto, CA, USA), then imported into XCMS (Version, Scripps Center, La Jolla, CA, USA) for data pre-processing. Processed data was annotated with chemical compound ID as aforementioned, then imported into R [12] 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 (PCA). Each MS feature was fitted to a linear model using \texttt{lm} function with treatment (NDF vs HDF) as the predictor. P-value for the significance of diet was generated by comparing these two models with \texttt{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.

**Correlation Analysis of the Microbiomic and Metabolomic Data**

To explore potential associations between the microbiomic compositions and abundance of metabolites in faeces or plasma, Spearman correlation was applied to the microbiomic data and individual SCFAs data, whilst unsupervised regularized Canonical Correlation Analysis (rCCA) with the shrinkage method was performed on the microbiomic and metabolomic data across the dietary groups using the mixOmics package. All analyses were conducted in R. Correlations were shown as heatmap with either Spearman's r or the rCCA similarity scores. Selected correlations with similarity score larger than 0.7 were further shown in scatter plots.

**Results**

**Parturition duration**

The parturition durations are shown in Table 1. HDF sows had significantly shorter parturition duration ($P < 0.05$) and mean birth interval ($P < 0.05$), relative to NDF ones.
Table 1

|                        | NDF       | HDF       | P-value |
|------------------------|-----------|-----------|---------|
| Parturition duration   | 303 ± 26  | 229 ± 19  | 0.03    |
| Mean birth interval    | 23.1 ± 3.5 | 14.5 ± 1.6 | 0.03    |

Data are shown as mean ± SEM.

Faecal And Plasma Scfas Concentration

In both faecal samples of d 110 and plasma samples at parturition, levels of total SCFAs, acetate, propionate, isobutyrate (P = 0.06 in plasma), valerate and isovalerate (P = 0.07 in faeces) were higher in HDF sows than those in NDF ones (all Ps < 0.05 unless otherwise stated). No significant difference in level of butyrate was found between HDF and NDF sows in either faecal or plasma samples. In plasma samples of d 107, levels of isobutyric acid (P = 0.09) and valeric acid (P = 0.02) were higher in HDF sows than those in NDF ones, where no significant difference in level of total SCFAs, acetic acid, propionic acid, butyric acid and iso-valeric acid were found between treatments (Table 2).
### Faecal and plasma SCFAs concentrations

| Metabolite   | Faeces | Plasma on d 107 | Plasma at Parturition | P-value |
|--------------|--------|----------------|-----------------------|---------|
|              | NDF    | HDF            | NDF                   | HDF     | NDF    | HDF     |         |
| Acetate      | 42.6 ± 3.4 | 53.0 ± 1.2 | 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 | 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 | 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 | 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 | 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 | 5.2 ± 0.4   | 5.4 ± 0.4   | 0.73 | 4.8 ± 0.3   | 7.4 ± 0.9   | 0.01 |
| Total SCFAs  | 70.4 ± 5.3 | 86.1 ± 2.0 | 365.0 ± 17.6 | 414.2 ± 20.9 | 0.38 | 262.7 ± 13.0 | 354.2 ± 24.5 | < 0.01 |

Faecal data in µmol/g, plasma data in µmol/L. Total SCFAs = the sum of acetate, propionate, butyrate, isobutyrate, valerate and isovalerate.

**Faecal And Plasma Metabolomics**

In total, 186 metabolites in positive mode and 170 in negative mode were annotated, whist 217 (positive) and 179 (negative) in plasma. Among these metabolites, 15 metabolites in faeces and 12 in plasma were found with significant difference (adjusted P < 0.05) between NDF and HDF sows. PCA score plots of faecal and plasma metabolome are shown as Fig. 2. Tendency of separation in both score plots indicates difference between the two groups in both faecal and plasma metabolome, and faecal NDF metabolome and plasma HDF metabolome was less diverse than their counterpart in the same sample type. Information of identified metabolites, such as metabolite name, RT, m/z, adduct, abundance in NDF and HDF sows, fold change and adjusted P-value, is listed in Table 3, respectively. While only nearly half of the faecal metabolites (7/15) showed higher abundance in HDF sows, levels of all identified plasma metabolites are higher in the HDF sows.
| Metabolite                        | Ion                  | RT(min) | M/Z      | Abundance       | Fold change |
|----------------------------------|----------------------|---------|----------|-----------------|-------------|
|                                  |                      |         |          | NDF (10^4)     | HDF (10^3)  |
| 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               |                      |         |          |                 |             |

RT, retention time. Data are shown as mean ± SEM; Fold change, refers to abundance (HDF)/abundance (NDF).
| Metabolite                     | Ion          | RT (min) | M/Z      | Abundance NDF (10^4) | Abundance HDF (10^3) | Fold change |
|-------------------------------|--------------|----------|----------|----------------------|----------------------|-------------|
| 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 | 12690.4 ± 306.3      | 16365.2 ± 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        |

RT, retention time. Data are shown as mean ± SEM; Fold change, refers to abundance (HDF)/abundance (NDF).

**Correlation**

Associations between abundance of specific taxa and that of SCFAs by Spearman Correlation analysis, and that metabolites by rCCA in faeces and plasma are shown as heatmaps in Figs. 3, 4 and 5, respectively. Across the diet groups, multiple taxa were correlated with SCFAs in abundance in faeces and plasma. Of note, more correlations were found in plasma than in faeces and the correlations found are not identical in faecal and plasma samples. In faeces, four genera were found associated with acetate, two genera with propionate. Three genera were found associated with butyrate, six with isobutyrate (Fig. 3A). In plasma, *Anaerovibrio* was correlated with acetate, while *Sphaerochaeta, Sutterella* and *Bacteroides* were correlated with propionate (Fig. 3B). The taxa, such as *Cellulosilyticum, Lachnoclostridium*, were found associated with intermediate metabolites related to energy metabolism, such as malic acid and 2-keto-gluconic acid as well as acetyl carnitine in plasma. Indole metabolites, such
as norharmane in faeces (Fig. 4), imidazoleacetic acid in plasma (Fig. 5), were also correlated with bacterial taxa, including *Anaerovibrio*, which was also correlated with abundance of betaine.

**Discussion**

In this study, HDF diet improved the parturition progress, relative to NDF diet. HDF sows had a substantial deduction (24%) in the parturition duration and mean birth interval relative to NDF ones, which is in line with our previous study [16] and others [17].

For mammals, maternal uterine contractility directly determines fetal expulsion [18], and uterine contractility is an energy-demanding process, thus the parturition process would be affected by host energy status [19]. In human-being, glucose is of importance for the myometrium as energy source during parturition, and ATP production increases 2- to 3-fold during uterine contraction [20]. Our own study has shown that the increased energy intake in late gestation improved parturition process, using pigs as a model [21]. It has also been reported that pigs fed high-fibre diet had a more constant level of blood glucose, indicating a more sustained energy supply during parturition [22]. Glucose can be produced by SCFAs, such as propionate and acetate through TCA cycle [23]. In addition to glucose, SCFAs can also be an important energy resource contributing to nearly 30% of the energy requirements of pigs [22, 24], while 10% in human being and 80% in ruminants [23]. In this study, supportively, we did find the markedly increased plasma concentrations of SCFAs in HDF sows at parturition. In the current study, acetate is the most abundant SCFA, followed by propionate and butyrate in both faeces and plasma, which is consistent with previous study [25]. The molar ratio of acetate, propionate and butyrate in faeces is 66:23:11 in sows in this study, a little different with that in human faeces, 60:20:20 [9]. Functionally, acetate can be used to produce propionate and butyrate [5], and involved in liver lipogenesis and lipolysis in adipose tissue [26]. Moreover, propionate and butyrate can regulate glucose homeostasis [5].

Of note, most of SCFAs, 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 level [27], whereas inulin intake by mice showed the increasing level of butyrate in faeces, but not in plasma [28]. Alfalfa-containing diet also increased butyrate level in the caecal digesta of pigs [29] and caecal levels of mucosal genes involved in SCFA sensing and absorption [29]. In our previous study, sows fed with dietary fibre levels by guar gum and cellulose for the whole gestation had increased faecal level of butyrate [30]. Therefore, it is speculated that this inconsistency could be due to the heterogeneity in fibre types and difference in species, and pathophysiological status of the subjects. In this study, moreover, butyrate failed to increase but valerate did. A previous study pointed out that butyrate and valerate have competitive metabolic pathways [31], this may be a reason for this result, but the precise mechanism is not sufficiently clear. Regardless of diet, when it comes to parturition, the plasma concentrations of acetate and total SCFAs decreased, relative to d 107 of gestation. The lower levels of SCFAs at parturition further suggest the expenditure of SCFAs for energy supply, while HDF sows means the sufficient intake of dietary fibre for bacterial fermentation and constant production of SCFAs.
In addition to SCFAs, using non-targeted metabolomics analysis, we also found some intermediate metabolites were increased in HDF sows, which related to energy metabolism, such as betaine and dimethylglycine (DMG), which is in line with a study on pigs consumed high-fibre rye [32]. Previous study has reported that betaine improves energy utilization, especially when energy intake is insufficient [33]. It should be noted that betaine is catabolized via a series of enzymatic reactions to donate methyl groups and DMG is an intermediate metabolite. The underlying mechanism is that betaine is catalysed by betaine homocysteine methyltransferase to form DMG [34]. In this study, the increased DMG in the plasma of sows fed HDF diet coincided with the higher betaine. In addition, the result of plasma metabolomics showed a higher concentration of propionic acid in HDF sows, which supported our SCFAs data. Furthermore, dietary fibre can serve as the metabolism substrate for specific bacteria producing secondary bile acids [35]. Our untargeted metabolomic analysis showed the 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 activities of gut bacteria [36]. Results from different studies on effect of consumption of dietary fibre on bile acids are rather inconsistent. Wheat-bran fibre decreased the faecal levels of lithocholate and deoxycholate in humans [37, 38], whilst soluble beta-glucans increased faecal levels of primary and secondary bile acids [39].

Considering the crucial role of gut microbiome on fermentating dietary fibre and production of SCFAs and other metabolites, the composition of gut microbiota was determined using 16S rRNA sequencing. We found HDF intake in the late gestation leaded to the comprehensive changes of gut microbiome, with lower abundance of Firmicutes, higher abundance of Bacteroidetes and lower ratio of Firmicutes to Bacteroidetes, which is in accordance with a previous study in rats [40]. Abundance of Phylum Synergistetes, digesting fibre to produce acetic acid [31], also increased. Genera Turicibacter and Terrisporobacter showed decreased abundance in HDF sows, as reported in pigs and children consuming inulin-enriched diet [29, 41, 42]. The changes of multiple genera involving in fibre degradation are positively correlated with the levels of specific SCFAs in faeces and plasma, suggesting their roles in the production of SCFAs. The family Rikenellaceae and genus Cellulosilyticum degrades carbohydrates [43, 44], and Cellulosilyticum was positively correlated with acetate and isobutyrate in faeces, but not in plasma in this study. Lachnoclostridium degrades complex polysaccharides to produce SCFAs [45], its positive correlation with acetate and isobutyrate was found. Alloprevotella and Pyramidobacter from the Synergistetes phylum produce acetate [45, 46], but only a positive correlation between Pyramidobacter and faecal valerate was found in this study. Anaerovibrio was reported with lipolytic activity producing glycerol for propionate synthesis [31], 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 SCFAs.

Our correlations analyses showed the positive correlations between phyla Succinivibrio, Butyribrio and Isobutyrate and valerate, butyribrio and butyrate in the faeces. Also we found the positive correlations between Succinivibrio, Cellulosilyticum and pseudouridine, which is a metabolite standing for cell turnover of the host. The family Prevotellaceae, genus Anaerovibrio and imidazoleacetic acid involving in bacterial metabolism of tryptophan, Lachnoclostridium and an intermediate of energy metabolism, 2-
keto-Gluconic acid, were also observed by rCCA. Moreover, the strong positive correlation found between *Anaerovibrio* and plasma level of betaine suggested the role of gut microbiome 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 intake of dietary fibre in late gestation improved parturition process. This improvement may be associated with the altered gut microbiome and production of SCFAs, as well as other metabolites involving in energy metabolism and host physiology. However, the further investigations are needed, whether other mechanism remains about the improvement of parturition process by dietary fibre, also fibre types and gut microbiome differences across species need to be concerned.

**Abbreviations**

SCFAs, short chain fatty acids; d 90, days 90 of gestation; NDF, normal dietary fibre; HDF, high dietary fibre; NMDS, Non-metric Multi-dimensional Scaling;

**Declarations**

**Ethics approval and consent to participate**

All institutional and national guidelines for the care and use of laboratory animals were followed. The experimental procedures were approved by the Animal Care and Use Committee of Sichuan Agricultural University (Permit No. DKY-B20121602).

**Consent to publication**

All authors provide their consent to this publication.

**Availability of data and materials**

The datasets analyzed in the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no conflicts of interest.

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Authors’ contributions

In this work, Lianqiang Che and Yang Liu designed the study. Yang Liu and Nan Chen carried out the animal and laboratory experiments. Yannan Jiang, Ruinan Zhang, Zhengfeng Fang, Yan Lin, Shengyu Xu, Bin Feng, Yong Zhuo, De Wu, Peter Kappel Theil and Lianqiang Che analyzed the data. Yang Liu and Pingping Jiang wrote the manuscript and Lianqiang Che helped to revise the manuscript.

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References

1. Dijk AJV, Rens BTTM, Lende TVD, Taverne MAM. Factors affecting duration of the expulsive stage of parturition and piglet birth intervals in sows with uncomplicated, spontaneous farrowings. Theriogenology. 2005;64(7):1573-90.

2. Oliviero C, Heinonen M, Valros A, Peltoniemi O. Environmental and sow-related factors affecting the duration of farrowing. Anim Reprod Sci. 2010;119(1):85-91.

3. Guillemet R, Hamard A, Quesnel H, Père MC, Etienne M, Dourmad JY et al. Dietary fibre for gestating sows: Effects on parturition progress, behaviour, litter and sow performance. Animal. 2007;1(6):872-80.

4. Wang Y, Zhou P, Liu H, Li S, Zhao Y, Deng K et al. Effects of inulin supplementation in low- or High-Fat diets on reproductive performance of sows and antioxidant defence capacity in sows and offspring. Reprod Domest Anim. 2016;51(4):492-500.

5. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F, Institute Of Medicine DOMA, Wallenberg L et al. From dietary ber to host physiology: Short-Chain fatty acids as key bacterial metabolites. Cell. 2016;165(6):1332-45.

6. Hussain SK, Dong TS, Agopian V, Pisegna JR, Durazo FA, Enayati P et al. Dietary protein, fiber and coffee are associated with small intestine microbiome composition and diversity in patients with liver cirrhosis. Nutrients. 2020;12(5):1395.

7. Mathilde, Sciellour L, Etienne, Labussière, Olivier, Zemb et al. Effect of dietary fiber content on nutrient digestibility and fecal microbiota composition in growing-finishing pigs. Plos 2018;13(10):e0206159.

8. Havlik J, Marinello V, Gardyne A, Hou M, Edwards CA. Dietary fibres differentially impact on the production of phenolic acids from rutin in an in vitro fermentation model of the human gut microbiota. Nutrients. 2020;12(6):1577.
9. Kumar J, Rani K, Datt C. Molecular link between dietary fibre, gut microbiota and health. Mol Biol Rep. 2020;47(29):6229-37.

10. LeBlanc JG, Chain F, Martín R, Bermúdez-Humarán LG, Courau S, Langella P. Beneficial effects on host energy metabolism of short-chain fatty acids and vitamins produced by commensal and probiotic bacteria. Microb Cell Fact. 2017;16(1):79.

11. Schönfeld P, Wojtczak L. Short- and medium-chain fatty acids in energy metabolism: The cellular perspective. J Lipid Res. 2016;57(6):943-54.

12. Team RRDC. A language and environment for statistical computing. Computing. 2013;1:12-21.

13. Dixon P. VEGAN, a package of R functions for community ecology. J Veg 2003;14(6): 927-30.

14. S. Pollard SDAM. Multiple Testing Procedures: the multtest Package and Applications to Genomics. In: Gentleman R., Carey V.J., Huber W., Irizarry R.A., Dudoit S, editors; Springer. New York: 2015. p. 249-271.

15. Che L, Hu L, Zhou Q, Peng X, Liu Y, Luo Y et al. Microbial insight into dietary protein source affects intestinal function of pigs with intrauterine growth retardation. Eur J Nutr. 2019;59:327-44.

16. Wang YS, Zhou P, Liu H, Li S, Zhao Y, Deng K et al. Effects of inulin supplementation in low- or High-Fat diets on reproductive performance of sows and antioxidant defence capacity in sows and offspring. Reprod Domest Anim. 2016;51(4):492-500.

17. Bilkei Papp G. The effect of increased fibre content fed in the previous week on the parturition of sows. Magyar Állatorvosok Lapja. 1990:597-601.

18. Rizzo A, Angioni S, Spedicato M, Minoia G, Mutinati M, Trisolini C et al. Uterine contractility is strongly influenced by steroids and glucose metabolism: An in vitro study on bovine myometrium. Gynecol Endocrinol. 2011;27(9):636-40.

19. Feyera PTFK. Impact of sow energy status during farrowing on farrowing kinetics, frequency of stillborn piglets and farrowing assistance. J Anim Sci. 2018;96(6):2320-31.

20. Jrg C, Matthews SG, Gibb W, Lye SJ. Endocrine and paracrine regulation of birth at term and preterm. Endocr Rev. 2000;21(5):514.

21. Che L, Hu L, Wu C, Xu Q, Zhou Q, Peng X et al. Effects of increased energy and amino acid intake in late gestation on reproductive performance, milk composition, metabolic, and redox status of sows. J Anim Sci. 2019;97(7):2914-26.

22. Serena A, Jørgensen H, Bach Knudsen KE. Absorption of carbohydrate-derived nutrients in sows as influenced by types and contents of dietary fiber. J Anim Sci. 2009;87(1):136-47.
23. Gijs DB, Karen VE, Groen AK, Koen V, Dirk-Jan R, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res. 2013;54(9):2325-40.

24. Inoue D, Tsujimoto G, Kimura I. Regulation of energy homeostasis by GPR41. Front Endocrinol. 2014;5:

25. Lunn J, Buttriss JL. Carbohydrates and dietary fibre. Nutrition Bulletin. 2010;32(1): 21-64.

26. Den Besten G, Lange K, Havinga R, Van Dijk TH, Gerding A, Van Eunen K et al. Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids. Am J Physiol-Gastr L. 2013;305(12): 900-10.

27. Birkeland E, Gharagozlian S, Birkeland Kl, Valeur J, Mge I, Rud I et al. Prebiotic effect of inulin-type fructans on faecal microbiota and short-chain fatty acids in type 2 diabetes: A randomised controlled trial. Eur J Nutr. 2020: 1-14.

28. Igarashi M, Morimoto M, Suto A, Nakatani A, Kimura I. Synthetic dietary inulin, Fuji FF, delays development of diet-induced obesity by improving gut microbiota profiles and increasing short-chain fatty acid production. PeerJ. 2020;8(2): e8893.

29. Jiawei, Wang, Chunfu, Qin, Ting, He et al. Alfalfa-containing diets alter luminal microbiota structure and short chain fatty acid sensing in the caecal mucosa of pigs. J Anim Sci Biotechno. 2018;9(2):11.

30. Zhuo Y, Feng B, Xuan Y, Che L, Wu D. Inclusion of purified dietary fiber during gestation improved the reproductive performance of sows. J Anim Sci Biotechno. 2020;11(1): 47.

31. Ramos AFO, Terry SA, Holman DB, Breves G, Pereira LGR, Silva AGM et al. Tucumã oil shifted ruminal fermentation, reducing methane production and altering the microbiome but decreased substrate digestibility within a RUSITEC fed a mixed hay-concentrate diet. Front Microbiol. 2018;9:1647.

32. Bertram HC, Malmendal A, Nielsen NC, Straadt IK, Larsen T, Knudsen KEB et al. NMR-based metabonomics reveals that plasma betaine increases upon intake of high-fiber rye buns in hypercholesterolemic pigs. Mol Nutr Food Res. 2010;53(8):1055-62.

33. Schrama JW, Heetkamp MJ, Simmins PH, Gerrits WJ. Dietary betaine supplementation affects energy metabolism of pigs. J Anim Sci. 2003;81(5): 1202-9.

34. Cools A, Maes D, Buyse J, Kalmar ID, J-A V, Janssens GPJ. Effect of N,N-dimethylglycine supplementation in parturition feed for sows on metabolism, nutrient digestibility and reproductive performance. Animal. 2010;4(12):2004-11.

35. Makki K, Deehan EC, Walter J, Backhed F. The impact of dietary fiber on gut microbiota in host health and disease. Cell Host Microbe. 2018;23(6):705-15.
36. Javitt NB, Kok E, Carubbi F, Blizzard T, Byon CY. Bile acid synthesis. Metabolism of 3 beta-hydroxy-5-cholenoic acid in the hamster. J Biol Chem. 1986;261(27):12486.

37. Alberts DS, Einspahr JG, Earnest DL, Krutzsch MF, Lin P, Hess LM et al. Fecal bile acid concentrations in a subpopulation of the wheat bran fiber colon polyp trial. Cancer Epidem Biomar. 2003;12(3):197-200.

38. Reddy BS, Engle A, Simi B, Goldman M. Effect of dietary fiber on colonic bacterial enzymes and bile acids in relation to colon cancer. Gastroenterology. 1992;102(5):1475-82.

39. Ghaffarzadegan T, Zhong Y, F K H Llenius F, Nyman M. Effects of barley variety, dietary fiber and β-glucan content on bile acid composition in cecum of rats fed low- and high-fat diets. J Nutr Biochem. 2017;53:104-10.

40. Ferrario C, Statello R, Carnevali L, Mancabelli L, Milani C, Mangifesta M et al. How to feed the mammalian gut microbiota: Bacterial and metabolic modulation by dietary fibers. Front Microbiol. 2017;8:

41. Jae-Young K, Min KY, In-Sung K, Jeong-A. K, Da-Yoon Y, Bishnu A et al. Effects of the brown seaweed laminaria japonica supplementation on serum concentrations of IgG, triglycerides, and cholesterol, and intestinal microbiota composition in rats. Front Nutr. 2018;5:

42. Josephine H, Nicolucci AC, Heidi V, Alana S, Jon M, Reimer RA et al. Effect of Prebiotic on Microbiota, Intestinal Permeability, and Glycemic Control in Children with Type 1 Diabetes. J Clin Endocr Metab. 2019;104(10):4427-40.

43. Pitta DW, Pinchak WE, Dowd SE, Osterstock J, Gontcharova V, Youn E et al. Rumen bacterial diversity dynamics associated with changing from bermudagrass hay to grazed winter wheat diets. Microb Ecol. 2010;59(3):511-22.

44. Cai S, Shao N, Dong X. Cellulosilyticum.

45. Peng X, Wang R, Hu L, Zhou Q, Liu Y, Yang M et al. Enterococcus faecium NCIMB 10415 administration improves the intestinal health and immunity in neonatal piglets infected by enterotoxigenic Escherichia coli K88. J. Anim. Sci. Biotechno. 2019;10(1):72.

46. Pan X, Xue F, Nan X, Tang Z, Wang K, Beckers Y et al. Illumina sequencing approach to characterize thiamine metabolism related bacteria and the impacts of thiamine supplementation on ruminal microbiota in dairy cows fed High-Grain diets. Front Microbiol. 2017;8:1818.

**Figures**
Figure 1

α-diversity (A), β-diversity using Bray-Curtis distance (B) and unifrac distance (C) of the gut microbiome, and genera with differential abundance between HDF and NDF sows (D).
Figure 2

PCA score plots of faecal (A) and plasma (B) metabolome
Figure 3

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

Figure 4

rCCA of the gut bacterial taxa and metabolites in faeces. rCCA Similarity Scores are coloured according to the scale listed on the right.
Figure 5

rCCA of the gut bacterial taxa and metabolites in plasma. rCCA Similarity Scores are coloured according to the scale listed on the right.

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