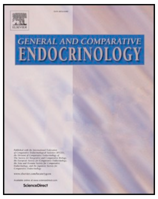
General and Comparative Endocrinology 277 (2019) 141–147

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen



Crude fiber modulates the fecal microbiome and steroid hormones in pregnant Meishan sows

Xueyuan Jiang[a,1], Naisheng Lu[a,1], Yun Xue[a,b,1], Suli Liu[a,1], Hulong Lei[a], Weilong Tu[a], Yang Lu[a], Dong Xia[a,*]

[a] *Shanghai Engineering Research Center of Breeding Pig, Shanghai Academy of Agricultural Sciences, Shanghai, PR China*
[b] *Shanghai Animal Disease Control Center, Shanghai, PR China*

ARTICLE INFO

Keywords:
Crude fiber
Steroid hormones
Pregnancy
Fecal microbiome
Meishan sows

ABSTRACT

The beneficial effects of dietary fiber on the reproductive performance and welfare of sows have been discussed broadly, but few researches examined the causal changes and the association of gut microbiota and the steroid hormones, the main regulators of reproductive function. To shed light on this, thirty-six Meishan sows were allocated into 2.5% crude fiber (CF) group and 7.5% CF group respectively for an entire farrowing interval. On the 90th day of gestation, the saliva and fresh stool of sows were individually collected in the morning (06:00–07:00) for steroid hormones, short-chain fatty acids (SCFAs) and microbiome analysis. In addition, the parameter of pregnant behavioral and farrowing performance was recorded and evaluated. We observed that, as compared with the 2.5% CF treatment, 7.5% CF significantly increased the litter size ($p = 0.01$), reduced the stereotypic behaviors including sham chewing, rolling tongue and licking ground ($p = 0.02, 0.04, 0.01$) at later gestation stage, but increased lying time ($p = 0.00$). In coincide with this, 7.5% CF diet increased the salivary progesterone ($p = 0.00$), fecal estradiol and progesterone ($p = 0.01, 0.02$) level, fecal water and SCFAs content ($p = 0.02, 0.03$), decreased the salivary and fecal cortisol ($p = 0.01, 0.00$) level. Further, 7.5% CF diet increased the fecal microbiota richness (ACE, $p = 0.04$; Chao, $p = 0.07$) and diversity (Shannon, $p = 0.01$; Simpson, $p = 0.04$), the proportion of genus *Ruminococcus*, *Butyrivibrio*, *Lactobacillus* and *Fibrobacter* ($p = 0.02, 0.05, 0.04, 0.00$), whereas reduced the proportion of genus *Clostridium*, *Streptococcus*, *Bacteroides* and *Escherichia-Shigella* ($p = 0.00, 0.00, 0.04, 0.04$). These results indicate that, fibrous diet can regulate the steroid hormones secretion and modulate the gut with more cellulose-degrading and probiotic bacterium, but less opportunistic pathogens, and this may contribute to the improvement of reproductive performance and welfare in sows.

1. Introduction

There are growing concerns about the contribution of dietary fiber to the reproductive performance and welfare in sows keeping. High fiber diet increased the survival rate of embryo in early gestation stage (Ferguson et al., 2007), and improved the farrowing performance including the litter size and total litter weight at birth and weaning (Sun et al., 2015; Veum et al., 2009). Numbers of studies observed that dietary fiber reduced stereotypic and aggressive behavior, but increased rest time (Budiño et al., 2014; Sapkota et al., 2016). While the underlying mechanism remains unknown.

Dietary fiber is composed of plant polysaccharides and lignin, which are generally not hydrolyzed by endogenous enzymes in the gastrointestinal tract, but can be fermented by the intestinal bacterium. Many of the intestinal bacterium are involved in the main metabolic pathways, such as carbon metabolism and amino acid synthesis (Li et al., 2008). And, the intestinal bacterium and their metabolism products can affect the metabolism, immunity and even the hormone secretion of their host (Lee and Hase, 2014). It is worth noting that aberrations in the gut microbiota may result in metabolic diseases such as obesity, metabolic syndrome, and diabetes (Ley et al., 2006; Peterson et al., 2008). However, the homeostasis of gut microbiota is dynamic and highly susceptible to numerous factors, such as diet composition, host genetics, and even different stage of gestation (David et al., 2014; Koren

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Abbreviations: CF, crude fiber; SCFAs, short-chain fatty acids; CP, crude protein; DE, digestive energy; E_2, estradiol; P_4, progesterone

[*] Corresponding author at: Institute of Animal Husbandry & Veterinary Sciences, Shanghai Academy of Agricultural Sciences, No. 2901, BeiDi Road, Minhang District, Shanghai 201106, PR China.

E-mail address: xiadong@saas.sh.cn (D. Xia).

[1] These authors contributed equally to the work.

https://doi.org/10.1016/j.ygcen.2019.04.006
Received 11 October 2018; Received in revised form 11 March 2019; Accepted 1 April 2019
Available online 02 April 2019
0016-6480/

et al., 2008). For instance, a long-term low protein diet changed the gut microbial composition and metabolite profiles, including amino acids and glucose metabolism-related pathways (Zhou et al., 2016). High fiber diet obviously increased the number of cellulolytic bacterium in the gut microbiome (Nenci et al., 2007). Yet, despite the clear importance of the gut microbiome, our understanding of the causal changes or the relationship of the gut microbiome and reproductive performance of sows is to date very limited.

Meishan pigs, a Chinese indigenous breed and well known for its high fertility (mean litter size: 15–16 piglets), is traditionally raised on diet containing a high proportion of roughage such as green grass, residue of vegetable and bran of grain, this means the diet has a high level of crude fiber. Whereas in last decade, a lot of animal feed industry have been developed in China, it is convenient for Meishan pigs to have commercial feeds, which usually contain crude fiber below 3%. According to the farm productive records, there is an increasing tendency of the percentage of litter size less than 15. Whether the low crude fiber feed caused the reduction of litter size in Meishan sow remains unknown. Therefore in this study, Meishan sows were treated with diets containing 2.5% crude fiber (CF, similar ratio of CF as the commercial feed) and 7.5% CF respectively for an entire reproductive cycle to analyze the effect of dietary fiber on the secretion of steroid hormones, pregnant behaviors, the farrowing performance and the gut microbiome in Meishan sow.

2. Methods

2.1. Animals and sampling

All animal care and experimental protocols were in accordance with the Guidelines of the Institutional Animal Care and Use Committee of the Institute of Animal Husbandry and Veterinary Sciences, Shanghai Academy of Agricultural Sciences, and were in compliance with standard international regulations.

Thirty-six Meishan sows (2nd ~ 3rd parity) from the National Conservation Farm of Meishan Pigs (Jiading, Shanghai), balanced for parity and bodyweight (143.12 ± 4.24 kg), were allocated into 2.5% CF group and 7.5% CF group, eighteen sows in each group. Diets for the two groups were formulated with similar levels of crude protein (CP) and digestive energy (DE), as indicated in Table 1. After the piglets were weaned from the previous parity, sows were immediately moved into individual gestation crate 2.30 m × 0.63 m with partly slat floor (2.00 m concrete solid floor plus 0.30 m concrete slatted floor). Sows were fed individually with 1.0 kg of gestation diet twice daily at 07:00 and 17:00 respectively till the 90th day of gestation, and thereafter were fed on lactation diet ad libitum till the end of lactation. Approximately 10 days before the expected time of parturition, sows were moved to the farrowing pens (1.80 m × 2.20 m) equipped with floor heating for piglets. All the thirty-six sows were used for the analysis of farrowing performance (eighteen sows in each group), including the litter size, number of alive birth and birth weight. Diets were presented in pellet form. All the pigs had normal epidemic prevention and free access to nipple drinker.

On the 90th day of gestation, nine sows from each group were randomly selected for salivary and fecal sample collection in the morning (06:00–07:00). Saliva was collected using cotton swab (Thomsson et al., 2014), in brief, held the cotton swab with forceps and allowed the sow to chew on the cotton swab until it was soaked. Then the saliva was recovered by centrifuging the cotton swab at 3000 × g in room temperature for 5 min (GTR10-1 centrifuge, Era Beili Centrifuge Co., Ltd., Beijing, China) and stored at − 20 °C until analyzed. Fresh stool was individually collected via rectal massage without contamination with the barn floor. Each fecal sample was mixed homogeneously individually over ice and sampled for different analysis. The fecal sample for microbiome pyrosequencing analysis was filled in a tube with ethanol (1:3) and then stored at − 20 °C until DNA extraction. The fecal samples for short-chain fatty acids (SCFAs) and steroid hormones analysis were kept frozen at − 20 °C.

Table 1
Feed compositions and nutrient levels.

Feed (%)	Gestation feed		Lactation feed	
	2.5% CF	7.5% CF	2.5% CF	7.5% CF
Compositions				
Corn	65.00	60.8	61.80	52.00
Barley (include bran)	21.00	0.00	15.00	0.00
Full-fat soybean	10.00	9.20	17.20	16.00
Imported fish meal	0.00	0.00	2.00	2.00
Alfalfa meal	0.00	20.40	0.00	20.0
Soybean oil	0.00	5.60	0.00	6.00
Phytase	0.02	0.02	0.02	0.02
Sodium sulfate	0.20	0.20	0.20	0.20
Limestone	1.08	0.40	1.25	0.58
Premix	4.00	4.00	4.00	4.00
Nutrient levels				
DE (Mcal/Kg)	3.26	3.25	3.34	3.33
CP (%)	12.39	12.38	15.81	15.28
CF (%)	2.50	7.50	2.50	7.50

Crude fiber (CF), digestible energy (DE), crude protein (CP). The premix provided the following amounts per kilogram diet: Mn 63.6 mg, Cu 25 mg, Fe 150 mg, Zn 172.5 mg, I 0.14 mg, Se 0.3 mg.

2.2. Behavior and farrowing performance investigation

The video camera (DS-7608N-E2/8P, Hikvision, Hangzhou) was installed on the ceiling above the gestation barn to view the entire pen. Nine sows from each group were randomly selected for behavior observation respectively. The behavior of each sow during the gestation day 81 to day 85 was recorded and stored. Through the video playback, the frequency and lasting time of each behavior (Dedecker et al., 2014), including lying, standing, sham-chewing (mouth empty while moving jaw in a repetitive chewing motion), rolling tongue and licking the ground during the lighting time (07: 00 to 17: 00) were observed and registered by a trained observer. In addition, the total litter size and number of alive born pigs, birth weight and weaning weight of piglets in each experimental sow were accurately recorded.

2.3. Determination of fecal SCFAs

For the SCFAs detection, one gram of each fecal sample was diluted with distilled water, homogenized, and centrifuged (Heraeus Instruments, Düsseldorf, Germany) at 11,900 × g for 15 min. The supernatant was filtrated through a 0.22 μm filter, then 1.0 μL filtrate was injected into the gas chromatograph (7890B, Agilent Technologies, CA, USA) equipped with a flame ionization detector and a capillary column (30 m × 0.32 mm, 0.3 mm film thickness; Varian Inc., USA). To measure the SCFAs, 1.5 mM crotonic acid was used as an internal standard. The column, injector and detector temperatures were 120 °C, 180 °C and 180 °C respectively. Hydrogen gas, produced by a gas generator (Parker ChromGas, Parker Hannifin Corporation, MN, USA), was the carrier gas used at a flow rate of 30 mL/min. A standard SCFAs mixture containing acetic acid, propionic acid, butyric acid, isobutyric acid, pentanoic acid and isopentanoic acid was used for calculation, and the results were expressed as mmol/g of fresh fecal sample.

2.4. Determination of salivary and fecal steroid hormones concentration

As compared to blood sampling, salivary and fecal sample collection is non-invasive with less interference from acute stressors. Salivary steroid hormones can reflect the biological activities of steroid hormones in plasma (Thomsson et al., 2014), fecal steroid hormones concentration can stand for the systemic steroid hormone synthesis and secretion especially for periodic investigation (Fujita et al., 2001; Rolland et al., 2005; Burgess et al., 2012). Thus for avoiding blood sampling stress, here we measured the salivary and fecal steroid hormones. The fecal steroid hormones measurement were followed the protocol described previously (Fujita et al., 2001). In brief, fecal samples were frozen-dried for 72 h, the sample weight before and after freeze dried was recorded for the calculation of stool water content and 0.10 g of the fecal powder was extracted in 3 mL of 80% aqueous methanol by vortex for 15 min. Following extraction, the suspension was centrifuged, and the supernatant was recovered for hormones measurement.

The progesterone (P_4), estradiol (E_2) and cortisol content measurements were performed using the commercial EIA kits (Cayman, P_4, Item No. 582601, intra-assay CV $<$ 7.3%, inter-assay CV $<$ 16.4%, sensitivity is 10 pg/mL. E_2, Item No. 582251, intra-assay CV $<$ 12.3%, inter-assay CV $<$ 5.5%, sensitivity is 15 pg/mL. Cortisol, Item No. 500360, Intra-assay CV $<$ 5.1%, Inter-assay CV $<$ 6.7%, sensitivity is 110 pg/mL). All samples were run in triplicate.

2.5. *Pyrosequencing analysis of fecal microbiome*

The total genomic DNA was extracted from the stool samples using the commercially available DNA extraction kit, according to the manufacturer's instructions (Ultra clean fecal DNA isolation kit, Solarbio Co., Ltd., CHN). The concentration of the extracted DNA was determined using a Nano-Drop 1000 spectrophotometer (Thermo Scientific Inc., Wilmington, DE, USA). The amplification of fecal DNA was performed by using a barcode-tagged primer set for pyrosequencing of the bacterial 16S rRNA gene. This primer set targeted the V4 and V5 hypervariable regions of the 16S rRNA genes using the 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′) primer set. The PCR was performed using 4 μL 5 × FastPfu Buffer, 2 μL dNTPs (2.5 mM), 0.4 μL FastPfu Polymerase, 0.8 μL of each primer (5 μM), and 10 ng of fecal DNA as the template. The amplification program consisted of an initial denaturation at 95 °C for 5 min, 27 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s, and a final extension at 72 °C for 10 min. The amplification products from each sample were evaluated by electrophoresis in 2% agarose gel and purified using the QIAquick PCR purification kit (Qiagen, Valencia, CA). The products were quantified using QuantiFluorTM-ST fluorescent quantitation system (Promega, Madison, WI, USA), and then mixed in equivalent proportions. Sequencing was performed using Illumina Miseq PE250 according to the manufacturer's instructions.

A quality control of sequences was conducted and only high-quality sequences were used for subsequent analyses. The operational taxonomic units (OTU) picking with 97% similarity cut off was compiled with Qiime using default parameters. Taxonomic classification was performed based on the OTU database. The richness estimators (ACE and Chao) and the diversity indices (Shannon and Simpson) were measured with Mothur program (http://www.mothur.org) and Rarefactuin software.

2.6. *Statistical analysis*

The data were analyzed with SPSS 17.0 using a randomized block design, considering the CF as the main effect. The data variations were explored using the Shapiro-Wilk normality test, *p*-values greater than 0.05 indicate that data are homogenous in variance. The effects of CF on the behavior and farrowing performance, fecal SCFAs, fecal steroid hormones and microbial community of the sows were tested for significance using the Student's *t*-test, with significant differences defined as $p < 0.05$. Results are presented as means ± standard error (SE).

3. Results

3.1. *Farrowing performance*

As shown in Table 2, sows fed on 7.5% CF diet had more litter size than sows fed on 2.5% CF diet (14.57 ± 0.84 vs 11.00 ± 0.83, $p = 0.01$), and more pigs born alive (14.43 ± 0.95 vs 10.90 ± 0.82, $p = 0.01$). But no statistic difference of the mean birth weight and the mean daily weight gain during lactation period were observed between the two groups. In addition, there was no dead piglet during the sucking and weaning period in the two treatments, thus the weaning litter size kept the same as the number of pigs born alive.

Table 2
Farrowing performance and body weight gain.

Items	2.5% CF	7.5% CF	*p* value
Litter size	11.00 ± 0.83	14.57 ± 0.84	0.01
Alive born pigs per litter	10.90 ± 0.82	14.43 ± 0.95	0.01
Birth weight (kg)	1.11 ± 0.02	1.07 ± 0.17	0.24
Bodyweight gain during lactation (g/d)	186.25 ± 8.63	192.52 ± 9.58	0.27

Crude fiber (CF). Values were represented as means ± SE, $p < 0.05$ means significant difference between 2.5% CF group and 7.5% CF group, N = 18.

3.2. *Behavior and fecal water content*

From the video camera record, the stereotypic behaviors including sham chewing, rolling tongue and licking ground during the lighting time (07: 00 to 17: 00) of the sows fed on 7.5% CF diet were significantly less than the sows fed on 2.5% CF diet ($p = 0.02$, Table 3). And the frequency of standing and the ratio of the time spent on standing to lying in the sows fed on 7.5% CF diet were fewer than the sows fed on 2.5% CF diet ($p = 0.00$). The fecal water content of 7.5% CF treated sows was significantly higher than that of the 2.5% CF treated sows ($p = 0.02$).

3.3. *Steroid hormones level*

The EIA assay illustrated that, the 7.5% CF treatment significantly up-regulated the salivary P_4 level ($p = 0.00$, Fig. 1a), down-regulated the salivary cortisol level ($p = 0.01$, Fig. 1c), but did not cause statistic changes of the E_2 level in the saliva ($p = 0.38$, Fig. 1b) as compared with the 2.5% CF treatment.

The concentration of fecal P_4 ($p = 0.01$, Fig. 1d) and E_2 ($p = 0.02$, Fig. 1e) of the 7.5% CF treatment were significantly higher than that of the 2.5% CF treatment. Whereas, the fecal cortisol level was significantly lower in the 7.5% CF group than that in the 2.5% CF group ($p = 0.00$, Fig. 1f).

3.4. *Microbiome diversity and composition*

Pyrosequencing profiled a total of 1,558,719 valid reads that were assigned to 1,384 OTUs after screening them with strict criteria. Number of OTUs, coverage and statistical estimates of species richness for each group at a genetic distance of 3% are presented in Table 4. The treatment of 7.5% CF diet significantly increased the indices of OTUs

Table 3
Behaviors and fecal water content.

Behaviors	2.5% CF	*7.5% CF*	*p* value
Sham chewing (times/h)	0.76 ± 0.15	0.32 ± 0.12	0.02
Rolling tongue (times/h)	0.14 ± 0.08	0.00 ± 0.00	0.04
Licking ground (times/h)	0.24 ± 0.10	0.00 ± 0.00	0.01
Total stereotypic behavior (times/12 h)	6.40 ± 1.60	2.00 ± 1.03	0.02
The ratio of standing /lying	0.20 ± 0.01	0.11 ± 0.01	0.00
Standing (times/12 h)	3.47 ± 0.11	2.38 ± 0.08	0.00
Fecal water content (%)	59.11 ± 0.89	63.09 ± 1.06	0.02

Crude fiber (CF). Nine sows from each group were randomly selected for behavior observation (N = 9), values were represented as means ± SE, $p < 0.05$ means significant difference between 2.5% CF group and 7.5% CF group.

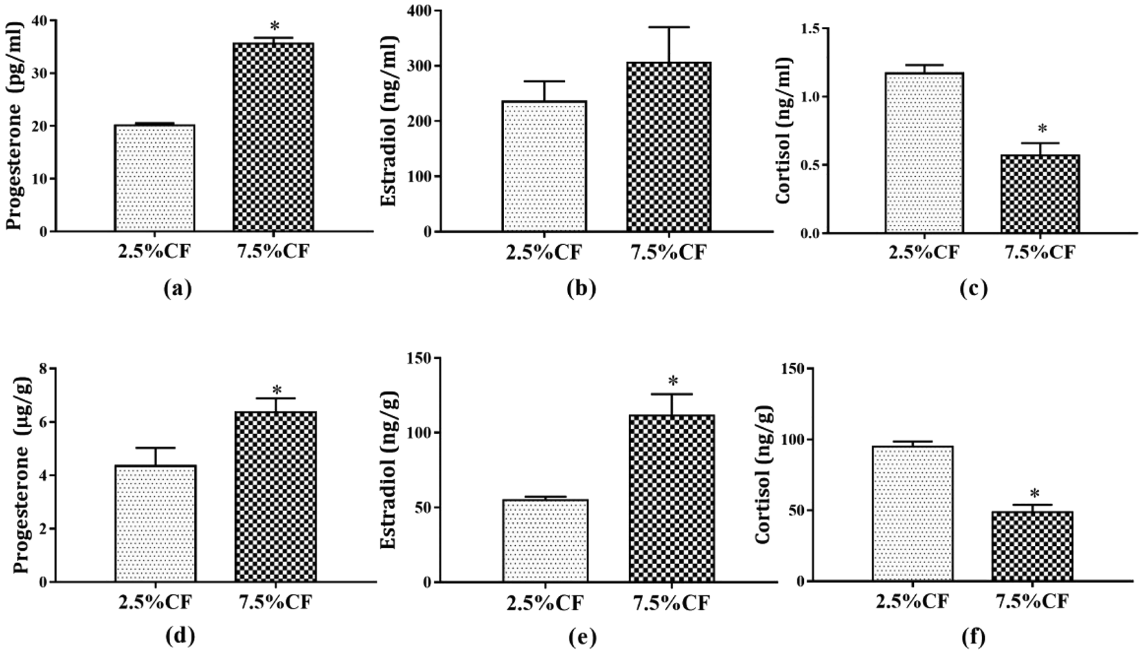

Fig. 1. Saliva and stool steroid hormone concentrations. Saliva progesterone (a), estradiol (b) and cortisol (c) concentration were expressed as picogram or nanogram per mL saliva. Fecal progesterone (d), estradiol (e) and cortisol (f) concentration were expressed as nanogram or microgram per gram feces. Crude fiber (CF), the data were represented as means ± SE (N = 9), * indicates significant difference between 2.5% CF group and 7.5% CF group, $p < 0.05$.

($p = 0.02$), ACE ($p = 0.04$) and Shannon ($p = 0.01$), while decreased the Simpson value ($p = 0.04$), when compared with the 2.5% CF diet treatment.

At the phylum level (Fig. 2a), we observed that the majority proportion of sequences (> 85%) were attributed to *Firmicutes* (53.58%) and *Bacteroidetes* (31.80%), and followed by *Spirochaetes* (7.13%), *Tenericutes* (2.86%) and *Proteobacteria* (2.23%). Among them, the relative abundance of *Firmicutes* was significantly lower in 7.5% CF group ($p = 0.05$), but the relative abundances of *Bacteroidetes*, *Spirochaetes*, *Tenericutes*, *Proteobacteria* and *Fibrobacteres* were significantly higher in 7.5% CF group than that in the 2.5% CF group ($p = 0.04$, 0.01, 0.01, 0.03, 0.00).

At the genus level (Fig. 2b), the proportions of *Ruminococcus*, *Butyrivibrio*, *Lactobacillus* and *Fibrobacter* in the fecal microbiome were more ($p = 0.02$, 0.05, 0.04, 0.00) in the 7.5% CF group than that in 2.5% CF group, and the proportions of *Clostridium*, *Streptococcus*, *Bacteroides*, Unclassified *Bacteroidales* and *Escherichia – Shigella* were less in 7.5% CF group than that in the 2.5% CF group ($p = 0.00$, 0.00, 0.00, 0.04, 0.04).

Table 4
Stool bacterial richness and diversity index.

Item	2.5% CF	7.5% CF	*p* value
Coverage (%)	99.50 ± 0.00	99.50 ± 0.00	0.72
OTUs	753.75 ± 14.02	830.67 ± 22.29	0.02
ACE	868.12 ± 17.92	949.22 ± 26.62	0.04
Chao	887.87 ± 19.73	960.56 ± 27.19	0.07
Shannon	5.02 ± 0.02	5.15 ± 0.02	0.01
Simpson	0.016 ± 0.00	0.01 ± 0.00	0.04

Crude fiber (CF). The operational taxonomic units (OTUs) were defined with 97% similarity. The coverage percentages, richness estimators (ACE and Chao), and diversity indices (Shannon and Simpson) were calculated. The data were expressed as mean ± SE, N = 9, $p < 0.05$ means significant difference between 2.5% CF group and 7.5% CF group.

3.5. *Fecal SCFAs concentration*

The stool SCFAs concentrations in the 7.5% CF diet treated sows, include propionic acid, butyric acid, isobutyric acid, pentanoic acid and isopentanoic acid, and the total SCFAs concentrations were obviously higher than that in the 2.5% CF diet treated sows ($p = 0.02$, 0.01, 0.00, 0.02, 0.00, 0.03, Table 5). However, no statistic difference of the fecal acetic acid content was observed between the two treatments.

4. Discussion

Present study for the first time observed that, 2.5% CF diet treatment decreased the litter size of Meishan sows compared with the 7.5% CF diet treatment. Our data are in agreement with previous researches that a high fiber diet (containing 50% unmolassed sugar beet pulp) increased the survival rate of embryo in early gestation stage in gilts (Landrace × Large White) (Ferguson et al., 2007). Veum et al. (2009) treated the sows with additional ground wheat straw to the gestation diet for 3 reproductive cycles, and observed that the additional ground wheat straw improved the farrowing performance including the litter size and total litter weight at birth and weaning. Sun et al., (2015) reported that the high dietary fiber increased lactation feed intake and improved number of piglets weaned per litter in multiparous Landrace sows. All these suggest that the dietary fiber is important for the farrowing performance of sows.

Previous studies indicated that high fiber diet could eliminate the stereotypic behaviors in gestation pigs (Ferguson et al., 2007; Veum et al., 2009; Dedecker et al., 2014). Consistently, here the 7.5% CF treated sows had less stereotypic behaviors and more resting time than the 2.5% CF treated sows at the later gestation stage. As we know, constipation is another discomfort symptom for the pregnant sows, as sow approaching farrowing the intestine is less active and the water absorption increases, dry or even firm stool is often founded in pregnant sows at the later stage. Here we observed that the stool water content in the 7.5% CF diet treated sows was higher than that in the 2.5% CF diet treated sows. Similar results were achieved by the previous study, pregnant sows fed on a diet contain 7.0% CF in the later stage of gestation had fewer constipation symptoms than the 3.8% CF treated sows

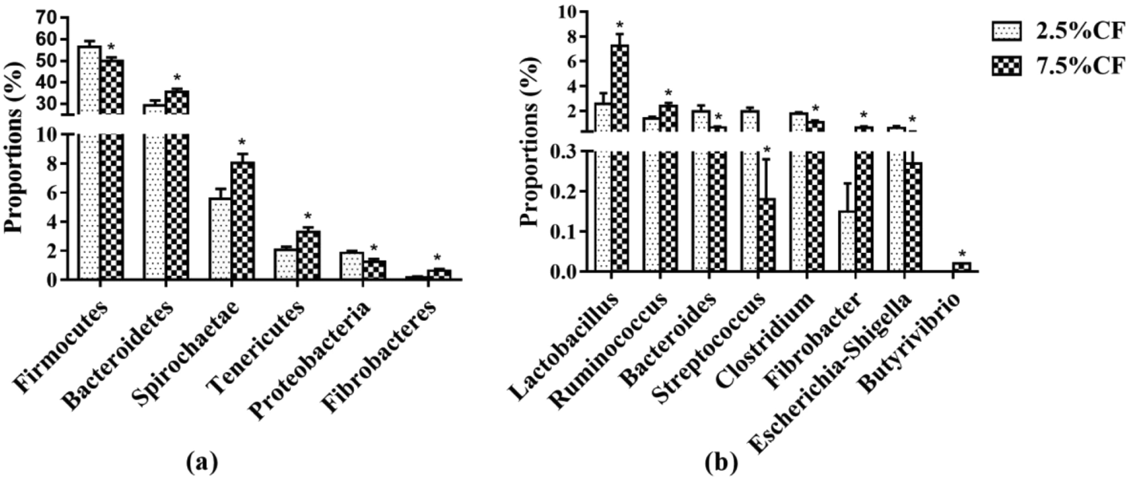

Fig. 2. Stool microbiome composition. Microbiome composition at phylum level (a) and genus level (b), only list significant different genus. Crude fiber (CF). The high-throughput 16S rRNA gene-based pyrosequencing technique was used to investigate the effects of a high fiber diet on stool microbial composition of Meishan sows. The data were represented as means ± SE (N = 9), * indicates significant difference between 2.5% CF group and 7.5% CF group, $p < 0.05$.

Table 5
Fecal short-chain fatty acids concentrations.

SCFAs (mmol/g)	2.5% CF	7.5% CF	*p* value
Acetic acid	65.47 ± 3.88	74.76 ± 3.80	0.12
Propionic acid	21.96 ± 2.35	29.19 ± 1.34	0.02
Butyric acid	5.32 ± 0.55	9.33 ± 0.90	0.01
Isobutyric acid	0.89 ± 0.10	1.98 ± 0.12	0.00
Pentanoic acid	1.40 ± 0.18	2.14 ± 0.15	0.02
Isopentanoic acid	2.08 ± 0.11	4.21 ± 0.37	0.00
Total SCFAs	101.35 ± 8.10	125.27 ± 5.92	0.03

Crude fiber (CF). Short-chain fatty acids (SCFAs). The data were represented as means ± SE (N = 9), $p < 0.05$ means significant difference between 2.5% CF group and 7.5% CF group.

(Oliviero et al., 2009). All this suggest that the high fiber diet might eliminate the constipation severity in pregnant sows.

Interestingly, parallel to the alleviation of stereotypic behaviors and constipation symptom in the 7.5% CF treated sows, the salivary and fecal cortisol concentrations were obviously decreased, when compared with the 2.5% CF treated sows. Sun et al. (2015) achieved a similar study that high level of konjac flour diet decreased the non-feeding oral behavior and the plasma concentration of cortisol at 1 h postprandial of multiparous Landrace sows on day 90 of gestation. In mammals, the physiological stress is typically characterized by the increased level of cortisol in blood. For instance, three weeks of feed restriction increased the cortisol level in growing pigs (Metges et al., 2015). The pregnant animal is suffering from a number of stress including the development of fetus, changes of hormones, metabolism and immunity, and the cortisol level of pregnant animal is higher than empty ones (Manojlović-Stojanoski et al., 2012; Nepomnaschy et al., 2004; Kanitz, et al., 2012). Here the 7.5% CF diet treatment reduced the stereotypic behavior and the cortisol level in saliva and stool, and the 7.5% CF diet treated sows had more lying time, and less constipation symptom than the 2.5% CF diet treated sows, illustrate that the high fiber could alleviate the stress of pregnant sows.

In mammals, the increased secretion of cortisol may inhibit or impair the reproductive function via disrupting the hypothalamic-pituitary-gonads axis (Salak-Johnson, 2017). Acute infusion of cortisol suppressed the pulsatile luteinizing hormone secretion in the ovariectomized ewe (Breen and Karsch, 2004). Administration of dexamethasone (a kind of synthetic glucocorticoids) decreased the secretion of P_4 in pregnant sheep (Breen and Karsch, 2004). Conversely, females with low blood cortisol level achieved better reproductive performance than the high ones (Jang et al., 2015; Song et al., 2015). Here we observed that 7.5% CF diet treated sows had lower salivary and fecal cortisol levels, but higher salivary and fecal P_4 concentration at the gestation day 90 and improved farrowing performance than the 2.5% CF diet treated sows. This suggests that the high fiber diet could alleviate the pregnant stress, and consequently reduce the cortisol inhibiting action on the synthesis of P_4 and E_2, and these may account for the improvement of the reproductive performance in sows.

As compared to blood sampling, salivary and fecal sample collection is non-invasive with less interference from acute stressors (Sheriff et al., 2010). Salivary steroid hormones can reflect the steroid hormones biological activities in plasma. Fecal steroid hormone concentrations represent the steroid hormone production over time, and the changes in fecal steroid concentrations could indicate the endocrinal status in blackbuck (Archunan and Rajagopal, 2013) and goats (SankarGanesh et al., 2014). The fecal E_2 level was higher during estrus than that in diestrus, and increased level of P_4 during post-estrus were observed in stool. Present data partly agreed with this, fecal P_4 and cortisol levels were in parallel with the difference of salivary P_4 and cortisol concentration between the two treatments, while the 7.5% CF treatment obviously increased the fecal E_2 level in contrast to the 2.5% CF treatment, but no significant change of the salivary E_2 level was found between the two treatments. Research indicated that the insoluble fiber could affinity to the estrogens in intestinal gut and consequently influenced the estrogen metabolism by interrupting the enterohepatic circulation of the estrogens (Aubertin-Leheudre et al., 2008). And, whether this account for the inconsistent changes of estradiol concentration in saliva and stool remain unclear, further studies are needed.

Dietary fiber can alter the gut microbiota composition (Song et al., 2015; Glade and Meguid, 2016; Jiang et al., 2017). Consistently, here the pyrosequencing analysis showed that the 7.5% CF diet obviously increased the diversity and the richness of the fecal microbiome, enriched the proportion of *Butyrivibrio*, *Fibrobacter*, *Lactobacillus* and *Ruminococcus* in stool microbiota. Researches indicated that *Butyrivibrio*, *Fibrobacter* and *Ruminococcus* can degrade the dietary fiber into SCFAs (Chassard et al., 2011; Louis and Flint, 2009). Previous studies proved that the fecal SCFAs contents are positively related to the dietary CF contents (Beyer-Sehlmeyer et al., 2003; Canh et al., 1998). In the present study, 7.5% CF diet obviously increased the fecal SCFAs contents. SCFAs act as an important energy and signaling molecules, they can directly bind to their specific G-protein-coupled receptor (GPR) 41 and GPR43, inhibit histone deacetylation, play positive actions on the homeostasis of carbohydrate and lipid metabolism, gastrointestinal motility and immunity. A double-blind, randomized, placebo-controlled study on volunteers illustrated that rectal administration of butyric acid improved the intestinal motility, reduced the abdominal pain or discomfort (Koh et al., 2016). The stimulating action of butyric acid on the intestinal motility may account for the elimination constipation symptom in the 7.5% CF diet treated sows. In addition, our previous in vitro trial illustrated that butyric acid can regulate steroidogenesis via the cAMP signaling pathway in porcine granulosa cells (Lu et al., 2017). Yet, whether the increased SCFAs contribute to the change of fecal steroid hormones in the 7.5% CF diet treated sow remains unclear.

Usually, *Butyrivibrio* and *Lactobacillus* are considered as the probiotics in gut microbiome, oral administration of *Butyrivibrio* alleviated overall symptoms of *Campylobacter coli* infection in mice (Ohkawara et al., 2006). High population of *Lactobacillus* in gut can inhibit the growth of pathogenic bacteria (Pfeiler and Klaenhammer, 2007; Valeriano et al., 2014). Administration of *Lactobacillus Heleveticus R0052* and *Bifidobacterium Longum R0175* can prevent stress-induced changes in neurogenesis, barrier integrity, and stress reactivity (Ait-Belgnaoui et al., 2012). Liang et al. (2015) observed that *Lactobacillus Helveticus NS8* down-regulated the secretion of corticosterone in Sprague Dawley rat. Zijlmans et al. (2015) observed that pregnant women with high self-reported stress had high salivary cortisol concentrations and lower relative abundances of *Lactobacillus* in the fecal microbiome. Mudd et al. (2017) observed in piglets that the fecal *Ruminococcus* population was negatively predicted the serum cortisol level. Present data agree with above, accompanied with the increasing of genus *Butyrivibrio*, *Fibrobacter*, *Lactobacillus* and *Ruminococcus* in the stool microbiota, the fecal cortisol concentration was decreased in the 7.5% CF treated sows, and these suggest that the gut microbiome may influence the cortisol synthesis and metabolism.

As we know, the pathogenic infection may evoke an immunity response and stimulate the secretion of glucocorticoids in the host. *Escherichia-Shigella* is a harbor pathogenic strain of *E. coli*, which can produce Shiga-toxins and cause diarrhea (Hodges and Gill, 2010). Weaning piglets challenged with *Escherichia coli* lipopolysaccharide increased the serum cortisol level (Guo et al., 2017). In turn, stress may enrich the replication of pathogenic bacterium in the gut. For instance, weaning is one of the most stressful events for piglets involving physiological, nutritional, and cognitive-behavioral responses (Campbell et al., 2013). The serum cortisol level was increased during the weaning period when the piglets are often infected with pathogenic bacteria (Moeser et al., 2007; Tuchscherer et al., 2017). The population of *Streptococcus* (an emerging zoonotic pathogen) in the gut microbiome was increased in weaning piglets (Su et al., 2008). Besides the weaning time, the pregnancy is another stress stage for the females, especially in the later stage of pregnancy, the fecal and the blood cortisol levels, together with the proportion of *Streptococcus* and *Proteobacteria* in the fecal microbiome composition were higher than those in the early stage. The phylum *Proteobacteria* contains multiple pathogens and is often associated with inflammatory conditions (Koren et al., 2012). Consistently, here parallel to the decrease of fecal cortisol concentration in the 7.5% CF diet treated group, the population of potential pathogenic bacteria were decreased in the stool microbiota including genus *Bacteroides*, *Clostridium*, *Streptococcus* and *Escherichia-Shigella*. *Bacteroides* can synthesize anti-stress proteins so that can survive and replicate under stress condition (Hochart-Behra et al., 2014; Sovran et al., 2016). The high proportion of *Bacteroides* in gut microbiome also was found in depressed patients who had a higher cortisol level than normal (Kelly et al., 2015). Genus *Clostridium* contains common free-living bacteria and pathogens, and it was found positively correlated with fecal cortisol level (Vlčková et al., 2018). All these further illustrate, there might be an interaction between the gut microbiome and the cortisol.

5. Conclusions

In compared with the 2.5% CF treatment, 7.5% CF diet decreased stereotypic behaviors and constipation symptom, moreover increased resting time and farrowing performance in Meishan sows. Meanwhile, the 7.5% CF diet decreased the salivary and fecal cortisol level, increased salivary P_4 level and fecal concentrations of P_4, E_2 and SCFAs. The 7.5% CF treated sows had more proportion of cellulose-degrading bacterium and probiotic bacterium, but less proportion of opportunistic pathogens in the fecal microbiota than that of the 2.5% CF treated sows. Present data suggest that there are interactions between the gut microbiome and the steroid hormones, especially the cortisol, while the mechanism involved should be elucidated in further study.

Acknowledgments

We wish to express our gratitude to Mr. Bo Wang and the stuff of the National Conservation Farm of Meishan Pigs for their assistance with animal care. This work was funded by National Key Research and Development Program of China (2016YFD0500502), Shanghai Key Project for Agro Scientific Research 2014 (7-2-6), Shanghai Youth Talent Project for Agro System 2018 (1-23).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2019.04.006.

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