INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 11, No. 5, p. 32-42, 2017

RESEARCH PAPER OPEN ACCESS

# Influence of mulberry forage on gastrointestinal microbial composition and diversity in pigs

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**Key words:** Protein-rich forage, Mulberry leaves, Gastrointestinal bacteria, Pig.

http://dx.doi.org/10.12692/ijb/11.5.32-42 Article published on November 12, 2017

## Abstract

This study was undertaken to evaluate the effects of mulberry forage on changes in bacterial communities in various segments of the gastrointestinal tract of pigs (jejunum, ileum and cecum). A total of 40 healthy pigs were divided into 5 groups and 1 group as the control group was fed standard diet, the other 4 groups were fed standard diet containing different levels of mulberry leaves. Intestinal content was collected from the jejunum, ileum and cecum from the 5 groups. Bacterial community compositions were analyzed using 16S rRNA gene-targeted metagenomicapproach. In our study, regardless of the diet, Firmicutes, Proteobacteria and Bacteroidetes were the major components (>93%) of intestinal bacterial communities. Firmicutes and Proteobacteria predominated in the jejunum and ileum, and Firmicutes and Bacteroidetes predominated in the cecum. Furthermore, we also found that phylum Firmicutes, Bacteroidetes and class Clostridia, Bacilli were enriched in the mulberry diet group, while phylum Proteobacteria and class Gammaproteobacteria showed a higher abundance in the standard diet group. Our results revealed that although the intestinal bacteria varied due to the different composition of diet, substituting the commercial concentrate with mulberry forage did not result in a gastrointestinal disturbances in our study. Therefore, mulberry forage could be a valuable alternative protein-rich forage in pig feeding and could economize the pig production.

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## Introduction

With the increased demand for animal production and the scarcity of concentrates in many developing countries, there is an obvious demand for sufficient and inexpensive livestock feed(Li *et al.,* 2017). For sustainable intensification of pig industry, it is imperative to find local high-protein alternatives to reduce feeding costs.

As we know, mulberry has been cultivated for thousands of years, and mulberry leaves have long been the major feed for the silkworm(Liu and Willison, 2013). Previous studies have shown that the forage mulberry has a high protein content (18 to 25 % in DM),low neutral detergent fiber content (García *et al.,* 2008)and high *in vivo* DM digestibility (Ba *et al.,* 2005), which suggested that they have the potential to be used as a protein-rich forage supplement for animal production (Benavides, 2002; Sanchez, 2002) and play a valuable role in world agriculture.

The swine gastrointestinal tract harbours a diverse and dense population of microorganisms, and the microorganisms have a significant impact on the growth and health of pigs(Isaacson and Kim, 2012). Maintaining animal health and performance through prevention of gastrointestinal tract disorders is important for the swine industry (Pieper *et al.,* 2015). However, little is known about how mulberry leaves could influence the swine intestinal bacterial community structure. Thus, the objective of the present study was to determine the effects of substituting the commercial concentrate with different levels of the mulberry forage on bacterial composition and diversity in the jejunum, ileum and cecum of pigs.

## Materials and methods

*Animals and sampling*

A total of 40 healthy Xiangcun Black pigs, a Chinese local breed, with initial body weight70±1kg were used in a 60-d feeding study. They were divided into five groups (n=8) and each group was fed a different diet. The control group was fed standard diet, the other 4 groups were fed standard diet containing different levels of mulberry leaves (Table 1). The animals were fedtwice daily and had ad libitum access towater. To investigate the effects of mulberry leaves on the intestinal bacterial community, 5pigs were selected randomlyin each group, and totally 25pigs were selected. Animals were sacrificed according to the institutional animal care guidelines. Samples were collected from 25 pigs and analyzed. For sampling, the pigs were sacrificed and 5 to 10 cm sections of the jenumum, ileum, or cecum were tied off and stored at -80°C until genomic DNA was extracted.

*DNA extraction, 16S rRNA amplification from the microbial consortium*

Total genomic DNA was extracted from intestinal luminal contents using a MoBio Ultra Clean™Soil DNA isolation kit (San Diego, CA, USA) following the manufacturer's instructions. Finally, the DNA was eluted with TE buffer. The amount and purity of DNA were determined by using a NanoDrop® Spectrophotometer ND-1000 (Thermo Fisher Scientific, USA) based on the absorbency of A260 and the ratio of A260/A280, respectively. The extracted total microbial DNA was stored at -80°C prior to analysis.

The variable V4 region of the bacterial 16S rRNA gene was amplified with the general 16S rRNA gene primers 515F and 806R containing the specific barcode sequence. The forward primer (515F) was 5'-*GTTTCG*GTGCCAGCMGCCGCGGTAA-3', where the sequence of the barcode is shown in italics. The reverse primer (806R) was 5'-*GTGAAA*GGACTACHVGGGTWTCTAAT -3', where the sequence of the barcode is shown in italics. All PCR reactions were carried out in 30 μLs with 15μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM of forward and reverse primers and approximately 10 ng template DNA. Thermal cycling consisted of initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 60 s. Finally, extension occurred for 10 min at 72 °C.

*PCR Product quantification, qualification and purification*

We mixed the same volume of 1X loading buffer (containing SYB green) with PCR products and ran electrophoresis on a 2 % agarose gel for detection. PCR products were mixed in equidensity ratios. Then, mixture PCR products were purified with a GeneJET Gel Extraction Kit (Thermo Scientific).

*Sequencing of rDNA*

Sequencing libraries were generated using the NEB Next® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) following the manufacturer's recommendations, and index codes were added.

The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. Finally, the library was sequenced on an Illumina MiSeq platform.

*Sequence analysis*

Paired-end reads from the original DNA fragments were merged using FLASH (Magoč and Salzberg 2011), a very fast and accurate analysis tool that was designed to merge paired-end reads when at least some of the reads overlap the read generated from the opposite end of the same DNA fragment. Paired-end reads were assigned to each sample according to the unique barcodes.

Sequences analyses were performed by the UPARSE software package (Uparse v7.0.1001, http://drive5.com/uparse/) (Edgar 2013) using the UPARSE-OTU and UPARSE-OUT ref algorithms. In-house Perl scripts were used to analyze alpha (within samples) and beta (among samples) diversity. Sequences with ≥97% similarity were assigned to the same OTUs. We picked representative sequences for each OTU and used the RDP classifier (Version 2.2, http://sourceforge.net/projects/rdp-classifier/) (Wang *et al.* 2007) to annotate taxonomic information for each representative sequence. To compute Alpha Diversity, we rarified the OTU table and calculated three metrics: Chao1 (estimates the species abundance), Observed Species (estimates the number of unique OTUs found in each sample), and the Shannon index. Rarefaction curves were generated based on these three metrics.

A graphical representation of the relative abundance of bacterial diversity from phylum to species can be visualized using a Krona chart (Ondov *et al.* 2011).

## Results

*Diversities of bacterial communities*

In this study, at a >97 % sequence identity threshold, in jejunum group samples, MGJ.9 group showed the highest OTUs (485), MGJ.12 showed the lowest value (406).

**Table 1.** Ingredient and chemical compositions of diets for experimental pigs.

| Item | Experimental group (% of diet dry matter) | | | | |
|---|---|---|---|---|---|
| | CG | MG.3 | MG.6 | MG.9 | MG.12 |
| Ingredients | | | | | |
| Corn | 67.52 | 66.77 | 65.65 | 64.72 | 63.80 |
| Soya bean | 18.00 | 17.33 | 16.50 | 15.73 | 14.90 |
| Wheat bran | 12.00 | 10.50 | 9.60 | 8.40 | 7.29 |
| Mulberry forage | 0 | 3.00 | 6.00 | 9.00 | 12.00 |
| Calciumbicarbonate | 0.50 | 0.60 | 0.60 | 0.65 | 0.66 |
| Limestone | 0.68 | 0.50 | 0.35 | 0.20 | 0.05 |
| Salt | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Mineral and vitamin premix | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Diet composition (g/kg DM) | | | | | |
| CP | 170.27 | 141.39 | 140.87 | 140.29 | 139.62 |
| EE | 32.89 | 27.47 | 27.55 | 27.61 | 27.68 |
| CF | 24.90 | 23.77 | 27.04 | 30.17 | 33.35 |
| NDF | 332.19 | 274.61 | 273.41 | 271.69 | 270.29 |
| ADF | 406.90 | 333.49 | 328.49 | 323.20 | 318.24 |

CP: Crude potein; EE: Ether extracts; CF: Crude fiber; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; MG: mulberry diet group; CG: control group.

In ileum group samples, MGI.9 showed the highest OTUs (395), MGI.12 showed the lowest value (197). In cecum group samples, the highest number of OTUs was found in MGC.6 group (577), MGC.12 showed the lowest value (375).The Shannon diversity index and the Chao1 richness index showed similar comparative trends in predicting the number of OTUs in all samples. In jejunum and ileum group samples, MGJ.9 and MGI.9 groups had a more diverse bacterial community composition compared with the other groups (Table 2).

**Table 2.** Statistics and alpha diversity of all samples.

| Sample name | Qualified reads | Q20 | OTU (97 %) | Chao1 (97 %) | Shannon (97 %) |
|---|---|---|---|---|---|
| CGJ | 40,645 | 97.81 | 452 | 624.99 | 2.18 |
| MGJ.3 | 44,107 | 97.91 | 421 | 651.98 | 3.48 |
| MGJ.6 | 43,396 | 97.75 | 420 | 647.82 | 4.14 |
| MGJ.9 | 42,458 | 97.83 | 485 | 691.09 | 4.39 |
| MGJ.12 | 45,922 | 97.94 | 406 | 573.05 | 3.93 |
| CGI | 39,448 | 97.57 | 258 | 376.17 | 2.17 |
| MGI.3 | 40,884 | 97.72 | 240 | 436.59 | 3.16 |
| MGI.6 | 43,234 | 97.64 | 262 | 424.22 | 3.24 |
| MGI.9 | 40,395 | 97.61 | 395 | 524.96 | 3.25 |
| MGI.12 | 40,872 | 97.59 | 197 | 341.81 | 2.15 |
| CGC | 47,206 | 97.90 | 555 | 723.08 | 5.27 |
| MGC.3 | 45,618 | 97.88 | 534 | 699.03 | 5.80 |
| MGC.6 | 41,105 | 98.02 | 577 | 808.21 | 6.03 |
| MGC.9 | 35,393 | 98.02 | 444 | 563.93 | 4.02 |
| MGC.12 | 40,160 | 97.97 | 375 | 527.84 | 3.83 |

MG: Mulberry diet group; CG: Control group; J: Jejunum; I: Ileum; C: Cecum.

*Bacterial community structure*

Fig. 1 slowed the classification of the sequences at the phylum level in each group. The bacterial taxa were distributed in 10 different phyla in all samples (the proportion ranged from 99.85 to 99.99 %), including Firmicutes, Proteobacteria, Bacteroidetes, Cyanobacteria, Fusobacteria, Actinobacteria, Spirochaetes, TM7, Euryarchaeota, Tenericutes. The majority of bacterial sequencesin all samplesbelonged to these three phyla Firmicutes, Proteobacteria and Bacteroidetes.

**Table 3.** Statistical analysis of microbial communities with diet composition (phylum level).

| | Parameter | Jejunum | | Ileum | | Cecum | |
|---|---|---|---|---|---|---|---|
| | | CP | CF | CP | CF | CP | CF |
| Microbial diversity | OTU number | 0.265 | -0.048 | -0.091 | 0.046 | 0.422 | -0.886* |
| | Chao1 estimator | -0.138 | -0.383 | -0.342 | -0.117 | 0.319 | -0.776 |
| | Shannon diversity | -0.932* | 0.575 | -0.571 | -0.274 | 0.198 | -0.856 |
| Bacterial composition | Firmicutes | -0.945* | 0.580 | -0.376 | 0.394 | 0.417 | -0.669 |
| | Proteobacteria | 0.966** | -0.565 | 0.399 | -0.311 | 0.767 | -0.911* |
| | Bacteroidetes | 0.868 | -0.623 | 0.139 | -0.656 | -0.583 | 0.832 |
| | Cyanobacteria | -0.442 | 0.061 | -0.415 | -0.341 | 0.899* | -0.182 |
| | Fusobacteria | 0.999** | -0.429 | -0.208 | -0.573 | 0.235 | -0.941* |
| | Actinobacteria | -0.696 | -0.058 | -0.440 | 0.247 | -0.514 | 0.841 |
| | Spirochaetes | 0.967** | -0.443 | -0.368 | -0.224 | 0.335 | -0.834 |
| | TM7 | -0.710 | 0.811 | -0.479 | 0.177 | -0.374 | -0.525 |
| | Euryarchaeota | -0.514 | 0.305 | 0.995** | -0.381 | -0.159 | -0.714 |
| | Tenericutes | -0.813 | 0.745 | -0.633 | 0.131 | -0.068 | -0.842 |

* Correlation between two parameters is significant at the level of 0.05 (two tailed), ** Correlation between two parameters is significant at the level of 0.01 (two tailed), CP: Crude protein, CF: Crude fiber.

When sequences were analyzed at the class level, more than 98.06 % of the sequences could be classified in all samples. Clostridia, Gammaproteobacteria, Bacilli, Bacteridia, Alphaproteobacteria, Erysipelotrichi, Chloroplast, Fusobacteria, Actinobacteria and Coriobacteria were the top 10 dominant classes in all samples. Among them, Clostridia, Gammaproteobacteria, Bacilli, and Bacteridia were the most four dominant bacterial classes in all samples (Fig. 2).

When sequences were analyzed at the genus level (the lowest level assigned), around 41.98 to 93.79 % of the sequences could be classified. The top 10genera included*Escherichia*, *Lactobacillus*, *Streptococcus*, *SMB53*, *Turicibacter*, *Eubacterium*, *Paracoccus*, *Prevotella*, *Roseburia*, and *Serratia*. The majority ofbacterial sequences in these 3 groups belonged to these genus *Escherichia*, *Lactobacillus*, *Streptococcus*, and *SMB53* (Fig. 3).

*Dynamics of the bacterial community structure in the different groups*

At the phylum level, in jejunum group samples, we found that the relative abundances of Firmicutes in MGJ groups were significantly higher than that of CGJ group, while the relative abundances of Proteobacteria were significantly lower in MGJ groups when compared with CGJ group. In cecum group, the abundance of Proteobacteria in CGC group was significantly higher than those of MGC.6, MGC.9, and MGC.12 groups, among them, MGC.12 group showed the least value. However, for Bacteroidetes, it showed significantly less abundance in CGC group, and the abundances of Bacteroidetes in MGC.9 and MGC.12 groups were higher than those of MGC.3 and MGC.6 groups (Table S1).

**Table S1.** Bacterial compositions and comparative analysis of these bacteria (phylum level).

| Taxa | Relative abundance % (CG group) | Relative abundance % (MG.3 group) | Relative abundance % (MG.6 group) | Relative abundance % (MG.9 group) | Relative abundance % (MG.12 group) |
|---|---|---|---|---|---|
| Jejunum group samples | | | | | |
| Firmicutes | 25.00b | 59.26ab | 78.42a | 78.82a | 72.88a |
| Proteobacteria | 68.69a | 32.20b | 18.25b | 16.36b | 21.33b |
| Bacteroidetes | 2.99a | 1.62ab | 0.21b | 1.17ab | 0.34b |
| Spirochaetes | 0.06a | 0.02ab | 0.005b | 0.01ab | 0.02ab |
| Ileum group samples | | | | | |
| Proteobacteria | 50.60a | 41.52a | 45.35a | 6.07b | 45.92a |
| Bacteroidetes | 1.16a | 2.25a | 0.16b | 1.27a | 0.11b |
| Spirochaetes | 0.01b | 0.02ab | 0.05a | 0.02ab | 0.01b |
| Cecum group samples | | | | | |
| Proteobacteria | 6.24a | 4.71ab | 3.93b | 3.12bc | 1.43c |
| Bacteroidetes | 4.91b | 7.76b | 5.68b | 14.12a | 13.65a |

At the class level, in jejunum group samples,there was a decrease in the relative abundance of Clostridia in CGJ group when compared to MGJ groups.

Among MGJ groups, the MGJ.12 group showed the highest value.Besides that, the relative abundances of Gammaproteobacteria, Bacteroidia and Fusobacteria in CGJ group were significantly higher than those of MGJ groups. In ileum group samples, compared to the MGI groups, Clostridia was lower in CGI group. In cecum group samples, we found that the relative abundances of Clostridia and Alphaproteobacteria in CGC, MGC.3 and MGC.6 groups were significantly higher than those of MGC.9 and MGC.12 groups.

For Gammaproteobacteria, the CGC group showed the highest value, while the MGC.12 group showed the least value. For Bacilli, the MGC.12 group showed the highest value, while the CGC group showed the least value (Table S2).

**Table S2.** Bacterial compositions and comparative analysis of these bacteria (class level).

| Taxa | Relative abundance % (CG group) | Relative abundance % (MG.3 group) | Relative abundance % (MG.6 group) | Relative abundance % (MG.9 group) | Relative abundance % (MG.12 group) |
|---|---|---|---|---|---|
| Jejunum group samples | | | | | |
| Clostridia | 10.66b | 40.59ab | 36.61ab | 46.55ab | 56.41a |
| Gammaproteobacteria | 67.75a | 30.02b | 17.23b | 8.76b | 19.50b |
| Bacteroidia | 2.97a | 1.60ab | 0.21b | 1.15ab | 0.26b |
| Fusobacteria | 2.09a | 0.07b | 0.04b | 0.08b | 0.02b |
| Ileum group samples | | | | | |
| Clostridia | 4.63b | 20.62a | 27.49a | 21.39a | 14.38a |
| Bacilli | 42.52ab | 28.34b | 25.97b | 69.88a | 31.44b |
| Cecum group samples | | | | | |
| Clostridia | 56.28a | 50.75a | 49.13a | 20.85b | 19.23b |
| Gammaproteobacteria | 4.77a | 4.36ab | 3.33ab | 2.83bc | 1.27c |
| Bacilli | 28.52c | 30.91c | 37.85bc | 56.63ab | 61.85a |
| Alphaproteobacteria | 0.17ab | 0.11ab | 0.19a | 0.03b | 0.05b |

At the genus level, in jejunum group samples, we found that the relative abundances of *Escherichia* and *Prevotella* in CGJ group were significantly higher than those of MGJ groups. For *Streptococcus*, the CGJ group showed the least value, MGJ.6 group showed the highest value. For *SMB53* and *Serratia*, the relative abundances were significantly higher in MGJ groups. In ileum group samples, the relative abundances of *SMB53*in MGI groups were significantly higher than that of CGI group. In cecum group samples, for *Escherichia*, the MGC.12 group showed the least value, and there was no significant difference between other groups. For *Lactobacillus* and *Prevotella*, the MGC.12 group showed the highest value, while the CGC group showed the least value. For *Streptococcus*, *SMB53* and *Turicibacter*, the relative abundances were higher in CGC group (Table S3).

**Table S3.** Bacterial compositions and comparative analysis of these bacteria (genus level).

| Taxa | Relative abundance % (CG group) | Relative abundance % (MG.3 group) | Relative abundance % (MG.6 group) | Relative abundance % (MG.9 group) | Relative abundance % (MG.12 group) |
|---|---|---|---|---|---|
| Jejunum group samples | | | | | |
| *Escherichia* | 66.04a | 21.96b | 8.94b | 4.14b | 4.14b |
| *Streptococcus* | 0.47c | 3.21bc | 25.47a | 9.23b | 5.28b |
| *SMB53* | 1.11b | 8.55a | 13.04a | 13.94a | 19.98a |
| *Prevotella* | 0.51a | 0.16a | 0.04b | 0.08b | 0.03b |
| *Serratia* | 0.07b | 0.84ab | 2.91ab | 1.09ab | 5.55a |
| Ileum group samples | | | | | |
| *SMB53* | 2.38c | 11.85b | 23.96a | 10.33b | 9.19b |
| Cecum group samples | | | | | |
| *Escherichia* | 3.94a | 4.09a | 2.52ab | 2.70a | 1.12b |
| *Lactobacillus* | 22.22b | 29.32b | 35.35ab | 56.06a | 60.40a |
| *Streptococcus* | 4.04a | 0.95ab | 1.07ab | 0.35b | 0.73b |
| *SMB53* | 12.36a | 6.39ab | 8.26a | 0.76b | 1.15b |
| *Turicibacter* | 2.06a | 0.50b | 0.55b | 0.13b | 0.43b |
| *Prevotella* | 1.08b | 3.71b | 1.64b | 7.83a | 10.26a |

*Correlations between diet composition and specific groups of microbes*

In jejunum group samples, pearson's correlation analysis indicated that the Shannon diversity was negatively correlated to the level of diet crude protein ($P$<0.05). For the bacterial composition, we found that diet crude protein was positively correlated with relative abundances of Proteobacteria, Fusobacteria, and Spirochaetes($P$<0.01), while Firmicutes showed a significant negative correlation with diet crude protein($P$<0.05).

The diet crude fiber showed no significant correlation with the microbial diversity and bacterial composition

(*P*>0.05) (Table 3).

In ileum group samples, the microbial diversity and bacterial composition showed no significant correlation with both diet crude protein and crude fiber except the relative abundance of Euryarchaeota. It was positively affected by diet crude protein (*P*<0.01) (Table 3).

In cecum group samples, negative correlations were observedbetween diet crude fiber and the bacterial OTU number, the relative abundances of Proteobacteria and Fusobacteria (*P*<0.05). The diet crude protein was positively correlated with the relative abundance of Cyanobacteria (*P*<0.05) (Table 3).

**Discussion**

The intestinal microorganisms have been the subject of study for many decades because of their importance in the health and well being of animals(Dowd*et al.,* 2008). In our study, regardless of the diet, we found that the majority (>93%) of the bacteria in the pig jejunum, ileum and cecum are from three phyla: Firmicutes, Proteobacteria and Bacteroidetes, which indicated that these bacteria were stable in pigs, and play a critical role in the microbial ecology of the pig gut.

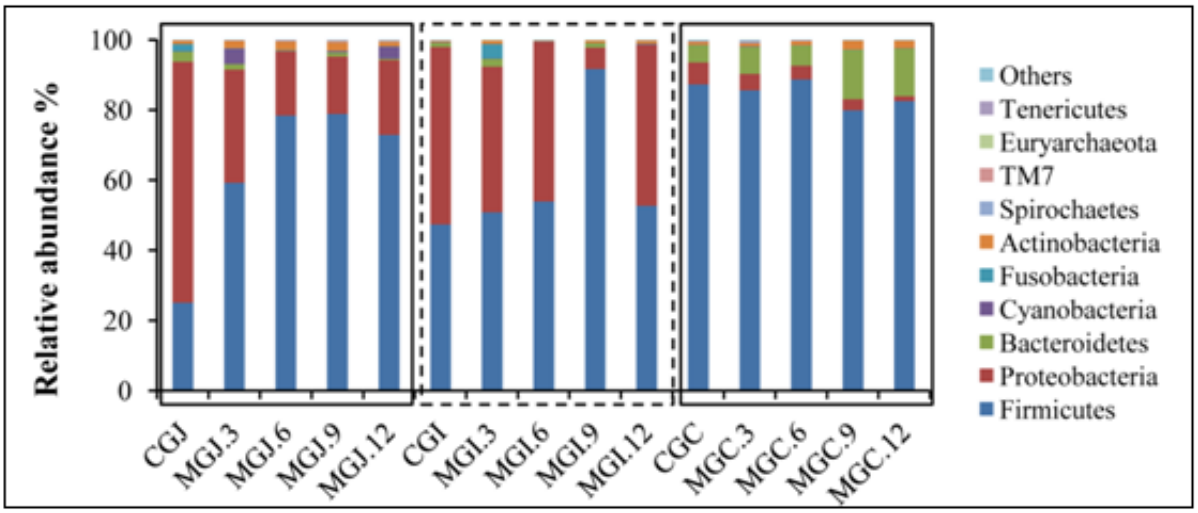


**Fig. 1.** Bacterial composition of the communities in those samples (Phylum level).

This report is inagreement with the dominant phyla reported in manyother studies of pig gut microbiota as well as in our present study (Kim *et al.*,2011; Isaacson and Kim, 2012; Pieper *et al.*,2015). Furthermore, we also found that there were site-specific differences in the microbial composition in various segments of the gastrointestinal tract of pigs.Dissimilarity between these gastrointestinal tracts might be due to the different functional capacity of the small and large intestines of pig (Yang *et al.,* 2016).

In the jejunum and ileum, the compositions of the bacteria were similar to those previously described with most bacteria being in the phyla Firmicutes and Proteobacteria(Isaacson and Kim, 2012; Zhao *et al.,* 2015).In the cecum, bacteria in the phylum Firmicutes was the most dominant followed by bacteria in the phylum Bacteroi

detes. These results were similar to the results of Isaacson *et al.*, they found that the majority of the bacteria in the cecum were classified in the phyla Firmicutes and Bacteroidetes(Isaacson and Kim, 2012).

Previous study has showed that the carbohydrates, such as xylan and cellulose, are the principal energy substrates for large intestine microbial fermentation (Yang *et al.,* 2016; Knudsen and Hansen, 1991), and Bacteroidetes is known to aid the digestion of complex carbohydrates (Spence*et al.,* 2006).

In the small intestine of pigs, transit of fluid and materials is rapid (Clemens*et al.*,1975).

These conditions are unfavourable for the establishment of microbial growth. However, in the large intestine, materials are retained for longer time, which allows prolific microbial growth (Decuypere and Van der Heyde, 1972). Previous studies have also shown that the microbial density in the small intestine is lower than that of cecum (Jensen, 1988).Consistent with the study of Jensen (1988), we also found that the bacterial diversities in jejunum and ileum were lower than that of in the cecum in this study.

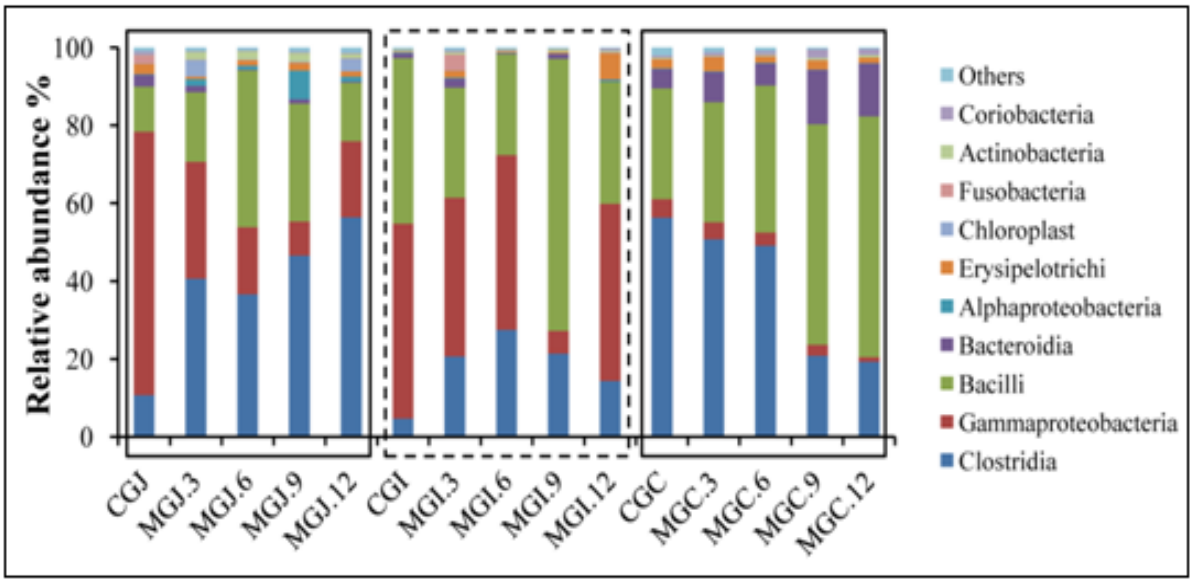


**Fig. 2.** Bacterial composition of the communities in those samples (Class level).

The gastrointestinal bacteria is dynamic and previous studies have shown that their density and composition subject to changesbased on diet, such as the type and inclusion level of dietary fiber in diets (Dowd *et al.,* 2008; Kim *et al.,* 2011). In our study, we found that the relative abundances ofphylum Firmicutes and class Clostridia in MGJ group and phylum Bacteroidetes and class Bacilliin MGC groupwere significantly higher than those of CGJ or CGC group.

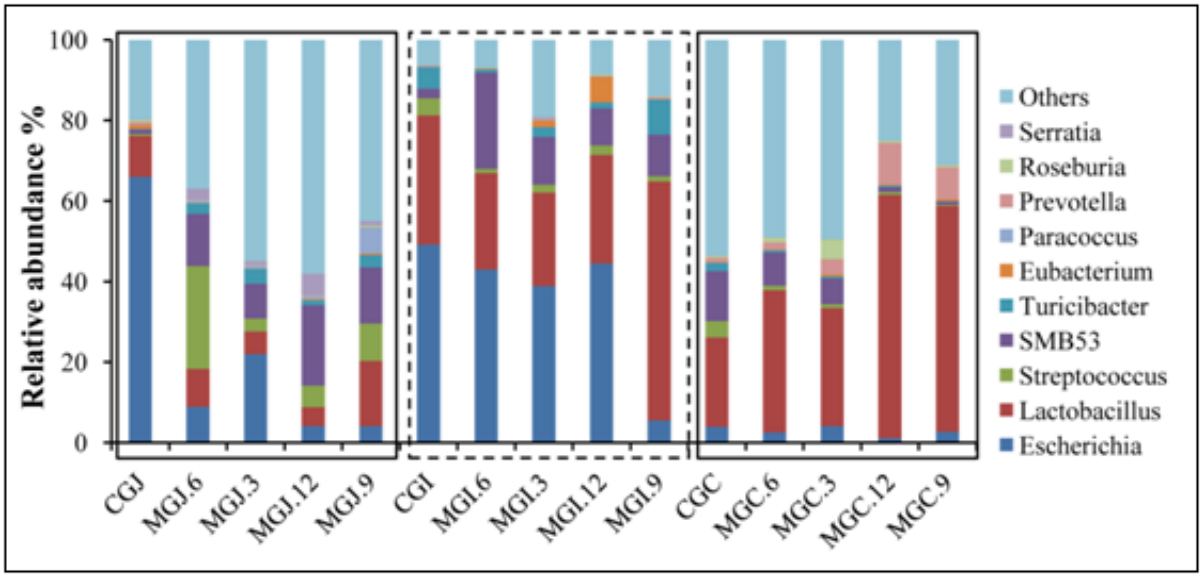


**Fig. 3.** Bacterial composition of the communities in those samples (Genus level).

This phenomenon was likely attributable to the higher fiber content in the mulberry leaves diet.Members of the Bacteroidetes have demonstratedutilization of a wide range of carbohydrate, includingplant cell wall, glycoproteins and so on (Salyers, 1979). The class Clostridia is well known as a typical cellulolytic class, they are reported as the important plant biomass degraders(Kataeva*et al.,* 2002; Doi, 2008), and many bacteria in class Bacilli are strongly associated with lignocellulose degrading (Li *et al.,* 2006; Lin *et al.,* 2012). Previous studies have also showed that bacteria in these two classes could form a stable lignocellulose degrading microbial consortium(Wongwilaiwalin*et al.*, 2010).

## Conclusion

In this study, the main conclusion is that although some of the bacterial contents varied because of the different compositions of diet, the core bacterial compositions in the jejunum, ileum and cecumwere not affected by substituting the commercial concentrate with different levels of the mulberry forage. Therefore, we can conclude that mulberry forage have the potential to be used as a protein-rich forage supplement for pig production. Further studies are needed toevaluate the effects of mulberry forage on pig performance, nutrient digestibility and intestinal morphology.

## Acknowledgments

This work was supported by theOpen Fund Project of Key Laboratory of Subtropical Agro-ecological Processes, Institute of Subtropical Agriculture, Chinese Academy of Sciences(No. ISA2015202), and the National Natural Science Foundation of China(No. 31500006).

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