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Effect of dietary *Bacillus subtilis* on proportion of Bacteroidetes and Firmicutes in swine intestine and lipid metabolism

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ABSTRACT. The ratio of Bacteroidetes and Firmicutes bacterial groups in the gut can affect the ability to absorb nutrients. We investigated the effect of probiotic *Bacillus subtilis* supplementation of diets on growth performance, fat deposition, blood lipids, copy numbers, and percentage of Bacteroidetes and Firmicutes in cecal contents, as well as mRNA expression of key lipid metabolism enzymes in the liver and adipose tissue of finishing pigs. Twenty-four Duroc x Meishan crossbreed 8-week-old pigs (10.28 ± 0.59 kg) were randomly allocated to two dietary treatments: maize-soybean meal-based diets with *B. subtilis* (probiotic group) and without *B. subtilis* (control group). The probiotic diet led to a significant increase in the average daily gain and feed conversion ratio of pigs weighing 10 to 110 kg. The mean backfat depth was increased while leaf lard weights were decreased by probiotic supplementation. Ingestion of probiotics decreased the serum triglyceride and glucose concentrations, but did not change the levels of total cholesterol and free fatty acids in the serum. The mRNA expressions of fatty acid synthase (FAS) and acetyl-CoA carboxylase α (ACCα) in the liver were down-regulated by the dietary probiotic supplement. Conversely, the gene expressions of FAS

and ACCα in the adipose tissue increased. The probiotic diet decreased the copy numbers and percentage of Bacteroidetes, while it increased the percentage of Firmicutes in the cecal contents. We conclude that the addition of *B. subtilis* improves growth performance and up-regulates lipid metabolism in subcutaneous fat of finishing pigs. We conclude that *B. subtilis* affects lipid metabolism through regulation of the proportion of Bacteroidetes and Firmicutes in the gut.

Key words: Probiotics; *Bacillus subtilis*; Bacteroidetes; Firmicutes; Lipid metabolism; Growth performance

INTRODUCTION

Trillions of microbes live in the animal gut, helping to break down otherwise indigestible feed ingredients (Gill et al., 2006). Substantial evidence suggests that bacteria in the gut play an important role in animal energy metabolism. Transplanting the gut microbiota from normal mice into germ-free recipients increases their body fat without any increase in food consumption (Backhed et al., 2004).

Two groups of bacteria are dominant in the swine gut, the Bacteroidetes and Firmicutes, consisting of more than 90% of all phylogenetic types in the human, mouse and pig gut (Eckburg et al., 2005; Ley et al., 2005, 2006). Recent studies have reported that percentage body fat correlates with the abundance of Bacteroidetes and Firmicutes in the gut microbiota in humans, mice (Ley et al., 2005, 2006; Turnbaugh et al., 2006) and swine (Guo et al., 2008a). The obese mice ob/ob have a 50% reduction in the abundance of Bacteroidetes and a proportional increase in Firmicutes compared to their lean (+/+) littermates (Ley et al., 2005). The abundance of Bacteroidetes is decreased in obese pigs (Guo et al., 2008a) compared to lean pigs, and the proportion of Bacteroidetes increases with weight loss (Ley et al., 2006). These changes in gut microbial communities are division-wide and not attributable to differences in food consumption or dietary calorie content (Ley et al., 2005, 2006). These findings suggest that the manipulation of gut microbial communities could be an alternative treatment for obesity.

Probiotics are defined as live microorganisms that produce a health benefit when administered to animals, including humans (Sanders, 2008). The use of probiotic microorganisms for the prevention or therapy of gastrointestinal disorders is an obvious measure and perhaps the most usual application of probiotics. In addition, evidence shows that probiotics can regulate human abdominal adiposity. Probiotics decrease the percent increase in body weight ($P = 0.0331$) and fat pad mass ($P < 0.01$) in diet-induced overweight rats (Kang et al., 2010). Similar results were obtained in adults with obese tendencies in a randomized controlled trial (Kadooka et al., 2010). However, such experiments in animal production are rare. Probiotics as a green growth promoter has been widely used mainly in piglets and weaned piglets, because of their antagonistic effect against pathogens, adjusting microbial community and immune regulation (Jasek et al., 1992; Abe et al., 1995; Kyriakis et al., 1999), as well as secretion of digestive enzymes to help host digestion. Its impact on the proportion of Bacteroidetes and Firmicutes in the pig intestine and lipid metabolism has not been investigated. From this perspective, it is worth investigating the effects of probiotics on microbial composition in the swine intestine.

Our team was the first to report on the real-time polymerase chain reaction (PCR)

quantification of the predominant bacterial divisions in the distal gut of Meishan and Landrace pigs (Guo et al., 2008a). Our previous results demonstrated that body fat was closely related to the percentages of Bacteroidetes division and Bacteroides spp in the gut microbiota of common pig breeds, and that differences in gut microbial ecology in obese versus lean animals could be analogous, although the trend for Firmicutes percentage was not significant. Thus, manipulating gut microbial communities could control fat storage in pigs.

Spores of *B. subtilis*, *B. licheniformis*, *B. cereus*, and *B. clausii* are used as direct-fed microbials for animals and humans (Spinosa et al., 2000). The *B. subtilis* spore cell "is metabolically dormant and as close to indestructible as any cell found on earth; nonetheless, the spore retains the ability to revive almost immediately when nutrients return to the environment" (Driks, 2002). *B. subtilis* is on the list of direct-fed microorganisms reviewed by the Food and Drug Administration Center for Veterinary Medicine, and found to present no safety concerns when used in direct-fed microbial products. In addition, Bacillus belongs to the class Firmicutes.

Therefore, the aim of this study was to examine the impact of *B. subtilis* on the proportions of Bacteroidetes and Firmicutes in swine intestine and fat metabolism, providing the experimental basis for the application of probiotic treatment of human obesity. In addition, excessive deposition of fat not only reduces the efficiency of feed utilization and production costs but also affects pork quality and flavor. This study also aimed to examine probiotics in potential applications as a pig fat deposition regulator.

MATERIAL AND METHODS

Animals and diets

Before the trial start, suckling pigs (Duroc x Meishan, N = 24) were fed a non-antibiotic diet up to 10.28 ± 0.59 kg, and they were then randomly divided into 2 dietary treatment groups with 12 replicate pens per treatment and 1 pig per pen. Treatment 1 was a diet containing no antibiotics (control group, N = 12), and treatment 2 consisted of 20 g/kg probiotics (*B. subtilis*, 2.0 x 10^{10} CFU/g) added to the control diet (probiotic group, N = 12). All diets were formulated according to National Research Council (NRC, 1998) requirements for pigs. The compositions of the basal diets are shown in Table 1. All pigs had free access to water and food. Feed intake and body weight were recorded every 2 weeks. The trial was conducted on the pig farm of Sichuan Agricultural University (Ya'an, P.R. China). All experimental procedures and housing were approved by the Sichuan Province Committee on Laboratory Animal Care.

Tissue sampling

The protocol for sample collection was approved by the Sichuan Province Committee on Laboratory Animal Care.

Each pig was removed from the test, stunned, bled, and slaughtered at approximately 110 kg liveweight. Blood was taken from overnight-fasted pigs via the anterior vena cava, and serum was obtained by centrifuging whole blood at 1500 *g* for 15 min at 4°C. The liver and subcutaneous fat in the back were rinsed in ice-cold phosphate buffer, pH 7.2, minced with scissors, frozen in liquid nitrogen, and stored at -80°C prior to analysis.

Table 1. Formulation and calculated nutrient content of basal diets, fed basis.

Ingredients (%)	Content			
	10-20 kg	20-50 kg	50-80 kg	80-110 kg
Extruded corn	25.00	-	-	-
Rice	25.00	-	-	-
Full-fat soybean	9.50	-	-	-
Yellow maize	25.46	80.03	87.67	89.92
Soybean meal	9.50	17.39	9.95	8.00
Dicalcium phosphate	1.10	0.50	0.32	0.15
Calcium carbonate	1.01	1.09	1.00	1.10
Salt	0.20	0.19	0.25	0.25
Mineral premix[a]	0.25	0.25	0.25	0.25
L-lysine-HCl	0.34	0.30	0.30	0.18
DL-methionine	0.06	0.04	0.03	0.00
L-threonine	0.11	0.05	0.06	-
DL-tryptophan	0.02	-	0.02	0.01
Choline chloride	0.10	0.10	0.10	0.10
Sweetening agent	0.03	0.01	0.01	0.01
Phytase	0.01	0.01	0.01	0.01
Complex enzyme	0.01	0.01	-	-
Vitamin premix[b]	0.05	0.03	0.03	0.03
Fish meal	2.00	-	-	-
Acidifier	0.25	-	-	-
Calculated composition (%)[c]				
Digestible energy (Mcal/kg)	3.43	3.32	3.32	3.34
Crude protein	16.22	14.23	11.52	10.86
Calcium	0.77	0.58	0.48	0.47
Total phosphorus	0.57	0.41	0.36	0.32
Non-phytate phosphorus	0.38	1.21	0.18	0.15
TID lysine[d]	0.99	0.80	0.64	0.51
TID methionine + TID cysteine	0.56	0.45	0.38	0.34
TID threonine	0.63	0.50	0.42	0.35
TID tryptophan	0.18	0.15	0.12	0.10

[a]Provided per kilogram of diet: 5 mg Cu (as copper sulfate); 80 mg Fe (as ferrous sulfate); 3 mg Mn (as manganese oxide); 80 mg zinc (as zinc oxide); 0.25 mg Se (as sodium selenite); 0.14 mg I (as potassium iodide). [b]Provided per kilogram of premix: 10,000,000 IU vit. A; 2,000,000 IU vit. D3; 25,000 IU vit. E; 30 mg vit. B12; 16,000 mg riboflavin; 35,000 mg niacin; 25,000 mg pantothenic acid; 5,000 mg menadione. [c]Calculated values based on food composition and the true ileal digestibility data of NRC (1998); [d]TID = true ileal digestible.

For sampling, the pigs were sacrificed after 12-16 h fasting, with body weight recorded before slaughter. Immediately after death, luminal content of the cecum was collected. Samples were transferred to sterile tubes, snap-frozen in liquid nitrogen, and stored at -80°C until analysis.

Body fat and carcass quality data collection

Hot carcass weight was collected before chilling to calculate dressing percent. Fat thickness opposite the first rib, last rib, and last lumbar vertebra was measured to calculate average backfat thickness. The cross-section of the longissimus muscle (LM) in the right side of the carcass was traced onto acetate paper and the LM area was measured using a compensating planimeter.

Measurement of serum lipid and glucose

The concentrations of triglyceride (TG), total cholesterol (TC) and glucose in the serum samples were analyzed with a SHIMADZU CL8000 Clinical Chemistry Analyzer using a colorimetric method (enzyme) following manufacturer instructions (NingBo RuiYuan Bio-

technology Co., Ltd., Zhejiang, China). Serum-free fatty acid concentrations were measured with a spectrophotometer (148-4 ZDSYS-04) using a colorimetric method (chemistry) following manufacturer instructions (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China).

Bacterial strains

The two bacterial strains used in this study were obtained from the American Type Culture Collection (ATCC, USA), including *Bacteroides thetaiotaomicron* (ATCC 29741T) and *Eubacterium rectale* (ATCC 33656T). Both strains were grown anaerobically in brain heart infusion broth (Oxoid, Basingstoke, UK) at 37°C overnight.

Extraction of DNA from pure cultures and cecal samples

Genomic DNA from the above bacterial strains was extracted from the cultures with a TaKaRa MiniBest bacterial genomic DNA extraction kit (TaKaRa, Dalian, China), according to manufacturer instructions. DNA was extracted from cecal luminal contents by a TIANamp Stool DNA kit (Tiangen Biotech Co., Ltd. Beijing, China). The cecal samples were thawed on ice, and about 200 mg cecal contents were weighed out for each extraction, according to manufacturer instructions. The final elution volume was 100 µL, and the concentration was determined by Multiskan Spectrum (Thermo Electron Corp., Finland).

PCR conditions

The primers specific for groups used are listed in Table 2. These primers were synthesized commercially by Sangon Biotech (Shanghai, China). Real-time quantitative PCR was carried out with a 7900HT Fast Real-Time PCR System (Applied Biosystems, USA) using optical grade 384-well plates in a final volume of 10 µL. Analyses were performed in triplicate, and the mean values were calculated. Each PCR mixture was composed of 5 µL Power SYBR PCR Master Mix (Applied Biosystems), 100 nM of each group-specific primer (Table 2), and 1 µL DNA in each reaction for detecting Bacteroidetes, Firmicutes, and all bacteria. The amplification program consisted of 1 cycle of 50°C for 2 min; 1 cycle of 95°C for 10 min; 40 cycles of 95°C for 15 s, and 60°C for 1 min. A melting curve analysis was done after amplification. Data analysis was performed using the Sequence Detection Software (version 2.3) supplied by Applied Biosystems. The threshold cycle values and baseline settings were determined by automatic analysis settings.

Standard curve

An amplicon containing target regions of the Firmicutes and all bacteria primers (Table 2) was produced using primers (Suzuki et al., 2000; Fierer et al., 2005) with DNA extracted from *E. rectale* ATCC 33656T, and another amplicon containing target regions of the Bacteroidetes and all bacteria primers (Table 2) was produced using primers (Suzuki et al., 2000) with DNA extracted from *B. thetaiotaomicron* ATCC 29741T. The amplified products were run on a 1.5% agarose gel with a no-template control to confirm the specificity of the amplification, and the products purified were then ligated into pMD18-T vectors (TaKaRa),

followed by transformation into JM109 competent *Escherichia coli* cells with the ligation mixture (TaKaRa). Plasmid standards were extracted using the E.Z.N.A.™ Plasmid Mini kit (Omega Bio-Tek, USA) with DNA concentrations detected by spectrophotometer (Coulter DU 800, Beckman, USA). Standard curves were generated using triplicate 3- or 4-fold dilutions of plasmid DNA. We used at least five nonzero standard concentrations in each assay, and the plasmid DNA concentrations ranged from 3×10^{-5} to 3×10^{-2} ng DNA per reaction. Target copy numbers for each reaction were calculated from the standard curves.

Table 2. Sequences of oligonucleotide primers.

Gene	Primer name and sequence (5'-3')	Size of product (bp)	Annealing temperature (°C)	Reference
Bacteroidetes	F: GGARCATGTGGTTTAATTCGATGAT R: AGCTGACGACAACCATGCAG	126	60	(Guo et al., 2008b)
Firmicutes	F: GGAGYATGTGGTTTAATTCGAAGCA R: AGCTGACGACAACCATGCAC	126	60	(Guo et al., 2008b)
All bacteria	F: ACTCCTACGGGAGGCAGCAG R: ATTACCGCGGCTGCTGG	200	60	(Fierer et al., 2005)
ACCα	F: GGTGATGGTCTATATCCCTCCTC R: GATTTCTACGGTCCCTTCTGGT	147	55	(Gallardo et al., 2008)
FAS	F: CTACATCGAGTGCATCAGACAGG R: GAACAGGAAGAGGCTGTGGTT	147	55	(Jun et al., 2006)
HSL	F: GTGAAGGACAGGACAGTGAGG R: GAGGTAAGGCTCGTGGGATTT	169	55	(Lei et al., 2005)
ATGL	F: GCACCTTCATTCCCGTGTAC R: CGAGAGATGTGCAAGCAGGG	160	60	(Deiuliis et al., 2008)
β-actin	F: TCGCACTTCATGATCGAGTTG R: CGACGGCCAGGTCATCAC	138	55	(Lee et al., 2006)

ACCα = acetyl-CoA carboxylase α; FAS = fatty acid synthase; HSL = hormone-sensitive lipase; ATGL = adipose triglyceride lipase.

Test specificity of the real-time PCR assays for Bacteroidetes and Firmicutes

Quantitative PCR products were selected at random in the Bacteroidetes and Firmicutes assays and cloned using the procedure described above. For each of the two PCR assays, 15-20 positive clones were reamplified using plasmid-specific primers, purified using the E.Z.N.A.™ PCR purification kit (Omega Bio-Tek), and sequenced on an Applied Biosystems 3700 automated sequencer using the BigDye Terminator kit (Applied Biosystems). The sequences were assigned to taxonomic groups using the RDP classifier program (Cole et al., 2005).

Statistical analysis

Statistically significant differences were determined by independent-sample *t*-tests (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $P < 0.05$ or $P < 0.01$. The linear regression method was used to analyze the correlation between the percentages of bacterial groups and backfat thickness.

RESULTS

Growth performance data are presented in Table 3. There were pronounced improvements ($P < 0.05$) in average daily gain (ADG) and average daily feed intake (ADFI) as a result

of the addition of probiotics to the diet. Although feed intake and gain (F/G) increased concomitantly, F/G was significantly decreased ($P < 0.05$) by the addition of probiotics.

Table 3. Growth performance of pigs fed the experimental diets.

	Control	Probiotics
Initial body weight (kg)	10.16 ± 0.48	10.42 ± 0.32
Final body weight (kg)	110.23 ± 0.26	110.07 ± 0.23
ADFI (g)	1806.38 ± 29.83	1826.23 ± 22.76[a]
ADG (g)	576.97 ± 7.61	597.47 ± 8.17[a]
F/G	3.131 ± 0.023	3.057 ± 0.029[a]

[a]Significantly different compared with the control group at $P < 0.05$ by independent-sample *t*-tests; ADFI = average daily feed intake; ADG = average daily gain; F/G = feed intake/gain.

Pigs were slaughtered at the same liveweight. Probiotics had no effects on the dressing percentage or leaf lard weight. However, the LM area and average backfat thickness were significantly increased ($P < 0.05$), while the leaf lard weight was significantly decreased ($P < 0.05$) by the addition of probiotics (Table 4).

Table 4. Effects of probiotics on carcass quality and body fat.

	Control	Probiotics
Dressing percentage (%)	69.23 ± 2.40	70.58 ± 4.11
Average backfat depth (cm)	5.07 ± 0.27	5.92 ± 0.15[a]
Longissimus muscle area (cm^2)	33.36 ± 2.15	36.98 ± 2.43[a]
Leaf lard weight (kg)	1.59 ± 0.13	1.39 ± 0.12[a]

[a]Significantly different compared with the control group at $P < 0.05$ by independent-sample *t*-tests.

Real-time PCR analysis was performed to quantify all bacteria, Firmicutes division and Bacteroidetes division in the cecal samples. The probiotic group had lower copy numbers of all bacteria and Bacteroidetes in the cecal contents, and higher copy number of Firmicutes compared to the control group. The percentage of each group (based on all bacteria) was coincident with changes in copy number. The percentage of Bacteroidetes in the probiotic group was lower ($P < 0.01$) by 64.33%, whereas the copy numbers of Firmicutes was not significantly different ($P = 0.290$) between the two groups. The percentage of Firmicutes in the probiotic group was higher than in the control group ($P < 0.01$) (Table 5). The relationship between the percentage of Bacteroidetes and backfat thickness was linear ($R^2 = 0.8071$) (Figure 1).

Table 5. Copy numbers and percentages of bacterial groups and all bacteria[a].

	Copies/g of cecal content		Percentage[b]	
	Control	Probiotics	Control	Probiotics
All bacteria	$(8.56 \pm 1.16) \times 10^{12}$	$(7.45 \pm 0.36) \times 10^{12}$		
Firmicutes	$(5.12 \pm 3.23) \times 10^{12}$	$(5.27 \pm 1.19) \times 10^{12}$	59.81 ± 10.21	78.79 ± 12.32
Bacteroidetes	$(8.42 \pm 1.27) \times 10^{11}$	$(2.61 \pm 2.07) \times 10^{11}$	9.84 ± 1.34	3.51 ± 1.02

[a]Means and standard deviations; [b]relative to all bacteria copies.

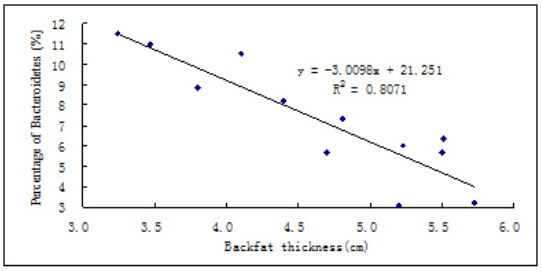

Figure 1. Correlation between backfat thickness and the percentages of Bacteroidetes ($P < 0.05$).

Probiotics significantly decreased serum TG and glucose concentrations and increased serum fatty acid synthase (FFA) level. However, there was no significant difference ($P > 0.05$) in serum TC between the control and probiotic groups (Table 6).

Table 6. Effects of probiotics on the serum lipids and glucose of pigs.

	Control group	Probiotics
TG (mM)	0.53 ± 0.21	0.45 ± 0.15[a]
TC (mM)	2.39 ± 0.27	2.34 ± 0.26
FFA (μM)	186 ± 12.35	219 ± 17.32[a]
Glucose (mM)	5.12 ± 0.45	4.76 ± 0.30[a]

[a]Significantly different compared with the control group at $P < 0.05$ by independent-sample *t*-tests; TG = triglycerides; TC = total cholesterol; FFA = free fatty acids.

In the liver, probiotics significantly ($P < 0.05$) decreased the mRNA levels of FAS and acetyl-CoA carboxylase α (ACCα), but their effects on these genes in the subcutaneous fat were opposite, with significant increases in the mRNA levels of FAS ($P < 0.01$) and ACCα ($P < 0.05$). The expressions of hormone-sensitive lipase and adipose triglyceride lipase (ATGL) in the liver were not significantly affected by probiotics. However, the mRNA level of ATGL in subcutaneous fat increased ($P < 0.01$) (Table 7).

Table 7. Effects of probiotics on the mRNA levels of fatty acid synthase (FAS), acetyl-CoA carboxylase α (ACCα), hormone-sensitive lipase (HSL), and ATGL (adipose triglyceride lipase) in the liver and subcutaneous fat tissue of pigs.

	Liver		Subcutaneous fat	
	Control	Probiotics	Control	Probiotics
FAS	31.32 ± 2.73	26.91 ± 5.27[a]	78.34 ± 4.31	180.67 ± 5.16[A]
ACCα	11.76 ± 1.32	9.18 ± 1.56[a]	19.12 ± 2.15	25 ± 3.27[a]
HSL	0.15 ± 0.01	0.18 ± 0.01	2.65 ± 0.23	3.23 ± 0.17
ATGL	0.21 ± 0.02	0.19 ± 0.02	9.78 ± 1.32	15.89 ± 2.78[a]

[a]Significantly different compared with the control group at $P < 0.05$ by independent-sample *t*-tests. [A]Significantly different compared with the control group at $P < 0.01$ by independent-sample *t*-tests.

DISCUSSION

Probiotic growth promoters are highly effective alternatives to antibiotics, particularly

in weanling and starter pigs. However, probiotics are seldom fed to finishing pigs. Our study found that probiotics improved ADG and ADFI and significantly decreased F/G ($P < 0.05$) in finishing pigs. Similar findings have been reported in studies when probiotics were given to piglets (Choi et al., 2011) and growing-finishing pigs (Gebru et al., 2010; Meng et al., 2010). There are also reports that probiotics have no positive effects on ADG and feed conversion of pigs (Kornegay and Risley, 1996; Estienne and Hartsock, 2005); this may be caused by different probiotic strains used in the experiments. These results show the enormous potential of probiotics as green growth promoters in finishing pig production.

In our study, Duroc x Meishan crossbreed pigs were used to analyze the effects of probiotics. Control and probiotic group pigs were fed the same basal diets formulated according to NRC (1998) in the same environment. The probiotic group pigs had a 16.77% higher backfat thickness compared to the control pigs (Table 4) but lower leaf lard weight. These results were consistent with similar findings reported for diet-induced overweight rats (Kang et al., 2010). However, our results showed that probiotics decreased the lipid accumulation in subcutaneous tissue. This indicates that microbes in the pig digestive tract exert opposite effects on abdominal and subcutaneous fat deposition.

In the current study, probiotics decreased the serum TG concentration and mRNA levels of lipogenic enzymes (FAS and ACCα) in the liver of pigs. These findings were in accordance with reports on humans consuming fermented milk (Agerbaek et al., 1995; Bertolami et al., 1999; Kobayashi et al., 2012; Komatsuzaki and Shima, 2012). In rodents, Taranto et al. (1998) showed that *Lactobacillus reuteri* caused a 40% reduction in TG. No significant differences were found for serum cholesterol level in our study. However, Sindhu and Khetarpaul (2003) showed that the cholesterol content was reduced in mice fed *Saccharomyces boulardii* and a tomato pulp diet. Furthermore, an increase in serum cholesterol concentrations was found in the livers of rats fed a diet supplemented with *Lactobacillus acidophilus* and *Streptococcus faecalis* (Fukushima et al., 1999). We also inspected the mRNA levels of lipogenic and lipogenesis enzymes in subcutaneous fat, because subcutaneous fat is the main fat deposition site of pigs, which is significantly different compared to humans and other animals. Interestingly, the influences of probiotics on fat metabolism enzyme mRNAs are opposite in subcutaneous fat *vs* liver. The mRNA levels of lipogenic enzymes were increased far more than lipogenesis enzymes by supplementation with probiotics. The mechanism of this phenomenon deserves further study.

The cecum was chosen for sampling in this study because it is an anatomically distinct structure, located between the distal small intestine and colon, which is colonized with sufficient quantities of bacteria (Turnbaugh et al., 2006).

Previous findings suggest that the manipulation of gut microbial communities could be an alternative treatment for obesity. Thus, the copy numbers of Bacteroidetes and Firmicutes in cecal contents were determined. Our findings showed that probiotics decreased the copy number and percentage of Bacteroidetes and increased the percentage of Firmicutes in cecal contents. We also demonstrated a linear relationship between the percentage of Bacteroidetes and backfat thickness. These results and backfat thickness data were in accordance with the theory that percentage body fat correlates positively with the abundance of Firmicutes in the gut microbiota in humans and mice (Ley et al., 2005, 2006; Turnbaugh et al., 2006) and swine (Guo et al., 2008a). This may be because the *B. subtilis* used in this study belongs to the Firmicutes group, and thus, adding *B. subtilis* resulted in substantial proliferation of Firmicutes in the pig intestine.

CONCLUSIONS

In conclusion, our results show the enormous potential of probiotics as green growth promoters in finishing pig production. We demonstrated that adding *B. subtilis* can manipulate the gut microbial communities and then regulate the site of fat deposition. This is the first report of the manipulation of the two major groups of obesity-related bacteria by probiotic supplementation. The results provide a reference for the use of probiotic treatment of obesity and a reference for improving the efficiency of pig production.

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