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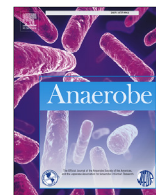

Anaerobes in the microbiome

Changes in faecal bacteria during fattening in finishing swine

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ABSTRACT

Body fat accumulation in mice and human is linked to the percentage of *Firmicutes* and *Bacteroidetes*, two bacterial phyla dominant in the large intestine. However, little is known about the relationship between the composition of the gut microbiota and fattening in pig. This study aimed to investigate the abundance of *Firmicutes*, *Bacteroidetes*, and *Bacteroides*, which is the major genus within *Bacteroidetes*, in porcine faeces during fattening. Ten 4-month-old crossbred pigs were given free access to commercial feed for fattening and water for 14 weeks. Daily feed intake and body weight were measured every 2 weeks. Faecal samples were collected at 0, 4, 8, and 14 weeks, and plasma samples were collected every 2 weeks. Daily feed intake increased until 8 weeks, and then decreased. Body weight increased with fattening during the experimental period. Feed efficiency showed high values at 0–4 and 6–8 weeks. The level of *Firmicutes* increased ($P < 0.05$), whereas those of *Bacteroides* and *Bacteroidetes* decreased ($P < 0.05$) with fattening. The total short chain fatty acid content in the faeces increased ($P < 0.05$) with fattening until 8 weeks and then decreased ($P < 0.05$) at 14 weeks. There were no significant relationships between the level of *Firmicutes* and feed intake or plasma leptin concentration. The levels of *Bacteroidetes* and *Bacteroides* correlated with feed intake, body weight, and plasma leptin or plasma urea nitrogen (PUN) concentration. Our results suggested that the level of *Firmicutes* increased and those of *Bacteroidetes* and *Bacteroides* decreased with increase in feed intake and body weight, similar to previous results obtained for mice and human. However, energy extraction from feed was not influenced by compositional alteration of gut flora, because daily gain and feed efficiency did not show high values towards the end of the fattening period. Manipulating the gut microbiota might help improve fattening performance, although further studies are necessary to understand the relationships between the composition of gut microbiota and energy absorption.

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1. Introduction

The gastrointestinal tract (GIT) of pigs is colonized with a dense and an enormously diverse microbiota comprising more than 400 different phylotypes [1]. The gut microbiota plays important roles in the host, and the large intestine microbiota contributes toward normal functioning of the gut, production of short chain fatty acids (SCFAs) as a source of energy, production of vitamins, and defence from pathogens in human [2]. Additionally, microbial fermentation in the GIT is important for energy homeostasis and weight management [3]. In pigs, the SCFAs produced in the large intestine supply up to 30% of the energy required for maintenance [4]. Thus, energy supply in the form of SCFAs within the large intestine is an important energy source and a factor affecting the productivity of pigs. Furthermore, the SCFAs produced by microbial fermentation in the large intestine act as signalling molecules. SCFAs, which are ligands for GPR41 and GPR43, stimulate the expression of leptin, a polypeptide hormone with pleiotropic effects on appetite and energy metabolism, in mouse adipocytes [5,6]. In swine, leptin suppressed feed intake [7], and leptin mRNA expression increased with age and body weight gain [8] as shown in mouse. Additionally, backfat thickness in pigs was positively associated with leptin mRNA levels [9]. Leptin is thought to be an important regulator of appetite, energy metabolism and body composition [10], and could affect growing and fattening performance in pig.

Bacteroidetes and *Firmicutes* are two phyla predominant in the large intestine of human [11], mouse [12], Guinea pig [13], pig [14],

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equine large intestine [15], cow rumen [16], and ostrich [17]. *Bacteroides* is the major genus in *Bacteroidetes*. The levels of *Bacteroidetes* and *Firmicutes* in the caeca differ between obese and lean mice. Compared to lean mice, genetically obese mice (*ob*/*ob* mice) showed 50% reduction in the level of *Bacteroidetes* and a proportional increase in the level of *Firmicutes* [12]. The microbiota of obese mice helps in increasing energy generation from ingested diet [18]. Ley et al. [11] reported that obese subjects presented lower level of *Bacteroidetes* in overweight and obese subjects. Contrary to this, Schwiertz et al. [19] reported higher level of *Bacteroidetes* in overweight and obese subjects. In humans, there is still no consensus in the literature about the levels of different bacterial species in the gut of obese and lean subjects [20]. Group-specific primer sets for use in real-time PCR for *Bacteroides* spp [21]. and the two major phyla *Firmicutes* and *Bacteroidetes* [22] in the mammalian large intestine have been developed and validated until date. Bacterial composition was studied using these primer sets [22]. Lean and obese pigs have different levels of *Firmicutes* and *Bacteroidetes* [22]. Some previous studies on human, mice and pig showed that the level of gut microbes differs depending on body fat accumulation [11,12,22]. In case of swine production, feed efficiency is very important in growing and fattening. It is desirable to have a large amount of meat with less feed consumption. Improved understanding of the relationship among gut microbiota, body fatness and energy extraction from feed will help effectively improve porcine growth and fattening performance. The present experiment was performed to investigate the changes in the levels of faecal microbes in association with fattening and growth in pigs.

2. Material and methods

2.1. Animals and management

Ten 4-month-old crossbred (Landrace X Large white) and castrated pigs (average body weight, 40.2 ± 1.7 kg) were used in this study. These animals were caged individually at Mie University, Tsu, Japan. Animals were allowed a dietary adaptation period of 1 week before initiating the experiment. They were fed a commercial diet for finishing (Nippon Formula Feed Mfg. Co. Ltd. Yokohama, Japan) throughout the experiment; the ingredients and nutrient composition of the diet are listed in Table 1. The diet was free from intestinal microbiota modifiers such as antibiotics or probiotics. The diet and water was available *ad libitum* throughout the adaptation and experimental periods. The diet was provided every morning at 9:00 or after sampling on the day of faeces or blood sampling. Dietary intake and body weight were measured every 2 weeks. Animal handling was performed according to the Mie University guidelines.

2.2. Sample collection

Faecal material present in the rectum was aseptically collected by wearing disposable plastic gloves at 0, 4, 8, and 14 weeks and immediately stored at −80 °C until analysis. Blood samples were collected from each pig via jugular venipuncture into heparinized tubes at 09:00 every 2 weeks, and then centrifuged at 1400 × *g* for 15 min at 4 °C to obtain plasma, which was stored at −80 °C for determining plasma leptin and urea nitrogen (PUN) concentrations.

2.3. Measurement

Plasma leptin concentration was determined using a radioimmunoassay kit (Multi-Species Leptin RIA kit; Linco Research, St. Charles, MO). PUN was determined using commercial kit (Urea nitrogen B-Test Wako; Wako Pure Chemical Industries, Ltd. Osaka, Japan). The concentrations of acetic acid, propionic acid, and butyric acid in faecal samples were analysed by ion-exchange high-performance liquid chromatography, as described by Furuya et al. [23]; the concentration of total SCFAs was equal to the sum of the concentrations of acetic acid, propionic acid, and butyric acid.

2.4. Extraction of DNA from faeces

Frozen faecal material was thawed at room temperature. The total DNA from the faecal material was extracted using the UltraClean Faecal DNA Isolation kit (MO BIO Laboratories, Inc. Carlsbad, CA). The extracted DNA was stored at −30 °C until analysis.

2.5. Real-time PCR

The DNA copy number of 16S ribosomal RNA gene of *Bacteroides*, *Bacteroidetes*, *Firmicutes,* and total bacteria was quantified by real-time PCR, as described by Guo et al. [22]. Real-time PCR was performed using the ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) with SYBR green PCR master mix (Applied Biosystems). Primers for the target groups used in this study are listed in Table 2. The reaction mixture (25 μL) for real-time PCR comprises 1 μL of the template, 12.5 μL of the SYBR green master mix, 50 nM forward and reverse primer (each), and 2.5 μL of sterilized Milli-Q water. The PCR conditions for DNA quantification were 50 °C for 2 min, 95 °C for 10 min, and 40 cycles of 95 °C for 15 s and 60 °C for 1 min. This was followed by melting curve analysis. The levels of *Bacteroidetes, Firmicutes,* and *Bacteroides* were expressed as the copy number per total number of bacteria. Standard DNA was prepared as follows. The 16S rRNA gene of *Eubacterium limosum* was used as the standard DNA for *Firmicutes.* The 16S rRNA gene of *Bacteroides thetaiotaomicron* was used as the standard DNA for *Bacteroidetes* and *Bacteroides*. The 16S rRNA gene of *Escherichia coli* was used as the standard DNA for total bacteria. These 16S rRNA gene fragments were cloned with a TA Cloning Kit (Invitrogen, Carlsbad, CA), as described in the manufacturer's protocol. PCR products from these cloned DNA were used as the standard. Amplified standard DNAs were purified using a QIAquick PCR purification kit (QIAGEN, Inc. Valencia, CA) and quantified with spectrophotometry (at 260 nm); then, 10-fold serial dilutions were prepared just before real-time PCR.

Table 1
Composition of the experimental diet.

Item	
Ingredients (%)	
Maize	78
Wheat bran	9
Soybean meal	8
White fish meal	3
Calcium phosphate, Calcium carbonate, Salt	2
Composition (%DM[a])	
Crude Protein	14.6
Crude fat	3.6
Crude fibre	3.0
Crude ash	3.8
Calcium	0.74
Phosphate	0.65
DCP[b]	11.4
TDN[c]	76.1

[a] DM: Dry matter.
[b] DCP: Digestible crude protein.
[c] TDN: Total digestible nutrients.

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