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Research Letter

RESEARCH LETTER – Taxonomy & Systematics

A comparison of faecal microbial populations of South African Windsnyer-type indigenous pigs (SAWIPs) and Large White × Landrace (LW × LR) crosses fed diets containing ensiled maize cobs

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One sentence summary: A comparison of faecal microbial populations was carried out to explain differences in digestion of high-fibre diets in South African Windsnyer-type indigenous pigs and Large White × Landrace crosses.

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ABSTRACT

Faecal microbial communities in South African Windsnyer-type indigenous pigs (SAWIPs) and Large White × Landrace (LW × LR) crosses were investigated using high-throughput sequencing of the 16S rDNA genes. The faecal microbial communities in LW × LR crosses and SAWIPs fed control (CON) and high maize cob (HMC) diets were evaluated through parallel sequencing of 16S rDNA genes. *Butrivibrio*, *Faecalibacterium* and *Desulfovibrio*, although present in LW × LR pigs, were absent from the SAWIP microbial community. *Bacteroides*, *Succiniclasticum*, *Peptococcus* and *Akkermansia* were found in SAWIPs but not in LW × LR crosses. The ratios of *Bacteroidia* to *Clostridia* on the CON and HMC diets were similar (0.37 versus 0.39) in SAWIPs but different (0.24 versus 0.1) in LW × LR crosses. The faecal microbial profiles determined were different between the LW × LR and SAWIP breeds but not between pigs fed the CON and HMC diets. The composition of faecal bacterial communities in SAWIPs was determined for the first time. The differences in microbial communities detected may explain the enhanced ability of SAWIPs to digest fibrous diets compared with the LW × LR crosses.

Keywords: agriculture; fermentation; genomics; intestinal microbiology; metagenomics

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INTRODUCTION

Pig production faces the challenge of effectively utilizing fibrous feeds that are readily available (Montagne *et al.* 2012; Urriola and Stein 2012). In South Africa, for example, maize cobs are a cheap feed ingredient with potential for greater exploitation in pig diets. We recently reported that South African Windsnyer-type indigenous pigs (SAWIPs) digested and utilized diets containing maize cobs better than the Large White × Landrace crossbred (LW × LR) pigs (Kanengoni *et al.* 2014, 2015). This hardy breed plays an important role in the rural populations in Southern Africa in that it thrives on high-fibre diets and its characterization is critical in alleviating poverty in resource-poor communities. There has been little research investigating the effect of maize cobs on the dynamics of intestinal microbial communities in grower pigs. The microbes' metabolic processes have a direct influence on the host's gut health and nutrient availability, and this should be considered when formulating diets with unconventional ingredients. Culture-dependent methods are inadequate to elucidate microbial profiles in the hindgut since only 1% of intestinal microbes are culturable (Hugenholtz, Goebel and Pace 1998). Molecular techniques are therefore key to unravelling the complex intestinal microbial communities in humans and animals (O'Flaherty and Klaenhammer 2010; Kau *et al.* 2011; Gravitz 2012). We hypothesized that the SAWIP intestinal microbiota allows utilization of fibrous diets more efficiently compared with those of Large White crosses. The objective of this study was to evaluate the influence of breed and diet on the faecal microbiome in commercial and indigenous South African breeds fed diets containing ensiled maize cobs using high-throughput sequencing of the 16S rDNA genes.

MATERIALS AND METHODS

Animals, diets, housing and experimental design

Six LW × LR crossbreds and five SAWIPs from a group of 16 LW × LR pigs and 10 SAWIPs that underwent a growth performance and digestibility study (Kanengoni *et al.* 2014, 2015) were randomly selected for faecal microbial evaluation. These pigs were the progeny of SAWIP and LW × LR sows from the Agricultural Research Council-Irene pig breeding units that were bred naturally and farrowed vaginally. The piglets were weaned at 4 and 5 weeks (for the LW × LR crosses and SAWIPs, respectively) and raised indoors on similar diets under the same management system. The sows and piglets were not exposed to antimicrobials during the rearing process. As the SAWIPs and LW × LR pigs differ in their mature body weights, the pigs started the trial at a similar level of physiological maturity (0.10 of adult body weight). The concept of physiological age was used previously in experimental animals (Kanengoni *et al.* 2004; Morel, Lee and Moughan 2006). The SAWIPs weighed 17 ± 3.4 kg at 90 days and the LW × LR crossbred pigs weighed 29 ± 3.6 kg at 70 days at the onset of the trial. They were slaughtered at finisher weights (58 ± 8.8 kg for SAWIPs aged 146 days; 86 ± 8.8 kg for LW × LR pigs aged 126 days). During the trial, the pigs were individually housed in environmentally controlled houses (temperature 22–25°C) within pens measuring 2 × 1.5 m (LW × LR pigs) and 1.5 × 0.9 m (SAWIPs) per animal. Pigs had been blocked by weight and breed and randomly assigned to control (CON; without maize cobs) and high maize cob (HMC; containing 200 g of ensiled maize cob kg^{-1}) diets (Supplementary Table S1). Both diets were formulated to provide 14 MJ kg^{-1} of digestible energy (DE), 180 of g crude protein (CP) kg^{-1} and 11.6 g of lysine kg^{-1} per kg of dry matter (DM) of feed, which meet and exceed the requirements of growing pigs (NRC, 1998). The pigs were fed daily each morning, and 10% extra feed adjustments were made for animals that finished their daily allocation. The pigs were weighed weekly, and daily feed intake was calculated based on feed offered less leftovers. The feeders were checked and adjusted twice each day to ensure *ad lib* access to fresh feed and minimal wastage. Water was freely available through nipple drinkers. At the end of the growth and digestibility study, the pigs were humanely slaughtered at an abattoir <1 km away. The results of the growth and digestibility study have been reported elsewhere (Kanengoni *et al.* 2014, 2015). The research was approved by the Animal Ethics Committee of the Agricultural Research Council, Animal Production Institute (ARC-API, Ref: APIEC12/018).

Sampling, DNA extraction and PCR amplification

Pigs were processed in accordance with routine abattoir procedures including an ante-mortem inspection and 1 h rest prior to slaughter. The animals were electrically stunned and exsanguinated within 10 s of stunning. Post-dehairing and evisceration, faecal samples were collected aseptically from the rectum and immediately frozen at –20°C until processed for DNA isolation. A 250 mg aliquot from each faecal sample was thawed and DNA was extracted using the PowerSoil®DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA). The V3 and V4 regions of the 16S rDNA gene in these samples were amplified using the GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA, USA). Primers S-D-Bact-0341-b-S-17 (3′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-5′) and S-D-Bact-0785-a-A-21 (3′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-5′) that include adaptors complementary to Illumina forward, reverse and multiplex sequencing primers were used (Klindworth *et al.* 2013). PCR amplification was carried out in a total volume of 25 μL, which contained DNA (20 ng μL^{-1}), primers (200 nM) and DreamTaq Green PCR master Mix (Thermo Scientific, Waltham, MA, USA). The thermocycling conditions applied were as previously described (Don *et al.* 1991). PCR amplicons were separated on a 1% agarose gel and purified using a Qiaquick PCR purification kit (Qiagen, Alameda, CA, USA). Genomic DNA and PCR amplicons were quantified using a Qubit® 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA).

Library preparation and Illumina sequencing

Purified PCR products were used as templates to perform an index PCR. In this step, dual indices and Illumina sequencing adaptors were attached to PCR products using the Nextera XT Kit. The index PCR product purification and multiplexing was done following the Illumina 16S sample preparation guide (16S Sample Preparation Guide, 15044223; Illumina. San Diego, CA, USA). The library was sequenced using the Miseq version 2 platform (Illumina) with 300 by 300 bp paired-end V3 reagent chemistry (Illumina MS-102-3003) at the Agricultural Research Council Biotechnology Platform in Pretoria, South Africa (ARC-BTP).

Sequence processing and analysis

A combination of *de novo* and reference-based operational taxonomic unit (OTU) identification was carried out using the open˙reference calling method implemented within the Quantitative Insights Into Microbial Ecology (QIIME) software package. A default similarity level of 97% was used to cluster

sequences into individual OTUs, and a single representative sequence from each clustered OTU was used to align to the Greengenes database (version: gg˙13˙5) (Caporaso *et al.* 2010). Taxonomic classification for each OTU was determined with the Ribosomal Database Project (RDP) Classifier using a minimum confidence cut-off of 0.8. The OTUs with <100 sequences across all samples were excluded from further analysis. Estimates of distance matrices for both alpha and beta diversity calculation and a per-sample summary of OTU representation at various taxonomic levels were also calculated.

Statistical analysis

A comparison of bacterial species diversity and richness was conducted using rarefaction analysis. Group- and individual animal-based rarefaction curves were calculated. The similarities and dissimilarities between the groups were evaluated by unweighted (Supplementary Fig. S2; based on the presence or absence of taxa) and weighted (Fig. 2; based on relative abundance) UniFrac-based principal co-ordinates analysis. Abundant species were defined at an empirical cut-off of percentage abundance >1%. Comparisons were made with the 95% significance level. The significant differences of taxa (Phylum, Class, Order, Family and Genus) between CON and HMC diets and the two breeds were determined using a modified χ^2 test which includes a false discovery rate (FDR) determination to obtain a *P*-value for the null hypothesis.

RESULTS AND DISCUSSION

In the digestibility study, we reported that apparent total tract digestibility coefficients of crude protein (CP), acid detergent fibre (ADF), hemicellulose and neutral detergent fibre (NDF) in SAWIPs and LW × LR pigs fed HMC-based diets were greater ($P < 0.05$) than in the CON group (Kanengoni *et al.* 2015). We also reported that the SAWIPs had greater ($P < 0.05$) NDF digestibility coefficients than the LW × LR crosses although the latter had greater concentrations of total volatile fatty acids (VFAs) ($P < 0.05$) than the SAWIPs. To investigate these observations further, this study sought to evaluate the influence of breed and diet on the faecal microbiota composition and whether these could provide some insights into the differences in digestibility of nutrients between the LW × LR and SAWIP breeds.

Pigs fed high-fibre diets require 3–5 weeks to adapt to the digestibility of non-starch polysaccharide monomers (Longland *et al.* 1993). Pigs investigated in the current study were on the same diet for at least 8 weeks, so the intestinal microbes were well adapted and in a stable flux. Pyrosequencing of 16S rDNA amplicons resulted in a total of 280 000 high-quality sequences for SAWIPs and 320 000 for LW × LR pigs, with an average of 55 091 sequences (range = 51 000–56 000) per sample. These sequences were classified to OTUs ($n = 835$) and into Bacteria (99.3%) and Archea (0.7%) kingdoms. The majority of the OTUs detected in these two breeds are similar to those previously described from other pig breeds and other countries (Leser *et al.* 2002; Upadrasta *et al.* 2013; Pajarillo *et al.* 2015). To our knowledge, the 16S rDNA analysis of intestinal microbial communities in the SAWIP breed and similar indigenous breeds in the Southern Africa region has not been done previously. We however also acknowledge that it is difficult to compare studies because of the many variables involved, including differences in faecal sampling methods, DNA extraction and PCR amplification methods, and the variable regions of the 16S rRNA gene analysed among experiments (Wu *et al.* 2010). In addition, differences in age, genetics and living environments of individuals across studies may also contribute to the development of the gut microbiota and cannot be ignored (Hooda *et al.* 2012). Overall 99.3% of the OTUs from the LW × LR pigs on the CON diet, 99.6% of the OTUs from LW × LR pigs on the HMC diet, 98.85% of the OTUs from SAWIPs on the CON diet and 99.74% of the OTUs from SAWIPs on the HMC diet were assigned to the phylum level. Notably <50% of the OTUs detected in the SAWIPs and LW × LR pigs could be assigned to the genus level. Similarly Leser *et al.* (2002) and Pajarillo *et al.* (2015) reported that only 17% and 32% of the identified phylotypes in the gastrointestinal tract of Danish and Duroc pigs, respectively, belonged to known species. Lamendella *et al.* (2011) also reported that unclassified *Firmicutes* and gammaproteobacteria were among the top six abundant bacterial groups in pigs. These results are suggestive of a highly complex porcine intestinal microbial community, with the majority of the bacterial genera within this environment yet to be characterized.

Rarefaction analysis revealed that in all samples the depth of sequence analysis achieved was sufficient to cover the entire species diversity. All curves that were derived reached a plateau, reflecting diminished chances of finding new phylotypes with continued sampling (Fig. 1A and B; Supplementary Fig. S1). A comparison of species diversity in samples grouped based on both breed and diet showed significant differences in the diversity of microbial communities recovered from SAWIPs and LW × LR pigs fed the CON diet but not the HMC diet (Fig. 1A). In addition, there were significant faecal bacterial community diversities detected between individual animal-based samples (Fig. 1B). Weighted UniFrac analysis showed that individual pig differences and clustering of the gut microbial communities occurred based on breed (SAWIP; LW × LR) but not on diet (CON; HMC) (Fig. 2). However, there were single outliers in both breeds that did not cluster well with the other samples. It has been reported that each individual pig harbours its own specific and unique bacterial composition, even if the animals receive the same diet, stay in the same environment and are siblings (Hill *et al.* 2005). Although rarefaction estimates showed differences in the number of OTUs identified between breeds, diet and their interactions, there were no overall significant differences in microbial diversity. We presume that due to the small number of pigs used and the presence of outliers in the current study data set, such differences could not be detected (Fig. 2A and B). To confirm these possible differences it is necessary to study a large number of pigs.

Overall the OTUs detected in the LW × LR and SAWIP breeds were assigned to 14 phyla, 20 classes, 22 orders, 38 families and 41 genera (Table 1). Notably the genera *Butrivibrio*, *Faecalibacterium*, *Desulfovibrio* and unidentified *Enterobacteriaceae*, although present in LW × LR crosses were absent from SAWIPs. The SAWIP OTUs on the other hand included *Bacteroides*, unidentified RF16, *Succiniclasticum*, *Peptococcus*, unidentified *Dethiosulfovibrionaceae*, unidentified *Cerasicoccaceae* and *Akkermansia* that were absent from LW × LR pigs. *Faecalibacterium prausnitzii* species are a major representative of the *Firmicutes* phylum, and an acetate-consuming and butyrate-producing gut microbe, and they have been reported to have anti-inflammatory properties (Khan *et al.* 2012; Hooda *et al.* 2012). *Butyrivibrio* species are the predominant cellulolytic bacteria in the rumen (Varel, Fryda and Robinson 1984). *Desulfovibrio*, a member of the *δ-proteobacteria*, belongs to the group of the sulphate-reducing bacteria that derive their energy from anaerobic respiration, and are able to use a wide variety of inorganic compounds as electron acceptors (Lobo *et al.* 2007). Members of the *Bacteroides* genus are an important group in terms of pectin degradation, due to their high numbers

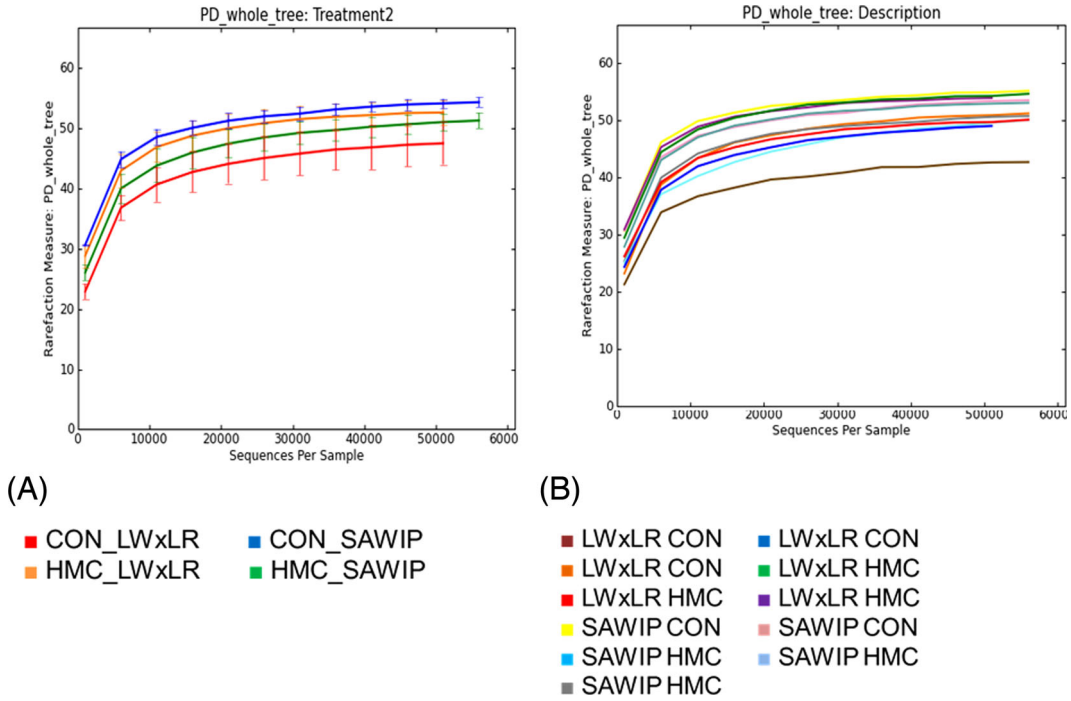

Figure 1. Rarefaction curves calculated based on phylogenetic diversity (PD) and observed species metrics for Large White × Landrace (LW × LR) pigs and South African Windsnyer-type indigenous pigs (SAWIPs) fed control (CON) and high maize cob inclusion (HMC) diets based on breed × diet interactions (**A**) and individual animals (**B**).

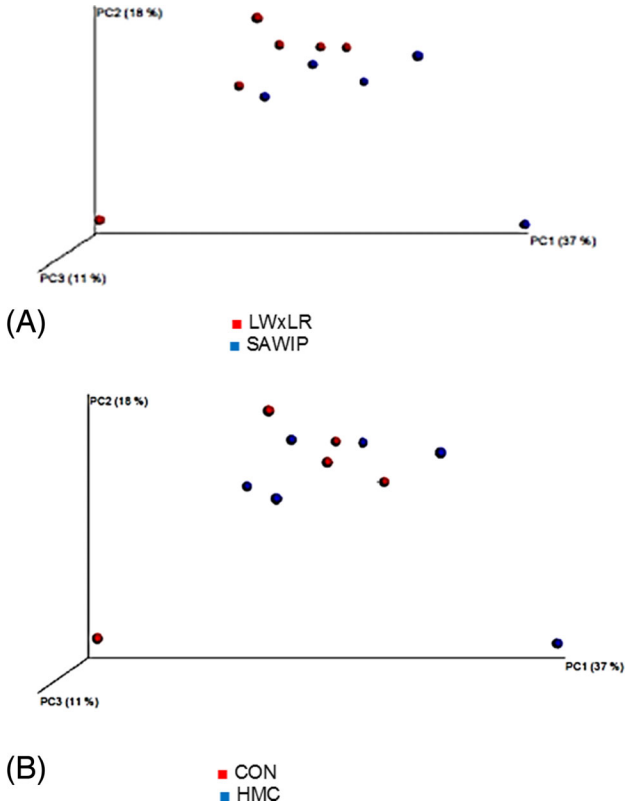

Figure 2. Weighted UniFrac-based principal co-ordinates plots of (**A**) the Large White × Landrace (LW × LR) and South African Windsnyer-type indigenous pig (SAWIP) breeds and (**B**) the control (CON) and high maize cob inclusion (HMC) diets.

and nutritional versatility (McCarthy, Kotarski and Salyers 1985). *Succiniclasticum ruminis* specializes in fermenting succinate and converting it quantitatively to propionate (Van Gylswyk 1995). *Akkermansia* belongs to the mucus-colonizing bacteria, capable of utilizing mucus as a sole carbon and nitrogen source (Derrien *et al.* 2004). *Akkermansia* spp. have been suggested as biomarkers for a healthy intestine (Png *et al.* 2010; Swidsinski *et al.* 2011). Information on the genotypic or the phenotypic characteristics of the other bacteria is not readily available as far as we could determine. Although the reasons for these differences are not clearly defined, it is known that genotype influences intestinal bacterial diversity in pigs, which might have an impact on their ability to utilize fibrous diets (Varel, Jung and Pond 1988; Pajarillo *et al.* 2014).

Irrespective of breed, the dominant phyla detected were *Firmicutes* (67.7%), *Bacteroidetes* (14.8%) and *Spirochaetes* (13.5%), whereas *Fibrobacteres*, TM7, *Verrucomicrobia*, *Cyanobacteria*, *Synergistetes*, WPS-2, *Tenericutes*, *Planctomycetes*, *Proteobacteria*, *Actinobacteria* and *Euryarchaeota* were below 1% and accounted for 4.1% of total reads (Table 1). The *Euryachaeota*, a member of the Archea kingdom, comprised 0.7% of the phyla of which LW × LR pigs had 0.5% and the SAWIPs had 0.2%. The LW × LR crosses had higher proportions of *Methanosphaera* (0.45% versus 0.18%) and *Methanobrevibacter* (0.50% versus 0.16%) than the SAWIPs. Among the *Firmicutes* phylum, *Clostridia* (58.8%) and *Bacilli* (8.4%) were the most dominant classes, whereas the *Bacteroidetes* phylum comprised only the *Bacteroidia* class (14.8%).

Crucially, the SAWIP and LW × LR breeds are obese and lean pigs, respectively, and differ in energy metabolism. A relationship between the intestinal tract methanogenic communities and the fatness or leanness of the host has been proposed (Su *et al.* 2014). Methanogens use hydrogen, formate, methanol and acetate to reduce carbon dioxide to methane, which

Table 1. Overall taxonomic classification and proportions (%) of OTUs from LW × LR pigs and SAWIPs independent of diet groupings.

Phylum	Class	Order	Family	Genus	LW × LR (%)	SAWIP (%)
Actinobacteria (0.1%)	*Coriobacteriia* (0.1%)	*Coriobacteriales* (0.1%)	*Coriobacteriaceae*	–	0.10	0.04
				Collinsella	0.05	0.02
Bacteroidetes (14.8%)	*Bacteroidia* (14.8%)	*Bacteroidales* (14.8%)	*Prevotellaceae*	*Prevotella*	2.37	2.08
			Paraprevotellaceae	CF231	0.23	0.18
				Prevotella	0.42	0.18
				YRC22	1.10	1.50
			BS11	–	0.22	0.66
			Bacteroidaceae	*Bacteroides*	0.00	0.08
			RF16	–	0.00	0.02
			Porphyromonadaceae	*Parabacteroides*	0.12	3.94
			p-2534-18B5	–	0.15	0.40
			S24-7	–	5.10	8.74
Cyanobacteria (0.1%)	4C0d-2 (0.1%)	YS2 (0.1%)	–	–	0.05	0.06
Euryarchaeota (0.6%)	*Methanobacteria* (0.7%)	*Methanobacteriales* (0.7%)	*Methanobacteriaceae*	*Methanosphaera*	0.45	0.18
				Methanobrevibacter	0.50	0.16
Fibrobacteres (0.2%)	*Fibrobacteria* (0.2%)	*Fibrobacterales* (0.2%)	*Fibrobacteraceae*	*Fibrobacter*	0.15	0.28
Firmicutes (67.7%)	*Bacilli* (8.4%)	*Lactobacillales* (7.1%)	*Streptococcaceae*	*Streptococcus*	1.63	5.58
			Lactobacillaceae	*Lactobacillus*	4.63	2.58
		Turicibacterales (1.3%)	*Turicibacteraceae*	*Turicibacter*	1.12	1.52
	Clostridia (58.8%)	*Clostridiales* (58.8%)	*Lachnospiraceae*	*Lachnospira*	0.95	0.08
				Coprococcus	0.60	0.58
				Other	0.05	0.14
				Epulopiscium	0.00	0.00
				Dorea	0.17	0.30
				Blautia	0.35	0.28
				Shuttleworthia	3.57	0.52
				Roseburia	0.30	0.20
				Butyrivibrio	0.03	0.00
			Ruminococcaceae	*Oscillospira*	0.88	1.78
				Faecalibacterium	0.03	0.00
				Ruminococcus	2.57	2.70
				–	7.90	12.62
			Clostridiaceae	SMB53	4.92	1.74
				Clostridium	3.55	2.20
				–	22.87	12.90
			Veillonellaceae	*Anaerovibrio*	0.03	0.02
				Phascolarctobacterium	0.07	0.04
				Succiniclasticum	0.00	0.02
				Megasphaera	0.73	0.08
			Christensenellaceae	–	0.15	1.82
			Mogibacteriaceae	–	0.15	0.20
			Peptostreptococcaceae	–	0.80	0.28
			Peptococcaceae	*Peptococcus*	0.00	0.04
	Erysipelotrichi (0.5%)	*Erysipelotrichales* (0.5%)	*Erysipelotrichaceae*	*Catenibacterium*	0.03	0.02
				[*Eubacterium*]	0.03	0.02
				L7A_E11	0.13	0.06
				Bulleidia	0.07	0.08
				p-75-a5	0.23	0.18
Planctomycetes (0.9%)	*Planctomycetia* (0.9%)	*Pirellulales* (0.9%)	*Pirellulaceae*	–	0.55	1.28
Proteobacteria (0.6%)	*Betaproteobacteria* (0.2%)	*Tremblayales* (0.2%)	–	–	0.38	0.02
	Deltaproteobacteria (0.0%)	*Desulfovibrionales* (0.0%)	*Desulfovibrionaceae*	*Desulfovibrio*	0.03	0.00
	Epsilonproteobacteria (0.1%)	*Campylobacterales* (0.1%)	*Campylobacteraceae*	*Campylobacter*	0.03	0.06
	Gammaproteobacteria (0.3%)	*Aeromonadales* (0.1%)	*Succinivibrionaceae*	*Succinivibrio*	0.08	0.06
		Enterobacteriales (0.2%)	*Enterobacteriaceae*	–	0.30	0.00
Spirochaetes (13.5%)	*Spirochaetes* (13.5%)	*Spirochaetales* (13.5%)	*Spirochaetaceae*	*Treponema*	12.48	14.66
Synergistetes (0.0%)	*Synergistia* (0.0%)	*Synergistales* (0.0%)	*Dethiosulfovibrionaceae*	–	0.00	0.08
Tenericutes (0.1%)	*Mollicutes*(0.1%)	RF39 (0.1%)	-	–	0.05	0.12
TM7 (0.1%)	TM7-3 (0.1%)	CW040 (0.1%)	F16	–	0.15	0.06
Other (0.6%)	Other (0.6%)	Other (0.6%)	Other	Other	0.55	0.62
Verrucomicrobia (0.6%)	*Opitutae* (0.0%)	*Cerasicoccales* (0.0%)	*Cerasicoccaceae*	–	0.00	0.04
	Verruco-5 (0.4%)	WCHB1-41 (0.4%)	RFP12	–	0.35	0.42
	Verrucomicrobiae (0.2%)	*Verrucomicrobiales* (0.2%)	*Verrucomicrobiaceae*	*Akkermansia*	0.00	0.46
WPS-2 (0.1%)	– (0.1%)	– (0.1%)	–	–	0.08	0.10

Table 2. Relative abundances (%) of faecal bacterial genera in LW × LR and SAWIP breeds fed CON and HMC diets.

	HMC		CON		P-values		
Genus	SAWIP	LW × LR	SAWIP	LW × LR	Breed	Diet	Breed × Diet
Oscillospira	0.9	1.2	3.1	0.5	0.654	0.885	0.02
Unidentified *Clostridiaceae*	14.1	15.3	11.1	30.5	0.654	0.885	0.534
Unidentified *Christensenellaceae*	1.9	0.1	1.7	0.2	0.129	0.953	0.534
Prevotella	1.6	4.1	2.9	0.7	0.904	0.885	0.534
SMB53	1.9	3.9	1.4	6.0	0.654	0.885	0.794
Unidentified *Bacteroidales*	1.6	1.7	1.4	0.4	0.686	0.885	0.794
Unidentified *Ruminococcaceae*	13.6	8.1	11.2	7.7	0.654	0.885	0.794
Akkermansia	0.8	0.0	0.0	0.0	0.654	0.885	0.794
Parabacteroides	6.5	0.2	0.1	0.03	0.654	0.885	0.794
Streptococcus	6.3	2.5	4.5	0.7	0.654	0.885	0.794
Lactobacillus	0.5	5.3	5.7	4.0	0.713	0.889	0.794
YRC22	1.2	1.9	2.0	0.3	0.819	0.889	0.794
Unidentified *Pirellulaceae*	1.3	0.4	1.1	0.8	0.654	0.961	0.794
Clostridium	2.8	3.4	1.3	3.6	0.654	0.953	0.823
S24-7	7.7	5.6	10.2	4.6	0.654	0.969	0.865
Turicibacter	1.8	0.7	1.1	1.5	0.800	0.961	0.896
Ruminococcus	2.6	3.1	2.9	2.0	0.904	0.944	0.946
Unidentified *Clostridiales*	9.0	9.1	8.6	6.2	0.819	0.885	0.946
Unidentified *Lachnospiraceae*	4.1	4.1	3.2	3.9	0.904	0.953	0.984
Treponema	14.4	11.1	15.1	13.9	0.881	0.961	0.984

translates to an energy loss for the animal (Johnson and Johnson 1995; Monteny, Groenestein and Hilhorst 2001). The higher proportions of *Methanosphaera* and *Methanobrevibacter* in the LW × LR pigs than in SAWIPs suggest that the SAWIP breed may retain more energy. Similarly Landrace pigs exhibited significantly more methanogen diversity than Erhualian pigs (Luo *et al.* 2012). Erhualian pigs are Chinese indigenous pigs that have not been selected for lean growth and tend to be obese (Luo *et al.* 2012), making them somewhat similar to the SAWIPs.

Although in this study the abundance of *Firmicutes*, *Bacteroidetes* and *Spirochaetes* between breeds and diets was not significantly different, the ratios of *Bacteroida* to *Clostridia* in each breed are suggestive of genotype influences. The ratio of *Bacteroidia* to *Clostridia* in SAWIPs on the HMC diet (0.37) was similar to that of those on the CON diet (0.39). In LW × LR pigs fed the HMC diet, the ratio of *Bacteroidia* to *Clostridia* was higher than that of those fed the CON diet (0.24 versus 0.1). Feeding the HMC diet did not result in significant differences in proportions of any bacterial taxa in either the LW × LR pigs or SAWIPs compared with pigs fed the CON diet. Consistent with our observations, other researchers have previously also reported no increase in cellulolytic bacteria in sows fed a 20% maize cob diet (Varel and Pond 1985). Table 2 shows the differences in the abundance of the genera between the breeds and the diets. There was a breed × diet interaction ($P < 0.05$) for the *Oscillospira* genus, which increased in LW × LR pigs as the diet changed from CON to HMC while it decreased in SAWIPs. *Verrucomicrobia opitutae* was detected only in SAWIPs on the HMC diet. There were no differences in breed, diet and breed × diet interactions in the other families. We previously reported that the LW × LR crosses consumed more feed weight wise and per metabolic body weight ($BW^{0.75}$) than SAWIPs at the finisher stage ($P < 0.05$) (Kanengoni *et al.* 2014). The observed differences in proportions of microbes between the two breeds could be due to different amounts of substrate passing through the gut. However, it could equally be due to the type of substrate. Hooda *et al.* (2012) proposed that gut microbial communities will shift due to substrate preference or metabolic cross-feeding.

The functional capabilities of the genus *Oscillospira* have not been determined, but it is likely that it plays a role in ffibre fermentation due to its presence in numerous rumen systems and its greater abundance in hosts that are fed fresh forage (Mackie *et al.* 2003). The phylum *Verrucomicrobia* has a widespread distribution, and is known to be one of the most common and diverse phyla in soil, aquatic habitats and in the gut of eukaryotes (Lee *et al.* 2009; Kielak *et al.* 2010). However, since members of this phylum have been difficult to culture, studies on understanding its role have been limited (Kielak *et al.* 2010). Some members of the *Verrucomicrobia* phylum have been reported to oxidize methane as a sole source of carbon and energy, making them the only known aerobic methanotrophs outside the *Proteobacteria*, and the only extreme acidophilic methanotrophs known (Dunfield *et al.* 2007; Islam *et al.* 2008). A related bacterium, strain *VeGlc2* of the order *Verrucomicrobiales*, was shown to ferment glucose to acetate, propionate, succinate and CO_2 through the Embden–Meyerhof–Parnas pathway (Janssen 1998).

CONCLUSION

In conclusion, 16S rDNA profiling analysis of intestinal microbial communities in the SAWIP breed is presented here for the first time. Analysis of faecal microbial communities in this study revealed differences occurring between the LW × LR and SAWIP breeds but not between the CON and HMC diets. These results suggest that the enhanced ability of the SAWIP to digest fibrous diets in comparison with the LW × LR breed may be a consequence of the differences in their intestinal microbial communities. A greater understanding of the roles of the intestinal microbes in utilization of fibrous diets in the SAWIPs could allow the design of rational and innovative approaches to formulate feeds.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSLE online.

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Conflict of interest. None declared.

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