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Effect of xylanase and xylo-oligosaccharide supplementation on growth performance and faecal bacterial community composition in growing pigs.

T.A. Sutton[1,2,*], H.V. Masey O'Neill[3], M.R. Bedford[4], K.McDermott[1], H.M. Miller[1].

[1] Faculty of Biological Sciences, University of Leeds, LS2 9JT, United Kingdom

[2] ABN – A Division of AB Agri Ltd., 64 Innovation Way, Peterborough, PE2 6FL, United Kingdom

[3] AB Agri Ltd. – 64 Innovation Way, Peterborough, PE2 6FL, United Kingdom

[4]AB Vista – A Division of AB Agri Ltd., Woodstock Court, Blenheim Road, Marlborough, Wiltshire, SN8 4AN, United Kingdom

Corresponding author: *E-mail address: Tegan.Sutton@abagri.com

ABSTRACT: This study was conducted to investigate the effects of xylanase (XYL) and xylo-oligosaccharide (XOS) supplementation on the growth performance and faecal bacterial community composition in growing pigs over time. In this 35-day trial, a total of 464 grower pigs with an average initial body weight (BW) of 14.5kg (SD ± 1.56 kg) were blocked into mixed sexed pens of 4 to 5 pigs balanced for BW, sex and litter origin. Pens were randomly allocated to 1 of 4 dietary treatments in a 2 × 2 factorial treatment arrangement with 2 concentrations of supplementary XYL (0 and 0.15 g/kg) and XOS (0 and 0.20 g/kg). On a weekly basis, pen feed intake and pigs were weighed to calculate pig performance. Faecal samples from 32 male pigs were collected on days 1, 14 and 35 of the trial and analysed to profile the bacterial communities through 16S ribosomal ribonucleic acid (rRNA) sequencing of the V4 region on the MiSeq platform (Illumina). There was no effect of XYL on pig

performance, nor was there an interaction between XYL and XOS. Pigs supplemented with XOS had a poorer gain to feed ratio during the first week (Day 1 to 7) of the trial ($P < 0.01$). During the second week of the trial (Day 8 to 14), pigs fed XOS showed an improved average daily gain compared to those without XOS ($P < 0.01$), but there were no performance effects in the overall trial period (Day 1 to 35). Alpha diversity increased over time ($P < 0.05$), and as an index of bacterial community compositions, beta diversity also changed over time ($P < 0.05$), but there was no overall effect of treatment on alpha or beta diversity. Despite no overall treatment effect, certain operational taxonomic units (OTUs) associated with *Muribaculaceae_ge* and *Prevotellaceae NK3B31 group* were higher in all 3 dietary treatments compared to the unsupplemented control diet ($P < 0.05$). In summary, XYL and XOS had limited effect on pig performance in this trial. Faecal bacterial communities significantly changed over time but despite influencing certain OTUs, treatment had no overall effect on faecal bacterial community composition. Supplementation of XYL or XOS, individually or simultaneously, increased the abundance of OTUs belonging to the *Muribaculaceae* and *Prevotellaceae* families which are associated with carbohydrate metabolism, indicating that these bacteria are likely involved in the mechanistic pathways of XYL and XOS.

Keywords: Grower pig, Pig performance, Xylanase, Xylo-oligosaccharide (XOS)

Abbreviations: ADFI, Average daily feed intake; ADG, Average daily gain; BW, Body weight; BXU, Birch xylan units; FCR, Feed conversion ratio; FTU, Phytase unit; G:F, Gain : feed ratio; GIT, Gastro-intestinal tract; NMDS, Non-metric multidimensional scaling; NSPs, Non-starch polysaccharides; OTUs, Operational taxonomic units; PCR, Polymerase chain reactions; rRNA, Ribosomal ribonucleic acid; XOS, Xylo-oligosaccharides; XYL, Xylanase.

1. Introduction

The fibre component of cereals is primarily composed of complex carbohydrates found in plant cell walls called non-starch polysaccharides (NSPs). The amount and type of NSPs vary among cereal grains, with xylans, β-glucans and cellulose being the most prominent (Choct, 1997). Cereals such as wheat and rye contain large amounts of soluble and insoluble NSPs, with the main soluble component being xylan (Choct, 1997). Within the gastrointestinal tract (GIT) of monogastrics, solubilised xylans are anti-nutritive as they increase digesta viscosity which in turn reduces nutrient digestibility and host growth (Olukosi et al., 2007). Further to this, valuable nutrients are trapped within cells rendering them inaccessible to the host due to a lack of endogenous enzyme production to degrade plant cell walls (Masey O'Neill et al., 2014). Supplementation of exogenous β-1,4-xylanases can alleviate these antinutritive effects by hydrolysing the xylan polysaccharide, thus decreasing digesta viscosity, releasing entrapped nutrients and improving the nutritive value of feed and host growth (Masey O'Neill et al., 2014). A less well-known mechanism of xylanase (XYL) and its benefits on performance, is the indirect provision of fermentable xylo-oligosaccharides (XOS) from the hydrolysis of the xylan backbone (Masey O'Neill et al., 2014). These are short-chain xylo-oligomers that resist digestion and are fermented in the hindgut where they have prebiotic effects by selectively stimulating beneficial bacteria like bifidobacteria or lactobacilli, and reducing pathogenic bacteria like *Escherichia coli* (Hsu et al., 2004; Liu et al., 2018). Furthermore, XOS can also be manufactured commercially via the hydrolysis of corncobs and can be formulated directly into the diet of the host. As an emerging new-generation prebiotic, studies using XOS have shown promising improvements in performance, nutrient digestibility, gut structure and gut bacterial community composition in broilers and weanling pigs (Liu et al., 2018; Ribeiro et al., 2018). However, little attention has been given to growing pigs, hence this study investigated

the effect of XYL and XOS supplementation on the performance and faecal bacterial community composition of growing pigs over time.

2. Materials and methods

Study protocols were approved by the University of Leeds Pig Research Centre and ethical approval was granted by the Animal Welfare and Ethical Review Body.

2.1. Animals and housing

At 7 weeks of age, 464 grower pigs ((Large White x Landrace females) x JSR Pietrain-based Geneconverter 900 sire line) with an average initial body weight (BW) of 14.5 kg (SD ± 1.56 kg) were used in this randomised complete block design with 24 replicates and 4 to 5 mixed-sex pigs per pen for a 35-day feeding study. All pigs were weighed at the start of the trial and blocked into pens balanced for litter origin, sex and BW. Pens within each replicate were randomly allocated to 1 of 4 dietary treatments described below. The trial was conducted over 2 batches with 12 replicates in each batch. Pigs were housed in conventional fully slatted weaner-grower facilities where each pen (155 × 129 cm) had 2 nipple drinkers and 1 single-space feeding trough.

2.2. Experimental design and dietary treatments

Pigs were fed with a 1-phase feeding program from day 1 to 35 of the trial and had *ad libitum* access to pelleted feed and water. All dietary treatments were manufactured at Roslin Nutrition Ltd. (Scotland) and formulated to meet or exceed the National Research Council nutrient recommendations for 11-50 kg pigs (NRC, National Research Council. 2012). Dietary treatment compositions with calculated and analysed nutrient concentrations are presented in Table 1. All diets included 0.10 g/kg phytase [Quantum Blue, AB Vista, Marlborough, UK; 5000 phytase units (FTU)/g] to give an expected activity of 500 FTU/kg of feed. Inclusion of

XYL [endo-1,4-β-xylanase, Econase® XT, AB Vista, Marlborough, UK; 160000 Birch Xylan Units (BXU)/g)] in the XYL and XYL*XOS dietary treatment groups provided an expected activity of 24000 BXU/kg of feed. Supplementation of XOS (XOS 35, 35%, Longlive Biotechnology Corporation, China) in the XOS and XYL*XOS dietary treatment groups provided 0.07 g/kg of pure XOS in the feed. Inclusion rates of XYL and XOS were according to the supplier's recommendations at the time of the trial. Dietary treatments were arranged in a 2 × 2 factorial treatment arrangement with 2 concentrations of supplementary XYL (0 and 0.15 g/kg) and 2 concentrations of XOS (0 and 0.20 g/kg) to give 4 experimental treatments; Control (0 g/kg XYL and XOS), XYL (0.15 g/kg), XOS (0.20 g/kg) and XYL*XOS (0.15 and 0.20 g/kg, respectively).

2.3. Measurements and sampling

2.3.1. Pig performance and health observations

On a weekly basis (Days 1, 7, 14, 21, 28, 35) all pigs were weighed individually and average daily gain (ADG) was calculated. Weekly feed intake was recorded on a pen basis and pen average daily feed intake (ADFI) was calculated. ADG and ADFI data were used to calculate weekly average gain to feed ratio (G:F). The timing of any pig mortality or removal from the trial was recorded, along with the pig BW to allow for ADFI and G:F adjustments. Health condition of digestive function was assessed by recording daily pen faecal scores on a scale of 1 to 4 by the same personnel (1 = firm faeces, 2 = soft faeces, 3 = mild diarrhoea, 4 = severe diarrhoea). Pen health scores were recorded daily on a scale of 1 to 4 by the same personnel (1 = no signs of ill health, 2 = some signs of ill health, 3 = clear indications of ill health, 4 = seriously ill pigs). Pen cleanliness scores were recorded daily on a scale of 1 to 4 by the same personnel (1 = clean pigs, 2 = light contamination with faecal material, 3 = contamination with faecal material, 4 = heavy contamination with faecal material).

2.3.2. Faecal collection

Faecal samples were collected from 1 individual male pig per pen on days 1, 14 and 35. Faeces were collected immediately after defecation and placed on ice before being stored frozen (-80°C) until analysis. Of the collected samples, 32 were selected for bacterial community analysis (8 replicates per treatment). Those selected for analysis had a BW close to that of the pen average, had not received antibiotic treatment and were littermates within replicates.

2.4. Analysis

2.4.1. Phytase and XYL recovery

Phytase and XYL recovery were determined at ESC (Ystrad Mynach, Wales, UK) according to the manufacturer's enzyme-linked immunosorbent assay for Quantum Blue and Econase XT. All diets were analysed for standard nutrients at DM Scientific (East Lothian, Scotland, UK).

2.4.2. DNA extraction and bacterial community analysis

Total bacterial DNA was extracted from the faecal samples (0.2 g) using the QIAamp DNA Stool Mini Kit (QIAGEN®, Hilden, Germany) as per the manufacturer's protocol, with 2 modifications. To maximise cell lysis, the faecal samples underwent bead beating (Tissue Lyser LT, Qiagen; 0.2 g of 0.1 mm silica beads) for 5 minutes at a maximum speed of 50 rps and were incubated at an increased temperature of 95°C. Extracted DNA quantity and quality was measured spectrophotometrically (NanoDrop ND-1000).

Extracted DNA was sent to the Environmental Genomics Facility at the University of Southampton for next generation sequencing following the Illumina 16S Metagenomic sequencing library preparation protocol. Polymerase chain reactions (PCR) were used to

amplify the V4 region of the bacterial 16S ribosomal ribonucleic acid (rRNA) gene using the modified 515F (Parada et al., 2016) (*TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG*GTGYCAGCMGCCGCGGTAA) and 806R (Apprill et al., 2015) (*GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG*GGACTACNVGGGTWTCTAAT) primer set (overhang sequences correspond to Illumina adapters shown in italics). The 25 µl PCR reaction consisted of 2.5 µl microbial DNA (5 ng/ul), 5 µl forward primer (515F), 5 µl of reverse primer (806R) and 12.5 µl KAPA HiFi HotStart ReadyMix. The amplification was performed using the following program: 95ºC for 3 minutes, 25 cycles of (30 seconds at 95ºC, 30 seconds at 55ºC, 30 seconds at 72ºC), 72ºC for 5 minutes, before being held at 4ºC. Amplification was confirmed using a Bioanalyser 1000 chip. AMPure XP beads were used to purify the 16S V4 amplicon away from the primers and primer dimer species. Nextera XT v2 index adaptors were attached using a further 8 cycles of PCR. AMPure XP beads were used to clean the final library before quantification. All AMPure clean up steps and the setup of the indexing PCR were carried out on a liquid handling robot (Biomek 4000). Libraries were quantified by a fluorometric quantification method using double stranded DNA binding dyes, normalised and pooled. Pooled libraries were denatured with NaOH and diluted with hybridisation buffer before heat denaturation and MiSeq sequencing.

2.4.3. Bioinformatics

Mothur (v.1.41.1) was used to process the sequence reads and the MiSeq standard operating procedure was followed (Kozich et al., 2013). Briefly, contigs were created by combining the forward and reverse reads, any ambiguous bases or contigs smaller or larger than 200-300 base pairs were removed. Duplicate sequences were merged, and unique sequences were aligned to the SILVA reference database (v.132). Only contigs that aligned between position 11894 and 25319 were selected with a maximum homopolymer length of 8. Sequences were pre-clustered

allowing for 1 difference in every 100 base pairs of sequence. Chimeras and sequences that aligned to Archaea, Eukaryota, chloroplasts or mitochondria were removed from the dataset. Sequences were clustered into operational taxonomic units (OTUs) with 97% similarity, before quantifying the number of OTUs within each group and their taxonomy. A BIOM file was then created to transfer the data into R (v. 1.1.463).

2.5. Statistical analysis

2.5.1. Pig performance and health observations

The pen served as the experimental unit for all growth performance data (BW, ADG, ADFI, F:G). Data points smaller than the 25^{th} quartile – 1.5 × interquartile range or greater than the 75^{th} quartile + 1.5 × interquartile range were identified as outliers and removed. Data were tested for normality by visualisation of histograms and the Shapiro-Wilk test for normality, whilst the Levene's test was used to assess the homogeneity of variance. Any data showing non-normal distribution or unequal variance were inversely transformed prior to analysis. Transformed data were back-transformed for inclusion into the data tables. Performance data were analysed as a 2-way ANOVA using the statistical package JMP® (Version *14.1*. SAS Institute Inc., Cary, NC, 1989-2019) (SAS, 2020). The statistical model included the fixed effects of XYL, XOS and their interaction, and replicate and batch as random variables. The initial BW of pigs was included as a covariate for BW and ADG analysis. Main effects were analysed individually when interactions were non-significant. Average pen faecal, health and cleanliness scores were analysed by the non-parametric Kruskal-Wallis one-way ANOVA. Significant differences were classed as $P < 0.05$ and trends as $P < 0.10$.

2.5.2. Bacterial community composition analysis

Individual pigs served as the experimental unit for the bacterial community composition analysis. A general linear model was used to determine the effects of treatment and time on

194 bacterial abundance at the level of the phylum and genus. *Post-hoc* differences were identified
195 using a Tukey's test (JMP®). Number of OTUs, Chao1 (Chao, 1984) and Shannon-Weiner
196 (Shannon, 1948) alpha diversities were measured using the Phyloseq package (v.1.22.3) in R
197 (McMurdie and Holmes, 2013). A general linear model (lme4) was used to determine the
198 effects of treatment and time on alpha diversity and number of OTUs. Models were reduced
199 using analysis of deviance. Beta diversity was analysed using the packages Vegan (v.2.5.3) and
200 DESeq2 (v.1.18.1). A permutational multivariate ANOVA (PERMANOVA - adonis) was used
201 to assess community similarities across treatment and time. A non-metric multidimensional
202 scaling (NMDS; axis = 2) plot using Bray-Curtis distances was used to plot beta diversity.
203 DESeq2 analysis identified the fold change of OTUs which differed significantly between 2
204 groups. DESeq2 was performed on un-rarefied data and *P* values presented were corrected for
205 multiple testing (Benjamin-Hochberg correction).

206

207 ## 3. Results

208 ### 3.1. Phytase and XYL recovery

209 The analysed phytase activity in the feed (FTU/kg) of the control, XYL, XOS and XYL*XOS
210 dietary treatments were 457, 658, 561 and 613, respectively. The analysed XYL activity in the
211 feed (BXU/kg) in the control, XYL, XOS and XYL*XOS supplemented diets were <2000,
212 19700, <2000 and 20800, respectively. Hence, the XYL dietary treatment had a recovery of
213 82%, whilst the XYL*XOS treatment had a recovery of 87%. Recovery was lower than
214 expected but similar in both XYL treatments.

215 ### 3.2. Pig Performance and health observations

216 Pig growth performance and health scores are presented in Table 2. There was no effect of
217 XYL on any of the performance parameters throughout the trial, nor was there an interaction

between XYL and XOS, hence only main effects are presented. However, XOS supplementation increased ADFI ($P < 0.05$) and decreased G:F ratio ($P < 0.01$) during the first week of the trial (Day 1 to 7). During the second week of the trial (Day 8 to 14), XOS supplementation increased ADFI compared to treatments without XOS ($P < 0.05$), which led to a higher ADG ($P < 0.01$) and BW at day 14 ($P < 0.05$) but G:F ratio was not affected. There was also a trend for XOS supplemented pigs to have a higher ADFI between day 15 to 21 ($P = 0.087$). There was a trend for XYL fed pigs to have a higher G:F ratio between day 29 to 35 compared to those without XYL ($P = 0.085$). There was no significant effect on overall performance (Day 1 to 35) of either XYL, XOS or their combination. Growth performance was slightly lower than expected for this unit with an ADG of 0.60 kg/d from day 1 to 35 across all treatments. There was no difference between treatments for average pen faecal, health or cleanliness scores from day 1 to 35.

3.3. Bacterial community composition analysis

The majority of faecal bacteria belonged to the phyla Firmicutes (51%) and Bacteroidetes (40%), jointly making up 91% of the bacterial community (Table 3). Changes of the bacterial community over time and between dietary treatments were estimated at the phylum and genus level. There were 6 phyla with a relative abundance greater than 1% in a minimum of one treatment or time group average (Table 3). There was no effect of treatment on the relative abundance of OTUs at the phyla level, nor was there an interaction between dietary treatment and timepoint (Table 3). There was a trend for the abundance of Tenericutes to be lowest in the control group and highest in the XOS group ($P = 0.059$). The abundance of Tenericutes also decreased from day 1 to 14, and then preceded to increase from day 14 to 35 ($P < 0.001$). Spirochaetes tended to follow the same trend as that of Tenericutes over time ($P = 0.091$).

There were 33 genera with a relative abundance greater than 1% in a minimum of one treatment or time group average (Table 3). Of the 33 genera, 17 were from the phylum Firmicutes, 11 from Bacteroidetes, 2 from Actinobacteria, 1 from Tenericutes, 1 from Actinobacteria and 1 from Epsilonbacteraeota. The abundance of *Prevotellaceae_NK3B31_group* was highest in the XOS group (P <0.05). Of the 33 genera, the abundance of 21 significantly changed over time (P < 0.05). Many genera decreased in abundance from day 1 to 14, namely, *Phascolarctobacterium, Rikenellaceae_RC9_gut_group, Mollicutes_RF39_ge, Ruminococcaceae_unclassified, Prevotellaceae_NK3B31_group, Ruminococcaceae_NK4A214_group* and *Prevotella_1*. Moreover, the abundance of many genera also increased from day 1 to 14, namely, *Prevotella_7, Dialister, uncultured bacteria, Acidaminococcus, Mitsuokella, Oribacterium* and *Streptococcus*.

The number of OTUs and alpha diversity (Chao1 and Shannon) are presented in Table 4. The number of OTUs and Chao1 diversity was higher at day 35 compared to day 1 and 14 (P < 0.001). Shannon indices were greater at day 35 compared to day 14, but not day 1 (P < 0.01). Dietary treatment did not affect the number of OTUs, however, XOS supplementation tended to have higher numbers of OTUs compared to the other dietary treatments (P = 0.078). Chao1 diversity was not significantly different between dietary treatments, however, Shannon indices tended to be the lowest in the XYL treatment group and highest in the XOS treatment group (P = 0.089). There was no time * diet interaction for the number of OTUs or Shannon indices. However, there was a trend for Chao1 (P = 0.064), such that the control and XYL treatments increased in diversity over time, whereas a reduction in diversity was observed at day 14, with a subsequent increase at day 35 for the XOS and XYL*XOS treatments.

There was no diet * timepoint interaction for beta-diversity. The beta diversity of bacterial communities changed over time ($P < 0.001$; Figure 1) but was unaffected by treatment. An NMDS plot of the similarity of bacterial communities at each timepoint shows the divergence of day 14 samples from day 1 samples, whilst the samples at day 35 cluster more closely together than other time points (Figure 1).

Due to the significant effect of time on bacterial community composition, DESeq2 was used to identify the individual OTUs which showed the greatest change in abundance between timepoints. Of interest, from day 1 to 14 (Figure 2), OTUs associated with *Veillonella* and *Megamonas* from the *Veillonellaceae* family increased by 24.2 and 10.0 log2 fold from a base mean of 17.61 and 74.12, respectively (base mean; mean counts of all samples normalised for sequencing depth; $P < 0.001$). The greatest decrease in abundance from day 1 to 14 was for OTUs associated with the genus *Prevotella_2*, where abundance decreased by 23.7 log2 fold from a base mean of 9.14. Of the top 10 decreases in abundance from day 1 to 14, all genera were from the *Prevotellaceae, Muribaculaceae* and *Rikenellacea*e families, which all belong to the order Bacteroidales.

Although there was no overall treatment effect for beta diversity, DESeq2 analysis between the control group and the 3 dietary treatments was conducted to identify if certain OTUs were affected. Each dietary treatment (XYL, XOS and XYL*XOS) showed an increased abundance of OTUs associated with *Muribaculaceae_ge* ($P < 0.05$) and *Prevotellaceae_NK3B31_group* ($P < 0.001$) compared to the control diet (Table 5). Moreover, all 3 dietary treatments showed a reduction in OTUs associated with *Prevotella_9* ($P < 0.001$) and *Alloprevotella* ($P < 0.001$) compared to the control diet.

290

291 ## 4. Discussion

292 This study aimed to investigate the effects of XYL and XOS supplementation on the growth
293 performance and faecal bacterial community composition of growing pigs over time. The
294 nutritive value of cereals can be improved with XYL supplementation by increasing nutrient
295 digestibility via the degradation of the plant cell walls and release of trapped nutrients as well
296 as reducing digesta viscosity (Passos et al., 2015). Furthermore, the degradation of plant cell
297 walls produces short-chain oligomers called XOS as an end-product of xylan degradation *in*
298 *vivo*. The oligomers produced during the hydrolysis of plant cell walls reach the hindgut and
299 exert prebiotic effects by acting as substrates for selective bacteria, thus influencing the GIT
300 bacterial community composition and subsequent energy provision for the host (Courtin et al.,
301 2008; Ribeiro et al., 2018). Provision of XOS to the hindgut can be indirect via the
302 supplementation of XYL or direct via the addition of commercially produced XOS into the
303 diet. It was predicted that the supplementation of XYL, XOS and their combination would
304 improve pig performance, however, there was no effect on overall growth performance in the
305 current study.

306

307 The effect of XYL supplementation on pig performance is inconsistent in the literature, most
308 likely due to differences in the duration of the studies, age of pigs trialled, xylanase
309 concentration and the type and quantity of substrate used within the diet formulation (Barrera
310 et al., 2004). A recent study found that 0.05 and 0.10 g/kg of XYL supplementation in a corn-
311 soybean meal-based diet linearly increased ADG and feed conversion efficiency in weanling
312 pigs (Lan et al., 2017). Moreover, others have found a higher concentration of XYL (0.5 g/kg)
313 in a corn-soybean meal-based diet improved ADG and feed conversion ratio (FCR) in heavier

pigs of 27 to 68 kg (Fang et al., 2007). However, in the current study, despite a trend for improved G:F efficiency in the final week of the trial (Day 29 to 35), overall, pigs fed XYL supplemented diets showed a similar performance to those without XYL and therefore had no overall beneficial effects on growth performance. This agrees with a study where weanling pigs (10 to 24 kg) receiving diets composed of corn, rye, wheat and soybean meal supplemented with 5 different concentrations of a *Bacillus circulans* XYL between 0 and 32 000 U kg^{-1} did not show any improvements in growth performance (Olukosi et al., 2007). Albeit, there were differences in the trials, for instance, the current study used a wheat-soybean meal diet and a *Trichoderma reesei* XYL. The age of the animal studied can also affect the pig performance response to XYL, for instance, including 0.10 g/kg of XYL in the first 2 weeks post-weaning (3 to 5 weeks of age) has been shown to decrease BW, ADG and feed efficiency (Lu et al., 2019). However, XYL supplementation from 2 weeks post-weaning led to an improved final BW and overall ADG up to 6 weeks post-weaning (5 to 9 weeks of age). The current study used pigs of 7 to 12 weeks of age, however, the same benefits of XYL inclusion were not shown despite this older age.

The majority of research on XOS has been conducted in broilers where some studies have shown no effect on bird performance (Craig et al., 2019), whilst others have shown beneficial effects on growth and immunity (Zhenping et al., 2013; Suo et al., 2015; Ribeiro et al., 2018). Despite this, there is a scarcity of research focusing on the effect of XOS in pigs. In the current study, XOS fed pigs had a higher ADFI but similar ADG compared to those without XOS, leading to a poorer G:F ratio in the first week of the trial (Day 1 to 7). However, during the second week of the trial from day 8 to 14, XOS increased ADFI by 0.11 kg/d which lead to an extra 60 g/d of growth and an increased BW of 0.35 kg at day 14, however this benefit was not maintained throughout the trial. It is suffice to conclude that XOS had limited effect on overall

pig performance in the current study. This agrees with a recent weanling pig study (Yin et al., 2019) which reported no performance benefits when using a lower concentration of XOS at 0.10 g/kg (40% XOS, 0.10 g/kg supplied 0.04 g/kg of pure XOS). However, beneficial effects of XOS supplementation have been observed in piglets where ADG increased by 17% and G:F efficiency by 14% compared to unsupplemented diets (Liu et al., 2018). There were however multiple differences between the studies, for instance, the current study used a wheat-soybean meal diet, 7-week-old pigs and 0.20 g/kg of a 35% XOS product supplying 0.07 g/kg of pure XOS, compared to a corn-soybean meal diet, 3-week-old piglets and 0.20 g/kg of a 50% XOS product supplying 0.10 g/kg of pure XOS (Liu et al., 2018). Together, this indicates that a concentration of 0.10 g/kg of pure XOS improves the performance of young pigs, but highlights that concentrations of 0.04 g/kg (Yin et al., 2019) and 0.07 g/kg of pure XOS may not be a high enough concentration to elicit these benefits. Furthermore, the performance differences reported could be because the microbial community of the 7-week-old pigs in the current trial was more stable and less susceptible to change under the influence of XOS compared to a newly weaned pig with a higher plasticity microbiota, resulting in XOS having more of an effect in the younger pig.

Moreover, there was no difference between dietary treatments for average pen faecal, health or cleanliness scores from day 1 to 35. The average pen faecal score from day 1 to 35 across all treatments was 2.47, thus between the observations of ‘soft’ and ‘mild diarrhoea’, which may have contributed to the slightly lower growth performance than expected for this unit.

To investigate the prebiotic effect of XYL and XOS in pigs, faecal bacterial community composition was studied by sequencing the V4 region of the 16S rRNA gene between dietary treatment groups and over time. Over 90% of faecal bacteria belonged to the Firmicutes and

Bacteroidetes phyla. Similar bacterial compositions to those found in this study have been observed in the literature (Kim et al., 2011; Holman et al., 2017). An interesting observation was the abundance of Tenericutes. The phylum Tenericutes consists of the class Mollicutes and are gram-negative bacteria that lack a cell wall (Zhan et al., 2017). Tenericutes have been identified as an opportunistic phylum, for example, a study showed that Tenericutes tended to increase with the inclusion of dietary flavonoid supplementation in dairy cows (Zhan et al., 2017). The abundance of Tenericutes was lower at day 14 compared to day 1 or 35 in the current study and showed a tendency to be lower in the control group compared to the XOS group. Interestingly, Tenericutes were also one of the most dominant phyla after dietary XOS intervention for 6 months in pigs (Pan et al., 2019). At the genus level, the majority of these changes can be explained by the change in abundance of *Mollicutes_RF39_ge* which belongs to the Tenericutes phyla. This may indicate that the abundance of bacterial competitors of *Mollicutes_RF39_ge* were highest at day 14 and in the control group, leading to the lower abundance observed, however, the functional roles of *Mollicutes_RF39_ge* remain unclear (Turnbaugh, 2017).

Of the 33 genera identified to have an abundance >1%, the only genus affected by dietary treatment was that of the *Prevotellaceae_NK3B31_group* which was higher in the XOS group compared to the control, XYL or XYL*XOS groups. Belonging to the *Prevotellaceae* family, the abundance of *Prevotellaceae_NK3B31_group* have been reported to be enriched in low FCR pigs compared to high FCR pigs (Quan et al., 2019), albeit there were no efficiency improvements with XOS supplementation in the current study. The abundance of 21 genera changed from day 1 to 14. These results may be explained by a change in diet, since the pigs transitioned from a highly digestible weaner diet at day 1 to a more indigestible cereal-based

grower diet, possibly explaining the flux in bacterial abundance while the bacterial community adjusted.

Alpha diversity defines the diversity within a particular ecosystem and is commonly used as an indicator of species richness and evenness using Chao1 and Shannon indices measures, respectively (Pan et al., 2019). Dietary treatment had no significant effect on alpha diversity, which agrees with other studies investigating XOS (Pourabedin et al., 2017; Pan et al., 2019) and alternative prebiotics (Berding et al., 2016; Li, 2017). Albeit, XOS did show a trend for an increased number of OTUs and species evenness, indicating there is potential for XOS to increase alpha diversity. To this point, XOS has been shown to increase species richness (Chao1) in weanling pigs (Yin et al., 2019). Early bacterial colonisation and succession in the GIT are vital for the establishment of specific bacterial community compositions and subsequent host health. The age of the host has a notable effect on the diversity and bacterial community of the microbiome, with stability generally reached after 5 weeks of age (Thompson et al., 2008). Supporting this, in a trial with 3-month-old pigs, time had no effect on alpha or beta diversities over a 12-week sampling period, indicating that the bacterial communities had stabilised by this later age (Umu et al., 2015). The current study demonstrated that time influenced alpha diversity, in particular, the number of OTUs and species richness which was greater at day 35 compared to day 1 and 14, indicating that the bacterial communities may have been continuing to adapt over time.

Beta diversity defines the heterogeneity of species composition between different communities along the environmental gradient, thus reflecting the species diversity between communities (Pan et al., 2019). The beta diversity of bacterial community composition was not affected by dietary treatment; however, it did change over time. As shown in the NMDS graph, the

411 communities at day 14 diverged from day 1, whilst the communities at day 35 clustered more
412 tightly, indicating a more homogenous bacterial community composition at the end of the trial.
413 These results may be explained by a change in diet since the pigs transitioned from a highly
414 digestible weaner diet at day 1 to a more indigestible cereal-based grower diet. These changes
415 in raw material content could have altered the quantity and type of material that reached the
416 hindgut, highlighting the importance of diet in shaping gut bacterial communities (Frese et al.,
417 2015). This, in turn, likely caused disruption to the bacterial community. By day 35, it is likely
418 the community had specialised in fermenting more indigestible materials. Similar results have
419 been seen in weanling pigs, where the composition of bacterial communities significantly
420 diverged over two weeks after weaning, demonstrating bacterial community composition
421 change over time irrespective of treatment (Looft et al., 2012). Moreover, clustering tendencies
422 have been observed between different doses of XOS in pigs, however, the growth stage at
423 which XOS was added was postulated to have been more of a driving force to shape the gut
424 microbiota structure than XOS dosage which played a comparable insignificant role (Pan et
425 al., 2019).

426 To explore the time effect further, DESeq2 analysis was conducted to identify the greatest
427 changes in abundance between day 1 and 14. Both *Veillonella* and *Megamonas* from the
428 *Veillonellaceae* family were found to increase with time. *Veillonellaceae* are gram-negative
429 bacteria known for lactate fermentation (Bonder et al., 2016). *Veillonellaceae* is considered to
430 be a pro-inflammatory family of bacteria as sufferers of irritable bowel disease and irritable
431 bowel syndrome show increases in their abundance (Gevers et al., 2014; Shukla et al., 2015),
432 thus possibly indicating some gut dysfunction at day 14 in the current study. To this point, the
433 abundance of the genera *Megasphaera, Dialister* and *Mitsuokella* which belong to the
434 *Veillonellaceae* family were all identified as having a >1% relative abundance in the current
435 study, with the latter 2 genera showing a significant increase in abundance at day 14. With the

main function of the *Veillonellaceae* family being lactate utilisation (Daly et al., 2012), it is reasonable to assume that an increase of lactate could have been present in the gut at day 14, hence explaining the large increase in lactate-utilising bacterial abundance in the faeces. A potential reason for lactate presence could include an increased level of starch fermentation, whereby bacteria degrade starch into small polysaccharides or other metabolic intermediates such as lactate, thus consequently increasing the abundance of lactate-utilising bacteria to prevent its accumulation (Duncan et al., 2004; Trachsel, 2017). Interestingly, the dietary switch from the digestible weaner diet to a more indigestible grower diet at day 1 resulted in a 28.6% increase in dietary starch levels, from 350 to 450 g/kg. Most starch is usually digested in the upper GIT of monogastrics, but an overload of dietary starch into an immature digestive system or an increased level of resistant starch may lead to increased starch fermentation in the hindgut. Dissections and consequent GIT sample collection were not within the scope of this study, however, investigating starch digestion and lactate concentration along the GIT as well as its interplay with lactate-utilising bacteria and other metabolites in pigs would be interesting for future studies. This is of particular importance when considering cross-feeding, as bacteria like *Prevotella* spp. or *Bifidobacterium* spp. may produce lactate as a metabolic intermediate of starch fermentation (Duncan et al., 2004; Trachsel, 2017), whilst species like M*egasphaera elsdenii* and *Anaerostipes caccae* utilise the lactate and produce butyrate, thus conferring additional health and energy benefits to the host (Muñoz-Tamayo et al., 2011).

Members of the *Prevotellaceae, Muribaculaceae* and *Rikenellacea*e families, which all belong to the order Bacteroidales, showed the greatest decline in abundance from day 1 to 14. *Prevotella* spp. are adapted to metabolise a wide range of complex carbohydrates, and therefore provide benefits to the host via the production of SCFAs (De Filippo et al., 2010; Dou et al., 2017). Moreover, *Muribaculaceae* are involved in complex carbohydrate degradation

(Ormerod et al., 2016; Lagkouvardos et al., 2019) and *Rikenellacea*e are also known to ferment carbohydrates and proteins (Su et al., 2014; Xin et al., 2019). As pigs age, the gut matures and becomes more efficient at degrading less digestible material, it is therefore expected that the abundance of bacteria capable of degrading complex carbohydrates would increase. With the change to a more indigestible cereal-based grower diet occurring at day 1, it is likely that the microbiota was in a state of flux at day 14 whilst adapting to the change in quantity and different substrates reaching the hindgut.

Although there was no overall treatment effect on bacterial community structure, some treatment effects were observed which are worth mentioning. Supplementation of XYL decreased OTUs associated with *Veillonellaceae_unclassified* when compared to the control group. Interestingly, similar results have also been reported in a weanling pig study (Lu et al., 2019), whereby XYL decreased the abundance of *Veillonellaceae* and also tended to decrease *Megasphaera*, both of which are members of the lactate-utilising *Veillonellaceae* family (Daly et al., 2012). This repeated observation may indicate that the mechanistic pathway of XYL could suppress the growth of *Veillonellaceae* families.

Furthermore, OTUs classified as *Prevotella_9* and *Alloprevotella* were shown to decline in all 3 dietary treatments when compared to the control, whilst the same OTUs associated with *Muribaculaceae_ge* and the *Prevotellaceae NK3B31 group* increased. *Muribaculaceae_ge* and the *Prevotellaceae NK3B31 group* belong to the *Muribaculaceae* and *Prevotellaceae* family, both of which have been associated with the degradation of complex carbohydrates, including xylan (De Filippo et al., 2010; Ormerod et al., 2016; Quan et al., 2019; Xin et al., 2019). This indicates that specific OTUs associated with both *Muribaculaceae_ge* and the *Prevotellaceae*

NK3B31 group may be involved in the mechanistic pathways of XYL and XOS in the gut. In addition, the combination of XYL and XOS would be expected to have an enhanced effect via a dual approach. The increase in *Muribaculaceae_ge* abundance (21.37 log2 fold) in the XYL*XOS treatment group was indeed higher than the increases seen in the single XYL and XOS treatment groups (14.89 and 15.14 log2 fold, respectively), albeit no performance benefits were seen.

A series of experiments investigating the prebiotic activity of XOS in broilers showed improvements in bird performance and shifts in microbial populations in the upper GIT tract (Ribeiro et al., 2018). The authors postulated that even if all the supplemented XOS was converted to SCFAs at 100% efficiency, this would not solely be responsible for the improvements observed. Hence, it was suggested that XOS was acting as a signal to the xylan-degrading bacteria to increase in abundance and activity, thus improving xylan digestibility and efficiency of overall digestion. This stimulatory concept has been shown in broilers (Bautil et al., 2020) and described elsewhere in the literature (Bedford, 2018; Petry and Patience, 2020). Despite the supplements in the current trial appearing to increase the abundance of some bacterial families associated with carbohydrate metabolism, xylan degradation along the GIT was not measured within the scope of this project and no performance benefits were observed. A longer feeding period or an earlier introduction might allow for a longer 'training' period, in which the bacterial communities would become xylan-degrading specialists and ultimately confer performance benefits to the host.

5. Conclusion.

Overall, Xylanase and Xylo-oligosaccharide supplementation had a limited effect on pig performance and faecal bacterial community composition. Results of this trial and comparison with the literature suggest a higher concentration of pure xylo-oligosaccharides (0.10 g/kg) in younger pigs may be necessary to observe performance benefits. The degradation of xylan along the gastro-intestinal tract coupled with bacterial community composition analysis would be of interest for future similar trials.

6. Declaration of interest:

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8. Literature

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Table 1: Dietary treatment composition with calculated and analysed nutrient levels.

Raw material	Control	XYL [a]	XOS [b]	XYL*XOS
Ingredient, g/kg				
Wheat	719.9	719.7	719.7	719.5
Soybean meal	225.0	225.0	225.0	225.0
Soya oil	19.8	19.8	19.8	19.8
Dicalcium phosphate	11.1	11.1	11.1	11.1
Vitamin-mineral premix [c]	5.0	5.0	5.0	5.0
Limestone	4.5	4.5	4.5	4.5
L-Lysine HCl, 784 g/kg	4.4	4.4	4.4	4.4
Sodium bicarbonate	3.7	3.7	3.7	3.7
Salt	3.6	3.6	3.6	3.6
Threonine, 980 g/kg	1.3	1.3	1.3	1.3
DL-Methionine, 980 g/kg	0.90	0.90	0.90	0.90
XYL	0.00	0.15	0.00	0.15
XOS	0.00	0.00	0.20	0.20
Phytase [d]	0.10	0.10	0.10	0.10
L-Tryptophan, 980 g/kg	0.07	0.07	0.07	0.07
L-Valine, 965 g/kg	0.06	0.06	0.06	0.06
Calculated nutrient composition				
Net energy (MJ/kg)	10.43	10.43	10.43	10.43
Standardised ileal digestible lysine (g/kg)	11.0	11.0	11.0	11.0
Apparent total tract digestible phosphorus (g/kg)	3.8	3.8	3.8	3.8
Analysed nutrient composition				
Dry matter (g/kg)	875.0	873.0	876.0	876.0
Ash (g/kg)	45.0	42.0	43.0	44.0
Crude protein (g/kg)	183.0	188.0	187.0	187.0
Crude fibre (g/kg)	26.0	23.0	23.0	21.0
Calcium (g/kg)	6.30	5.90	5.70	6.20

[a] XYL; Xylanase - Endo-1,4-β-xylanase, Econase® XT, AB Vista, Marlborough, UK. [b] XOS; Xylo-oligosaccharide - XOS 35, Longlive Biotechnology Corporation, China. [c] Vitamin

726 premix, active substance per kg of diet: 10,000 IU Vitamin A (retinyl acetate), 2250 IU Vitamin
727 D_3 (cholecalciferol), 75 mg Vitamin E (alpha tocopheryl acetate), 0.91 mg Vitamin K_3
728 (menadione), 1.6 mg Vitamin B_1 (thiamine mononitrate), 4 mg Vitamin B_2 (riboflavin), 8.9 mg
729 Pantothenic acid (calcium-D-pantothenate), 2.4 mg Vitamin B_6 (pyridoxine hydrochloride), 25
730 ug Vitamin B_{12} (cyanocobalamin), 30 mg Nicotinic acid, 0.5 mg Folic acid, 100 ug Biotin, 100
731 mg Iron (sulphate monohydrate), 15 mg Copper (sulphate pentahydrate), 45 mg Manganese
732 (sulphate monohydrate), 80 mg Zinc (sulphate monohydrate), 1 mg Iodine (calcium iodate
733 anhydrous), 0.25 mg Selenium (selenite) and 1500 mg Magnesium phosphate. [d] Phytase;
734 Quantum Blue, AB Vista, Marlborough, UK
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Table 2: Main effects of XYL and XOS on grower pig performance and health observations [a]

	Treatments					*P*-Value		
	XYL[b]		XOS[c]		SEM			
Inclusion (g/kg)	0	150	0	200		XYL	XOS	XYL*XOS
BW[d] (kg)								
Day 1	14.5	14.6	14.5	14.6	0.31	0.467	0.635	0.363
Day 7	17.2	17.2	17.3	17.2	0.08	0.589	0.221	0.628
Day 14	20.7	20.6	20.5	20.8	0.17	0.619	<0.050	0.803
Day 21	24.9	24.6	24.6	24.9	0.22	0.213	0.180	0.475
Day 28	29.8	29.2	29.4	29.6	0.31	0.132	0.587	0.294
Day 35	35.6	35.2	35.2	35.6	0.32	0.305	0.391	0.644
ADG[e] (kg/d)								
Day 1 to 7	0.38	0.37	0.38	0.37	0.012	0.644	0.375	0.419
Day 8 to 14	0.52	0.51	0.48	0.54	0.020	0.626	<0.010	0.753
Day 15 to 21	0.60	0.55	0.58	0.57	0.024	0.109	0.564	0.551
Day 22 to 28	0.70	0.66	0.69	0.67	0.026	0.176	0.385	0.223
Day 29 to 35	0.81	0.83	0.81	0.83	0.021	0.497	0.754	0.163
Day 1 to 35	0.60	0.59	0.59	0.60	0.009	0.250	0.458	0.637
ADFI[f] (kg/d)								
Day 1 to 7	0.76	0.75	0.73	0.78	0.036	0.818	<0.050	0.821
Day 8 to 14	1.08	1.02	1.00	1.11	0.042	0.315	<0.050	0.733
Day 15 to 21	1.08	1.05	1.04	1.09	0.024	0.401	0.087	0.574
Day 22 to 28	1.37	1.33	1.35	1.35	0.018	0.246	0.745	0.437
Day 29 to 35	1.46	1.42	1.44	1.44	0.013	0.131	0.919	0.604
Day 1 to 35	1.15	1.12	1.12	1.15	0.020	0.295	0.254	0.897
G:F[g]								
Day 1 to 7	511	513	542	482	19.7	0.913	<0.010	0.306
Day 8 to 14	511	522	507	527	31.0	0.617	0.348	0.527
Day 15 to 21	552	528	552	528	20.6	0.283	0.297	0.670
Day 22 to 28	516	501	521	496	20.8	0.443	0.207	0.497
Day 29 to 35	559	589	572	576	14.9	0.085	0.801	0.215
Day 1 to 35	531	535	538	528	11.7	0.731	0.392	0.833
Faecal score - day 1 to 35[h]	2.47	2.49	2.41	2.49	0.020		0.567	
Health score - day 1 to 35[i]	0.10	0.10	0.08	0.11	0.010		0.916	
Cleanliness score - day 1 to 35[j]	1.13	1.14	1.16	1.17	0.010		0.590	

[a] Data are means of 24 replicate pens of 4-5 pigs. [b] XYL; Xylanase. [c] XOS; Xylo-oligosaccharide. [d] BW; Body weight. [e] ADG; Average daily gain. [f] ADFI; Average daily feed intake. [g] G:F; Gain to feed ratio. [h] Faecal score; 1 = firm faeces, 2 = soft faeces, 3 = mild diarrhoea, 4 = severe diarrhoea. [I] Health score; 1 = no signs of ill health, 2 = some signs of ill health, 3 = clear indications of ill health, 4 = seriously ill pigs. [j] Cleanliness score; 1 = clean pigs, 2 = light contamination with faecal material, 3 = contamination with faecal material, 4 = heavy contamination with faecal material.

Table 3: The average relative abundance (>1%) at the Phyla and Genera level in pig faeces over time and between diets (n=32).

Relative abundance (%)		Day			SEM	Diet[2]				SEM	*P* Value		
Phylum[1]	Genus[1]	1	14	35		CON	XYL	XOS	XYL*XOS		Timepoint	Diet	Timepoint X Diet
Firmicutes		49.86	52.33	49.80	1.060	51.14	51.31	50.00	50.21	1.230	0.163	0.835	0.528
Bacteroidetes		39.40	39.69	40.25	0.930	38.90	39.08	39.99	41.14	1.074	0.806	0.439	0.175
Tenericutes		2.63^{a}	1.59^{b}	2.45^{a}	0.207	1.80	2.24	2.73	2.12	0.239	<0.001	0.059	0.902
Spirochaetes		2.11	0.96	1.78	0.376	1.47	1.97	2.00	1.02	0.434	0.091	0.338	0.779
Proteobacteria		2.06	1.89	1.98	0.186	2.21	1.93	1.88	1.87	0.215	0.809	0.654	0.937
Actinobacteria		1.62	1.90	1.17	0.670	1.75	1.74	1.22	1.55	0.309	0.155	0.593	0.356
Bacteroidetes	*Prevotella_9*	12.59	14.04	11.25	0.921	12.43	13.42	10.56	14.09	1.063	0.107	0.109	0.904
Firmicutes	*Lactobacillus*	7.24	7.55	7.45	1.002	7.16	8.39	6.71	7.40	1.157	0.976	0.769	0.072
Bacteroidetes	*Muribaculaceae_ge*	6.54^{a}	5.4^{a}	8.60^{b}	0.596	6.44	6.38	6.93	7.64	0.688	<0.001	0.544	0.854
Firmicutes	*Lachnospiraceae_unclassified*	5.69	5.26	4.28	0.501	5.54	4.36	5.28	5.12	0.579	0.130	0.511	0.680
Firmicutes	*Megasphaera*	5.46	6.02	6.27	0.597	6.00	6.60	5.83	5.24	0.690	0.616	0.579	0.987
Firmicutes	*Phascolarctobacterium*	4.17^{a}	1.43^{b}	1.63^{b}	0.324	1.93	2.64	2.52	2.54	0.374	<0.001	0.529	0.682
Bacteroidetes	*Rikenellaceae_RC9_gut_group*	3.46^{a}	1.68^{b}	2.39^{c}	0.208	2.60	2.46	2.72	2.25	0.240	<0.001	0.554	0.995
Bacteroidetes	*Alloprevotella*	2.91	3.90	2.98	0.363	3.44	3.19	3.01	3.42	0.419	0.103	0.874	0.616
Tenericutes	*Mollicutes_RF39_ge*	2.59^{a}	1.56^{b}	2.41^{a}	0.207	1.77	2.21	2.67	2.10	0.238	<0.001	0.070	0.863
Firmicutes	*Ruminococcaceae_UCG-002*	2.56^{ab}	1.85^{a}	2.64^{b}	0.216	2.02	2.22	2.85	2.30	0.250	<0.05	0.116	0.950
Firmicutes	*Ruminococcaceae_unclassified*	2.35^{a}	1.73^{b}	2.05^{ab}	0.130	2.00	1.88	2.25	2.04	0.150	<0.010	0.369	0.928
Firmicutes	*Subdoligranulum*	2.30	2.38	1.60	0.235	2.08	2.41	1.80	2.08	0.271	<0.05*	0.488	0.205
Bacteroidetes	*Prevotella_7*	2.28^{a}	4.17^{b}	3.70^{ab}	0.442	2.94	3.37	3.83	3.39	0.510	<0.010	0.678	0.614
Bacteroidetes	*Prevotellaceae_unclassified*	2.12	2.35	2.02	0.275	2.06	1.85	2.65	2.09	0.318	0.684	0.333	0.754
Bacteroidetes	*Prevotellaceae_NK3B31_group*	2.05^{a}	0.92^{b}	0.80^{b}	0.193	1.09	1.04	1.83	1.08	0.222	<0.001	<0.05*	0.182
Firmicutes	*Dialister*	2.01^{a}	5.54^{b}	3.70^{c}	0.446	4.05	4.25	2.94	3.76	0.515	<0.001	0.297	0.488
Bacteroidetes	*Prevotella_2*	1.96	2.08	1.92	0.217	1.96	2.13	1.93	1.94	0.251	0.870	0.933	0.846
Spirochaetes	*Treponema_2*	1.90	0.76	1.59	0.362	1.24	1.74	1.81	0.88	0.417	0.077	0.353	0.726
Firmicutes	*Ruminococcaceae_ge*	1.88	1.81	2.00	0.165	1.80	1.95	1.96	1.88	0.190	0.702	0.932	0.063
Firmicutes	*Ruminococcaceae_UCG-014*	1.70	1.44	1.73	0.136	1.67	1.34	1.81	1.67	0.157	0.247	0.195	0.856
Firmicutes	*Faecalibacterium*	1.16^{ab}	1.65^{a}	0.89^{b}	0.200	1.52	1.22	1.11	1.09	0.231	<0.050	0.519	0.912
Bacteroidetes	*Uncultured*	1.06^{a}	1.60^{b}	1.17^{a}	0.116	1.15	1.28	1.34	1.34	0.134	<0.010	0.708	0.895
Firmicutes	*Ruminococcaceae_NK4A214_group*	0.87^{a}	0.42^{b}	0.58^{b}	0.075	0.57	0.56	0.71	0.65	0.087	<0.001	0.611	0.605
Bacteroidetes	*Bacteroidales_unclassified*	0.86	0.80	0.97	0.179	1.05	0.75	1.05	0.65	0.207	0.783	0.395	0.272
Actinobacteria	*Olsenella*	0.82^{a}	0.59^{ab}	0.39^{b}	0.101	0.65	0.78	0.39	0.58	0.116	<0.050	0.117	0.240
Firmicutes	*Acidaminococcus*	0.79^{a}	2.39^{b}	2.28^{b}	0.350	1.82	1.61	1.80	2.05	0.404	<0.050	0.902	0.723
Bacteroidetes	*Prevotella_1*	0.79^{a}	0.20^{b}	0.42^{ab}	0.110	0.58	0.44	0.46	0.42	0.127	<0.001	0.821	0.358
Firmicutes	*Clostridiales_unclassified*	0.71^{ab}	0.45^{a}	0.92^{b}	0.103	0.74	0.49	0.84	0.69	0.119	<0.010	0.207	0.873
Firmicutes	*Mitsuokella*	0.63^{a}	2.15^{b}	1.39^{ab}	0.281	1.21	1.60	1.12	1.63	0.324	<0.001	0.581	0.712
Firmicutes	*Oribacterium*	0.53^{a}	1.07^{b}	0.75^{a}	0.092	0.96	0.72	0.72	0.74	0.106	<0.001	0.323	0.152
Epsilonbacteraeota	*Campylobacter*	0.48	0.45	0.30	0.192	0.84	0.21	0.36	0.23	0.221	0.777	0.160	0.849
Actinobacteria	*Collinsella*	0.23	0.66	0.22	0.146	0.59	0.30	0.22	0.36	0.169	0.060	0.461	0.612
Firmicutes	*Streptococcus*	0.03^{a}	0.74^{b}	0.94^{b}	0.147	0.84	0.30	0.64	0.49	0.170	<0.001	0.156	0.501

[1]Phyla and genera with >1% abundance in a minimum of one treatment or time group average. * No significant post-hoc (Tukey). [a-c] Means within a row that do not share a common superscript are significantly different ($P < 0.05$).

Table 4: Number of OTUs and alpha diversity measures of bacterial community across time and diet [a]

	Number of OTUs [b]	Chao1	Shannon
Day			
1	838.6[a]	1380.1[a]	4.6[ab]
14	813.8[a]	1346.1[a]	4.5[a]
35	940.8[b]	1572.1[b]	4.7 [b]
Diet [c]			
CON	845.6	1389.4	4.6
XYL	836.2	1387.2	4.5
XOS	916.5	1505.8	4.7
XYL*XOS	859.4	1448.7	4.6
***P* value**			
Day	< 0.001	< 0.001	<0.01
Diet	0.078	0.115	0.089
Day * Diet	0.262	0.064	0.975

[a-b] Means within a column that do not share a common superscript are significantly different ($P < 0.05$). [a] Data are means of 8 replicates pigs. [b] OTU; Operational Taxonomic Unit. [c] CON; Control diet, XYL; Xylanase 0.15 g/kg, XOS; Xylo-oligosaccharides 0.20 g/kg, XYL*XOS; Xylanase 0.15 g/kg and Xylo-oligosaccharides 0.20 g/ kg.

Table 5: DESeq2 analysis of the operational taxonomic units (OTUs) in Xylanase (XYL), Xylo-oligosaccharide (XOS) and XYL*XOS treatment groups compared to the dietary control (CON) group (n=32).

OTU Number	Genus	Base mean[a]	Log 2 fold change	*P* - Value	OTU Number	Genus	Base mean	Log2 fold change	*P* - Value
Increased from CON to XYL					**Decreased from CON to XYL**				
544	*Prevotellaceae_NK3B31_group*	20.9	20.26	<0.001	223	*Prevotella_9*	58.0	-27.08	<0.001
270	*Muribaculaceae_ge*	2.9	14.89	<0.050	452	*Alloprevotella*	5.3	-18.28	<0.001
					4219	*Ruminococcaceae_UCG-004*	0.3	-16.27	<0.050
					428	*Veillonellaceae_unclassified*	14.9	-6.73	<0.001
					41	*Campylobacter*	426.3	-2.53	<0.050
Increased from CON to XOS					**Decreased from CON to XOS**				
544	*Prevotellaceae_NK3B31_group*	20.9	22.93	<0.001	354	*Prevotella_9*	27.3	-25.58	<0.001
317	*Prevotella_7*	10.2	22.79	<0.001	652	*Prevotellaceae_NK3B31_group*	9.3	-24.22	<0.001
255	*Prevotella_9*	10.4	21.35	<0.001	408	*Alloprevotella*	5.1	-21.19	<0.001
609	*Dorea*	6.3	21.28	<0.001					
270	*Muribaculaceae_ge*	2.9	15.14	<0.010					
353	*Treponema_2*	27.7	10.97	<0.001					
Increased from CON to XYL*XOS					**Decreased from treatment CON to XYL*XOS**				
544	*Prevotellaceae_NK3B31_group*	20.9	22.83	<0.001	286	*Prevotella_7*	33.3	-25.56	<0.001
255	*Prevotella_9*	10.4	22.66	<0.001	698	*Prevotella_9*	9.2	-23.93	<0.001
609	*Dorea*	6.3	21.86	<0.001	408	*Alloprevotella*	5.1	-21.35	<0.001
317	*Prevotella_7*	10.2	21.61	<0.001	303	*Bacteria_unclassified*	47.2	-6.41	<0.010
270	*Muribaculaceae_ge*	2.9	21.37	<0.001					

[a] Base mean; mean counts of all samples normalised for sequencing depth.

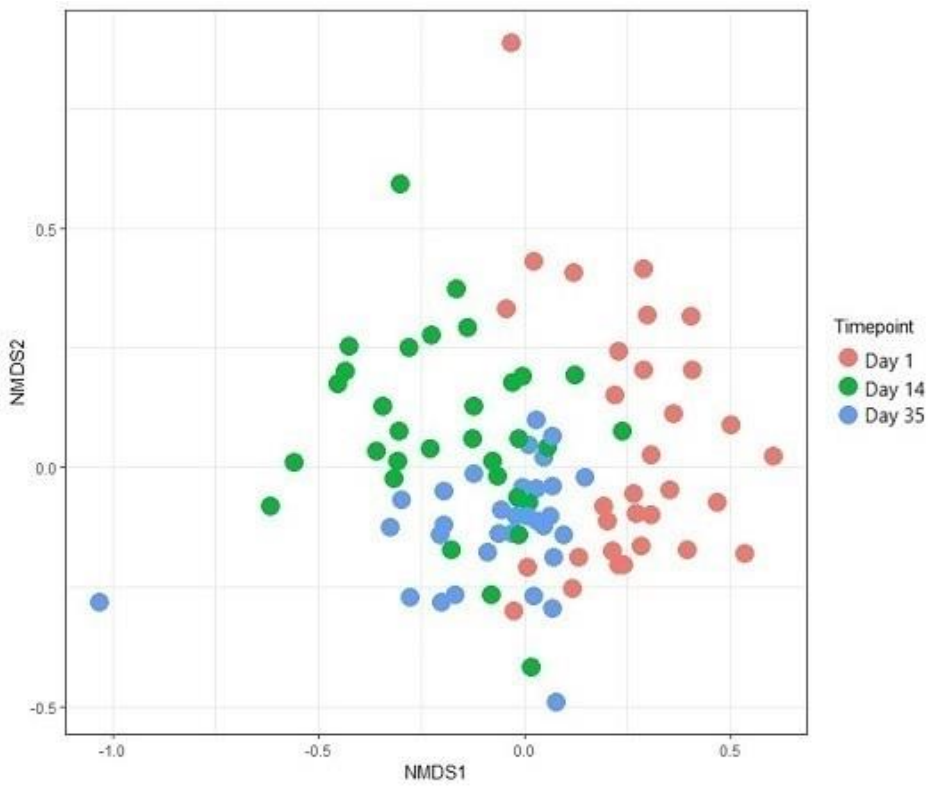

Figure 1: Non-metric multidimensional scaling (NMDS) plot of Bray-Curtis similarity coefficients from 16S rRNA gene sequence data from individual pigs over time (n=32, $P < 0.001$).

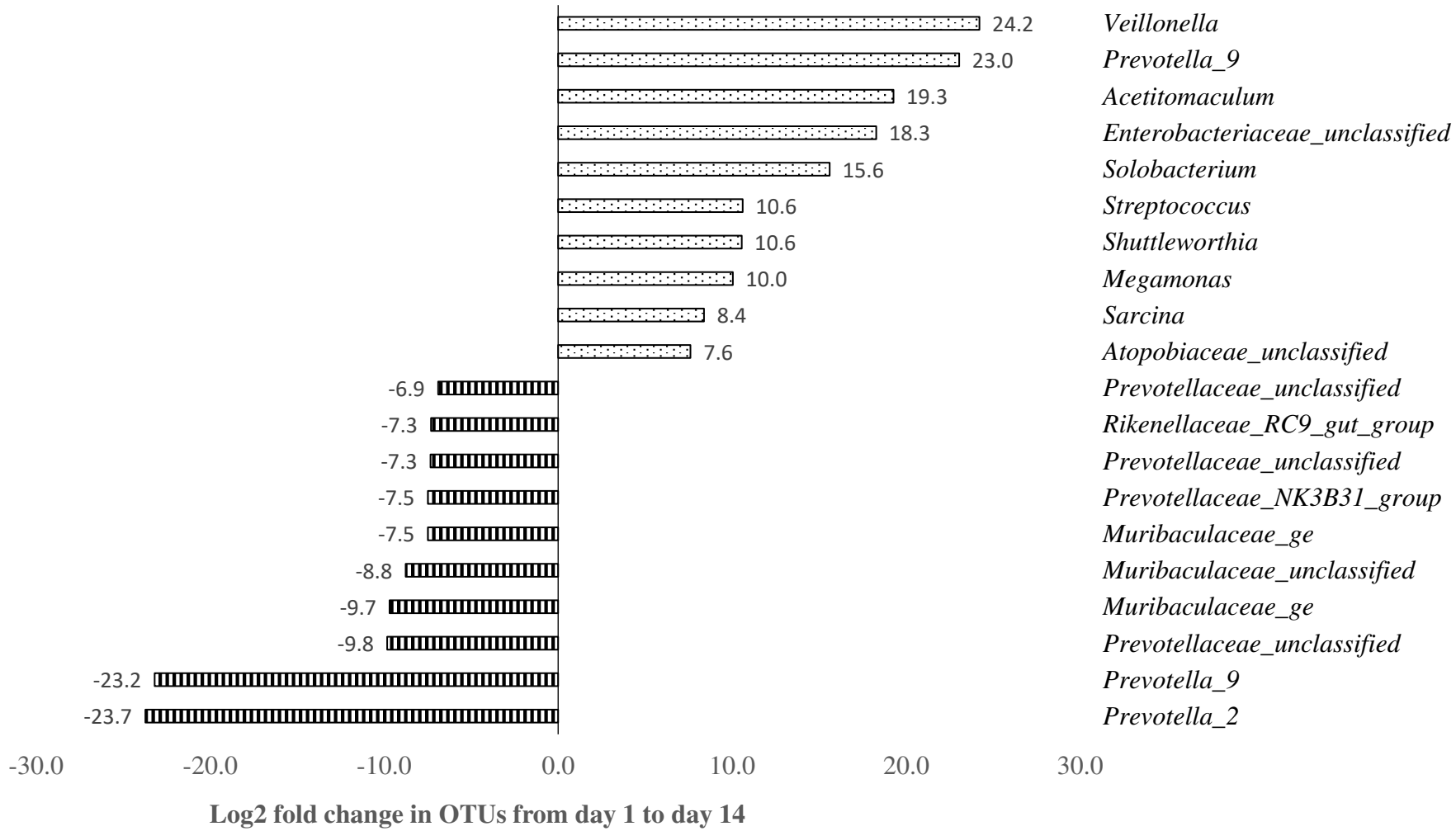

Figure 2: DESeq2 analysis of the operational taxonomic units (OTUs) from day 1 to 14 that showed the greatest change in abundance (n=32).