In vitro fermentation of arabinoxylan from oat (*Avena sativa* L.) by Pekin duck intestinal microbiota

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Abstract

Arabinoxylan (AX) is abundant in cereal grains used as feed for ducks. However, the duck intestinal microbes responsible for the degradation of AX are not fully understood. In this study, oat AX was degraded and utilized by different duck intestinal microbiota in vitro. Changes in short-chain fatty acids (SCFAs), branch-chain fatty acids, and the pH resulted from a 72-h AX fermentation in intestinal samples were measured. The addition of AX increased the concentration of isobutyric acid and decreased the concentrations of SCFAs. The pH values decreased significantly in the intestinal samples. Gut microbiota were assessed using high-throughput sequencing of the 16S ribosomal RNA gene, and the results indicated that AX stimulated the growth of *Megamonas* and *Bifidobacterium* species, with *Megamonas* exhibiting the greatest stimulation. Overall, the results suggest that oat AX is utilized by specific bacteria in duck intestines, providing the theoretical basis for the impacts of AX on animal health.

Keywords Arabinoxylan · Microbiome · Hydrolysis · 16S rRNA · Fermentation

Introduction

Cereal grains are an important food source for monogastric animals. In most cereal grains, the cell walls of the endosperm cells and the aleurone layers consist of approximately 60–70% arabinoxylans (AXs) (Izydorczyk and Biliaderis 1995). AX, a constituent of hemicelluloses, is comprised of a xylose backbone with arabinose side chains. AXs have both negative and positive effects on animals that consume them. For instance, AX lowers the nutrient and energy digestibility of animal diets, but AX fermentation in the gut can modulate the gut environment, promote beneficial bacteria growth (Jha and Berrococo 2015), and stimulate

immunostimulatory and protective effects against avian coccidiosis (Akhtar et al. 2012).

Although the structure of AX from different sources can be diverse, the most common AXs consist of a linear backbone of β -1,4-xylopyranosyl units that are substituted with a single α -L-arabinofuranoside at the 2'-OH or 3'-OH. Additionally, ferulic acid can be esterified to the arabinose side chains, creating cross-links with other cell wall components (Cleemput et al. 1993). Similar to other non-starch polysaccharides, AX is resistant to digestion by endogenous enzymes of monogastric animals but can be utilized to a great extent by animal gut microbiota (Choi et al. 2015; Jha and Berrococo 2015; Mendis et al. 2016). For poultry species, non-digestible carbohydrates are mainly fermented by bacteria in the small intestine (ileum) and caecum (Stanley et al. 2015; Prayoonthien et al. 2018).

In vitro gut batch fermentation models provide a direct assessment of both gut microbial species and their related functionality. These models also reduce various interference factors associated with animal experiments and reduces costs (Polli 2008; Park et al. 2017). Several previous studies have utilized in vitro analysis methods, in which intestinal cultures, nutrients, and prebiotics were anaerobically mixed to assess the intestinal performance (Carlson et al. 2016; Jiao et al. 2014; Li et al. 2012,

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2016; Prayoonthien et al. 2018). Soluble cereal AXs were allowed to in vitro ferment by human faecal microbiota; and the results showed that AX had a slow fermentation rate (Rumpagaporn et al. 2015). A study on the growth and metabolism of human faecal microbiota with supplementation of xylan in vitro showed an increase in *Bacteroides ovatus* a decrease in *Bifidobacterium adolescentis*, and the formation of propionic acid was enhanced (Adamberg et al. 2018). The effect of xylan on fermentation characteristics of ileal chime from pigs was assessed in vitro, and xylan led to a higher total gas production and increased total short-chain fatty acids (SCFAs) (Bauera et al. 2010).

The predominant species associated with the degradation of xylan in ruminants and human intestines have been well documented (Grootaert et al. 2009). For instance, xylanolytic organisms were isolated from human faecal samples and bovine rumen, and the most abundantly represented genera were *Bacteroides* spp. (Chassard et al. 2007; Zhang et al. 2014; Despres et al. 2016) and *Prevotella* spp. (Dodd et al. 2010; Emerson and Weimer 2017), which belong to the Bacteroidetes phylum (Dodd et al. 2011).

Duck is an important meat resource in the Chinese livestock industries. The intestinal microbes involved in the degradation of AX in duck are not yet known. In the present study, the fermentability of oat AX by small intestinal, caecal, and large intestinal microbiota from Pekin ducks was assessed using in vitro batch culture fermentation. Moreover, AX degradation, SCFA and BCFA production, and changes to intestinal microbial populations and species diversity were determined following the addition of AX during different intestinal periods.

Materials and methods

Substrates

Oat (*Avena sativa*) grains were harvested in Australia in 2014 and supplied by Hebi Dayong Animal Husbandry Co., LTD (Hebi, Henan Province, China). The oat hull was ground into a powder; and was then treated with α -amylase (*Bacillus licheniformis*, Megazyme, Wicklow, Ireland) and alkaline protease (Novozyme Biological Co. Tianjin, China). AX was extracted from the oat hulls using the methods described by Akhtar et al. (2012). AX is soluble and contains the two monosaccharides xylose and arabinose, which are present at a ratio of 10:1. The main AX structure includes a β -1,4-linkage xylose backbone, which is substituted with α -2- and α -3-arabinose. The molecular weight of AX is 3.63×10^4 kDa, based on size exclusion chromatography analyses.

Duck intestinal sample preparation and batch culture AX fermentation

Five 38-day-old Pekin ducks were used in this study. All ducks were healthy and were fed an antibiotic-free maize and soybean diet containing 10% cotton-seed meal. The study was approved by the Laboratory Animal Care and Usage Committee of the Feed Research Institute of the Chinese Academy of Agricultural Sciences.

The schematic overview of the experimental design is shown in Fig. 1. The intact small intestines, caeca, and large intestines from the five ducks were collected aseptically and placed into sterile sample bags (10" $\times$ 15", Labplas Inc., Ste-Julie, QC, Canada) in an anaerobic chamber (BACTRION EZ-2, Shel-Lab Co., Cornelius, USA). The contents of the small intestines were mixed into a single pooled sample, weighed and subsequently diluted to a 1:10 concentration in anaerobic phosphate-buffered saline (PBS) (0.1 M, pH 6.8). The samples were then homogenized in the sterile sample bags to yield 10% (wt/vol) samples. The samples were filtered through sterile four-layered medical gauze, and the resultant filtrates were then used as the inoculum. The contents of the ceca and large intestines from the five ducks were processed according to the same procedure. The effects of AX on the composition of the small intestinal, caecal, and large intestinal microbiota from the ducks were evaluated using batch culture fermentation, as described in Lei et al. (2012) and Li et al. (2016). The basal medium composition was prepared according to the methods of Li et al. (2016), and 5.0 g/L AX was added to the basal medium as the sole carbon source. The basal medium contained the following: peptone, 3.0 g/L; yeast extract, 4.5 g/L; bile salts no. 3, 0.4 g/L; NaCl, 4.5 g/L; L-cysteine, 0.8 g/L; hemin, 0.05 g/L; KCl, 2.5 g/L; KH_2PO_4 , 0.4 g/L; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 4.5 g/L; $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, 0.2 g/L; resazurin, 0.25 g/L; Tween-80, 1 mL/L, trace element solution 2 mL/L, and the pH was adjusted to 6.5. The trace element solution contained the following: $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 3.0 g/L; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.32 g/L; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g/L; $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$, 0.18 g/L; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.1 g/L; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.18 g/L; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.01 g/L and $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, 0.092 g/L. The basal medium without any carbon substances was used as a control. Serum bottles (100 mL) contained 80 mL growth medium were autoclaved, and the hot bottles containing the medium were placed into the anaerobic chamber. After the medium cooled to ambient temperature, 20 mL of 10% small intestinal, caecal, and large intestinal slurries from the ducks were inoculated into 80 mL of growth medium and were then incubated at 37 °C for 72 h in an anaerobic chamber. The pH values of the basal medium without AX and with

Fig. 1 Schematic overview of the experimental design

AX were measured after they were autoclaved to eliminate the possibility of a pH drop in the medium. The pH was not controlled during the fermentation process. The experimental scheme was as follows: 3 intestinal sections (small intestines, ceca, and large intestines) $\times$ 2 environments (with and without AX), and three bottles per treatment. Samples were removed at 0, 3, 6, 9, 18, 24, 36, 48, 60, and 72 h for the analysis of carbohydrate degradation. Samples at 0 h and 72 h were used for analysis of the pH, SCFAs, BCFAs, and bacterial community structure.

Analysis of carbohydrate degradation

Thin-layer chromatography (TLC) was used to detect carbohydrate degradation of in vitro AX fermentation in duck intestinal microbiota. The samples were treated with ion exchange resin and were then loaded onto pre-coated silica gel-60 TLC aluminum plates (Merck KGaA, Darmstadt, Germany). After treatment with a solvent consisting of n-butyl alcohol: acetic acid: water (2:1:1), the plates were stained with orcinol agent (900 mg of orcinol, 25 mL of

H₂O, 375 mL of anhydrous ethanol, and 50 mL of concentrated sulfuric acid) and were observed after incubation at 105 °C for 5 min.

Analysis of pH, SCFAs and BCFAs

The pH value of the sample after a 72 h fermentation period was measured using a pH probe (PB-21, Sartorius, Göttingen, Germany). For SCFA and BCFA quantification, the 72 h fermented samples were centrifuged at 13,200×g for 10 min, and the supernatant was collected. The SCFA and BCFA concentrations were measured via HPLC analysis, which included a Bio-Rad Aminex HPX-87H Ion Exclusion Column (300 mm×7.8 mm, Bio-Rad Laboratories Inc., Hercules, CA, USA) and a diode-array detector. The analysis was performed at 40 °C with a mobile phase of 5 mM H₂SO₄ at a flow rate of 0.6 mL/min and was measured OD_{215nm} (Li et al. 2016).

DNA extraction from in vitro culture fluids and PCR amplification

Bacterial DNA samples from the in vitro fermentation were extracted using an E.Z.N.A.[®] Stool DNA kit (Omega Bio-tek, Norcross, GA, USA) following the provided protocols. DNA quality and concentrations were measured with a NanoDrop 2000 (NanoDrop Technologies Inc., DE, USA).

The variable V1-V3 region of 16S rRNA was amplified using a PCR protocol and the following primers: 338F 5'-ACTCCTACGGGAGGCAGCAG-3'; 806R 5'-GGA CTACHVGGGTWCTAAT-3'. The PCR mixture (20 µL) contained 4 µL of 5× FastPfu Buffer (TransGen AP221-02, TransGen Biotech, Beijing, China), 2 µL of dNTPs (2.5 mM), 0.8 µL of each primer (5 µM), 0.4 µL of FastPfu (TransGen Biotech, Beijing, China), 0.2 µL of BSA (20 mg/mL), 10 ng of template DNA, and nuclease-free water, which was added to reach a 20-µL volume. PCR amplification was carried out on a gene amplification instrument (ABI GeneAmp 9700, Foster City, USA), and the cycling conditions were as follows: 95 °C for 3 min, followed by 28 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s, and a final extension at 72 °C for 10 min. The purified PCR products were pooled in equimolar concentrations, and paired-end sequencing (2×300) was performed on an Illumina MiSeq platform (MiSeq Reagent Kit v3) according to the standard protocols. The sequencing data were deposited in the NCBI sequence read archive (NCBI-SRA), with SRA accession number SRP151851.

Sequence and statistical analysis

All 16S rRNA gene sequences were processed on a bioinformatics cloud platform (i-sanger of Majorbio Inc., Shanghai,

China). Raw FASTQ files were de-multiplexed, and QIIME (version 1.17) was used to conduct quality filtration using the following criteria: (1) the 300-bp reads were truncated at any site receiving an average quality score < 20 over a 50-bp sliding window, and truncated reads that were shorter than 50 bp were discarded. (2) Exact barcode matching, a two-nucleotide mismatch in primer matching, and ambiguous characters were used to identify and remove non-quality reads. (3) Only sequences with at least 10-bp overlaps were assembled, and reads that could not be assembled were discarded. Operational units (OTUs) were clustered based on a 97% similarity cutoff implemented in the program UPARSE (version 7.1 <http://drive5.com/uparse/>), and chimeric sequences were identified and removed using UCHIME (version 4.2.40, http://drive5.com/usearch/manual/uchime_algo.html). The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier (<http://rdp.cme.msu.edu/>) in comparison to the Silva (SSU115) 16S rRNA database, and a confidence threshold of 70% was applied (Amato et al. 2013). Mothur (version v.1.30.1) was used to determine the alpha diversity (Chao and Shannon diversity indices). Using R (R Development Core Team 2012), a principal coordinate analysis (PCoA) was applied to the resulting distance matrices to generate two-dimensional plots.

Welch *t* tests were used to evaluate the statistical significance of the relative abundance of bacteria at the phylum and genus taxonomic levels, based on a significance level of *P* < 0.05. Sample comparisons were corrected using the FDR procedure (Benjamini and Hochberg 1995).

Results

Degradation of AX by duck intestinal samples

The degradation of AX by the small intestinal, caecal, and large intestinal microbiota was determined by TLC, and the results are presented in Fig. 2. AX was partly degraded into oligosaccharides by small intestinal, caecal, and large intestinal microbiota, but the rate of degradation (the time point of the apparent emergence of oligosaccharides on the TLC plates) varied among the three samples. A complex medium was used in the batch fermentation; therefore trace amounts of oligosaccharides inevitably appeared at the time of inoculation; these were absorbed by bacteria during the 3–6 h in vitro fermentation. AX was degraded the fastest by caecal microbiota, and oligosaccharides appeared after a 3 h incubation. AX degraded continuously over time, with degradation stabilizing after 18 h (Fig. 2b). AX was degraded by large intestinal microbiota more slowly than by caecal microbiota, and oligosaccharides were apparent on the TLC plates after a 9 h incubation. The products of fermentation by the large intestinal

Fig. 2 TLC results of arabinoxylan degradation by duck intestinal samples during batch fermentation. The small intestinal, caecal, and large intestinal slurries from the ducks were inoculated into the growth medium with AX and were then incubated at 37 °C for 72 h in an anaerobic chamber. Samples were removed at 0, 3, 6, 9, 18, 24, 36, 48, 60, and 72 h for analysis of carbohydrate degradation by TLC. **a** Small intestinal sample; **b** caecal sample; and **c** large intestinal sample

microbiota exhibited no significant changes after 36 h (Fig. 2c). AX can be degraded by small intestinal microbiota, but the rate of degradation was the slowest among the three samples. Oligosaccharides clearly appeared on the TLC plates after 36 h of incubation (Fig. 2a). No oligosaccharides were observed in the samples without AX, with the exception of oligosaccharides derived from the

Table 1 pH values after 72-h fermentation by three intestinal samples

Intestinal samples	pH		
	Control	Arabinoxylan	<i>p</i> value
Small intestinal	6.84 ± 0.22	5.29 ± 0.02	< 0.001
Cecal	6.79 ± 0.08	5.05 ± 0.06	< 0.001
Large intestinal	6.70 ± 0.07	4.82 ± 0.03	< 0.001

basal medium, which subsequently disappeared after 6 h of batch fermentation.

pH value, and SCFA and BCFA production during in vitro fermentation

The pH values associated with the fermentation of AX were lower than in the groups without AX in all three samples, and the differences were significant ($p < 0.01$) (Table 1). Large intestinal microbiota exhibited pH values ranging from 6.70 to 4.82, and the lowest pH value was detected after fermentation. The SCFA and BCFA concentrations associated with batch fermentation by the three intestinal samples after 72 h of fermentation are shown in Fig. 3. Compared to those of the basal medium, the concentrations of isobutyric acid were significantly increased, and the SCFA concentration included acetic, propionic, butyric, and succinic acids, which decreased in all AX samples. An isobutyric acid concentration of 5.25 mg/mL was produced by caecal microbes using AX, but only a 0.47 mg/mL isobutyric acid concentration was produced in the group without AX (Fig. 3).

Effects of AX fermentation on bacterial community structure

The 16S rRNA genes were sequenced using an Illumina MiSeq platform. Regarding the small intestinal, caecal, and large intestinal samples analyzed from in vitro fermentation for 72 h (3 different intestinal samples × 2 (with and without AX) × 3 = 18 analyzed samples), there were > 0.7 million sequences generated. The community structure of microbiota from the three duck samples was markedly varied, and AX supplementation in the culture medium changed the composition of the microbiota community. Compared to the starting of fermentation, the small and large intestinal samples without AX demonstrated an increased Shannon diversity index and Chao richness index ($p < 0.05$) (Table 2). Compared to the samples without AX, the Chao richness and Shannon diversity indices of the samples with AX decreased significantly ($p < 0.05$) in all three samples (Table 2). Therefore we speculate that several genera that can utilize AX are promoted.

We found that the initial intestinal samples (time zero) have three phyla-Firmicutes, Proteobacteria, and

Fig. 3 Concentration of SCFAs and BCFAs during batch fermentation. The small intestinal, caecal, and large intestinal slurries from the ducks were inoculated into the growth medium (with and without AX) and were then incubated at 37 °C for 72 h in an anaerobic chamber. Three bottles per treatment were used, and samples taken after

72 h were used for analysis of SCFAs and BCFAs. *SS* small intestinal sample without arabinosyl (AX), *SX* small intestinal sample with AX, *CS* caecal sample without AX, *CX* caecal sample with AX, *LS* large intestinal sample without AX, *LX* large intestinal sample with AX. The results shown are the average $\pm$ SD of three replicates

Bacteroidetes that represent more than 99.0% of the duck intestinal microbiota. Firmicutes was the most abundant bacterial phylum in the small intestine. Firmicutes and

Bacteroidetes were the major bacterial phyla in the caecum and large intestine. Table 3 shows the changes in the small intestinal, caecal, and large intestinal microbiota after

Table 2 Diversity indices calculated from 16S rRNA gene sequences based on a 97% sequence similarity threshold

	Intestinal samples	0 h	72 h-con	72 h-AX	p value
Chao richness index	Small intestinal	112.36 ± 8.10 ^b	137.47 ± 11.88 ^a	64.83 ± 10.78 ^c	< 0.001
	Cecal	226.60 ± 4.79 ^a	231.35 ± 11.91 ^a	208.73 ± 5.79 ^b	0.036
	Large intestinal	212.98 ± 24.17 ^a	156.93 ± 20.91 ^b	84.30 ± 13.00 ^c	0.001
Shannon diversity index	Small intestinal	1.67 ± 0.24 ^b	2.67 ± 0.11 ^a	1.68 ± 0.12 ^b	0.001
	Cecal	2.86 ± 0.16 ^a	3.14 ± 0.13 ^a	1.51 ± 0.21 ^b	< 0.0001
	Large intestinal	1.74 ± 0.19 ^b	2.59 ± 0.08 ^a	0.73 ± 0.05 ^c	< 0.0001

^{a,b,c}Different letters indicate significant differences between treatment at the same intestinal sample (a > b > c)

fermentation with and without AX. In the samples without AX, Proteobacteria increased significantly in the three samples, and Firmicutes, Proteobacteria, and Bacteroidetes were the most abundant bacterial phyla after fermentation. These phyla represented 99.8%, 96.8%, and 80.5% of the small intestinal, caecal, and large intestinal microbiota reads, respectively. Compared to the initial intestinal samples, the proportion of Firmicutes sequence reads of samples without AX increased from 38.3 to 69.5% in the caecal group ($p=0.010$) and from 59.5 to 77.8% in the large intestine group ($p=0.059$). However, the overall abundance of Firmicutes sequences decreased significantly (from 93.9 to 31.0%) in the small intestine group ($p<0.001$).

AX supplementation in the growth medium influenced the composition of duck intestinal microbiota community structures. The abundance of bacterial phyla differed in the intestinal microbiota with or without AX. Compared to the intestinal samples without AX, the proportion of Firmicutes sequence reads with AX significantly increased from 42.3 to 69.5% in the caecal group ($p=0.009$) and from 27.8 to 77.8% in the large intestine group ($p<0.001$). However, the overall abundance of Firmicutes sequences decreased slightly from 35.1 to 31.0% in the small intestine group ($p=0.116$). Proteobacteria with AX decreased significantly in the three samples, including decreases from 52.8 to 29.4% in the small intestine group ($p=0.011$), 43.7–1.5% in the caecal group ($p=0.006$), and 44.9–19.3% in the large intestine group ($p=0.002$). For Bacteroidetes with AX, variation trends associated with the three samples were not entirely similar. Bacteroidetes with AX increased significantly from 11.8 to 36.6% ($p=0.002$) in the small intestine group and decreased significantly from 7.8 to 1.3% ($p=0.020$) in the large intestine group. The proportion of Bacteroidetes sequence reads remained unchanged in the caecal group with AX.

At the genus level, *Megamonas* was dominant in the duck intestines, representing 24.7%, 27.5%, and 48.6% of the sequence reads of the small intestinal, caecal, and large intestinal microbiota at the starting point of fermentation. After fermentation, the proportion of *Megamonas* sequence reads from samples incubated without AX was decreased.

Megamonas experienced the greatest growth with supplementation of AX, and its representation increased significantly ($p<0.05$) in samples. Compared to the intestinal samples incubated without AX, *Megamonas* increased from 6.6 to 28.1% ($p=0.003$), 13.9–63.8% ($p=0.003$), and 8.4–76.9% ($p<0.001$) of all reads in the small intestinal, caecal, and large intestinal samples, respectively (Table 3). *Megamonas* was the most abundant genus in samples incubated with AX, which possibly drove AX degradation. Meanwhile, *Bifidobacterium* increased significantly ($p<0.05$) in samples incubated with AX from 0 to 72 h of fermentation. Compared to the intestinal samples incubated without AX, *Bifidobacterium* also increased from 0.1 to 2.9% ($p=0.026$), 1.3–18.0% ($p=0.014$), and 0.1–1.5% ($p=0.003$) in the small intestinal, caecal, and large intestinal samples, respectively.

Bacteroides and *Prevotella* showed a similar trend, and the portion of sequence reads of each from 0 to 72 h of fermentation increased in the small intestinal samples but decreased in the caecal intestinal samples. AX could increase the percentage of *Bacteroides* and *Prevotella* in the small intestinal and large intestinal samples. *Streptococcus*, *Faecalibacterium*, and *Lactobacillus* remained stable or decreased slightly across the three samples. In this study, *Escherichia* and *Shigella* could propagate in the basal medium, and the abundance of *Escherichia* and *Shigella* in the three samples increased significantly from 0 to 72 h of fermentation. After AX was added in the growth medium, *Escherichia* and *Shigella* showed a descending trend, with the abundance of *Escherichia* and *Shigella* decreasing significantly among the sequenced reads in the caecal samples ($p<0.05$). However, the variable abundance of *Escherichia* and *Shigella* in the small and large intestines did not differ detectably ($p>0.05$).

Differences in the microbial community profiles were analyzed among the different duck intestine samples after fermentation in the presence or absence of AX. The unweighted Unifrac beta diversity PCoA plot was used to measure dissimilarity among all sequenced reads at the OTU level. The results showed that at 0 h fermentation, both the large and small intestinal samples clustered together, whereas the caecum at time 0 was distinct. The

Table 3 Combined changes across batch fermentation samples and identified abundant taxa

Targeted taxa (phylum/and genus)	Small intestine			Cecum			Large intestine		
	0 h	72 h-con	72 h-AX	p value	0 h	72 h-con	72 h-AX	p value	p value
<i>Firmicutes</i>	93.070 ^a	35.120 ^b	30.960 ^b	0.027	38.270 ^b	42.280 ^b	69.530 ^a	0.004	0.001
<i>Megamonas</i>	24.710 ^{ab}	6.591 ^b	28.070 ^a	0.009	27.470 ^b	13.900 ^b	63.800 ^a	0.003	<0.001
<i>Streptococcus</i>	40.920 ^a	0.035 ^c	0.296 ^b	0.002	0.048	0.025	0.077	0.430	0.006
<i>Faecalibacterium</i>	13.650 ^a	0 ^b	0.004 ^b	0.034	2.203 ^a	0.132 ^b	0.748 ^b	0.021	0.196
<i>Lactobacillus</i>	3.460 ^a	0.001 ^b	0.003 ^b	0.012	0.024	0.010	0.002	0.087	0.070
<i>Sellimonas</i>	0.030 ^b	6.669 ^a	0 ^b	0.034	0.056	1.309	0.040	0.058	0.030
<i>Ruminiclostridium</i>	0.730 ^a	0 ^b	0 ^b	0.008	0.086	0.105	0.200	0.051	0.027
<i>Lachnospiraceae</i>	0.020 ^b	4.561 ^a	0.011 ^c	0.008	0.494 ^b	8.606 ^a	0.215 ^b	0.030	0.005
<i>Subdoligranulum</i>	0.040 ^b	0.139 ^b	0.444 ^a	0.042	0.641 ^b	1.254 ^a	0.715 ^b	0.005	0.023
<i>Dialister</i>	0.001 ^b	0.249 ^b	1.877 ^a	0.029	0.779	0.609	0.297	0.118	0.184
<i>Lachnospiraceae</i>	0	9.940	0	–	0.023 ^b	2.433 ^a	0.009 ^b	0.008	0.023
<i>Flavonifractor</i>	0	1.036	0	–	0.073 ^b	0.151 ^a	0.016 ^b	0.008	0.174
<i>Anaerotruncus</i>	0	0.785	0	0.278	0.099 ^b	2.538 ^a	0.119 ^b	0.006	<0.001
<i>Veillonellaceae</i>	0 ^b	0.005 ^b	0.111 ^a	0.021	0	0	0	–	0.009
<i>Phascolarctobacterium</i>	0	0	0	0	0.200 ^b	1.404 ^a	0.114 ^b	0.008	–
<i>Blautia</i>	0	0	0	0	0.102 ^b	0.707 ^a	0.004 ^c	0.032	0.012
<i>Proteobacteria</i>	3.010 ^c	52.830 ^a	29.360 ^b	0.011	4.739	43.689	1.500	0.006	0.002
<i>Escherichia-Shigella</i>	0.68 ^b	30.070 ^a	28.220 ^a	0.005	0.005 ^c	28.220 ^a	0.939 ^b	0.016	<0.001
<i>Enterobacter</i>	0.001	1.752	0.003	0.206	0	0	0	–	–
<i>Proteus</i>	0 ^b	19.000 ^a	0.232 ^b	0.024	0 ^b	9.997 ^a	0 ^b	0.022	0.024
<i>Sutterella</i>	0 ^c	1.262 ^a	0.438 ^b	0.024	0.073 ^b	3.964 ^a	0.004 ^c	0.006	0.007
<i>Desulfovibrio</i>	0	0	0	–	4.028 ^a	1.184 ^b	0.449 ^a	0.040	0.008
<i>Actinobacteria</i>	0.780 ^{bc}	0.200 ^b	2.892 ^a	0.027	0.288 ^c	2.317 ^b	18.350 ^a	0.014	0.003
<i>Bifidobacterium</i>	0.520 ^b	0.083 ^c	2.852 ^a	0.018	0.174 ^c	1.337 ^b	17.980 ^a	0.003	<0.001
<i>Collinsella</i>	0	0	0	–	0.046 ^c	0.734 ^a	0.200 ^b	<0.001	–
<i>Bacteroidetes</i>	2.54 ^c	11.820 ^b	36.580 ^a	0.002	56.130 ^a	10.840 ^b	10.490 ^b	0.886	0.020
<i>Bacteroides</i>	2.43 ^b	9.385 ^b	29.590 ^a	0.031	35.560 ^a	4.233 ^b	5.758 ^b	0.011	0.093
<i>Prevotellaceae</i>	0	1.640	4.494	0.154	5.599	0.507	0.064	0.105	–
<i>Prevotella</i>	0.011	0.060	2.481	0.338	2.459 ^{ab}	0.016 ^b	2.742 ^a	0.061	0.003
<i>Alistipes</i>	0.029	0	0	–	6.183 ^{ab}	2.956 ^a	0.267 ^b	0.003	0.030
<i>Parabacteroides</i>	0.044	0.131	0	0.060	2.179	1.740	1.231	0.066	0.046
<i>Barnesiella</i>	0.001 ^b	0.225 ^a	0 ^b	0.035	0	1.337	0.195	0.060	0.009
<i>Alloprevotella</i>	0.002 ^{ab}	0 ^b	0.017 ^a	0.034	0 ^b	0.181 ^a	0.033 ^a	0.027	0.027
<i>Fusobacteria</i>	0.121	0.010	0.189	0.186	0.205	0.781	0.007	0.059	0.036

Table 3 (continued)

Targeted taxa (phylum/and genus)	Small intestine			Cecum			Large intestine		
	0 h	72 h-con	72 h-AX	p value	0 h	72 h-con	0 h	72 h-con	72 h-AX
<i>Fusobacterium</i>	0.121	0.010	0.189	0.186	0.205	0.781	0.033 ^b	19.160 ^a	0.018 ^b
				p value					p value
				0.059					0.036

Welch *t* tests were used to evaluate the statistical significance of the relative bacterial abundance at phylum and genus taxonomic levels. Comparisons of samples were corrected using the FDR procedure

^{a,b,c} Different letters indicate significant differences between treatment at the same intestinal sample (a > b > c)

small intestine, large intestine and caecum samples at 72 h of fermentation without AX clustered together. Upon AX enrichment, there were three distinct clusters for each sample location that were distinct from each other.

In general, the results indicated that the samples differed significantly in response to fermentation with AX (Fig. 4). The results also indicated that duck intestinal microbial communities varied by AX treatment, and bacteria utilizing AX accumulated during the batch fermentation process.

Discussion

Depolymerization of AX requires complementary bacteria in the gut that produce endo- β -1,4-xylanase, β -xylosidase, and α -L-arabinofuranosidase (Despres et al. 2016). AX degradation produces oligosaccharides that exhibit different linkage compositions and various degrees of polymerization (Petersen et al. 2014). The current study confirmed that AX can be utilized by intestinal bacteria, and oligosaccharide mixtures were produced (Fig. 2). The AX levels were too high for intestinal bacteria; therefore, AX was not completely utilized after a 72 h of in vitro fermentation. Compared to that of the small and large intestinal groups, the rate of AX degradation was the fastest in the caecal sample, and the results indicated that the fermentation of AX mainly occurred in the caecum. Many bacteria have been found in the intestinal caecum of ducks, and this area is suitable for a variety of bacterial species because of the relatively low oxygen, partial pressure, and enzyme and bile salt concentrations (Best et al. 2017).

The predominant species associated with the degradation of xylan in ruminant and human intestines have been well documented (Chassard et al. 2007). In ruminant, they include xylanolytic bacteria such as *Butyrivibrio fibrisolvens* (Whitehead and Cotta 2001), *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Eubacterium ruminantium* (Hespell and Whitehead 1990), and *Prevotella ruminicola* (Dodd et al. 2011). Species of human intestinal bacteria include *Bacteroides fragilis*, *Bacteroides ovatus*, *Bacteroides thetaiotaomicron* (Van Laere et al. 2000) and *Roseburia intestinalis* (Mirande et al. 2010); these bacteria can metabolize carbohydrates. *Caldicoprobacter oshimai* isolated from sheep faeces (Yokoyama et al. 2010), *Bacillus subtilis* isolated from the gut of the termite *Reticulitermes santonensis* (Tarayre et al. 2013), and *Microbacterium trichothecenolyticum* isolated from the gut of *Gryllotalpa orientalis* (Kim et al. 2014) can utilize xylan. AX oligosaccharides accumulate after AX degradation, and these substances can be used by other bacteria such as *Lactobacillus cellobiosus*, *Lactobacillus paracasei*, and *Bifidobacteria* spp. (Grootaert et al. 2009).

Fig. 4 Principle coordinate analysis (PCoA) depicting shifts in bacterial communities among the intestinal samples subjected to batch fermentation alone or supplemented with arabinoxylan (AX). The small intestinal, caecal, and large intestinal slurries from the ducks were inoculated into the growth medium (with and without AX) and were then incubated anaerobically at 37 °C for 72 h prior to analysis. CS caecal sample without AX (inverted triangle), CO caecal intestinal

sample at 0 h (hollow circle), CX caecal sample with AX (diamond with stripe), LS large intestinal sample without AX (triangle), LO large intestinal sample at 0-h (circle), LX large intestinal sample with AX (diamond); SS small intestinal sample without AX (plus sign), SO small intestinal sample at 0-h (circle with stripe), SX small intestinal sample with AX (triangle)

In this study, *Megamonas* exhibited the most significant increase among the identified taxa; after fermentation its abundance in the AX groups increased from 24.7 to 28.1, 27.5 to 63.8, and 48.6 to 76.9% of the sequenced reads in the small intestinal, caecal, and large intestinal samples, respectively. Compared with the groups without AX supplementation, the portion of *Megamonas* also showed a significant increase (Table 3). This microbe belongs to phylum Firmicutes, which is associated with the caecal microbiota of adult poultry (Murphy et al. 2005; Polansky et al. 2015; Videnska et al. 2014). The results of a metagenomics analysis indicated an increased abundance of *Megamonas hypermegale* in the caecal microbiomes of broilers fed high-fibre diets and layers fed low-fibre diets (Walugembe et al. 2015). Furthermore, a metagenomics analysis of chicken caecal microbiota showed that *Megamonas* contained a gene cluster encoding a cellobiose phosphotransferase system, 6-phospho-beta-glucosidase, and endoglucanases, which were likely associated with the degradation of non-starch polysaccharides (Sergeant et al. 2014). Moreover, the elevated abundance of *Bifidobacterium* can also interact with xylanase-producing bacteria, thus utilizing AX-oligosaccharides for proliferation (Grootaert et al. 2009).

One major finding of the current study was the significant amounts of isobutyric acid that were detected in the three intestinal samples ($p < 0.05$) (Fig. 3). To the best of

our knowledge, little is known about the role of branched short fatty acids, such as isobutyric acid, in animal guts. It was reported that isobutyric acid improved insulin sensitivity through the regulation of adipocyte lipids and glucose metabolism (Heimann et al. 2016). High concentrations of isobutyric acid were produced in all three samples and were likely positively correlated with the relative abundance of *Megamonas*. When carbohydrate energy is low in the gut, bacteria will ferment protein and amino acids to obtain energy (Reimer et al. 2014). Isobutyric acid is the end-product of degradation of amino acids such as valine, leucine, and isoleucine, and increased concentrations can be the result of specific amino acid utilization (McCormack et al. 2017; Walsh et al. 2013; Zarling and Ruchim 1987).

The community structure investigation of the microbiome was just performed only at the end of the fermentation experiment in this study, and not in the exponential phase and when the bulk of AX hydrolysis is occurring. After fermentation for 72 h, *Megamonas* exhibited the greatest growth, however its primary carbon source (AX or other oligosaccharides, proteins etc) is currently unknown. Increased isobutyric acid was also detected after the utilization of certain non-starch polysaccharides such as polydextrose (Jie et al. 2000); thus, isobutyric acid could be the final product of arabinoxylan metabolism by *Megamonas*. Moreover, SCFAs and BCFAs from fermentation were almost

completely absorbed by different gut bacteria (Koecher et al. 2014). Isobutyric acid is the main concern regarding residual short-chain fatty acids after fermentation of arabinoxylan in vitro, mainly because isobutyric acid was barely absorbed by arabinoxylan-degrading bacteria, such as *Megamonas*. Since increased isobutyric acid was reported in this study, further research on isobutyric acid and its role in the regulation of *Megamonas* metabolism should be conducted. Our study suggests that AX can be degraded by specific bacteria that are associated with the duck intestinal microbiota. Further studies should be conducted to isolate the specific bacteria that utilize AX and investigate the metabolism of AX in these specific species.

Cereal grain AX can resist gastrointestinal hydrolysis and absorption and shows the character of a prebiotic which can benefit human health. AX can be degraded into AX-oligosaccharides by intestinal microbes, and AX-oligosaccharides have beneficial effects on the host by selectively stimulating the growth of certain bacteria such as *Bifidobacterium* and/or *Lactobacillus* species in the gut (Broekaert et al. 2011). Fermentation of AX-oligosaccharides by gut bacteria gives rise to the production of short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate, which contribute to a lower pH, inhibit the growth of harmful bacteria, and promote calcium and magnesium absorption (Broekaert et al. 2011; Wong et al. 2006). In this study, though AX was found to stimulate the growth of *Bifidobacterium* spp., AX could also increase the concentration of BCFAs rather than SCFAs. The prebiotic potential of AX for animal health will be assessed in the future.

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Compliance with ethical standards

Conflict of interest All the authors declare that there are no conflicts of interest.

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