Effect of arabinoxylo-oligosaccharides and arabinoxylans on net energy and nutrient utilization in broilers

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ABSTRACT

Arabinoxylo-oligosaccharides (AXOS) are hydrolytic degradation products of arabinoxylans (AX) that can be fermented by the gut microbiota, thus potentially displaying prebiotic properties. This study examined the effects of AX and AXOS on net energy (NE) and nutrient utilization in broilers. Ross 308 broilers (n = 90, 30 birds per treatment) were fed wheat-soybean diets supplemented with pure AX, AXOS produced by exposing the AX to xylanase in vitro (AXOS), or AX with xylanase (AX + E) from d 10 to 21. Performance parameters were measured from d 10 to 21. On d 15, 10 birds per treatment were allocated to closed-circuit net energy chambers to assess the impact of AX and AXOS on dietary energy utilization, through assessment of both metabolisable energy (ME) and NE. Ileal and caecal digesta samples were collected on d 21 to determine the effect of AX and AXOS on ileal and total tract dry matter digestibility, ileal digestible energy, digesta pH, short chain fatty acids (SCFA) and microbiota concentration. Feed conversion ratio was numerically the lowest in birds fed the diet supplemented with AXOS, which is 1.26 compared to 1.37 and 1.30 for AX and AX + E, respectively. Ileal dry matter digestibility was higher in birds fed AXOS than those fed AX (P = 0.047). Ileal digestible energy and total tract dry matter digestibility were higher in birds fed AXOS than those fed AX or AX + E (P = 0.004 and P = 0.001, respectively). Birds fed AXOS had higher ME intake (P = 0.049) and nitrogen retention (P = 0.001) and a strong trend of higher NE (P = 0.056), NE intake (P = 0.057) and retained energy (P = 0.054) compared to those fed AX. Ileal total SCFA, lactic and formic acid concentrations were higher in birds fed AXOS than those fed AX (P = 0.011, P = 0.012 and P = 0.023, respectively). Birds fed AXOS or AX + E had higher caecal total SCFA, acetic, butyric and isovaleric acid concentrations compared to those fed AX (P = 0.001, P = 0.004, P = 0.016 and P = 0.008, respectively), and caecal propionic acid concentration was higher in birds fed AX + E than those fed AX (P = 0.050). Ileal and caecal microbiota concentrations were numerically higher and pH was lower in birds fed AXOS and AX + E than those fed AX. Results from this study indicate that feeding AXOS directly is more efficient than AXOS generation in the gastrointestinal tract, and suggest that AXOS has a potential to be an efficacious prebiotic in broiler diets.

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1. Introduction

Xylans, also known as arabinoxylans (AX) and pentosans, are the most abundant hemicelluloses in the cell walls of monocotyledonous plants, such as cereals. The presence of these polysaccharides has a direct negative impact on energy availability of monogastric diets (Choct and Annison, 1990), largely due to the direct effect of soluble AX on increasing digesta viscosity. These negative effects can be combatted by supplementing the diet with endo-β1, 4-xylanases, which hydrolyses the xylan backbone. These enzymes cleave the internal β-
xylosidic glycosidic linkages uninterrupted by side chains to short-chain xylans or xylo-oligosaccharides (Jommuengbout et al., 2009), resulting in a mixture of low-molecular weight xylans, arabinose-substituted xylo-oligosaccharides (arabinoxylan-oligosaccharides, AXOS) and non-substituted xylo-oligosaccharides (XOS). Partial depolymerisation of AX by enzymes reduces molecular chains containing more than 5,000 sugars to just over 1,000 sugars. The resulting short-chain xylans and xylo-oligosaccharides can be utilized more efficiently by gut microbiota, which have a direct positive impact on the overall energy utilization of the cereals. Access of endogenous digestive enzymes to cell contents is also improved and there is reduced loss of endogenous amino acids, namely through modifications to pancreatic amylase and mucin secretion (Cowieson and Bedford, 2009; Meng et al., 2005).

Caecal and colonic bacteria generate energy by fermenting the end products of hydrolysis of polysaccharides. Selective fermentation of AXOS and XOS by intestinal bacteria positively influences the composition and activity of the gastrointestinal microbiota, improving health and performance of the host. Therefore, XOS fulfils the definition of a prebiotic. The potential prebiotic effects of XOS include optimizing colon function, increasing for changing composition of AX, short chain fatty acids (SCFA), increasing mineral absorption, immune stimulation and increased ileal villus length (Kim et al., 2011). Fermentation of XOS results in production of SCFA, including butyrate and lactate, which stimulates butyrate production. Butyrate fuels epithelial cells and increases intestinal epithelial integrity, which results in improved growth performance and positive changes to intestinal microbiota composition and metabolic activity (De Maeschalck et al., 2015; Sanchez et al., 2008). Indeed, Mäkeläinen et al. (2010a,b) reported that XOS were fermented with high specificity by strains of Blifidobacteria. This suggests that feeding poultry AXOS could potentially result in improved gut heath, energy and nutrient utilization. This study examines whether it is more advantageous for AX to be hydrolyzed into AXOS in situ via supplemental enzymes or to supplement diets with AXOS that has been prepared in vitro.

The anti-nutritive effects of soluble non-starch polysaccharides (NSP) on energy utilization are evidenced by the negative relationship between soluble AX and apparent metabolizable energy (AME) and that in situ degradation of cell wall NSP by enzymes increases AME (Hughes and Choct, 1999). The accuracy of methods used to measure metabolizable energy (ME) may be questionable as they do not take into consideration the efficiency of nutrient utilization. Additionally, AME is often corrected for nitrogen, but this system is unable to fully take into account the energy value of high protein ingredients and it partitions energy use into meat production, waste and heat production (Swick et al., 2013). As a result, net energy (NE) was used in this study to determine the true energy value of the diets, as this method takes into account energy lost as heat and differences in metabolic utilization of ME of nutrients for maintenance and production requirements (Noblet et al., 2010). It was predicted by van der Klis et al. (2010) that when a NE system is used instead of a ME system cost savings as high as €4.00 to 4.50 per tonne could be achieved without any detrimental impact on production performance. This suggests that using a NE system to accurately assess the amount of energy provided from polysaccharides and oligosaccharides in feed ingredients could have significant economic value. The aim of this study was to examine the effects of AX and AXOS on NE and nutrient utilisation in broilers. This was assessed by feeding broilers diets containing pure AX, AXOS produced by exposing AX to xylanase in vitro or AX in combination with xylanase from d 10 to 21.

2. Materials and methods

2.1. Extraction of AX and AXOS

This study examined whether it was more efficacious to feed broilers with a dietary supplement of AXOS produced in the laboratory or to feed broilers with intact AX with xylanase. Arabinoxylan was isolated from a starch milling by-product that contained 191 g AX, 681 g starch and 43 g crude protein per kg dry matter. The AX were extracted by adjusting the milling by-product to 60% to 70% ethanol (according to the water content), leaving the mixture at room temperature for a minimum of 24 h, centrifuging it at 13,000 × g for 15 min and then freeze-drying the residue. The AXOS was prepared from the resulting AX by hydrolysing it with 16,000 BXU/g xylanase (Econase XT 25, AB Vista Feed Ingredients, Marlborough, UK); the AX was suspended in citrate buffer (50 mmol/L, pH 5.4) containing 0.02% azide, the pH was adjusted to pH 2.5 with HCl and then the xylanase was added. The solution was then shaken at 50 °C for 24 h, centrifuged at 13,000 × g for 15 min and the residue was freeze-dried.

2.2. Birds and husbandry

Ross 308 male broilers (n = 90) were obtained from a commercial hatchery at day of hatch. Chicks were randomized by weight and placed in 120 cm × 75 cm floor pens, 15 pens of 6 birds per pen, 30 birds per treatment, bedded on clean wood shavings. All birds received vaccination against Marek's disease, infectious bronchitis and Newcastle disease at the hatchery under Australian code of practice for the distribution of broiler chickens. On d 10, birds were individually weighed and allocated to a pen. Pen allocation was randomised across the room. Total pen weight and mean chick body weight (BW) were calculated and diet allocation was arranged to ensure there was no significant difference in BW by pen across diets. Birds were allowed ad libitum access to the treatment diets and water for the duration of the trial. The room was thermostatically controlled to produce an initial temperature of 34 to 35 °C (50% to 60% relative humidity) upon arrival and reduced in steps of approximately 0.5 °C per day, reaching 22 to 24 °C by d 21. The lighting regimen used was 24 h light on d 1, with darkness increasing by 1 h a day until 6 h of darkness was reached, which was maintained throughout the remainder of the study. All birds sampled were euthanised by cervical dislocation on d 21 post hatch. This occurred after at least 6 h of light, to ensure maximum gut fill. Total pen weight and feed intake (FI) were determined on d 21 post hatch and were used to calculate feed conversion ratio (FCR). Mortality was recorded daily and any birds culled or dead were weighed. Institutional and national guidelines for the care and use of animals were followed and all experimental procedures involving animals were approved by the university's animal ethics review committee.

2.3. Dietary treatments

All birds were fed a standard wheat-soybean starter crumble diet from arrival to d 10 (Table 1). From d 10 to 21, birds were allocated to 1 of 3 dietary treatments: a standard wheat-soybean meal based grower diet supplemented with an additional 2% AX, 2% AXOS or 2% AX with 16,000 BXU/g xylanase (Econase XT 25, AB Vista Feed Ingredients, Marlborough, UK) (AX + E). Diets were composed of wheat, sorghum and soybean meal and were formulated to be adequate in all nutrients, based on the Ross 308 nutrient specifications. The diets were mixed in house using a ribbon mixer and cold-pelleted (3 to 3.5 mm, 50 to 70 °C).

The analysed nutrient values of the diets are presented in Table 2. Nitrogen content of the diets was determined with a
Table 1
Composition (%) of basal diets (DM basis).

| Ingredient                        | Starter | AX | AXOS | AX + E |
|-----------------------------------|---------|----|------|--------|
| Wheat                             | 56.8    | 63.1 |      |        |
| Soybean meal 45.2%                | 26.1    | 19.9 |      |        |
| Canola meal 37%                   | 8.0     | 10.0 |      |        |
| Meat and bone meal 53%           | 3.5     | 2.5  |      |        |
| Tallow                           | 1.5     | 2.4  |      |        |
| Limestone                         | 0.732   | 0.766 |      |        |
| Dicalcium phosphate (18P:21Ca)    | 0.039   | 0.059 |      |        |
| Salt                              | 0.199   | 0.159 |      |        |
| Sodium bicarbonate                | 0.150   | 0.150 |      |        |
| Premix¹                          | 0.200   | 0.200 |      |        |
| Choline CI 60%                    | 0.074   | 0.068 |      |        |
| L-lysine HCl 78.4%                | 0.257   | 0.245 |      |        |
| DL-methionine                     | 0.292   | 0.232 |      |        |
| L-threonine                       | 0.174   | 0.138 |      |        |
| Phytase 5,000 U/g²               | 0.010   | 0.010 |      |        |
| TiO₂                             | 0.50    | 0.50 |      |        |

¹ Vitamin-mineral concentrate supplied per kilogram of diet: retinol, 12,000 IU; cholecalciferol, 5,000 IU; tocopherol acetate, 75 mg; menadione, 3 mg; thiamine, 3 mg; riboflavin, 8 mg; niacin, 55 mg; panthothenate, 13 mg; pyridoxine, 5 mg; folate, 2 mg; cyanocobalamin, 16 μg; biotin, 200 μg; cereal-based carrier, 149 mg; mineral oil, 2.5 mg; Cu (sulphate), 16 mg; Fe (sulphate), 40 mg; I (iodide), 1.25 mg; Se (selenite), 0.3 mg; Mn (sulphate and oxide), 120 mg; Zn (sulphate and oxide), 100 mg; cereal-based carrier, 128 mg; mineral oil, 3.75 mg. ² Phyzyme XP (Feedworks, Australia).

Table 2
Analysed proximate composition (g/kg DM) of the experimental diets containing 2% AX, AXOS or AX + E.

| Item                           | Starter | AX | AXOS | AX + E |
|-------------------------------|---------|----|------|--------|
| Dry matter, g/kg              | 919.13  | 924.27 | 921.13 | 923.41 |
| Ash, g/kg                     | 59.58   | 60.06 | 60.12 | 59.58  |
| Protein                       | 242.17  | 254.26 | 255.37 | 254.71 |
| Total P                       | 7.38    | 6.52  | 6.54 | 6.53   |
| Total Ca                      | 11.01   | 10.05 | 9.96  | 10.07  |
| Gross energy, MJ/kg DM        | 20.24   | 19.97 | 20.01 | 19.80  |
| Fat                           | 67.66   | 65.25 | 65.73 | 65.87  |
| Starch                        | 340.40  | 367.09 | 375.27 | 381.88 |
| Soluble NSP                   | 16.16   | 16.37 | 17.52 | 20.30  |
| Insoluble NSP                 | 73.88   | 77.57 | 74.68 | 74.26  |
| Total AXOS, mg/kg DM          | 140.02  | 154.69 | 152.7 |        |
| X₁                            | 6.44    | 10.12 | 5.48 |        |
| X₂                            | 32.85   | 119.16 | 26.37 |        |
| X₃                            | 42.86   | 227.42 | 48.31 |        |
| X₄                            | 57.87   | 157.99 | 72.54 |        |

AX – arabinonxylan; AXOS – arabininoxyl-o-oligosaccharides; AX + E = AX + xylanase; NSP – non-starch polysaccharides; X₁ = combined xylene; X₂ = xylobiose; X₃ = xylotriose; X₄ = xylotetraose.
voltage 70 V. The 1-phenyl-3-methyl-5-pyrazolone (PMP)-derivatives were detected using positive selected-ion monitoring (SIM) of the dominate [M + H] + ions. These were determined prior to HPLC analysis by direct infusion of single standards. The following SIM values were used in the HPLC analysis: PMP- Xyl4, m/z 877.5; PMP- Xyl3, m/z 745.5; PMP- Xyl2, m/z 613.5 and PMP- Xyl, m/z 481.5.

2.4. Response variables

2.4.1. Net energy

On d 15, 2 birds per pen were allocated to 1 of 15 closed-circuit calorimeter chambers, 5 replicate chambers per dietary treatment. Birds were acclimatised to the calorimeter chambers for 4 days prior to collection of data and calculation of heat production (HP). All birds had ad libitum access to feed and water throughout the NE trial period. Feed intake and total excreta output during the 3-day period (d 19, 20 and 21) were measured. The total excreta collected was weighed and was thoroughly homogenized, and subsamples were taken for analysis of dry matter and gross energy, as described above for the diet analysis. As described by Swick et al. (2013), chambers were approximately 100 cm long, 76 cm high and 70 cm wide and were made of stainless steel. Each chamber housed a wire mesh cage that was approximately 89 cm long, 60 cm high and 61 cm wide. Water was used to seal the chambers, as highlighted by Farrell (1972). The pressure in the chamber was controlled using a barometric sensor connected to an electronic switch that activated a solenoid valve. Temperature and humidity in each chamber were monitored continuously using temperature and humidity sensors. Humidity was maintained at less than 70% for the run. Chamber air was circulated by a 28 L/min diaphragm pump through a bottle containing 2 L of 320 g/kg KOH solution and a bubbler assembly, to absorb the CO2 produced by the birds. The air was then passed through a trap containing 3 kg of dried silica, to absorb the humidity, and was then returned to the chamber. CO2 concentrations were maintained at less than 4 mL/L. Each chamber was equipped with a 490 L cylinder of medical grade O2 fitted with a regulator and a reducing valve to replenish the O2 as it was consumed. O2 consumption was calculated by subtracting the weight of the O2 cylinder at the end of each daily run from its weight at the beginning of the run. The conversion of weight to volume was based on the density of the O2, which was approximately 1.331 g/L at 20 °C and 101.325 kPa. Subsamples of the KOH from each chamber were collected at the end of each daily run to analyse O2 production by the birds. Recovery of the CO2 was determined based on a BaCl2 precipitation technique, as described by Annison and White (1961) and Swick et al. (2013). Apparent metabolisable energy was determined by the total excreta collection method. Total heat production was measured during the whole 3-day trial period and was estimated from the O2 consumed and CO2 produced by the birds, using the equation: Total heat (kcal) = 3.866 x O2 consumed (L) + 1.200 x CO2 produced (L). The respiratory quotient (RQ) of the trial period was calculated as the ratio of CO2 produced to the volume of O2 consumed. Heat increment (HI) was calculated by subtracting fasting heat production from the total heat production.

To correct for zero activity, a value of 450 kJ/(kg BW0.70 day) was used, which corresponds to the asymptotic heat production (at zero activity) during a 24-h fasting, as proposed by Noblet et al. (2015) with BW0.70 being metabolic body weight of the birds. Net energy was calculated as ME intake minus HI divided by feed consumed on an as-is basis.

2.4.2. Ileal and caecal pH

On d 21, 2 birds per pen were randomly selected and euthanized by cervical dislocation to measure ileum and caeca pH and collect ileum and caeca digesta for analysis of SCFA and microbiota concentration and dry matter and energy digestibility. Immediately post-euthanasia the ileum and caeca were removed intact, and a digital pH meter (Ecossan, Eutech Instruments, Singapore) with a spear tip piercing pH electrode (Sensorex S175CD) was directly inserted into the digesta in the lumen, whilst ensuring the pH electrode did not touch the intestinal wall, and pH was recorded. This was repeated 3 times, putting the probe in different areas of the section of tract each time. The probe was then rinsed with ultra-pure water.

2.4.3. Ileal and total tract digestibility

Following the pH measurements, the digesta was collected on an individual bird basis and was weighed. It was then freeze-dried and re-weighted to determine the dry matter content. The dried digesta was then ground into a fine powder, and the energy and TiO2 content was measured, using the same methods as described earlier for the diet and excreta analysis. Ileal dry matter digestibility and total tract dry matter digestibility was determined using the equation: Digestibility (%) = [1 – (TiO2 diet/TiO2 ileal digesta or excreta) x (DM ileal digesta or excreta/DM diet)] x 100. Ileal digestible energy was calculated by multiplying the percent digestibility by the diet energy content.

2.4.4. Ileal and caecal SCFA concentration

To determine the SCFA concentration in the ileal and caecal digesta, briefly, 1 mL of internal standard (0.01 mol/L ethylbutyric acid) was added to approximately 2 g of fresh homogenized digesta sample and the solution was then mixed and centrifuged at 38,625 x g at 5 °C for 20 min. Approximately 1 mL of the resulting supernatant, 0.5 mL of concentrated HCl and 2.5 mL of ether were then combined. An internal standard solution and a blank were also prepared using 1 mL of the standard acid mixture and 1 mL of water respectively in place of the supernatant. The mixture was then centrifuged at 2,000 x g at 5°C for 15 min and 400 mL of the resulting supernatant was combined with 40 mL of N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA). The samples were then heated at 80 °C for 20 min, left at room temperature for 48 h and were then analysed on a Varian CP3400 CX gas chromatograph (Varian Analytical Instruments, Palo Alto, CA, USA). Total SCFA concentration was derived as the sum of all the SCFA measured in the sample, expressed as μmol/g digesta.

2.4.5. Ileal and caecal microbiota

Immediately post-collection, approximately 1 g of digesta was snap-frozen in liquid nitrogen and stored at −20 °C for DNA extraction. PCR amplification of 16S ribosomal DNA was used to quantify the chromosomal DNA counts of the total microflora, Lactobacillus spp. and Enterobacteria spp. Template DNA samples were prepared from the digesta using Bioline Isolate II Plant DNA Kit (Bioline, Alexandria, NSW, Australia). For DNA preparation, approximately 200 mg of ileal digesta was accurately weighed and vigorously shaken with 0.2 g of 0.1 mm glass beads prior to the extraction step. For the caecal samples, 60 mg of digesta was processed by a Qiextractor automated DNA extractor robot (Qiagen, Australia). A NanoDrop ND-8000 UV spectrophotometer was used to assess the DNA purity in all the samples (Thermo Fisher Scientific, Waltham, USA). Only DNA elutions that emitted ratios of between 1.6 and 1.8 in 260/280 nm wavelength were used for PCR analysis. The quantitative PCR analysis was performed on a Rotorgene-6000 real-time PCR machine (Corbett, Sydney, Australia). Duplicate samples of 10 μL were used in each PCR reaction. Each sample was amplified in triplicate, with SensiMix SYBR No-ROX Kit (Bioline, Meridian Life Science, Memphis, USA) was used to amplify the 16S ribosomal DNA for analysis. A SensiMix SYBR No-ROX Kit was used to quantify the total bacteria, Enterobacteriaceae, and Lactobacilli. Species-specific
16 rRNA annealing primers were used as follows. Enterobacteriaceae F: 5'-CATTGACGTAACCCCAAGAAGC-3' and R: 5'-CTCTACGAGTACAGTT-3'. Lactobacillus spp. F: 5'-CACCCTACACAGTGAG-3' and R: 5'-AGGACTAGGGAACTTCCA-3' and total bacteria F: 5'-CCGGYCCAGCTTACCTACGG-3' and R: 5'-TTACCGGGCTGTGGCAC-3'. Serial dilutions of linearised plasmid DNA (PCR 4-TOPO Vector, Life Technologies, Carlsbad, USA) inserted with respective ampiclons were used to construct a standard curve. A threshold cycle average from the replicate samples was assigned for quantification analysis. The number of target DNA copies was calculated from the mass of the DNA, taking into account the size of the ampiclon insert in the plasmid. Bacteria numbers were expressed as \( \log_{10} \) (genomic DNA copy number)/g digesta.

2.5. Statistical analysis

All data were analysed using IBM SPSS statistics version 23. After Kolmogorov–Smirnov testing to confirm normality, one-way ANOVA was used to determine the equality of the means, with diet as the factor. Treatment means were separated using Tukey post-hoc test where appropriate. Statistical significance was declared at \( P < 0.05 \).

3. Results

3.1. Performance

The effect of diets containing AX, AXOS or AX + E on broiler performance from d 10 to 21 is illustrated in Table 3. Dietary treatment had no significant effect on bird performance.

3.2. Ileal and total tract digestibility

As illustrated in Table 4, dietary treatment had no significant effect on the dry matter content of the ileal digesta or excreta. Ileal dry matter digestibility was higher \( (P = 0.047) \) in birds fed AXOS than those fed AX. Ileal digestible energy and total tract dry matter digestibility was higher \( (P = 0.004 \) and \( P = 0.001, \) respectively) in birds fed AXOS than those fed AX or AX + E.

3.3. Net energy

The dietary treatment effects on energy balance and efficiency of energy utilisation are shown in Table 5. Metabolisable energy intake [kJ/(kg BW0.70 d)] was higher \( (P = 0.049) \) in birds fed AXOS than those fed AX. Retained nitrogen [g/(bird d)] was lower \( (P = 0.001) \) in birds fed AX than those fed AXOS or AX + E. Retained energy and NE intake [kJ/(kg BW0.70 d)] and net energy [kJ/(bird d)] presented a strong trend of being higher in birds fed AXOS or AX + E compared to those fed AX \( (P = 0.054, P = 0.057 \) and \( P = 0.056, \) respectively).

3.4. Ileal and caecal SCFA and microflora concentration

Dietary treatment had no significant effect on the pH or microbiota content of the ileum or caeca. Table 6 shows that total SCFA concentration, along with lactic and formic acid concentration, was higher in the ileum of birds fed the diets with AXOS than those fed AX \( (P = 0.011, P = 0.012 \) and \( P = 0.023, \) respectively). Table 7 shows that total SCFA, acetic, butyric and isovaleric acid concentrations were lower in the caeca of birds fed AX than those fed AXOS or AX + E \( (P = 0.001, P = 0.004, P = 0.016 \) and \( P = 0.008, \) respectively). Propionic acid concentration in the caeca was higher in birds fed AX + E than those fed AX \( (P = 0.050, \) and lactic acid concentration was higher in birds fed AXOS than those fed AX or AX + E \( (P = 0.005, \) Generally, microbiota content was numerically higher in birds fed AXOS and AX + E than those fed AX.

4. Discussion

Results from this study suggest that AXOS has the capacity to be an efficacious prebiotic in broiler diets, as highlighted by its positive effects on broiler performance, intestinal SCFA production and energy utilisation. An interesting observation from this study was that feeding AXOS prepared in vitro was generally more advantageous than feeding AX + E, particularly when observing ileal and total tract digestibility. This was probably because depolymerisation of NSP \( \text{in situ} \) is not instantaneous, hence AXOS generation in the gut via the use of enzymes is not as efficient as feeding AXOS directly.

The concept of using AXOS as a feed additive to reduce the reliance on in-feed antibiotics is noteworthy. However, in order to develop further in this research area, technologies will need to be developed that can amass large volumes of AXOS as it is highly costly to produce on a laboratory scale to conduct a feeding experiment as we have done here. Additionally, it may be even more profitable to produce specific sized AXOS \( \text{in situ} \), resulting in customized prebiotic activities in broiler diets. In order to do this, a deeper understanding of the gastrointestinal microbiota is required to determine the substrate requirements and hence tailor the prebiotic capabilities to reflect the specific function and activity of the microbiota.

Performance and energy utilisation was lower in birds fed the diet containing AX, presumably because more digestive and metabolic effort was required for the birds to utilise this diet, meaning it was less efficient at providing energy for maintenance and production. This may be partly because the weight and relative proportion of energetically active organs, such as the gastrointestinal tract and pancreas, was greater in birds fed this diet \( \text{Wu et al., 2004} \), which increased the total cost of maintenance. This was illustrated by Gao et al. \( \text{2008} \) who showed that supplementing a wheat-based diet with xylanase resulted in reduced relative weights of the duodenum, jejunum, colon and pancreas in 21-day-old broilers. Future analysis is therefore warranted in quantifying the additive effects of AXOS. Arabinoxyrans contributes towards heat production and this study showed that if AX is hydrolysed \( \text{in vitro} \) there was numerically reduced heat increment and total cost of maintenance. Apparent metabolizable energy systems are traditionally used to evaluate dietary energy utilisation in broilers, but this system does not take into consideration the efficiency of nutrient utilisation and partitioning into meat, waste (namely depot fat), losses of chemical energy in the solid, liquid and gaseous excreta and energy and chemical losses due to heat production during digestion and absorption \( \text{Swick et al., 2013} \). The NE:ME ratio presented in this study suggests that the net energy system may provide a more sensitive measure of energy utilisation compared to the ME system, but the low number of replicates used in this study means that this cannot be confirmed and requires further investigation. In order to improve energy utilization from

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**Table 3**

| Item                  | FL g | BWG g | FCR |
|-----------------------|------|-------|-----|
| AX                    | 1.01569 | 746.67 | 1.37 |
| AXOS                  | 967.00 | 766.43 | 1.26 |
| AX + E                | 987.55 | 760.36 | 1.30 |
| SEM                   | 11.52 | 4.77  | 0.02 |
| P-value               | 0.267 | 0.018 | 0.167 |

AX = arabinoxylan; AXOS = arabinoxylo-oligosaccharides; AX + E = AX + xylanase; FL = feed intake; BWG = body weight gain; FCR = feed conversion ratio.
Table 4
Effect of diets containing 2% AX, AXOS or AX + E on ileal and total tract digestibility in broilers from d 10 to 21.

| Item       | DM, % | Ileal digesta | Excreta | IDE, MJ/kg | Total tract DM digestibility, % |
|------------|-------|---------------|---------|------------|--------------------------------|
| AX         | 18.59 | 25.81         | 85.89b  | 17.16b     | 91.15b                         |
| AXOS       | 17.55 | 24.56         | 86.84a  | 17.34a     | 91.58a                         |
| AX + E     | 18.31 | 26.60         | 86.18ab | 17.06b     | 91.32b                         |
| SEM        | 0.25  | 0.48          | 0.23    | 0.08       | 0.10                           |
| P-value    | 0.169 | 0.313         | 0.047   | 0.004      | 0.001                          |

AX = arabinoxylan; AXOS = arabinoxyl-oligosaccharides; AX + E = AX + xylanase; IDE = ileal digestible energy.

*a,b Means within the same column with no common superscript differ significantly (P ≤ 0.05).

Table 5
Effect of diets containing 2% AX, AXOS or AX + E on energy utilization in broilers from d 10 to 21.

| Item       | AX    | AXOS   | AX + E | SEM    | P-value |
|------------|-------|--------|--------|--------|---------|
| Energy value (DM basis) |       |        |        |        |         |
| ME feed, kJ/kg | 13.51 | 13.48  | 13.47  | 0.15   | 0.995   |
| NE feed, kJ/kg | 10.50 | 10.88  | 10.63  | 0.16   | 0.662   |
| NE/ME       | 0.77  | 0.81   | 0.79   | 0.01   | 0.143   |
| Energy partition, kJ/(bird d) | 1.547 | 1.631  | 1.631  | 32.02  | 0.532   |
| NE     | 1.163 | 1.358  | 1.289  | 21.56  | 0.056   |
| HI      | 431   | 423    | 429    | 7.59   | 0.410   |
| Energy/nitrogen balance, kJ/(kg BW0.70 d) | 1.497 | 1.682a | 1.631b | 32.03  | 0.049   |
| ME intake | 1.090 | 1.240  | 1.186  | 25.92  | 0.057   |
| NE      | 814   | 817    | 831    | 3.55   | 0.122   |
| HI      | 499   | 402    | 413    | 4.59   | 0.081   |
| Retained N, g/(bird d) | 2.50b | 3.06b  | 3.46b  | 0.12   | 0.001   |
| RQ       | 1.03  | 1.02   | 1.02   | 0.79   | 0.363   |

AX = arabinoxylan; AXOS = arabinoxyl-oligosaccharides; AX + E = AX + xylanase; ME = metabolisable energy; NE = net energy; HI = heat increment; HP = heat production; RE = retained energy; RQ = respiratory quotient.

*a,b Means within the same column with no common superscript differ significantly (P ≤ 0.05).

Table 6
Effect of diets containing 2% AX, AXOS or AX + E on pH, SCFA concentration and log10 DNA enumeration of gut bacteria using 16S rDNA qPCR quantification in the ileum of broilers at d 21.

| Item       | pH    | SCFA, μmol/g | Microbiota, log10 counts/g digesta | Microbiota, log10 counts/g digesta |
|------------|-------|--------------|-----------------------------------|-----------------------------------|
|            |       | Total        | Lactic acid                       | Formic acid                       | Total anaerobic | Lactobacillus | Enterobacteriaceae |
| AX         | 6.64  | 21.25b       | 18.61b                            | 0.51b                            | 9.61           | 8.15          | 6.01               |
| AXOS       | 6.46  | 49.18a       | 43.58a                           | 1.42a                           | 9.08           | 8.47          | 6.14               |
| AX + E     | 6.53  | 38.79a       | 35.59a                           | 0.70a                           | 10.03          | 8.51          | 6.19               |
| SEM        | 0.04  | 6.65         | 6.01                             | 0.23                            | 0.11           | 0.09          | 0.04               |
| P-value    | 0.831 | 0.011        | 0.012                            | 0.023                           | 0.088          | 0.375         | 0.499              |

AX = arabinoxylan; AXOS = arabinoxyl-oligosaccharides; SCFA = short chain fatty acids.

*a,b Means within the same column with no common superscript differ significantly (P ≤ 0.05).

Table 7
Effect of diets containing 2% AX, AXOS or AX + E on pH, SCFA concentration and log10 DNA enumeration of gut bacteria using 16S rDNA qPCR quantification in the caeca of broilers at d 21.

| Item       | pH    | SCFA, μmol/g | Microbiota, log10 counts/g digesta | Microbiota, log10 counts/g digesta |
|------------|-------|--------------|-----------------------------------|-----------------------------------|
|            |       | Total        | Acetic acid                       | Propionic acid                    | Butyric acid     | Isovaleric acid | Lactic acid                   | Total anaerobic | Lactobacillus | Enterobacteriaceae |
| AX         | 6.29  | 46.42b       | 30.88b                           | 1.79b                            | 11.47b           | 0.05b          | 0.21b                          | 10.51          | 8.69          | 7.70               |
| AXOS       | 6.21  | 100.75b      | 65.47b                           | 5.05b                            | 22.48b           | 0.21b          | 0.66b                          | 10.53          | 8.90          | 7.80               |
| AX + E     | 6.27  | 105.28a      | 67.87a                           | 7.51a                            | 24.39a           | 0.19a          | 0.30a                          | 10.61          | 8.83          | 7.92               |
| SEM        | 0.02  | 15.44        | 9.76                             | 1.35                             | 3.29             | 0.04           | 0.11                           | 0.02           | 0.05          | 0.05               |
| P-value    | 0.869 | 0.001        | 0.004                            | 0.050                            | 0.016            | 0.008          | 0.005                          | 0.411          | 0.290         | 0.881              |

AX = arabinoxylan; AXOS = arabinoxyl-oligosaccharides; SCFA = short chain fatty acids.

*a,b Means within the same column with no common superscript differ significantly (P ≤ 0.05).
in cells is particularly integral in energy production and is vital for ATP production and biosynthesis of long chain fatty acid, as well as playing a role in improving growth performance and increasing intestinal epithelial cell division and villus height, width and area (Hudha et al., 2010). According to Cuche et al. (2000), fermentation of oligosaccharides into SCFA potentially triggers a neuro-hormonal response, through stimulating peptide YY, resulting in delayed gastric emptying and duodenal transit time rates and hence heightened diet digestion and nutrient absorption in the small intestine. Findings from this study suggest that microbial metabolites such SCFA have the potential to be indicators of generation and prevalence of fermentative oligosaccharides and could hence be used to measure the effects of xylanase on nutrient digestibility and retention.

Microbiota hydrolyse indigestible carbohydrates into oligosaccharides and then into monosaccharides, which they then ferment in the anaerobic environment of the gut. Arabinono-xylo-oligosaccharides selectively stimulate beneficial bacteria, namely Bifidobacteria, and non-digestible carbohydrates act as the main source of energy during microbial proliferation in the hindgut (Makelainen et al., 2010a, b). The impact of diet on microbiota was not significant in this study, likely due to the low number of replicates. Ileal and caecal lactic acid concentration was higher in birds fed AXOS and AX + E than those fed AX. Lactobacilli readily ferment AXOS into lactic acid which lowers pH, but in this study there was a lack of significant effect of dietary treatment on pH, which may be partly because the lactic acid was absorbed in the intestine or used as a substrate for lactate-utilizing bacteria. The SCFA produced by Lactobacilli can also directly stop harmful bacteria from reproducing, by acting as bacteriostatic agents, producing bacteriocins with microbicidal or microbiostatic properties and modifying the receptors used by pathogenic bacteria, which increases resistance against pathogenic microbes (Adil and Magray, 2012). Also, propionic and formic acid, which were stimulated by AXOS and AX + E, have high bacteriostatic properties due to their pH reduction activity both in feed and in the gastrointestinal tract, through pharmacological actions on microflora (Haque et al., 2009; Hernández et al., 2005). Reduced digesta viscosity in birds fed AXOS and AX + E likely increased the rate of digesta passage and hence stomach emptying decreased fermentation of the pathogenic microbial populations, enabling the beneficial bacteria to flourish. Bacteria compete with the host for nutrients within the tract, elicitting an immune response that can dictate appetite, muscle catabolism, disease prevalence and nutrient absorption (Bedford, 2000). It is important therefore to promote the growth of bacteria that can provide nutrients for the host and reduce the growth of bacteria that are detrimental to the host. Findings from this study suggest there is potential to use AXOS to aid and control this, but further investigation in this research area is required.

5. Conclusion

In conclusion, AXOS appear to be efficacious prebiotics that have positive effects on net utilization of dietary energy and bird performance. This appears to be largely due to the ability of AXOS to stimulate beneficial bacteria and SCFA production. In this study, it was found feeding broilers with AXOS that had been prepared in vitro was more effective than in situ AXOS production, particularly with regards to enhancing diet digestibility, high-lighting the potential for using AXOS as a feed additive in the future. Further studies are required to examine the effect of the source and structure of AXOS on their effects in broilers, and to investigate the response and impact of AXOS in different environmental conditions, namely focusing on pH, transit time and microflora composition.

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