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Original Research Article

Improving sorghum digestion in broilers by targeting fermentation of xylan

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ABSTRACT

This study was to examine if it is possible to accelerate sorghum digestion in broiler chickens by targeting fermentation of the xylan. Cobb 500 broilers ($n = 960$, 80 birds per treatment) were fed 12 sorghum-soybean meal-based dietary treatments fed as 3 phases (starter d 0 to 12, grower d 13 to 23, finisher d 24 to 35), with 8 replicate pens of 10 birds per treatment. For half of the treatments ($n = 6$), 10% of the sorghum in the diet was directly replaced with 10% wheat bran, as a source of fermentable fibre. The diets were supplemented with either 0, 50 or 2,000 mg/kg xylo-oligosaccharides (XOS), with or without xylanase application. Body weight gain (BWG), feed intake (FI) and feed conversion corrected for mortality (cFCR) was determined at d 0 to 35, and male and female body weight were measured on d 35. On d 35, ileum and caeca samples were collected from 2 birds per pen, for determination of caecal cellulase and xylanase activity, microbiota composition and short chain fatty acid (SCFA) concentration, and ileal XOS concentration. Supplementation with 2,000 mg/kg XOS caused increased BWG at d 0 to 35 ($P = 0.007$) and enhanced caecal propionic, valeric and succinic acid concentration ($P < 0.05$). Wheat bran increased FI ($P = 0.018$) and BWG ($P = 0.016$), as well as caecal *Bifidobacteria* concentration ($P < 0.001$). Ileal XOS concentration was greatest when feeding combined wheat bran, 2,000 mg/kg XOS, and xylanase, resulting in increased caecal total SCFA, acetic acid and butyric acid concentration, and xylanase and cellulase activity ($P < 0.05$). Results from this study present that feed efficiency in birds fed sorghum-based diets is improved as a consequence of supplementing with fermentable fibre, xylanase and XOS.

1. Introduction

There is continuing interest in using sorghum as an alternative to wheat in poultry diets, particularly in countries such as Australia where the cost of wheat is high. However, there are concerns around the lower digestibility of sorghum compared to wheat in broiler diets, resulting in suboptimal broiler performance. Despite these concerns, there is evidence of broilers offered sorghum-based diets performing equally or better than their counterparts fed wheat, in terms of body weight gain (BWG) and feed conversion ratio (FCR) (Liu et al., 2015; Torok et al., 2011; Selle et al., 2010; Ao and Choct, 2004). This suggests that there is potential to use sorghum more in the poultry industry, but consistency in response of broilers to sorghum-based diets must first be achieved.

Sorghum has a low non-starch polysaccharide (NSP) content; approximately 0.2% to 0.9% soluble NSP and 4.2% to 6.1% insoluble NSP (Bach Knudsen, 2014), primarily in the form of arabinoxylans. Accordingly, the response to NSP-degrading enzymes in sorghum-based diets tends to be low, and the outputs from studies testing these enzymes are usually equivocal. For example, Shakouri et al. (2009) observed that supplementing sorghum-based diets with

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xylanase and β-glucanase increased anaerobic bacteria and *Lactobacilli* concentration in the gizzard but had no effect on their levels in the ileum or caeca, or impact on performance. Moreover, Cadogan et al. (2005) observed increased weight gain from d 0 to 21 because of supplementing protease, amylase, and xylanase in sorghum-based diets, but these enzyme effects were not evident at d 42. Similarly, Liu et al. (2014) observed increased apparent digestibility coefficients of starch in the proximal ileum and nitrogen in the distal jejunum and proximal ileum as a consequence of protease, amylase and xylanase application, but this did not translate into improvements in growth performance or overall nutrient utilisation. This highlights that in some cases it may be economically beneficial to apply NSP-degrading enzymes to sorghum-based diets, but sometimes it is not, making it difficult for poultry nutritionists to expect consistent performance from xylanase supplemented sorghum-based diets.

Traditionally NSP has been considered as an anti-nutrient, in that it increases digesta viscosity and acts as a diluent and physical barrier to enzymes, reducing nutrient utilisation and thus growth performance and litter quality. However, recent research has highlighted that moderate levels of dietary NSP improve gastrointestinal organ development (González-Alvarado et al., 2007; Hetland et al., 2005) and secretion of enzymes, HCl and bile acids (Hetland et al., 2003). The consequence of this is improved nutrient digestibility (Amerah et al., 2009; Jiménez-Moreno et al., 2009), growth performance (González-Alvarado et al., 2010) and gastrointestinal health (Kalmendal et al., 2011; Montagne et al., 2003). Arabinoxylans are of particular interest, given their high concentration in feed ingredients commonly fed to poultry. Oligosaccharides derived from depolymerisation of NSP display prebiotic properties, in that they are selectively fermented by beneficial bacteria, resulting in reduced ability of pathogenic bacteria to proliferate and production of short chain fatty acids (SCFA), which act as an energy source (Bao and Choct, 2010; Shakouri et al., 2006; Yadav and Jha, 2019). Of particular interest are xylo-oligosaccharides (XOS), mannan-oligosaccharides, fructo-oligosaccharides and galacto-oligosaccharides, and the polymers they are derived from. Thus, there is potential for dietary NSP to be exploited as a gut health stimulator. This was illustrated by Jacobs and Parson (2013), who saw improvements in FCR as a consequence of supplementing sorghum-based diets with distillers-dried grains. The hypothesis behind this is that providing a rich source of fermentable NSP to an NSP-poor diet will increase the fermentation capacity of the microbiota, resulting in increased production of microbial enzymes, thus enhancing the ability of the bird to utilize dietary NSP. In view of this, it is possible that response to xylanase in sorghum-based diets could be improved if these diets were supplemented with a source of fermentable xylan substrates, through increasing fermentation of the arabinoxylan in the endosperm cell walls of sorghum, thus increasing nutrient release. Xylanase itself also breaks down long chain xylans into shorter chain oligomers, which can be utilised by gastrointestinal bacteria.

Xylanases cleave the internal β-xylosidic glycosidic linkages of xylan into XOS, which have been proven to induce positive prebiotic effects in broilers, such as optimizing colon function, increasing SCFA production, increasing mineral absorption and energy utilisation, immune stimulation and increased ileal villus length (Jommuengbout et al., 2009; Kim et al., 2011; Morgan et al., 2018). Of primary interest is the ability of XOS to stimulate xylan digestion and heighten development of a fibre-fermenting microbiome, particularly in younger birds, thus enhancing the ability of adult birds to effectively utilize dietary fibre (Bautil et al., 2020). Additionally, the beneficial technological features of XOS include stability at acidic pH, heat resistance, ability to achieve significant biological effects at low daily doses, low calorie content and no toxicity (Carvalho et al., 2013). Thus, there is currently heightened interest in supplementing poultry diets directly with XOS, as opposed to relying on in situ production because of xylanase application, or as a complementary supplement with xylanase, to increase the availability of XOS as a fuel for beneficial microbiota. Accordingly, the aim of this study was to examine if it is possible to accelerate sorghum NSP digestion by establishing xylan-degrading bacteria in the bird's microbiota, by supplementing sorghum-based diets with XOS, xylanase and wheat bran as a source of fermentable xylan.

2. Materials and methods

2.1. Experimental design

Cobb 500 mixed sex broilers ($n = 960 + 10$ spare birds) were obtained from a commercial hatchery at day of hatch. The chicks were randomised by weight and placed in 1.07 m^2 floor pens, 96 pens of 10 or 11 birds per pen, 80 birds per treatment, bedded on clean wood shavings. Pen allocation was randomised across the room. 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. Temperature settings followed Cobb 500 recommendations of 33 to 34 °C on arrival, followed by a gradual decrease by approximately 0.5 °C daily until a temperature of 21 to 22 °C was reached 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. 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 Animal Ethics Committee at University of New England, New South Wale, Australia.

2.2. Dietary treatments

There were 12 sorghum-based dietary treatments, fed as 3 phases (starter d 0 to 12, grower d 13 to 23, finisher d 24 to 35). The formulation for the control sorghum diet is presented in Table 1. For half of the treatments ($n = 6$), 10% of the sorghum in the diet was directly replaced with 10% wheat bran, as a source of fermentable

Table 1
Composition of sorghum-based control diets (%, as is).

Item	Starter	Grower	Finisher
Sorghum (11% CP)	60.83	67.21	69.24
Soybean meal (48% CP)	33.17	27.11	24.68
Canola oil	1.95	2.06	2.79
Limestone	1.14	1.09	1.02
Dicalcium phosphate (18P/21Ca)	0.97	0.86	0.72
Salt	0.20	0.21	0.19
Sodium bicarbonate	0.23	0.14	0.10
Choline chloride	0.08	0.08	0.07
Titanium dioxide	0.50	0.50	0.50
L-Lysine	0.31	0.27	0.24
DL-Methionine	0.30	0.24	0.22
L-Threonine	0.08	0.04	0.04
L-Valine	0.04	0.00	0.00
Quantum blue 5G (100 g)	0.01	0.01	0.01
Vitamin premix[1]	0.09	0.08	0.08
Mineral premix[2]	0.11	0.10	0.10

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

fibre. The diets were supplemented with or without xylanase (16,000 BXU Econase XT 5P, AB Vista, Marlborough, UK) and XOS (AB Vista, Marlborough, UK) at either 0, 50 or 2,000 mg/kg. Birds had ad libitum access to the treatment diets and water for the duration of the trial.

Prior to diet formulation, representative subsamples of the sorghum and soybean meal were analysed by near-infrared spectroscopy to predict proximate analysis, AA concentration, and AME using AMINONIR PROX, AMINONIR NIR, and AMINONIR NRG (Evonik Nutrition & Care, Hanua, DE), respectively. All dietary treatments contained the same batch of all ingredients, including the wheat bran. The sorghum was red sorghum, ground by a roller mill to approximately 3.5 mm. The analysed composition of the diets is presented in Tables 2–4. Starter diet was fed as crumble from d 0 to 7 and as pellets from d 8 to 12, and grower and finisher diet were fed as pellets. Pellets were cold-pelleted (65 °C).

Protein content of the dietary treatments was determined by measuring nitrogen using the combustion method (LECO Corp., St. Joeseph, MI, USA), using ethylenediaminetetraacetic acid (EDTA) as a calibration standard and multiplying the nitrogen value by a factor of 6.25. Diet gross energy content was determined using an adiabatic bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA), standardised with benzoic acid. Extractable fat content was analysed by the Soxhlet method (AOAC 2003.05), and dry matter was analysed by oven-drying at 105 °C to constant weight (AOAC 930.15). The constituent sugar components of the dietary NSP were determined by gas chromatography (Model CP3800, Varian Inc., Palo Alto, CA, USA) as alditol acetates, following the procedure of Englyst et al. (1994) with some modifications as described by Theander et al. (1995) and Morgan et al. (2018). The wheat bran contained 137 g/kg insoluble NSP and 20 g/kg soluble NSP; approximately 96% of the insoluble NSP and 76% of the soluble NSP was arabinoxylan. The sorghum contained 47 g/kg insoluble NSP and 4 g/kg soluble NSP; approximately 61% of the insoluble NSP and 21% of the soluble NSP was arabinoxylan.

2.3. Response variables

2.3.1. Bird performance

Starting total pen weight and mean chick body weight (BW) were determined, ensuring there was no significant difference in starting BW across dietary treatments. On arrival and on d 35 birds and feed were weighed per pen, to determine BWG and feed intake (FI). Number of mortalities and weights of dead birds was recorded daily, and feed conversion ratio corrected for mortality was calculated from d 0 to 35. On d 35, the number of males and females per pen were counted, and percentage males per pen was used as a co-variate during statistical analysis of the performance data. At d 35, 2 birds per pen (one male and one female) were euthanised by cervical dislocation and ileum and caeca digesta samples were collected and pooled per pen.

2.3.2. Caecal SCFA concentration

To determine SCFA concentration in the caecal digesta, 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 × *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 × *g* at 5 °C for 15 min and 400 mL of the resulting supernatant was combined with 40 mL of N-tert-butyldi-methlsilyl-N-methyltrifuoroacetamide (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 mmol/g digesta.

2.3.3. Caecal microbiota concentration

Analysis of microbiota composition was determined in duplicate in the d 35 cecal digesta samples. DNA extraction from the samples was performed using an Isolate II Plant DNA Kit (Bioline, Alexandria, NSW, Australia) and QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) with slight modification, as described by Keerqin et al. (2017) and Kheravii et al. (2017). The purity of the extracted DNA was assessed by a Nano-Drop ND-8000 UV spectrophotometer (Thermo Fisher Scientific, Waltham, USA). Only DNA elution that emitted ratios from 1.8 and above at a wavelength of 260/280 nm were used for PCR analysis. Following a 20 × dilution with sterilized water, the extracted DNA was analysed for total anaerobic bacteria, *Bacillus* spp., *Bacteriodes* spp., *Bifidobacterium* spp., *Ruminococcus* spp., *Lactobacillus* spp., and *Enterobacteria* spp. by quantitative real-time PCR analysis using a Rotorgene 6500 real-time PCR machine, and quantification was determined using Rotorgene 6000 series software 1.7 (Corbett, Sydney, Australia). A threshold cycle averaged from the duplicate samples was used for quantification analysis. The number of target DNA copies was calculated using a standard curve constructed with plasmid DNA cloned with the amplicons. Copy numbers of plasmid DNA were calculated according to its mass, taking into account the size of the plasmid with amplicon insert. The resulting values were expressed as log10 (genomic DNA copy number)/g digesta. The species-specific 16 rRNA primers utilised are described in detail by Kheravii et al. (2017).

Table 2
Analysed nutrient composition of starter treatments (d 0 to 12).

Item	Starter treatments											
Wheat bran, %	0	0	0	0	0	0	10	10	10	10	10	10
Xylanase, BXU/kg	0	0	0	16,000	16,000	16,000	0	0	0	16,000	16,000	16,000
XOS, mg/kg	0	50	2,000	0	50	2,000	0	50	2,000	0	50	2,000
Analysed nutrient composition												
Dry matter, %	86.24	88.06	88.03	87.80	87.99	88.27	87.65	88.10	88.21	88.56	88.16	88.02
Protein, %	22.46	21.99	22.40	21.87	22.71	22.47	22.31	23.21	21.83	22.40	22.77	22.49
Fat, %	4.58	4.48	4.51	4.53	4.45	4.52	4.03	3.98	4.24	3.93	4.20	4.21
Energy, MJ/kg	16.11	16.14	16.34	16.21	16.35	16.45	16.24	16.45	16.48	16.50	16.42	16.49
Soluble NSP, g/kg	10.35	12.06	13.15	11.58	12.59	14.36	15.39	16.28	16.72	16.09	15.95	16.62
Insoluble NSP, g/kg	63.84	65.44	67.92	68.72	69.02	69.83	88.06	88.99	88.20	87.22	86.13	84.22
Free OS, g/kg	43.45	44.16	47.72	44.44	45.79	47.97	49.26	50.13	51.70	48.31	49.59	50.26
Xylanase, BXU/kg	<2,000	<2,000	<2,000	14,300	13,300	13,500	<2,000	<2,000	<2,000	16,000	15,700	13,700

XOS = xylo-oligosaccharides; NSP = non-starch polysaccharide; OS = oligosaccharides.

Table 3
Analysed nutrient composition of grower treatments (d 13 to 23).

Item	Grower treatments											
Wheat bran, %	0	0	0	0	0	0	10	10	10	10	10	10
Xylanase, BXU/kg	0	0	0	16,000	16,000	16,000	0	0	0	16,000	16,000	16,000
XOS, mg/kg	0	50	2,000	0	50	2,000	0	50	2,000	0	50	2,000
Analysed nutrient composition												
Dry matter, %	89.48	89.51	89.28	89.36	89.48	89.24	89.29	89.74	89.71	89.40	89.55	89.12
Protein, %	19.61	19.92	19.82	19.76	19.58	19.92	20.13	20.12	20.39	20.66	20.31	20.45
Fat, %	4.78	4.87	4.83	4.93	4.79	4.81	4.46	4.46	4.48	4.58	4.49	4.51
Energy, MJ/kg	16.61	16.75	16.85	16.76	16.71	16.43	16.67	16.82	16.53	16.79	16.79	16.19
Soluble NSP, g/kg	13.16	16.37	15.32	13.52	14.13	12.46	15.08	13.96	14.19	17.77	14.76	15.13
Insoluble NSP, g/kg	60.16	62.62	56.61	56.70	54.25	50.94	70.48	72.79	76.46	77.36	75.07	72.79
Free OS, g/kg	38.26	39.76	39.40	39.63	39.93	36.88	44.30	44.00	44.74	45.36	44.10	44.77
Xylanase, BXU/kg	<2,000	<2,000	<2,000	16,200	14,000	14,900	<2,000	<2,000	<2,000	15,700	15,900	14,000

XOS = xylo-oligosaccharides; NSP = non-starch polysaccharide; OS = oligosaccharides.

Table 4
Analysed nutrient composition of finisher treatments (d 24 to 35).

Item	Finisher treatments											
Wheat bran, %	0	0	0	0	0	0	10	10	10	10	10	10
Xylanase, BXU/kg	0	0	0	16,000	16,000	16,000	0	0	0	16,000	16,000	16,000
XOS, mg/kg	0	50	2,000	0	50	2,000	0	50	2,000	0	50	2,000
Analysed nutrient composition												
Dry matter, %	88.71	89.17	87.98	88.65	88.58	86.54	87.57	88.18	86.40	87.70	87.81	86.66
Protein, %	18.66	19.13	18.50	18.56	18.79	18.60	19.22	19.21	18.87	19.37	19.37	19.12
Fat, %	5.43	5.41	5.31	5.37	5.28	5.44	4.86	4.83	4.86	4.95	4.93	4.85
Energy, MJ/kg	16.53	16.90	16.57	16.79	16.73	16.48	16.55	16.69	16.51	16.60	16.62	16.46
Soluble NSP, g/kg	12.57	13.21	12.89	12.24	13.54	12.43	12.39	15.34	14.29	13.91	14.05	12.21
Insoluble NSP, g/kg	52.55	60.68	56.45	52.79	52.21	51.33	60.81	64.99	58.72	64.33	63.03	57.45
Free OS, g/kg	37.72	36.27	36.39	36.20	37.02	36.65	35.98	36.38	27.48	38.51	38.24	38.75
Xylanase, BXU/kg	<2,000	<2,000	<2,000	14,200	12,700	15,800	<2,000	<2,000	<2,000	15,600	14,800	14,700
Titanium dioxide, %	0.57	0.51	0.53	0.54	0.50	0.54	0.54	0.51	0.54	0.55	0.53	0.56

XOS = xylo-oligosaccharides; NSP = non-starch polysaccharide; OS = oligosaccharides.

2.3.4. Ileal XOS concentration

TiO_2 marker was quantified in the feed and ileum digesta by UV-spectroscopy at 410 nm (Cary 50 Bio UV–Visible spectrophotometer equipped with a Cary 50 MPR microplate reader, Varian Inc., Palo Alto, CA), as illustrated by Short et al. (1996). The single sugars arabinose and xylose, and xylo-oligosaccharides xylobiose (X_2), xylotriose (X_3), xylotetraose (X_4), xylopentaose (X_5) and xylohexaose (X_6) were extracted from the samples using a multi-step solid phase extraction. Extracted XOS were derivatised using 1-phenyl-3-methyl-5-pyrazolone (PMP). Analysis of the PMP-XOS was carried out on an Agilent Single Quad LCMS equipped with Agilent ZORBAX SB-C18 column (3.0 mm × 150 mm, 1.8 μm) and separated using mobile phases A: 0.1% formic acid in H_2O, and B: 0.1% formic acid in acetonitrile, as described by Morgan et al. (2020). The quantity of each XOS fraction was then calculated as mg/g of TiO_2 marker.

2.3.5. Caecal xylanase and cellulase activity

Xylanase concentration in the caeca and diets was analysed by Megazyme endo-xylanase assay kit (K-XylX6), and cellulase activity in the caeca was analysed by Megazyme endo-cellulase kit (K-CELLG3) (Megazyme, Wicklow, Ireland, UK), using a UV-spectroscopy at 510 nm (Cary 50 Bio UV–Visible spectrophotometer equipped with a Cary 50 MPR microplate reader, Varian Inc., Palo Alto, CA).

2.4. Statistical analysis

All data were analysed using IBM SPSS statistics version 25. Pen represented the replicate unit for statistical analysis. After Kolmogorov–Smirnov testing to confirm normality, univariate analysis was used to evaluate the contribution of wheat bran, xylanase and XOS in the dietary treatments on the measured parameter. The percentage of male birds per pen was applied as a co-variate. Treatment means were separated using Tukey post-hoc test where appropriate. Statistical significance was declared at $P < 0.05$.

3. Results

3.1. Bird performance

Table 5 illustrates the impact of the dietary treatments on individual bird performance at d 0 to 35 and male and female BW at d 35. It shows that feeding wheat bran increased FI and BWG ($P = 0.018$ and $P = 0.016$, respectively), but this did not translate into an effect on cFCR. Additionally, feeding 2,000 mg/kg XOS resulted in higher BWG at d 0 to 35 compared to feeding 0 or 50 mg/kg XOS ($P = 0.007$). An interaction between wheat bran, xylanase and XOS was observed on male BW ($P = 0.012$). This showed that when wheat bran was absent, feeding xylanase without XOS resulted in lower male BW compared to feeding 0 or 50 mg/kg XOS without xylanase. When wheat bran was present, feeding 2,000 mg/kg XOS and xylanase resulted in higher male BW compared to feeding 0 or 50 mg/kg XOS without xylanase. An interaction between wheat bran and xylanase was observed on female BW ($P = 0.006$). This showed that in the presence of wheat bran, supplementation with xylanase increased female BW, but in the absence of wheat bran, xylanase had no impact on female BW. There was no significant effect of the dietary treatments on mortality percentage ($P > 0.05$, data not shown).

3.2. Caecal SCFA and microbiota concentration

Table 6 presents the effect of the dietary treatments on SCFA concentration in the caeca. An interaction between wheat bran and XOS was observed on caeca total SCFA and acetic acid concentration ($P = 0.003$ and $P = 0.002$, respectively). This showed that when wheat

Table 5
Effect of wheat bran, xylanase, and xylo-oligosaccharides (XOS) in sorghum-based diets on individual feed intake (FI), body weight gain (BWG) and feed conversion ratio corrected for mortality (cFCR) at d 0 to 35, and BW in male and female broilers at d 35.

Wheat bran, %	Xylanase, BXU/kg	XOS, mg/kg	FI, g	BWG, g	cFCR	Male BW, g	Female BW, g
0	0	0	3,708	2,377	1.56	2,840[a]	2,318
0	0	50	3,647	2,324	1.57	2,814[ab]	2,281
0	0	2,000	3,601	2,346	1.53	2,718[abc]	2,410
0	16,000	0	3,532	2,317	1.53	2,594[c]	2,273
0	16,000	50	3,515	2,283	1.54	2,741[abc]	2,262
0	16,000	2,000	3,729	2,396	1.56	2,655[abc]	2,293
10	0	0	3,660	2,321	1.58	2,607[bc]	2,117
10	0	50	3,666	2,372	1.55	2,779[bc]	2,270
10	0	2,000	3,773	2,482	1.52	2,812[ab]	2,339
10	16,000	0	3,661	2,336	1.57	2,811[ab]	2,392
10	16,000	50	3,697	2,409	1.54	2,737[abc]	2,393
10	16,000	2,000	3,797	2,479	1.53	2,835[a]	2,422
SEM			19.82	13.39	0.01	17.61	19.99
0	0						2,336[ab]
0	16,000						2,276[ab]
10	0						2,242[b]
10	16,000						2,402[a]
0			3,622[b]	2,341[b]			
10			3,709[a]	2,400[a]			
		0		2,338[b]			
		50		2,345[b]			
		2,000		2,428[a]			
P-value							
Wheat bran	0.018	0.016	0.426	0.270	0.059		
Xylanase	0.589	0.993	0.426	0.324	0.824		
XOS	0.069	0.007	0.224	0.474	0.638		
Wheat bran × Xylanase	0.374	0.580	0.638	0.005	0.006		
Wheat bran × XOS	0.620	0.078	0.133	0.166	0.137		
Xylanase × XOS	0.238	0.797	0.280	0.834	0.777		
Wheat bran × Xylanase × XOS	0.225	0.454	0.776	0.012	0.778		

[a-c] Means within the same column, within the same parameter, with no common subscript, differ significantly ($P < 0.05$).

Table 6
Effect of wheat bran, xylanase, and xylo-oligosaccharides (XOS) in sorghum-based diets on short chain fatty acid (SCFA) and microbiota concentration in the caeca in broilers at d 35.

Wheat bran, %	Xylanase, BXU/kg	XOS, mg/kg	Total	Acetic	Propionic	Butyric	Valeric	Succinic
0	0	0	51.22	34.88	3.99	8.92	0.60	1.09
0	0	50	79.44	53.00	6.15	15.00	0.93	2.12
0	0	2,000	64.38	42.65	5.24	11.26	0.74	2.73
0	16,000	0	69.03	47.42	5.52	10.72	0.74	2.46
0	16,000	50	72.30	48.55	5.39	13.04	0.74	2.49
0	16,000	2,000	73.51	48.25	5.27	14.88	0.84	2.46
10	0	0	57.83	39.66	4.79	8.73	0.63	1.93
10	0	50	61.77	40.07	4.42	12.49	0.73	1.73
10	0	2,000	76.33	49.72	5.57	15.07	0.82	2.69
10	16,000	0	54.23	37.37	3.77	8.60	0.54	1.79
10	16,000	50	63.14	42.52	4.87	11.22	0.66	1.80
10	16,000	2,000	97.90	66.64	6.04	17.81	0.94	4.26
SEM			2.14	1.44	0.16	0.51	0.02	0.18
0		0	60.13[b]	41.15[b]		9.82[ab]		
0		50	75.87[ab]	50.78[ab]		14.02[ab]		
0		2,000	68.95[b]	45.45[b]		13.07[ab]		
10		0	56.03[b]	38.52[b]		8.67[b]		
10		50	62.46[b]	41.30[b]		11.86[ab]		
10		2,000	87.12[a]	58.18[a]		16.44[a]		
		0			4.47[b]		0.62[b]	1.79[b]
		50			5.27[ab]		0.77[a]	2.06[ab]
		2,000			5.30[a]		0.83[a]	3.03[a]
P-value								
Wheat bran			0.953	0.936	0.279	0.983	0.369	0.704
Xylanase			0.084	0.044	0.715	0.384	0.980	0.184
XOS			<0.001	0.001	0.036	<0.001	0.003	0.019
Wheat bran × Xylanase			0.984	0.822	0.651	0.700	0.744	0.988
Wheat bran × XOS			0.003	0.002	0.103	0.034	0.127	0.288
Xylanase × XOS			0.139	0.140	0.842	0.107	0.123	0.872
Wheat bran × Xylanase × XOS			0.135	0.081	0.051	0.846	0.321	0.171

[a,b] Means within the same column, within the same parameter, with no common subscript, differ significantly ($P < 0.05$).

bran was present, feeding 2,000 mg/kg XOS resulted in higher total SCFA and acetic acid concentration in the caeca compared to feeding 0 or 50 mg/kg XOS. Application of XOS had no impact on caeca total SCFA or acetic acid concentration in the absence of wheat bran. Similarly, an interaction between wheat bran and XOS was observed on caecal butyric acid concentration ($P = 0.034$). This interaction showed that in the presence of wheat bran feeding 2,000 mg/kg XOS resulted in higher butyric acid concentration compared to feeding no XOS, but no effects of XOS were seen when wheat bran was absent. Propionic and succinic acid concentration in the caeca was higher in birds fed 2,000 mg/kg XOS compared to those fed 0 mg/kg XOS ($P = 0.036$, and $P = 0.019$, respectively). Caecal valeric acid concentration was higher in birds fed 2,000 mg/kg XOS compared to those fed 0 or 50 mg/kg XOS ($P = 0.003$). The dietary treatments had no impact on concentration of formic, isobutyric, isovaleric or lactic acid.

Table 7 presents the effects of the dietary treatments on and *Enterococcus* and *Bifidobacteria* abundance in the caeca. The dietary treatments had no effects on concentration of total anerobic bacteria, *Lactobacillus*, *Bacillus, Bacteroide* or *Ruminococcus* in the caeca ($P > 0.05$, data not shown), thus only data on *Enterococcus* and *Bifidobacteria* are presented. An interaction between wheat bran and xylanase was seen on caecal *Enterococcus* concentration ($P = 0.009$), showing that when wheat bran was present, xylanase caused *Enterococcus* concentration to reduce, and when wheat bran was absent, with xylanase inducing increased *Enterococcus* concentration. *Bifidobacteria* concentration in the caeca was increased by feeding wheat bran ($P < 0.001$).

3.3. Ileal XOS concentration

Table 8 presents the concentration of XOS, xylose and arabinose in the diets and ileum digesta. Interactions among wheat bran, xylanase and XOS were observed on ileal xylose, X_2, X_3 and X_4 concentration ($P < 0.001$, $P < 0.001$, $P = 0.001$ and $P = 0.016$, respectively). Xylose concentration was consistently higher when feeding 2,000 mg/kg XOS compared to no XOS, irrespective of xylanase or wheat bran presence. When birds were fed both xylanase and wheat bran, supplementation with 50 mg/kg XOS increased ileal xylose concentration, but this was not the case when feeding no xylanase or when feeding xylanase without wheat bran. The application of XOS had no impact on ileal X_2, X_3 or X_4 concentration when birds were fed both xylanase and wheat bran, but in the absence of wheat bran or when feeding wheat bran without xylanase, X_2, X_3 and X_4 concentration were consistently higher when feeding 2,000 mg/kg XOS compared to feeding 0 or 50 mg/kg XOS. An interaction between wheat bran and xylanase was observed on ileal X_5 and X_6 concentration ($P = 0.008$ and $P = 0.003$, respectively). This showed that when wheat bran was fed, supplementation with xylanase resulted in increased concentration of X_5 and X_6 in the ileum, but no effect of xylanase was observed in the absence of wheat bran.

3.4. Caecal xylanase and cellulase activity

Table 9 presents an interaction between wheat bran and XOS on caecal xylanase and cellulase activity ($P = 0.014$ and $P < 0.001$, respectively). When wheat bran was present, feeding 50 or 2,000 mg/kg XOS resulted in greater caecal xylanase activity compared to feeding no XOS. However, XOS had no impact on xylanase activity in the absence of wheat bran. Similarly, in the absence of wheat bran, XOS had no impact on caeca cellulase activity. However, in the presence of wheat bran, feeding 2,000 mg/kg XOS resulted in greater cellulase activity compared to feeding 0 or 50 mg/kg XOS.

4. Discussion

This study examined if it is possible to improve performance responses when feeding sorghum to broilers through establishing a gastrointestinal microbiota that is proficient at utilising dietary xylan, by feeding xylanase, XOS and a source of fermentable fibre.

Table 7
Effect of wheat bran, xylanase, and xylo-oligosaccharides (XOS) in sorghum-based diets on short chain fatty acid (SCFA) and microbiota concentration in the caeca in broilers at d 35.

Treatments			Microbiota (log_{10} counts/g digesta)	
Wheat bran, %	Xylanase, BXU/kg	XOS, mg/kg	*Enterococcus*	*Bifidobacteria*
0	0	0	9.18	8.43
0	0	50	9.17	8.68
0	0	2,000	9.07	8.57
0	16,000	0	9.15	8.71
0	16,000	50	9.47	8.61
0	16,000	2,000	9.36	8.97
10	0	0	9.21	8.80
10	0	50	9.43	9.21
10	0	2,000	9.38	9.15
10	16,000	0	9.14	9.42
10	16,000	50	8.99	9.08
10	16,000	2,000	9.14	9.20
SEM			0.04	0.06
0	0		9.14[b]	
0	16,000		9.33[a]	
10	0		9.34[a]	
10	16,000		9.09[b]	
0				8.66[b]
10				9.14[a]
P-value				
Wheat bran			0.835	<0.001
Xylanase			0.835	0.112
XOS			0.636	0.655
Wheat bran × Xylanase			0.009	0.942
Wheat bran × XOS			0.730	0.893
Xylanase × XOS			0.877	0.174
Wheat bran × Xylanase × XOS			0.205	0.504

[a,b] Means within the same column, within the same parameter, with no common subscript, differ significantly ($P < 0.05$).

Table 8
Effect of xylo-oligosaccharides, xylanase and wheat bran in sorghum based diets on the concentration of xylose (X_1), arabinose and xylobiose (X_2), xylotriose (X_3), xylotetraose (X_4), xylopentaose (X_5) and xylohexaose (X_6) in the ileum of broiler chickens at d 35.

Wheat bran, %	Xylanase, BXU/kg	XOS, mg/kg	XOS, mg/g marker													
			Diet							Ileum						
			Arabinose	X_1	X_2	X_3	X_4	X_5	X_6	Arabinose	X_1	X_2	X_3	X_4	X_5	X_6
0	0	0	6.84	0.56	0.72	0.58	0.21	0.06	0.03	13.64	0.87^{e}	0.51^{e}	0.43^{c}	0.13^{c}	0.07	0.04
0	0	50	8.30	1.71	2.12	1.70	0.60	0.18	0.08	15.19	1.15^{e}	0.91^{de}	0.60^{c}	0.21^{c}	0.13	0.07
0	0	2,000	10.05	22.36	46.62	36.05	12.46	3.99	1.20	8.34	7.46^{bcd}	13.83^{bcd}	10.83^{bc}	3.48^{bc}	0.80	0.26
0	16,000	0	9.08	0.70	0.49	0.34	0.10	0.03	0.02	13.02	2.29^{de}	3.01^{de}	2.06^{c}	0.71^{c}	0.21	0.12
0	16,000	50	9.57	3.56	5.57	4.64	1.60	0.37	0.20	11.61	4.28^{cde}	6.75^{cde}	3.87^{c}	0.81^{c}	0.29	0.13
0	16,000	2,000	9.03	23.28	62.13	53.40	17.05	4.40	1.36	12.57	15.77^{a}	30.66^{a}	24.05^{a}	6.21^{ab}	1.46	0.52
10	0	0	8.16	1.90	1.03	0.92	0.26	0.07	0.03	11.69	3.09^{cde}	4.62^{de}	3.93^{c}	1.42^{c}	0.60	0.08
10	0	50	8.30	3.21	5.15	4.54	1.39	0.36	0.13	12.94	2.70^{de}	3.87^{de}	3.34^{c}	1.27^{c}	0.43	0.09
10	0	2,000	10.58	28.41	55.86	48.66	14.89	4.06	1.25	11.77	12.93^{ab}	23.75^{ab}	18.23^{ab}	6.18^{ab}	1.66	0.48
10	16,000	0	8.88	3.41	1.73	1.28	0.32	0.09	0.03	16.07	8.49^{bc}	27.15^{a}	19.56^{ab}	5.13^{ab}	1.78	0.42
10	16,000	50	6.36	3.99	4.90	1.56	0.58	0.22	0.25	11.46	14.79^{a}	19.28^{abc}	17.34^{ab}	5.76^{ab}	1.51	0.50
10	16,000	2,000	5.41	18.08	38.24	27.09	7.89	1.78	0.56	13.17	15.16^{a}	25.75^{ab}	20.76^{ab}	7.06^{a}	2.25	0.81
SEM										0.48	0.66	1.35	1.11	0.34	0.09	0.03
0	0														0.33^{c}	0.12^{b}
0	16,000														0.65^{bc}	0.26^{b}
10	0														0.90^{b}	0.22^{b}
10	16,000														1.85^{a}	0.58^{a}
P-value																
Wheat bran								0.632	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
Xylanase								0.450	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
XOS								0.187	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
Wheat bran × Xylanase								0.455	0.093	0.130	0.102	0.047	0.008	0.003		
Wheat bran × XOS								0.390	0.006	0.016	0.049	0.451	0.569	0.609		
Xylanase × XOS								0.054	0.435	0.732	0.969	0.784	0.991	0.639		
Wheat bran × Xylanase × XOS								0.246	<0.001	<0.001	0.001	0.016	0.105	0.263		

[a-e] Means within the same column, within the same parameter, with no common subscript, differ significantly ($P < 0.05$).

Table 9
Effect of xylo-oligosaccharides, xylanase and wheat bran in sorghum based diets on xylanase and cellulase activity in the caeca of broiler chickens at d 35.

Wheat bran, %	Xylanase, BXU/kg	XOS, mg/kg	Xylanase, U/g	Cellulase, U/g
0	0	0	3.38	4.57
0	0	50	8.17	5.78
0	0	2,000	9.10	3.55
0	16,000	0	17.42	6.24
0	16,000	50	13.32	5.65
0	16,000	2,000	17.62	4.86
10	0	0	5.44	4.12
10	0	50	11.65	5.59
10	0	2,000	10.42	7.10
10	16,000	0	13.81	5.20
10	16,000	50	24.40	8.23
10	16,000	2,000	18.97	13.24
SEM			0.80	0.38
0		0	10.40^{b}	5.41^{b}
0		50	10.75^{b}	5.72^{b}
0		2,000	13.36^{ab}	4.21^{b}
10		0	9.63^{b}	4.66^{b}
10		50	18.03^{a}	6.91^{b}
10		2,000	14.70^{a}	10.17^{a}
P-value				
Wheat bran			0.023	<0.001
Xylanase			<0.001	<0.001
XOS			0.003	0.019
Wheat bran × Xylanase			0.771	0.061
Wheat bran × XOS			0.014	<0.001
Xylanase × XOS			0.586	0.184
Wheat bran × Xylanase × XOS			0.063	0.199

[a,b] Means within the same column, within the same parameter, with no common subscript, differ significantly ($P < 0.05$).

The sorghum used in this study contained a reasonable quantity of insoluble NSP, measured at approximately 50 g/kg, composed primarily of arabinoxylan. This suggested that it is necessary to target the xylan in non-viscous grains such as sorghum, as suggested by Choct (2006). This has been highlighted in numerous studies that have observed improvements in performance of birds fed sorghum-based diets by supplementing with xylanase, such as Tang et al. (2017) and Selle et al. (2010). However, in this study the effect of xylanase on d 0 to 35 performance was not consistent, and varied depending on the presence or absence of wheat bran or XOS. This discrepancy may because the diets used in this study contained comparatively less xylan and soluble NSP, so had lower viscosity inducing effects, meaning xylanase had less impact. In this study, feeding 2,000 mg/kg XOS resulted in a significant increase in XOS and xylose concentration in the ileum. The presence of monomeric pentose sugars, such as arabinose and xylose, signifies successful NSP degradation (Kim et al., 2016), but excessive levels are unfavourable, as they can increase osmosis in the lumen, resulting in diarrhoea, and thus issues with litter quality. In this study the heightened xylose concentration in the ileum did not translate into a negative impact on performance, suggesting this was not an issue here. Further research is warranted into how the monosaccharides and disaccharides generated may have impacted excreta moisture content.

In this study feeding 2,000 mg/kg XOS increased BWG. This is in agreement with Ribeiro et al. (2018) who observed increased BWG at d 0 to 42 when feeding XOS at both 100 and 1,000 mg/kg in both wheat- and corn-based diets. Ribeiro et al. (2018) also observed that supplementing 10 g/kg XOS to a corn-based diet had no positive effect on BWG, highlighting that excessive levels of XOS should be avoided. Clearly, the relatively high dosage of 2,000 mg/kg XOS used in this study was not too extreme. In wheat-based diets, Craig et al. (2020) saw no effect on BWG when feeding XOS at 0.25 or 1 g/kg, and De Maesschalck et al. (2015) observed no impact on d 0 to 39 BWG when feeding 2 g/kg XOS at d 1 to 13 and 5 g/kg XOS at d 14 to 29. This may be because these diets also contained wheat bran and rye, respectively, which likely yielded considerable quantities of xylose and XOS, thus no additional benefit was gained by

exogenous addition of XOS. Also, feeding more fermentable fibre from a young age likely meant these birds had established a microbiota that contained adequate xylan-degrading bacteria, and was thus better adapted to utilising dietary xylan as the bird aged (Bautil et al., 2020). In this study, the starter phase diets with wheat bran contained approximately 4 g/kg more soluble NSP compared to those fed the control diet, thus providing notably more fermentable fibre to the young birds. The benefits of this are illustrated by the greater caecal *Bifidobacteria* concentration at d 35 observed in this study. Palframan et al. (2003) suggested that *Bifidobacterium* species may possess an oligosaccharide uptake mechanism, specifically for XOS, providing a competitive advantage over strains that can only utilise monosaccharides. However, it must be noted that although total protein levels were similar between the diets with or without wheat bran, the differing sources of protein would have influenced the microbiota composition in varying ways (Hubert et al., 2019). The fact that the concentration of oligosaccharides in the ileum were increased in this study when only 10% wheat bran was fed does suggest that in xylan and/or XOS deficient diets this ingredient can provide an advantageous source of oligosaccharides, particularly in the presence of a xylanase. The presence of only 10% wheat bran and resulting approximate 1 g/kg difference in soluble NSP in the grower and finisher phase, addresses the importance of ensuring there is sufficient fermentable fibre available in sorghum-based broiler diets; this is notable given that modern diets are very nutrient dense, which naturally forces fibre rich ingredients out of the diet. This positive effect of feeding wheat bran on BWG in birds fed otherwise very fibre-poor diets noted in this work suggests that fermentable fibre may be a limitation for growth in sorghum-based diets.

Feeding a combination of wheat bran, xylanase, and 2,000 mg/kg XOS resulted in high ileal XOS concentration, which was associated with increased caecal total SCFA, acetic acid, and butyric acid concentration. The xylanase resulted in XOS release from the wheat bran, also likely increasing the release of higher molecular weight, soluble NSP. Thus the combination of the xylanase, wheat bran and 2,000 mg/kg of XOS provided both the signal (XOS) to "train" the microbiota in the hindgut to hydrolyse and ferment dietary xylan, and the fuel (polymeric soluble NSP) for fermentation to continue over a long period of time. It is believed this combination was responsible for the augmented SCFA concentration and heightened xylanase and cellulase activity noted, presenting a more favourable microbiota in the caeca and intestines, which relies on fibre for its sustenance, thus reducing competition with the host for nutrients. The consequence of this is increased SCFA production, as noted above. The outputs from this study agree with Ding et al. (2018), who found acetic acid and butyric acid increased as XOS supplementation level increased from 0 to 0.5 mg/kg. Stanley et al. (2012) and Biddle et al. (2013) showed that enhancing the abundance of butyrate-producing bacteria in the caeca was associated with increased degradation of plant materials and production of SCFA, and improved performance. In this study the best feed conversion and BWG was seen in birds fed 2,000 mg/kg and wheat bran, which also responsible for the highest SCFA concentration. Moreover, De Maesschalck et al. (2015) stated that the beneficial effects of XOS on bird performance could be explained by cross-feeding between lactate-producing bacteria and lactate-utilising butyrate-producing bacteria. Increased butyric acid levels in broiler digesta have also previously been observed as a consequence of xylanase application, and have been linked to enhanced performance (Masey O'Neill et al., 2014; Lee et al., 2017), which is in agreement with this study. It appeared that 50 mg/kg XOS was not sufficient to induce notable positive effects on SCFA production, or bird performance. This is in agreement with Singh et al. (2021), who observed that total caecal SCFA concentration was increased when feeding 100 mg/kg XOS, but not 50 mg/kg XOS, and saw no improvements in performance with either level. Further research is warranted to determine the optimum XOS level required to achieve the greatest SCFA production in broilers. Xylanase genes in the caeca (Al-Darkazali et al., 2017) appear to be stimulated by wheat bran, xylanase and XOS, as illustrated by significantly lower xylanase activity observed in the absence of these supplements.

5. Conclusion

Supplementing sorghum-based diets with fermentable fibre, XOS and xylanase was shown to be advantageous, resulting in enhanced SCFA production, presence of probiotic bacteria species, endogenous enzyme activity in the caeca, and bird performance. Further research is warranted into the optimum level and source of fermentable fibre and XOS to use in sorghum-based diets for broilers.

Author contributions

Natalie Morgan: conceptualisation, validation, formal analysis, investigation, methodology, writing- original draft, project administration; **Andrew Wallace:** investigation, validation, methodology, writing-review and editing; **Mike Bedford:** conceptualisation, validation, methodology, funding, writing-review and editing, project administration.

Declaration of competing interest

We declare that we have no financial or personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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