A new monocomponent xylanase improves performance, ileal digestibility of energy and nutrients, intestinal morphology, and intestinal microbiota in young broilers

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Primary Audience: Nutritionists; Poultry Producers; Researchers

SUMMARY

Supplementation of feed enzymes such as xylanases has been shown to lower intestinal digesta viscosity and improve nutrient digestion thereby resulting in better gut health due to the reduced availability of undigested nutrients for the growth of harmful bacteria. Oligosaccharides produced by xylanase in situ from dietary non-starch polysaccharides with potential prebiotic effects could modulate the gut microbiome. This paper reports the positive effects of a new monocomponent xylanase in improving performance and nutrient digestion and modulating the intestinal microbiota of broilers. A corn-wheat-soybean meal-based diet was formulated and used to develop 3 dietary treatments using different doses (0, 45,000 U/kg and 90,000 U/kg) of the xylanase. A total of 288, one-day-old male broiler (Ross 308) chicks were allocated to 36 cages (12 replicates per treatment and 8 chicks per cage) and offered the experimental diets from d 0 to 21 post-hatch. The data were subjected to ANOVA using the General Linear Models procedure of SAS. The results confirm that supplemental xylanase at both dose levels improved ($P < 0.05$) broiler performance and nutrient digestibility. Xylanase supplementation had significant ($P < 0.05$) effects in modulating the intestinal microbiota with a higher relative abundance of commensal bacteria such as *Lactobacillus* and a lower abundance of potentially non-beneficial bacteria such as *E. shigella* indicating a prebiotic mode of action.

Key words: xylanase, broilers, nutrient digestibility, intestinal morphology, gut microbiota, prebiotic

2023 J. Appl. Poult. Res. 32:100301
https://doi.org/10.1016/j.japr.2022.100301

DESCRIPTION OF PROBLEM

The beneficial effects of using exogenous enzymes in animal feed in increasing the digestibility and bioavailability of nutrients as well as in mitigating the negative effects of some antinutritional factors have already been established (Bedford and Partridge, 2010). Non-starch polysaccharides (**NSP**) are components of raw materials, which are not digested by poultry owing to their structural complexity

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and their resistance to hydrolysis in the digestive system (Nguyen et al., 2021). The antinutritive effects of soluble NSP have been attributed to their ability to increase digesta viscosity, thereby limiting the contact between digestive enzymes and nutrients (Choct and Annison, 1992; Abdollahi et al., 2013) and increasing pathogenic bacterial growth in the intestine (Bedford and Cowieson, 2012). Moreover, insoluble NSP trap the cell wall nutrients, restricting the accessibility of digestive enzymes. NSPases have been reported to improve the digestibility of entrapped nutrients by degrading the NSP present within the intact cell wall (Bedford, 2000). Arabinoxylans (**AXs**) comprise nearly half of the NSP in corn and corn by-products (Jaworski et al., 2015) with corn AX containing 0.1% DM soluble AX and 5.1% DM insoluble AX. Xylanase that hydrolyzes the β-1,4-glycosidic bonds of AXs is expected to mitigate the impact of corn-based NSP. In fact, xylanase supplemented in a corn-based diet increased soluble NSP production in the ileum, which could probably be the result of soluble AX fragments released from the insoluble AXs found in corn (Pedersen et al., 2015). Moreover, AXs create architecture around starch and protein granules within the aleurone, referred to as the "cage effect" of NSP. One possible mode of action of xylanase is the disruption of these "AX cages" through AX hydrolyzation, and, in turn, releasing stored starch and protein granules for endogenous enzymes to digest.

One potential strategy to improve digestibility and alleviate the negative effects of AX is the supplementation of xylanase. Beneficial effects of xylanase supplementation to poultry diets are well documented, ranging from improvements in nutrient digestibility and utilization, better animal performance, and welfare to gut health benefits (Bedford, 2000; Adeola and Cowieson, 2012; Van Hoeck et al., 2021a,b).

The majority of xylanase research in animal nutrition has focused on glycoside hydrolase (**GH**) families 10 and 11. GH11 and GH10 xylanases differ in their size, protein structure, and substrate binding site shapes which reflects in their capabilities to act on arabinoxylans with different substitutions. GH10 xylanases typically have high molecular mass and feature a (β/α)8-barrel fold, while GH11 xylanases have smaller molecular mass with a conserved β-jelly roll fold (Javier et al., 2018). The compact wide shallow structure of the substrate binding cleft of GH11 may facilitate more efficient substrate binding compared to GH10. This difference in the efficiency of substrate binding is critical to feed application because of the complex structure of the plant cell wall xylan. There are many studies available in the literature comparing the efficacy of GH10 and GH11 for degrading soluble and insoluble arabinoxylans. A study by Vardakou et al. (2003) using fungal enzymes belonging to GH10 and GH11 families showed a higher capability of GH10 to hydrolyse WU-AX to a higher degree and with higher catalytic efficiency compared to GH11, while a more detailed study by Bonnin et al. (2006) showed that GH11 xylanase exhibited a broader range of substrate selectivity. These results are also in agreement with the reported selectivity of other GH10 and GH11 xylanases: Hu et al. (2013) noted that GH11 is particularly effective on wheat AX, while GH10 may be more effective against highly substituted corn fiber (Rose and Inglett, 2011; Pedersen, 2015). Also, only 25% as much GH10 survived the intestinal tract compared with GH11, which is a practical concern when supplementing feeds (Pedersen, 2015). However, it has to be noted that the properties of xylanases are dependent on the source organism. There are many biomass-degrading organisms capable of secreting different types of GH10 and GH11 xylanases, which harbor different carbohydrate-binding modules; therefore, each xylanase enzyme must be evaluated separately.

Maintaining the balance of good gut health is central to achieving the best growth and feed efficiency. The gastrointestinal tract is populated with diverse assemblies of microbiota that play critical roles in the overall well-being of the animal; however, the diversity and activities of gut microbiota are affected by a plethora of factors including dietary factors such as the ingredient type, dietary composition as well as feed additives (Bedford and Cowieson, 2012). It has been shown that the ingredient type and inherent digestibility may influence the efficacy of NSP-degrading enzymes and intestinal

microbiota profile. Józefiak et al. (2010) showed that both the cereal type and the exogenous enzyme supplementation influenced cecal microbiota in broiler chickens and reduced potentially pathogenic *Enterobacteriaceae* populations. Glycanases, such as xylanases and β-glucanases, increased the intestinal lactic acid bacteria with a corresponding decrease in the population of pathogenic bacteria such as *E. coli* (Rodríguez et al., 2012). Supplementation of these exogenous enzymes was found to provide some protection against necrotic enteritis due to the breakdown of the dietary NSP and the consequent reduction in digesta viscosity in wheat- and barley-based diets (McDevitt et al., 2006). The current experiment was designed to study the effects of a novel monocomponent xylanase on performance, nutrient digestibility, intestinal morphology, and microbiota composition in broilers fed corn-wheat-soybean meal-based diets.

MATERIALS AND METHODS

Ethics Approval

The experimental procedures were approved by the Massey University Animal Ethics Committee (MUAEC 17/13) and complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes.

Xylanase Enzyme Product

The enzyme product used in the present study (Xygest HT, Kemin Industries Inc., Des Moines, IA) is an intrinsically thermostable, monocomponent xylanase expressed in *Pichia pastoris* and is a beta 1-4, endo-xylanase enzyme belonging to the GH11 family. The product has a minimum xylanase activity of 3,000,000 U/g on a corn starch-based carrier with high activity at a pH range of 5 to 7 and more than 65% of maximum activity under physiological temperature conditions (40−43˚C; Van Hoeck et al., 2021a).

Birds, Housing, and Dietary Treatments

A total of 288-day-old male broiler (Ross 308) chicks were obtained from a local hatchery, individually weighed, and allocated on a weight basis to 36 cages (8 chicks per cage) housed in an environmentally controlled room. The 3 dietary treatments were then randomly assigned to 12 replicate cages each. Experimental diets were offered from d 0 to 21 post-hatch.

The temperature was maintained at 32˚C during the first week and gradually decreased to approximately 23˚C by the end of the third week. The birds received 20 h of fluorescent illumination per day. Feed and water were available ad libitum throughout the 21-d experimental period. Birds were checked at least thrice daily, and any unusual aspect of bird behavior or condition was recorded. Sick or injured birds were weighed and removed from the study.

A control starter diet based on corn, wheat, and soybean meal was formulated (Table 1). From the control diet, 3 experimental diets were developed using different doses (0, 45,000 U/kg, and 90,000 U/kg) of the enzyme product. The enzyme was added to the diets in a mixer prior to pelleting. The enzyme was premixed with a portion of the diet (10 kg) before addition to the batch mixer. The control diet was mixed first followed by the enzyme-supplemented diets. The mixer was cleaned after making each treatment diet. All diets contained titanium dioxide (5 g/kg) as an inert marker for the determination of nutrient digestibility. All diets were steam-conditioned at 80˚C. The conditioning time of the mash was 30 s, and the conditioning temperature was measured at the outlet (close to the exit point) of the conditioner. After conditioning, the diets were pelleted using a pellet mill (Richard Size Limited Engineers, Orbit 15, Kingston-upon-Hull, UK) capable of manufacturing 180 kg of feed/h and equipped with a die ring (3-mm holes and 35-mm thickness). Representative samples were obtained for all diets (500 g/diet) and were tested for xylanase activity units using Kemin's internal method (Table 2).

Mycotoxin Analyses

The following values (μg/kg) were determined for different mycotoxins in the corn DDGS used in this study: Deoxynivalenol, 317; Fumonisin B1, 766; Fumonisin B2, 240; Fumonisin B3, 86; Zearalenone, 26. All other

Table 1. Percentage composition and calculated values of the starter diet (0−21 d post-hatch).

Ingredient	g/100 g
Corn	33.20
Soybean meal, 46%	32.88
Wheat	20.0
Canola meal	3.0
Corn DDGS[1]	5.0
Soybean oil	1.97
Dicalcium phosphate	0.92
Limestone	1.19
L-Lysine HCl	0.25
DL-Methionine	0.27
L-Threonine	0.11
Salt	0.23
Sodium bicarbonate	0.27
Vitamin premix[2]	0.10
Trace mineral premix[2]	0.10
Phytase[3]	0.01
Titanium dioxide	0.50
Provision (g/100 g as received)	
AME, Kcal/kg	2900
CP	23.2
Crude fat	4.34
Soluble NSP[4]	1.51
Insoluble NSP[4]	10.04
Total NSP[4]	11.55
Dig. Met + Cys	0.91
Dig. Lys	1.22
Dig. Thr	0.82
Dig. Try	0.22
Dig. Val	0.92
Dig. Leu	1.70
Dig. Ile	0.82
Dig. Arg	1.37
Calcium	0.96
Non-phytate phosphorus	0.48
Sodium	0.19
Chloride	0.19

[1]Corn dried distillers' grains with solubles.

[2]Supplied per kg diet: antioxidant, 125 mg; biotin, 0.2 mg; calcium pantothenate, 20 mg; cholecalciferol, 5,000 IU; cyanocobalamin, 0.02 mg; folic acid, 2.0 mg; menadione, 4 mg; niacin, 80 mg; pyridoxine, 5.0 mg; trans-retinol, 15,000 IU; riboflavin, 9.0 mg; thiamine, 4.0 mg; dl-α-tocopheryl acetate, 80 IU; choline, 0.45 mg; ascorbic acid, 100 mg; Co, 1.0 mg; Cu, 20 mg; Fe, 40 mg; I, 2.0 mg; Mn, 100 mg; Mo, 1.0 mg; Se, 0.15 mg; Zn, 100 mg.

[3]Supplied 1,000 FTU/kg diet; to deliver 0.15 g/100 g non-phytate phosphorus and 0.18 g/100 g calcium.

[4]Non-starch polysaccharides.

mycotoxins were less than 10 μg/kg. All concentrations were negligible compared to the tolerance levels for poultry in European Union regulatory guidelines (Guerre, 2016).

Measurements

Body weight and feed intake were recorded at weekly intervals. Mortality was recorded daily. Feed per gain values were corrected for the body weight of any bird that died or culled during the experiment. On d 21, 2 birds per cage were randomly selected, killed by cervical dislocation, and immediately dissected to obtain samples of ileal and cecal contents for microbiota analysis.

The birds euthanized for microbiota analysis were also used for intestinal morphological examinations. Sections from the middle of the duodenum and jejunum (about 5 cm in length) were excised and flushed with cold saline and immediately placed in formalin 10% fluid. Samples were transferred into 70% ethanol after 72 h. Each fixed sample was then processed on a tissue processor. The samples were dehydrated through graded alcohols (70%, 95%, and absolute alcohol) at ambient temperature, cleared in isopropyl alcohol, and impregnated with Histosec pastilles under pressure at 60˚C. The samples were embedded in wax and sectioned using rotary Microtome using Feather S35 disposable blades at a thickness of 5 μm. Samples were then stained with alcian blue and hematoxylin-eosin and examined by light microscopy. Four segments were fixed on each slide and, the slides were viewed on an Olympus microscope and the following variables were measured: villus height, crypt depth, goblet cell number, and epithelial thickness (2 birds per replicate, 10 readings per segment).

On d 21, all remaining birds per cage were euthanized by intravenous injection (1 mL per 2 kg body weight) of sodium pentobarbitone (Provet NZ Pty Ltd., Auckland, New Zealand) and ileal digesta were collected as described by Ravindran et al. (2005). The ileum was defined as that portion of the small intestine extending from the Meckel's diverticulum to a point ~40 mm proximal to the ileocecal junction. The ileum was then divided into 2 halves and the digesta was collected from the lower half toward the ileocecal junction. Digesta from birds within a cage were pooled, lyophilized, ground to pass through a 0.5 mm sieve, and stored at 4˚C until laboratory analysis. The diets and digesta samples were analyzed for DM,

Table 2. Theoretical (target) xylanase activity based on enzyme concentration and inclusion in the feed and recovered (measured) xylanase activity in the treatment diets post-pelleting.

Treatment	Target xylanase activity (U/kg)	Measured xylanase activity (U/kg)[1]
Control + 45,000 U/kg xylanase	45,000	41,695 ± 2,482
Control + 90,000 U/kg Xylanase	90,000	84,760 ± 4,834

[1]Mean ± standard deviation (n = 3). Unit definition = One unit of activity is the amount of enzyme that liberates 1 μg of xylose equivalents per minute and per g of enzyme product.

gross energy (**GE**), titanium (**Ti**), starch, fat, and nitrogen (**N**) by standard procedures. Apparent ileal digestibility coefficients of DM, N, fat, starch, and GE were determined using Ti as the inert marker.

Chemical Analysis

Dry matter was determined in a convection oven at 105˚C (AOAC, 2016; methods 930.15 and 925.10). Nitrogen was determined by the combustion method using a CNS-2000 carbon, nitrogen, and sulfur analyzer (LECO Corporation, St. Joseph, MI). Gross energy was determined using an adiabatic bomb calorimeter (Gallenkamp Autobomb, Weiss Gallenkamp Ltd, Loughborough, UK) standardized with benzoic acid. Crude fat was determined following the Soxhlet extraction procedure. The fat was dissolved by repeatedly washing the samples with petroleum-ether by refluxing in a Soxtec apparatus (Soxtec System HT 1043 Extraction Unit, Höganäs, Sweden). The solubilized fat was then collected in the distillation flask and the increase in the weight of the flask represented the dissolved fat. Starch was measured using a total starch assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) based on thermostable α-amylase and amyloglucosidase. Titanium was measured on a UV spectrophotometer following the method of Short et al. (1996).

Calculations

The coefficients of apparent ileal digestibility (CAID) of nutrients were calculated using the following formula:

$$\text{CAID of diet component} = [(\text{Diet component}/\text{Ti})\text{d} - (\text{Diet component}/\text{Ti})\text{i}]/(\text{Diet component}/\text{Ti})\text{d}$$

where (Diet component/Ti)d = ratio of diet component to Ti in the diet, and (Diet component/Ti)i = ratio of diet component to Ti in the ileal digesta.

Statistical Analysis

The data were subjected to ANOVA using the General Linear Models procedure of SAS (version 9.4; 2015; SAS Institute, Cary, NC). Cage means served as the experimental unit for all data (n = 12). Differences were considered to be significant at $P < 0.05$ and significant differences between means were separated by the Least Significant Difference test.

Microbiota Analysis

16S-rRNA Amplicon Sequencing. Genomic DNA was extracted from 0.25 g of thawed, mechanically homogenized cecal and ileal contents using the Geneaid Presto Stool gDNA Extraction Kit (Geneaid, Taiwan, #STLD100). DNA extracts were diluted 1:10 with molecular grade water and 20 μL was analyzed on a 1% agarose gel along with a high molecular weight marker (Fisher Scientific, USA, # 12-352-019) to assess DNA quality. Samples were quantified by Qubit HS assay (Invitrogen, USA, #Q32854) and normalized to 5 ng/μL with Nuclease-Free Water (Invitrogen, USA, #4387936). For the amplicon PCR 1 μL of each normalized DNA was added to 17 μL of AccuPrime Pfx SuperMix (Invitrogen, USA, #12344040) and 1 μL each of the barcoded forward (16Sf V3 AATGATACGGCGACCACCGAGATCTACACxxxxxxxxTATGGTAATTGGCCTACGGGAGGCAGCAG) and reverse (16Sr V4 CAAGCAGAAGACGGCATACGAGATxxxxxxxxAGTCAGTCAGCCGGACTACHVGGGTWTCTAAT) V3V4

Table 3. Primer sequences for 16S-rRNA amplicon sequencing.

Primer	Sequence
16Sf V3	AATGATACGGCGACCACCGAGATCTACACxxxxxxxxTATGGTAATTGGCCTACGGGAGGCAGCAG
16Sr V4	CAAGCAGAAGACGGCATACGAGATxxxxxxxxAGTCAGTCAGCCGGACTACHVGGGTWTCTAAT

"xxxxxxxx" represents the unique barcode sequence assigned to each sample.

amplicon primers Table 3 (Klindworth et al., 2013). The following thermocycler program was run: 95˚C for 2 min, then 30 cycles of 95˚C for 20 s, 55˚C for 15 s, 72˚C for 5 min, followed by a final extension cycle of 72˚C for 10 min, and hold at 4˚C. A clean-up was performed on the PCR products using SequalPrep Normalization Plate Kit (Applied Biosystems, USA, # A1051001). The library concentration was quantified by Qubit HS assay (Invitrogen, USA, #Q32854). The full library size of ~630 bp was verified on a PerkinElmer LabChip GX Touch HT instrument using the DNA High Sensitivity LabChip Assay. The libraries were pooled by equal molarity. The pooled library was diluted to 2 nM with 10 mM Tris pH 8.5 with 0.1% Tween 20 for sequencing. A final volume of 10 uL of the 2 nM pooled library was denatured to single-strand DNA with 10 μL 0.2N NaOH (pH > 12.5) by mixing and incubating at room temperature for 5 min. Illumina PhiX Control v3 (Illumina, San Diego, CA, USA, #FC-110-3001) was diluted to 2 nM with 10 mM Tris pH 8.5 with 0.1% Tween 20 then denatured with 10 μL 0.2N NaOH (pH > 12.5) by mixing and incubating at room temperature for 5 min. Pooled library was diluted to 8 pM and PhiX was diluted to 12.5 pM with ice cold HT-1. Finally, 800 μL of pool library and 200 μL of PhiX were combined to give a calculated spike of 20% PhiX. Samples were mixed and 600 μL was loaded into a thawed Illumina MiSeq V2 cartridge for sequencing on the Illumina MiSeq platform using paired-end sequencing with 250 nucleotide read length.

Analysis of NGS Data

The reads were processed by the standard Quantitative Insight into Microbial Ecology 2 (QIIME2 v.2020.6) pipeline (Bolyen et al., 2019). The samples were grouped by 2 categorical variables (treatment and bird). The preparation step, which includes denoising, chimera checking, pair-joining and clustering through dereplication, was performed using DADA2 (Callahan et al., 2016), producing a list of all candidates' Amplicon Sequence Variants (**ASV**) found in each sample. This list was filtered by excluding ASVs that appeared in less than 10 samples. All samples were then rarefied, using Faith PD metrics, and any possible loss of information was ruled out by examination of the rarefaction plots.

Alpha (within sample) and beta (between sample) diversity analyses were performed with QIIME2 (Bolyen et al., 2019) and UniFrac (Lozupone et al., 2005), respectively. Alpha diversity indices measured included, Shannon, Evenness, Faith's PD, and Observed Features (ASVs), significance was determined using Kruskal-Wallis tests. Beta diversity indices measured included, the Jaccard index, Bray-Curtis dissimilarity, and Weighted and Unweighted, UniFrac distance, significance was determined using PERMANOVA tests.

Table 4. Influence of xylanases supplementation on the weight gain (g/bird), feed intake (g/bird), feed per gain (g feed/g gain), and mortality (%) of broilers (0 to 21 d post-hatch)[1].

Treatment	Weight gain	Feed intake	Feed per gain	Mortality
Control	1064	1347[a]	1.265[a]	2.0
Control + 45,000 U/kg xylanase	1054	1307[b]	1.240[b]	-
Control + 90,000 U/kg xylanase	1069	1289[b]	1.206[c]	-
Pooled SEM	9.3	10.7	0.0083	1.20
Probability, *P*-value	0.519	0.0017	0.0001	0.38

[a,bc]Means in a column not sharing a common letter are significantly different ($P < 0.05$).

[1]Each value represents the mean of 12 replicates (8 birds per replicate).

Taxonomic classification was obtained using the embedded Naive Bayes fitted classifier, trained on the Silva_138 99% identity database (Yilmaz et al., 2014). The relative abundance of ASV units was determined as a percentage of total bacteria per treatment. The relative abundance of the top 1% and top 0.1% most abundant ASV in the ceca and ilea respectively was analyzed by ANOVA using the General Linear Models procedure of SAS (version 9.4; 2015; SAS Institute, Cary, NC) to determine any difference between treatments.

RESULTS AND DISCUSSION

Performance

The effect of xylanase supplementation on broiler performance during 0 to 21 d post-hatch is shown in Table 4. Mortality was negligible during the trial period. Only 2 birds, out of 288, died and they were in the group fed the control diet. Weight gain was unaffected ($P > 0.05$) by enzyme treatments. Enzyme supplementation, at both inclusion levels, reduced ($P < 0.05$) the feed intake and resulted in better ($P < 0.05$) feed per gain compared to the unsupplemented control diet. Differences ($P < 0.05$) were observed between the 2 enzyme inclusion levels, with the feed per gain being lower in birds receiving the diet supplemented with 90,000 U/kg.

Early generations of commercial xylanases contained several side-enzyme activities, albeit minor, of protease, amylase, cellulase, xylanase, and β-glucanase, besides xylanase. It is likely, therefore, that the secondary enzymes may have played a part in improving bird performance and nutrient availability (Adeola and Cowieson, 2012). When it comes to supplementation of monocomponent xylanase to corn-based diets, varying responses have been reported in broilers owing to several factors such as differences in diet formulation, corn quality variability, age of birds, the nutrient density of diets as well as changes in the microbial population of the gut. Interplay of all/some of these factors might result in improvements in feed conversion rate with or without significant changes in feed intake or nutrient digestibility with xylanase supplementation. Studies conducted in corn-soy diets using single xylanase reported a significant increase in body weight gain without any effect on feed intake suggesting a mechanism involving an increase in feed use efficiency (Cowieson et al., 2010; Masey O'Neill and Lui, 2011). Improvement in ileal digestible energy which accompanied the increase in body weight gain (Cowieson et al., 2010) supported the mechanism of increased feed use efficiency. Our earlier study with the same xylanase using a different type of feed formulation improved the body weight gain and FCR in 35-day-old broilers, but at a lower feed intake compared to the low energy negative control diet (Van Hoeck et al., 2021a). Another set of broiler studies using xylanase-supplemented wheat and corn diets showed no correlation between the superior growth performance observed in birds-fed wheat diets relative to birds-fed corn diets and the improvements in nutrient digestibility and retention (Kiarie et al., 2014). It was concluded that the changes in the cecal VFA production by wheat and corn diets supplemented with xylanase differently influence the gut microbial profile resulting in the differences in growth performance. Cowieson et al. (2010) reported that the

Table 5. Influence of xylanase supplementation on the coefficient of apparent ileal digestibility of nutrients and energy, and ileal digestible energy (kcal/kg dry matter) in 21-day-old broilers[1].

Treatment	Dry matter	Nitrogen	Starch	Fat	Gross energy	Ileal digestible energy[2]
Control	0.657[b]	0.769[b]	0.947[b]	0.879	0.692[b]	3050[b]
Control + 45,000 U/kg xylanase	0.702[a]	0.813[a]	0.970[a]	0.894	0.736[a]	3241[a]
Control + 90,000 U/kg xylanase	0.700[a]	0.814[a]	0.970[a]	0.891	0.735[a]	3238[a]
Pooled SEM	0.0046	0.0057	0.0030	0.0105	0.0047	20.6
Probability, *P*-value	0.0001	0.0001	0.0001	0.554	0.0001	0.0001

[a,b]Means in a column not sharing a common letter are significantly different ($P < 0.05$).

[1]Each value represents the mean of 12 replicates (8 birds per replicate).

[2]Coefficient of apparent ileal digestibility of energy x gross energy of diet (kcal/kg).

increase in ileal digestible energy by xylanase supplementation, although significant at 42 d, was not obvious at 21 d. Hence, it could be possible for the birds to translate the nutrient or energy digestibility improvements to body weight gain at a later stage of growth with prolonged enzyme treatment.

The xylanase evaluated in the present study is a monocomponent xylanase and the results demonstrate that this xylanase on its own is capable of achieving efficient performance. The enhancement in bird performance with xylanase addition can be attributed to the viscosity reduction, removal of cage effect by the degradation of cell wall NSP, releasing entrapped nutrients, and a prebiotic effect of the XOS degradation products generated.

Ileal Digestibility of Nutrients and Energy

The influence of xylanase supplementation on the CAID of nutrients and energy is summarized in Table 5. Supplemental xylanase, at both inclusion levels, improved ($P < 0.05$) the CIAD of DM, N, starch, and GE, and apparent ileal digestible energy (**AIDE**) compared to the control diet. No differences ($P > 0.05$) were seen between the 2 inclusion levels. The CIAD of fat was unaffected ($P > 0.05$) by supplemental xylanase.

Similar observations in ileal digestibility improvement of nutrients with the supplementation of xylanase have been reported in an array of broiler feed formulations. Wang et al. (2005) reported improvements in the apparent protein digestibility in broilers aged 21 and 42 d with added xylanases. Our findings confirm previous reports indicating the beneficial effects of xylanase on energy and protein utilization of young birds fed corn-based diets (Kocher et al., 2003; Stefanello et al., 2016). The observed improvements in nutrient digestibility by xylanase supplementation could be attributed to the increase in the activity of endogenous enzymes owing to the increased availability of substrates due to the disruption of cell wall arabinoxylans (Kocher et al., 2003). Improvements in the CIAD of N and AIDE are in agreement with those reported in 28-d broilers fed wheat-based diets (Cowieson and Masey O'Neill, 2013). The increase in AIDE with xylanase reflects the increased CIAD of starch and protein. Indeed, a previous broiler study, using the same xylanase at a lower dosage (30,000 U/kg), reported that supplementation could effectively compensate for the lower digestibility of organic matter, N, fat, starch, and GE in a nutrient-deficient negative control diet (Van Hoeck et al., 2021a). This increased nutrient digestion and utilization due to the efficient degradation of NSP by xylanase might be the reason for the improved feed per gain observed in the current study.

Intestinal Morphometry

The influence of supplemental xylanase on the morphology of duodenum and jejunum in 21-day-old broilers is summarized in Table 6. In the duodenum, the morphometry was unaffected ($P > 0.05$) by xylanase addition. In the jejunum, xylanase had no effect ($P > 0.05$) on the villus height and crypt depth but increased ($P < 0.05$) goblet cell numbers and epithelial thickness at 90,000 U/kg. An earlier broiler

Table 6. Influence of xylanase supplementation on the villus height (μm), goblet cell number (per 100 μm villus height), epithelial thickness (μm), and crypt depth (μm) of the duodenum and jejunum of 21-day-old broilers[1].

	Duodenum				Jejunum			
Treatment	Villus height	Goblet cell number	Epithelial thickness	Crypt depth	Villus height	Goblet cell number	Epithelial thickness	Crypt depth
Control	997	12.1	13.3	41.1	665	12.1[b]	11.9[b]	41.5
Control + 45,000 U/kg xylanase	1029	12.0	13.4	40.2	635	11.8[b]	11.7[b]	39.1
Control + 90,000 U/kg xylanase	1016	12.7	14.5	42.2	644	13.9[a]	13.1[a]	42.3
Pooled SEM	24.0	0.60	0.40	1.43	19.0	0.43	0.35	1.13
Probability, *P*-value	0.644	0.651	0.091	0.615	0.528	0.003	0.016	0.129

[a,b]Means in a column not sharing common letters are significantly different ($P < 0.05$).

[1]Each value represents the mean of 12 replicates (two birds per replicate, 10 readings per bird).

study using the same xylanase product at graded dosages similarly found an increase in ileal villus height at a higher dosage of xylanase (90,000 U/g) indicating a dose-dependent response of xylanase on gut morphology (Van Hoeck et al., 2021a).

The number of goblet cells in the villi is an indicator of intestinal health, as these cells produce mucin which acts as a barrier, preventing the adhesion of pathogenic bacteria to the intestinal epithelium (Calik and Erg¨un, 2015). An increase in goblet cell number could be due to the increase in commensal bacterial populations that stimulate goblet cells (Forder et al., 2007). Such an increase in goblet cell counts in the ileum has been reported in broilers fed dietary prebiotic (Mannan oligosaccharides) and synbiotics (Mannan oligosaccharides combined with *Bacillus subtilis* and *Bacillus licheniformis*) (Kridtayopas et al., 2019). Epithelial thickness was influenced by supplemental enzymes in the jejunum, with the epithelia of birds receiving 90,000 U/kg xylanase being thicker ($P < 0.05$) than those receiving 0 and 45,000 U/kg. The increase in epithelial thickness could be the result of an increased probiotic population as reported in some studies (Hayashi et al., 2018).

16S rRNA Sequencing

Species Richness and Diversity in Cecum and Ileum. Alpha diversity indices for cecal and ileal analysis showed similar values across all 3 treatments for both, with an apparent increase in species richness in the cecum when compared to the ileum as indicated by Shannon metrics. Beta diversity analysis also indicates a lack of significant difference in diversity between each treatment in both the cecum and ileum, as demonstrated by plotting the unweighted unifrac metric as principal coordinate graphs (Figures 1 and 2) and no clustering occurred.

- *Cecal diversity*. Looking at the diversity at the level of family, 4 ASV units were found to have significant differences ($P < 0.05$) in relative abundance, 3 of which are in the phylum Firmicutes and one within the phylum Proteobacteria (Figure 3). These four ASV units are an unclassified Esherichia-shigella species (Proteobacteria), an unclassified Oscillospiraceae species, an unclassified Streptococcus spp., and an unclassified Lactobacillus spp. (all 3 within phylum Firmicutes). The 2 most abundant ASV units in the cecal microbiota across all treatments were the unclassified Lactobacillus spp. (15.2%) and the unclassified Streptococcus spp. (14.8%).
- *Ileal diversity*. Looking at the ileal taxonomic diversity at the level of family, only 2 had ASV units with significant

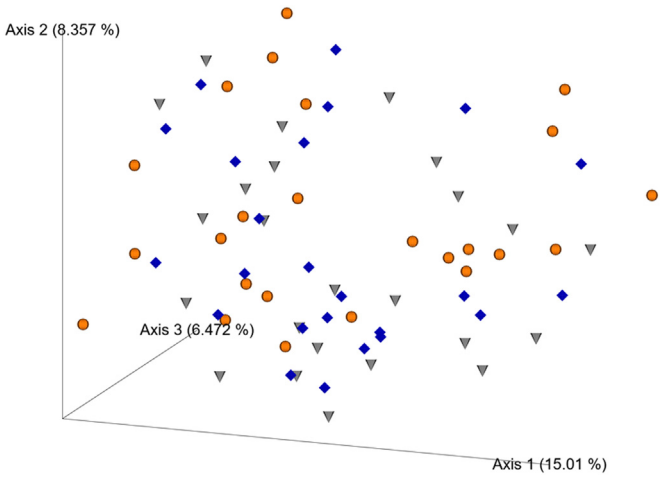

Figure 1. Microbiome diversity in the cecum. Unweighted UniFrac PCoA plot colored by treatment groups. Blue diamond is control, orange circle is control + 45,000 U/kg xylanase and grey triangle is control + 90,000 U/kg xylanase. No clustering was observed.

differences ($P < 0.05$) in relative abundance, both within the phylum of Firmicutes (Figure 4). The two ASV units were an unclassified Lactobacillus spp. and an unclassified member of the family Peptostreptococcaceae. The Lactobacillus spp. is the most dominant ASV unit in the ileum comprising of 47.3% of all identified bacteria.

The current work established distinct differences in the taxonomic composition of bacteria between the 2 sampling sites (ileum vs. cecum). As also observed in previous studies, the taxonomic composition along the gastrointestinal tract changes and marked differences in species diversity have been observed between the ceca and ileum, with the latter being less diverse (Glendinning et al., 2019; Richards-Rios et al., 2020). As reviewed by Oakley et al. (2014), the 3 most abundant phyla in the cecum are Firmicutes, Bacteroidetes, and Proteobacteria indicating the similarity of microbiota in this study to previous broiler reports. The most abundant phyla in the ileum were also the Firmicutes, specifically the family Lactobacilliaceae (Figure 4). Similar results in the composition of the taxa of ileal microbiota have been reported by Glendinning et al. (2019) and Richards-Rios et al. (2020). Glendinning et al. (2019) also observed the second most prevalent bacteria being a *Romboutsia* spp. from the family Peptrostreptococaceae. Similarly, the second most abundant bacteria in the current research were an unclassified member of the same family and further analysis may be able to determine if this unclassified bacterium is a *Romboutsia* spp. The present data are also supported by the results of Richard-Rios et al. (2020) where a time-course development of the ileal microbiota was determined during the growth cycle of the chicken. Bacteria from the family Lactobacilliaceae were found to be the most prevalent by d 14 and the slower-growing bacteria from the family Peptrostreptococaceae increased in abundance starting at d 21 (Richard-Rios et al., 2020), similar to the results observed on the current experiment where the microbiota was investigated at d 21.

Overall, in the present study, xylanase supplementation impacted the composition of the cecal and ileal microbiota. In both segments, the *Lactobacillus* spp. population significantly ($P < 0.05$) increased at both inclusion levels of xylanases. Interestingly, 45,000 U/kg xylanase produced a higher population of *Lactobacillus* spp. in the cecum, while conversely, 90,000 U/kg xylanase produced a higher

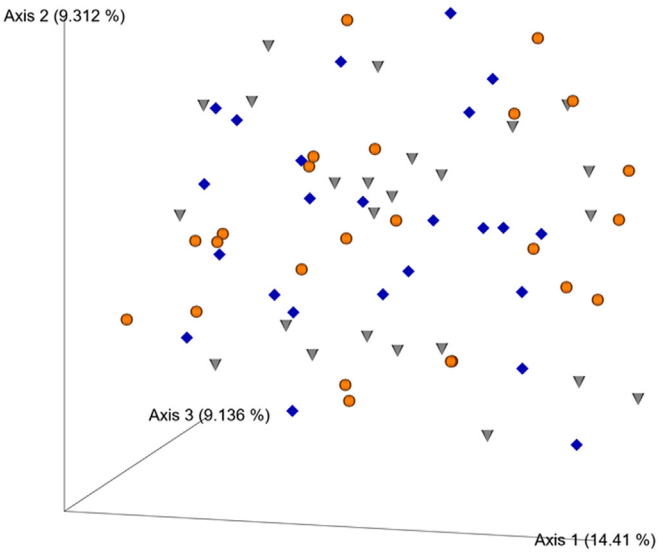

Figure 2. Microbiome diversity in the ileum. Unweighted UniFrac PCoA plot colored by treatment groups. Blue diamond is control, orange circle is control + 45,000 U/kg xylanase and grey triangle is control + 90,000 U/kg xylanase. No clustering was observed.

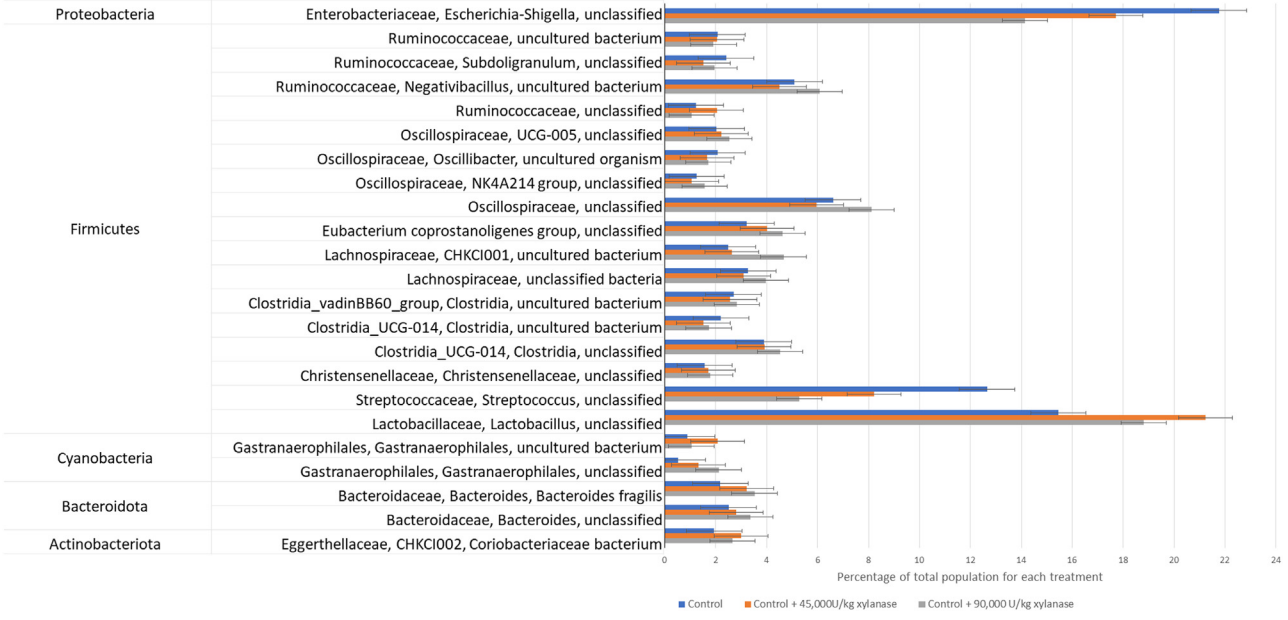

Figure 3. Relative abundance of the top 1% of identified species in the cecal microbiome as influenced by supplemental. All species are initially grouped by phylum. Blue bars represent the control with no added enzyme, orange bars are the control + 45,000 U/kg xylanase and grey bars are the control + 90,000 U/kg xylanase. Error bars represent standard error.

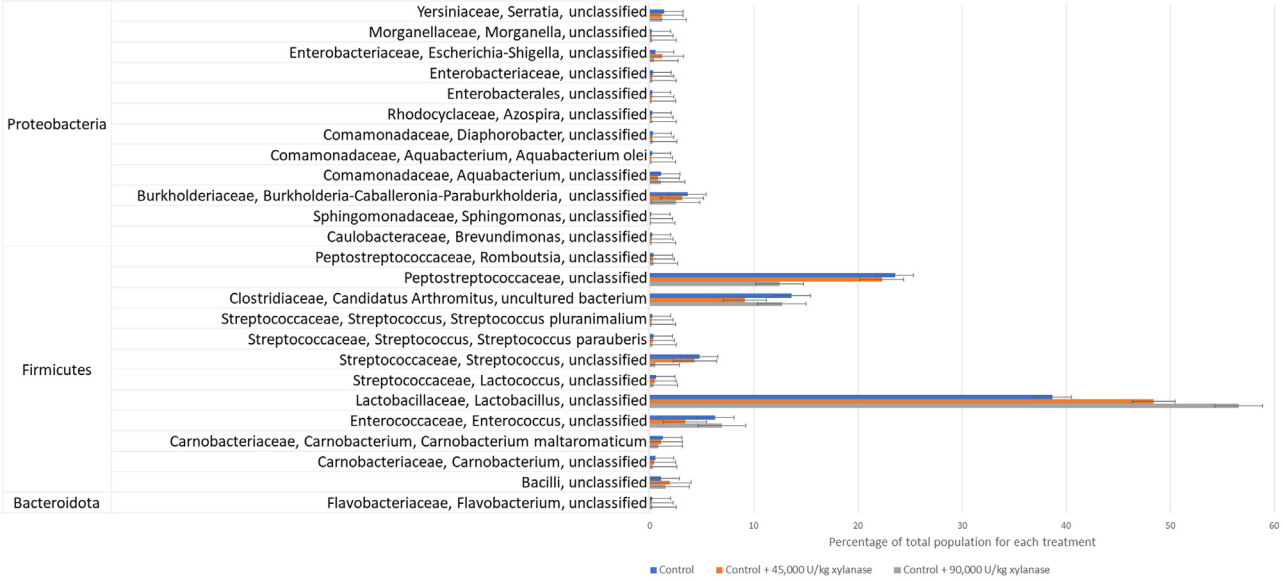

Figure 4. Relative abundance of the top 0.1% abundant of identified species in the ileal microbiome of chickens with xylanase treatments. All species are initially grouped by phylum. Blue bars represent the control with no added enzyme, orange bars are the control + 45,000 U/kg xylanase and grey bars are the control + 90,000 U/kg xylanase. Error bars represent standard error.

population of *Lactobacillus* spp. in the ileum. The cause of an increased abundance of *Lactobacillus* spp. for the 45,000 U/kg dosage while obvious is not statistically significant ($P > 0.05$) and could not be isolated to any single cause, further investigation would be necessary. Our results are in accordance with the results of a microbiota study in laying hens using the same novel xylanase. Van Hoeck et al. (2021b) reported a significant increase in populations of beneficial bacteria (Bacilli class; Enterococcaceae and Lactobacillales orders; *Merdibacter, Enterococcus*, and *Nocardiopsis* genera; *Enterococcus casseliflavus* species) with the addition of 45,000 U/kg xylanase to laying hen diet. Both the results of the current study and Van Hoeck et al. (2021b) demonstrate the ability of this xylanase to positively modulate the composition of intestinal microbiota.

Lactobacillus spp. have been reported to be associated with increased body weight, enhanced goblet cell numbers, and decreased *E. coli* colonization in the digestive tract among other positive health outcomes for poultry (Aliakbarpour et al., 2012, Li et al., 2017, Lokapirnasari et al., 2019). In vitro studies have established the inhibitory effect of Reuterin, a bacteriocin, produced by *Lactobacillus* spp. on the growth of *Salmonella* spp., *Shigella* spp., *Clostridium* spp., and *Listeria* spp. (Naidu et al., 1999). Increasing these types of useful bacteria along with substrates for their proliferation and metabolism could improve feed intake and nutrient utilization. *Escherichia−Shigella* in the crop and cecal contents were reported to have a negative effect on the weight gain of broilers and the fecal fat digestibility of broilers (Rubio et al., 2015).

Several studies have reported a higher abundance of lactobacilli and bifidobacteria in the intestinal microbiota of chickens fed prebiotic-supplemented diets (Gaggìa et al., 2010). Potential mechanisms proposed for the observed prebiotic-mediated intestinal microbiome changes include competitive exclusion of pathogenic bacteria (Callaway et al., 2008), production of antimicrobial molecules (Chen et al., 2007), host adaptive immune system stimulation (Babu et al., 2012), and improvements in intestinal morphology (Chee et al., 2010). The application of exogenous enzymes which could break down dietary NSP to short-chain oligosaccharides provides substrates to promote the growth of the commensal microbial population in the hindgut (Choct et al., 1996; Courtin et al., 2008). Xylo-oligosaccharides are chains of β-1,4-linked D-xylopyranoside units, produced by enzymatic degradation of arabinoxylans by endoxylanases. XOS supplementation (2 g/kg) has been reported to increase the relative abundance of the *Lactobacillus* genus in the cecum (Pourabedin et al., 2015). XOS supplementation to broiler diets, on top of a wheat-rye-based diet, improved the lactobacilli population in the hindgut. Chickens fed XOS supplemented diets showed an increased number of butyryl-CoA: acetate-CoA transferase gene copies in the ceca, which is known to play a key role in the bacterial butyrate production (De Maesschalck et al., 2015). A broiler study conducted using the xylanase, tested in the current work, showed an increase in butyrate production in the hindgut lending support for the in vivo prebiotic effect of XOS generated (Van Hoeck et al., 2021a). The reduction in the cecal relative abundance of the unclassified *Escherichia-Shigella* spp. in diets with 90,000 U/kg may be associated with the increase in *Lactobacillus* spp. (Figure 3). While the weight gain was unaffected by added xylanase, feed intake was lowered, and feed efficiency was improved (Table 4), indicating that the enzyme improves the intestinal environment to increase the abundance of beneficial *Lactobacillus* spp.

CONCLUSIONS AND APPLICATIONS

1. Supplementation of a novel monocomponent xylanase to a corn-wheat-soybean meal-based diet improved the feed efficiency, ileal nutrient digestibility, and ileal digestible energy in young broilers.
2. Xylanase supplementation showed benefits 'beyond' improved performance and digestion, including the modulation of intestinal microbiome toward a higher relative abundance of commensal bacteria such as *Lactobacillus* and lower abundance of potentially non-beneficial bacteria such as *Escherichia-Shigella.*

3. Speculatively, this observed shift in the gut microbiome toward increased populations of probiotic bacteria will have beneficial effects on intestinal health.

ACKNOWLEDGMENTS

The authors acknowledge Kemin Industries, Inc. for funding this project.

DISCLOSURES

B.L. Vasanthakumari, M. Di Benedetto, D. Gonzalez Sanchez, A. Wealleans are employees of Kemin Industries. The authors declare that we have no financial, commercial, legal, or professional relationship with other organizations, or with the people working with them, that could influence the content of this research paper.

REFERENCES

Abdollahi, M. R., V. Ravindran, and B. Svihus. 2013. Influence of grain type and feed form on performance, apparent metabolizable energy and ileal digestibility of nitrogen, starch, fat, calcium and phosphorus in broiler starters. Anim. Feed Sci. Technol. 186:193–203.

Adeola, O., and A. J. Cowieson. 2012. Board-invited review: opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. J. Anim. Sci. 89:3189–3218.

Aliakbarpour, H. R., M. Chamani, G. Rahimi, A. A. Sadeghi, and D. Qujeq. 2012. The Bacillus subtilis and lactic acid bacteria probiotics influences intestinal mucin gene expression, histomorphology and growth performance in broilers. Asian-Australas. J. Anim. Sci 25:1285–1293.

AOAC. 2016. Official Methods of Analysis. 18th ed. Association of Official Analytical Chemists, Washington, DC.

Babu, U. S., K. Sommers, L. M. Harrison, and K. V. Balan. 2012. Effects of fructooligosaccharide-inulin on Salmonella-killing and inflammatory gene expression in chicken macrophages. Vet. Immunol. Immunopathol. 149:92–96.

Bedford, M. R. 2000. Exogenous enzymes in monogastric nutrition - their current value and future benefits. Anim. Feed Sci. Technol. 86:1–13.

Bedford, M. R., and A. J. Cowieson. 2012. Exogenous enzymes and their effects on intestinal microbiology. Anim. Feed Sci. Technol. 173:76–85.

Bedford, M. R., and G. G. Partridge. 2010. Enzymes in Farm Animal Nutrition. CAB International, Wallingford, UK.

Bolyen, E., J. R. Rideout, M. R. Dillon. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37:852–857.

Bonnin, E., S. Daviet, J. F. Sorensen, O. Sibbesen, A. Goldson, N. Juge, and L. Saulnier. 2006. Behaviour of family 10 and 11 xylanases towards arabinoxylans with varying structure. J. Sci. Food Agric. 86:1618–1622.

Calik, A., and A. Ergün. 2015. Effect of lactulose supplementation on growth performance, intestinal histomorphology, cecal microbial population, and short-chain fatty acid composition of broiler chickens. Poult. Sci. 94:2173–2182.

Callahan, B., P. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods. 13:581–583.

Callaway, T. R., T. S. Edrington, R. C. Anderson, R. B. Harvey, K. J. Genovese, C. N. Kennedy, D. W. Venn, and D. J. Nisbet. 2008. Probiotics, prebiotics and competitive exclusion for prophylaxis against bacterial disease. Anim. Health Res. Rev. 9:217–225.

Chee, S. H., P. A. Iji, M. Choct, L. L. Mikkelsen, and A. Kocher. 2010. Functional interactions of manno-oligosaccharides with dietary threonine in chicken gastrointestinal tract. I. Growth performance and mucin dynamics. Br. Poult. Sci. 51:658–660.

Chen, Y. S., S. Srionnual, T. Onda, and F. Yanagida. 2007. Effects of prebiotic oligosaccharides and trehalose on growth and production of bacteriocins by lactic acid bacteria. Lett. Appl. Microbiol. 45:190–193.

Choct, M., and G. Annison. 1992. Anti-nutritive effect of wheat pentosans in broiler chickens: roles of viscosity and gut microflora. Br. Poult. Sci. 33:821–834.

Choct, M., R. J. Hughes, J. Wang, M. R. Bedford, A. J. Morgan, and G. Annison. 1996. Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. Br. Poult. Sci. 37:609–621.

Courtin, C. M., W. F. Broekaert, K. Swennen, O. Lescroart, O. Onagbesan, J. Buyse, E. Decuypere, T. Van de Wiele, M. Marzorati, W. Verstraete, G. Huyghebaert, and J. A. Delcour. 2008. Dietary inclusion of wheat bran arabinoxylooligosaccharides induces beneficial nutritional effects in chickens. Cereal Chem 85:607–613.

Cowieson, A. J., M. R. Bedford, and V. Ravindran. 2010. Interactions between xylanase and glucanase in maize-soy-based diets for broilers. Br. Poult. Sci. 51:246–257.

Cowieson, A. J., and H. V. Masey-O'Neill. 2013. Effects of exogenous xylanase on performance, nutrient digestibility, and caecal thermal profiles of broilers given wheat-based diets. Br. Poult. Sci. 54:346–354.

De Maesschalck, C., V. Eeckhaut, L. Maertens, L. De Lange, L. Marchal, C. Nezer, S. De Baere, S. Croubels, G. Daube, J. Dewulf, F. Haesebrouck, R. Ducatelle, B. Taminau, and F. Van Immerseel. 2015. Effects of xylo-oligosaccharides on broiler chicken performance and microbiota. Appl. Environ. Microbiol. 81:5880–5888.

Forder, R. E., G. S. Howarth, D. R. Tivey, and R. J. Hughes. 2007. Bacterial modulation of small intestinal goblet cells and mucin composition during early posthatch development of poultry. Poult Sci 86:2396–2403.

Gaggìa, F., P. Mattarelli, and B. Biavati. 2010. Probiotics and prebiotics in animal feeding for safe food production. Int. J. Food Microbiol. 141:S15–S28.

Glendinning, L., K. A. Watson, and M. Watson. 2019. Development of the duodenal, ileal, jejunal and caecal microbiota in chickens. Anim. Microbiome 1:17.

Guerre, P. 2016. Worldwide mycotoxins exposure in pig and poultry feed formulations. Toxins 8:350.

Hayashi, R. M, M. C. Lourenço, A. L. Kraieski, R. B. Araujo, R. Gonzalez-Esquerra, E. Leonardecz, A. F. da Cunha, M. F. Carazzolle, P. S. Monzani, and E. Santin. 2018. Effect of feeding Bacillus subtilis spores to broilers challenged with *Salmonella enterica* serovar Heidelberg Brazilian strain UFPR1 on performance, immune response, and gut health. Front. Vet. Sci. 5:13.

Hu, J., V. Arantes, A. Pribowo, and J. N. Saddler. 2013. The synergistic action of accessory enzymes enhances the hydrolytic potential of a "cellulase mixture" but is highly substrate specific. Biotechnol. Biofuels 6:112.

Javier, AL.-P., A. Anna, and K. Eva Nordberg. 2018. Structural considerations on the use of endo-xylanases for the production of prebiotic xylooligosaccharides from biomass. Curr. Protein Pept. Sci 19:48–67.

Jaworski, N. W., H. N. Lærke, K. E. Bach Knudsen, and H. H. Stein. 2015. Carbohydrate composition and in vitro digestibility of dry matter and nonstarch polysaccharides in corn, sorghum, and wheat and coproducts from these grains. J. Anim. Sci. 93:1103–1113.

Józefiak, D., A. Rutkowski, S. Kaczmarek, B. B. Jensen, R. M. Engberg, and O. Højberg. 2010. Effect of β-glucanase and xylanase supplementation of barley- and rye-based diets on caecal microbiota of broiler chickens. Br. Poult. Sci. 51:546–557.

Kiarie, E., L. F. Romero, and L. F. Ravindran. 2014. Growth performance, nutrient utilization, and digesta characteristics in broiler chickens fed corn or wheat diets without or with supplemental xylanase. Poult. Sci. 93:1186–1196.

Klindworth, A., E. Pruesse, T. Schweer, J. Peplies, C. Quast, M. Horn, and F. O. Glockner. 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res 41:e1.

Kocher, A., M. Choct, G. Ross, J. Broz, and T. K. Chung. 2003. Effects of enzyme combinations on apparent metabolizable energy of corn-soybean meal-based diets in broilers. J. Appl. Poult. Res. 12:275–283.

Kridtayopas, C., C. Rakangtong, C. Bunchasak, and W. Loongyai. 2019. Effect of prebiotic and synbiotic supplementation in diet on growth performance, small intestinal morphology, stress, and bacterial population under high stocking density condition of broiler chickens. Poult. Sci. 98:4595–4605.

Li, J., Z. Miao, W. Tian, Y. Yang, J. Wang, and Y. Yang. 2017. Effects of different rearing systems on growth, small intestinal morphology and selected indices of fermentation status in broilers. Anim. Sci. J. 88:900–908.

Lokapirnasari, W. P., T. B. Pribadi, A. Al Arif, S. Soeharsono, S. Hidanah, N. Harijani, R. Najwan, K. Huda, H. C. P. Wardhani, and N. F. N Rahman. 2019. Potency of probiotics *Bifidobacterium* spp. and *Lactobacillus casei* to improve growth performance and business analysis in organic laying hens. Vet. World. 12:8607.

Lozupone, C., and R. Knight. 2005. UniFrac: A new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. 71:8228–8235.

Masey O'Neill, H. V., and N. Lui. 2011. Effect of xylanase on performance and apparent metabolisable energy in starter broilers fed diets containing one maize variety harvested in different regions of china. Asian-Australas. J. Anim. Sci. 25:515–523.

McDevitt, R. M., J. D. Brooker, T. Acamovic, and N. H. C. Sparks. 2006. Necrotic enteritis; a continuing challenge for the poultry industry. Worlds Poult. Sci. J. 62:221–248.

Naidu, A. S., W. R. Bidlack, and R. A. Clemens. 1999. Probiotic spectra of lactic acid bacteria (LAB). Crit. Rev. Food Sci. Nutr. 39:13–126.

Nguyen, H. T., M. R. Bedford, and N. K. Morgan. 2021. Importance of considering non-starch polysaccharide content of poultry diets. Worlds Poult. Sci. J. 77:619–637.

Oakley, B. B., H. S. Lillehoj, M. H. Kogut, W. K. Kim, J. J. Maurer, A. Pedroso, M. D. Lee, S. R. Collett, T. J. Johnson, and N. A. Cox. 2014. The chicken gastrointestinal microbiome. FEMS Microbiol. Lett. 360:100–112.

Pedersen M.B. Department of Animal Science, Aarhus University; Aarhus, Denmark: 2015. Xylanases to improve the nutritional value of high fibre diets based on corn and wheat DDGS. Ph.D. Thesis

Pourabedin, M., L. Guan, and X. Zhao. 2015. Xylo-oligosaccharides and virginiamycin differentially modulate gut microbial composition in chickens. Microbiome 3:1–12.

Ravindran, V., L. I. Hew, G. Ravindran, and W. L. Bryden. 2005. Apparent ileal digestibility of amino acids in feed ingredients for broiler chickens. Anim. Sci. 81:85–97.

Richards-Rios, P., J. Fothergill, M. Bernardeau, and P. Wigley. 2020. Development of the ileal microbiota in three broiler breeds. Front. Vet. Sci. 7:17.

Rodríguez, M. L., A. Rebolé, S. Velasco, L. T. Ortiz, J. Treviño, and C. Alzueta. 2012. Wheat- and barley-based diets with or without additives influence broiler chicken performance, nutrient digestibility and intestinal microflora. J. Sci. Food Agric. 92:184–190.

Rose, D. J., and G. E. Inglett. 2011. A method for the determination of soluble arabinoxylan released from insoluble substrates by xylanases. Food Anal. Methods. 4:66–72.

Rubio, L. A., M. J. Peinado, R. Ruiz, E. Suárez-Pereira, C. Ortiz Mellet, and J. M. García Fernández. 2015. Correlations between changes in intestinal microbiota composition and performance parameters in broiler chickens. J. Anim. Physiol. Anim. Nutr. (Berl) 99:418–423.

Short, F. J. P., J. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium oxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Technol. 59:215–221.

Stefanello, C., S. L. Vieira, P. S. Carvalho, J. O. B. Sorbara, and A. J. Cowieson. 2016. Energy and nutrient utilization of broiler chickens fed corn-soybean meal and corn-based diets supplemented with xylanase. Poult. Sci. 95:1881–1887.

Van Hoeck, V., D. Wu, I. Somers, A. Wealleans, B. L. Vasanthakumari, A. L. Gonzalez Sanchez, and D. Morisset. 2021. Xylanase impact beyond performance: a prebiotic approach in broiler chickens. J. Appl. Poult. Res. 30:100193.

Van Hoeck, V., I. Somers, A. Abdelqader, A. L. Wealleans, S. Van de Craen, and D. Morisset. 2021. Xylanase impact beyond performance: a microbiome approach in laying hens. Plos One 16 p.e0257681.

Vardakou, M., P. Katapodis, M. Samiotaki, D. Kekos, G. Panayotou, and P. Christakopoulos. 2003. Mode of action of family 10 and 11 endoxylanases on water-Unextractable arabinoxylan. Int. J. Biol. Macromol. 33:129–134.

Wang, Z. R., S. Y. Qiao, W. Q. Lu, and D. F. Li. 2005. Effects of enzyme supplementation on performance, nutrient digestibility, gastrointestinal morphology, and volatile fatty acid profiles in the hindgut of broilers fed wheat-based diets. Poult. Sci. 84:875–881.

Yilmaz, P., L. W. Parfrey, P. Yarza, J. Gerken, E. Pruesse, C. Quast, T. Schweer, J. Peplies, W. Ludwig, and F. O. Glöckner. 2014. The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. Nucleic Acids Res 42:D643–D648.