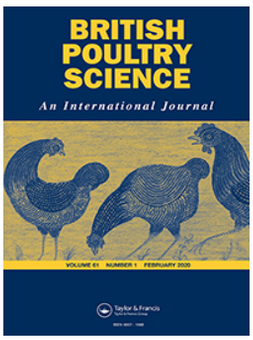

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Xylanase and xylo- oligosaccharide prebiotic improve the growth performance and concentration of potentially prebiotic oligosaccharides in the ileum of broiler chickens

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ABSTRACT

1. The objective of this study was to investigate the effect of supplementing broiler diets with xylanase or xylo- oligosaccharide (XOS) on growth performance, the concentration of non-starch polysaccharide (NSP) hydrolysis products in the ileum and concentration of short chain fatty acids (SCFA) in the caeca of broiler chickens.

2. In total, 500 male Ross 308 broilers were used in this 29-day (d) study. The treatments were organised into a 2 × 2 plus 1 factorial arrangement consisting of two additives (xylanase or XOS) at two levels (low or high) plus a control treatment with no additives. This gave five treatments with 100 birds in each treatment group. The diets were slightly deficient in protein by 20 g/kg and energy by 1 MJ/kg.

3. On d 14 and 28, two birds per pen were euthanised, the caeca content collected and analysed for short chain fatty acid (SCFA) concentration. On d 29, six birds per pen were euthanised and ileal digesta were collected and analysed for the concentration of NSP fractions.

4. On d 14, caecal acetic acid, iso-butyric acid, iso-valeric acid, n-valeric acid and total SCFA concentrations were significantly greater ($P \leq 0.05$) when diets were supplemented with XOS compared with xylanase.

5. Ileal concentration of arabinose, galactose and glucuronic acid (GlucA2) were significantly greater ($P \leq 0.05$) in the insoluble NSP fraction when diets were supplemented with a high level of xylanase, compared with the control treatment. Ileal concentration of fructose was significantly greater ($P \leq 0.05$) in the water soluble NSP when a high level of xylanase or low level of XOS were included in the diet compared with the control.

6. It was concluded that xylanase and XOS had similar effects on NSP concentration and SCFA in the caeca, although there was little effect on performance. This observation demonstrated further benefits of xylanase supplementation in wheat-based broiler diets beyond digesta viscosity reduction and the release of extra nutrients.

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Broilers; nutrient digestibility; performance; SCFA; XOS; xylanase

Introduction

Depression in growth performance caused by an increase in digesta viscosity is a common occurrence in broiler diets containing a large amount of non-starch polysaccharides (NSP; Jia et al. 2009). In order to overcome this, carbohydrase enzymes are often added to broiler diets to improve nutrient utilisation and increase productivity. Carbohydrases hydrolyse NSP, breaking it down into smaller oligosaccharides. This results in a decrease in digesta viscosity and the release of encapsulated nutrients (Knudsen 2014). In addition to these benefits, it has been suggested that the small oligosaccharides produced during NSP hydrolysis could have prebiotic properties (Courtin et al. 2008).

One way of investigating the production of small oligosaccharides is to measure the concentration of NSP hydrolysis products in the ileum of broilers, such as arabinose or xylose concentrations. A prebiotic is a small molecule which is fermented by beneficial bacteria, encouraging their growth, while discouraging the growth of pathogenic bacteria. Xylo-oligosaccharides (XOS) are associated with improvements in poultry performance (Al-Sultan et al. 2016) by modulating the gastrointestinal immune system, microbial populations (Jung et al. 2008; Yang et al. 2008) and increasing short chain fatty acid (SCFA) production, however, evidence for this has been inconsistent (Arsi et al. 2015; Gao et al. 2007).

To investigate the production of potentially prebiotic oligosaccharides during xylanase activity, monosaccharides were measured in the ileal digesta and compared to monosaccharides in digesta from birds supplemented with purified XOS. The objective of the trial was to investigate similarities in profiles of monosaccharides in digesta from birds receiving xylanase and those receiving XOS to illustrate that NSP hydrolysis products may have prebiotic-like effects similar to that of purified XOS.

NSP hydrolysis products are thought to be fermented by beneficial bacteria such as *Bifidobacter* and *Lactobacilli spp.*, producing SCFA (Lee et al. 2017). An increase in the concentration of SCFAs is often associated with an increase in the population of beneficial bacteria and a decrease in pathogenic bacteria (Engberg et al. 2004). In addition to this, SCFAs have been shown to influence growth performance in broilers. Butyrate, in particular, is regarded as an available energy source, increasing the energy available to the host for growth (Ravangard et al. 2017). Supplementing broiler diets with xylanase or XOS has been shown to affect the

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production of SCFA in the caeca of broiler chickens (Engberg et al. 2004; Lee et al. 2017). This could suggest that both additives have a similar mode of action. As such, the effect of xylanase or XOS on SCFA concentration in the caeca of broilers was investigated in the current study.

The objective of this experiment was to investigate the effect of supplementing wheat-based diets, which were deficient in energy and protein, with xylanase or XOS on growth performance, the concentration of NSP hydrolysis products in the ileum and the concentration of SCFA's in the caeca of broiler chickens.

Materials and methods

Animals and management

All the procedures in the experiment were approved by the SRUC Animal Experiment Committee.

Five hundred male Ross 308, one-d-old broilers were allocated to one of five treatments organised as a randomised complete block design. The birds were housed 10 in a pen, with ten pen replicates per treatment and provided with feed and water on an *ab libitum* basis throughout the experiment (0 to 29 d). The treatments followed a 2 × 2 plus 1 factorial arrangement with two different additives (xylanase or XOS) at two inclusion levels (high and low) plus the control. The low level of xylanase inclusion was 16,000 XU/kg and the high was 32,000 XU/kg. The low level of XOS inclusion was 0.25 g/kg and the high level was 1.0 g/kg. The lower level of each additive was based on the standard recommendation by the manufacturer while the higher level of each additive was chosen following a literature search (Wang et al. 2005; Zhenping et al. 2013; De Measschalck et al. 2015). The control diet was deficient in energy by 1 MJ/kg and protein reduced by 3%, to 20 g/kg CP however; all other nutrient requirements were met to ensure that any effects were induced by energy or protein deficiency or additive supplementation alone. The diets were formulated to be deficient in energy and protein to allow any improvements in growth or nutrient digestibility to become apparent, as previous work has indicated that xylanase supplementation may be more beneficial in nutrient-deficient diets (Francesch and Geraert 2009). The experiment was split into five treatments; (1) control, (2) control plus xylanase 16,000 BXU/kg, (3) control plus xylanase 32,000 BXU/kg, (4) control plus purified XOS 0.25 g/kg and (5) control plus purified XOS 1.0 g/kg. All of the experimental diets were provided in mash form and birds had *ad libitum* access to feed and water throughout the feeding trial. The xylanase used contained 160 000 U of endo- 1,4 β xylanase activity per gram. One unit of xylanase activity was defined as the amount of enzyme required to liberate 1 µmol of reducing sugars from xylan using a standardised test (Enzyme Services (ESC), Hengoed, Ystrad Mynack, UK). The xylanase (Econase XT) was supplied by AB Vista, Marlborough, UK. The XOS used in this study was purchased from Shandong Lifelong Bio-technology Co., China (XOS 35A). The ingredient and chemical composition of the control diet is shown in Table 1. Wheat bran was included in the diet during this study to increase the amount of NSP in the diet and maximise the potential for prebiotic oligosaccharide generation during NSP hydrolysis by xylanase.

Table 1. The calculated and analysed nutrient content of wheat-based diets supplemented with enzymes or prebiotic oligosaccharide.

Ingredient (g/kg)	Control
Wheat	556.5
Wheat Bran	192.5
Soybean meal	152.5
Soya oil	25.0
Limestone	12.5
Dicalcium Phos, 18%P	14.0
Sodium Bicarbonate	6.0
Lysine HCl	5.0
Methionine	2.0
Threonine	2.0
Valine	2.0
Vitamin & Mineral premix	5.0
TiO_2 Marker	25.0
Total	1000.0
Calculated content	
ME (MJ/kg)	11.41
Crude Protein (g/kg)	200.0
Calcium (g/kg)	10.0
Phosphorus (g/kg)	7.5
nPP (g/kg)	4.9
Na (g/kg)	2.1
Cl (g/kg)	3.5
Analysed nutrient content	
DM (g/kg)	877.1
AME (MJ/kg)	16.19
Na (g/kg)	1.9
Cl (g/kg)	3.1
Calcium (g/kg)	9.6
Phosphorus (g/kg)	6.3
N (g/kg)	31.3

Notes; TiO_2- titanium dioxide; Na- sodium; Cl- chloride; DM- dry matter; AME- apparent metabolizable energy; N- nitrogen

Growth performance

Feed and birds were weighed on d 0, 14 and 28. The data from feed and bird weights were used to calculate body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR).

Sample collection

On d 14 and 28, two birds per pen were euthanised by cervical dislocation and used to collect caeca content for SCFA analysis. Following euthanasia, the caeca were removed and the content was gently squeezed into a collection tube.

On d 29, the remaining six birds per pen were euthanised by cervical dislocation and ileal content was collected for nutrient digestibility and NSP analysis. Once located, the distal half of the ileum was removed and the contents were flushed with water into a collection pot. The ileal digesta from all six birds per pen was pooled and was collected on day 29, as opposed to day 28, due to practical reasons relating to volume of samples to be collected.

Short chain fatty acid analyses

Once the contents of the caeca were removed they were stored at -20°C and later analysed for SCFA concentration

as described by Khattak et al. (2018) using gas chromatography.

NSP fraction analyses

The ileal digesta samples collected from six birds per pen on d 29 were dried in a Unitherm force draft drying oven for 48 hours at 80°C. The samples were analysed for water extractable (WE) carbohydrate components and water unextractable (WU) NSP using HPLC, following the method of Englyst et al. (1994). The pre-caecal NSP concentration was calculated using the equation displayed in the calculations section.

Chemical analysis

The ileal digesta samples collected on d 29 were dried prior to conducting titanium and DM analysis according to the method of Short et al. (1996). Dry matter (DM) was determined using standard methods from AOAC (2006), whereby 1 g of the sample was dried in a uniform forced drying oven (Unitherm, Russel-Lindsay Engineering Ltd, Birmingham, England, UK) at 95°C for 24 hours. Nitrogen determination was carried out by the combustion method (Method 934.01; AOAC). Gross energy was determined using an isoperibol bomb calorimeter system using benzoic acid as an internal standard (Model 6200, Parr Instruments, Moline, Illinois, USA). Ileal digestibility was calculated using the index method described by Olukosi et al. (2007). The activity of xylanase in the diet was measured using a commercial test kit (Enzyme Services (ESC), Hengoed, Ystrad Mynack, UK). One BXU was defined as the amount of xylanase enzyme required to liberate 1 nmol of reducing sugars per minute from xylan at pH 5.3.

Calculations

Pre-caecal NSP concentration (g/100g DM intake) was calculated using the equation below:

$$\text{NSP Concentration} = \text{NSP conc. in digesta} \times \left(\frac{\text{Ti in diet}}{\text{Ti in digesta}} \right)$$

$$\text{NSP Conc. In digesta (\%)}$$

Statistics

Statistical analysis was conducted using the ANOVA function of Genstat (16th Edition). Data were analysed following the 2 × 2 plus 1 factorial arrangement with body weight as the blocking factor. When an interaction between additive type and inclusion level (excluding the no additive treatment) was significant, the means for growth performance and nutrient digestibility were separated using Tukey's test. The significant additive type × inclusion level interactions for ileal NSP concentration were detected using specific contrasts details of which are in Tables 5 and 6. Significance was set at $P \leq 0.05$ and trends at $P < 0.1$.

Results

Growth performance of broilers on days 14 and 28

The enzyme analysis results showed that diets 1, 4 and 5 contained xylanase activity below detection threshold. Diet 2 contained 16,600 BXU/kg and diet 3 contained 36,900 BXU/kg of xylanase activity.

On d 14 there was no additive type × inclusion level interaction for BWG, FI or FCR both on d 14 and 28 (Table 2). However, there were main effects of additive type and inclusion level on d 14. Body weight gain and feed intake were greater ($P < 0.05$) following xylanase supplementation compared with XOS supplementation. Feed conversion ratio was lower ($P < 0.05$) following additive supplementation at high inclusion level compared with low inclusion level. There was no effect of additive supplementation compared with the unsupplemented control.

On d 28, there were no significant ($P < 0.05$) main or interaction effects of additive type or inclusion level for BWG, FI or FCR. However, feed intake and FCR values were lower ($P < 0.001$) for broilers receiving xylanase or XOS compared with the control.

Nutrient digestibility in broilers aged 29 days and fed diets supplemented with xylanase or XOS

Nutrient digestibility in broilers aged 29 d and fed diets supplemented with xylanase or XOS is shown in Table 3. There was a significant additive type × inclusion level interaction for nitrogen (N) intake. Birds receiving diets containing the low level of xylanase had significantly ($P < 0.01$) lower N intake than those receiving the high level of xylanase. There was no effect of additive type or inclusion level on DM or N digestibility, however, there was trend for greater ileal digestible energy (IDE) in birds fed diets supplemented with xylanase compared to those with XOS. The DM, IDE and N digestibilities were lower ($P < 0.05$) in birds fed diets containing xylanase or XOS compared to the control. The N and gross energy intake were lower ($P < 0.05$) in birds fed diets containing xylanase or XOS compared to the control diets.

Short chain fatty acid concentration in the caeca of broiler chickens on days 14 and 28

Caecal SCFA concentration in response to xylanase or XOS supplementation on d 14 is shown is Table 4. There was no significant inclusion level or additive type × inclusion level interaction for SCFA concentration on d 14, however there was a main effect of ingredient type. The concentration of acetic acid, propionic acid, iso-butyric acid, iso- valeric acid and total SCFA were greater ($P < 0.05$) following XOS compared with xylanase supplementation. There was no effect of ingredient inclusion compared to the control treatment on acetic acid, n-butyric acid or n-valeric acid concentration in broilers on d 14. However, there was a trend ($P = 0.066$) for xylanase to decrease propionic acid concentration and XOS to increase propionic acid concentration.

Caecal SCFA concentration in response to xylanase or XOS supplementation on d 28 is shown in Table 4. There was no additive type × inclusion level interaction for SCFA concentration on d 28. There was no main effect of ingredient type or inclusion level on caecal SCFA concentration on day 28. The

Table 2. The growth performance of broilers fed diets deficient in energy and protein and supplemented with xylanase or XOS.

		Day 14			Day 28		
Additive	Inclusion level	BWG (g/bird)	FI (g/bird)	FCR	BWG (g/bird)	FI (g/Bird)	FCR
No Additive		388.1	596.2	1.54	1313.3	2953.9	2.26
Xylanase	Low	381.1	594.1	1.56	1294.5	2707.1	2.10
	High	394.3	579.2	1.47	1320.0	2546.5	1.93
XOS	Low	356.7	561.5	1.59	1261.6	2536.5	2.03
	High	375.1	561.7	1.51	1306.4	2604.7	2.00
	SEM	10.449	12.08	0.038	29.202	86.161	0.078
P-value of control vs additive[1]		0.390	0.111	0.752	0.592	<0.001	0.009
Means for main effect of additive type (AT)							
Xylanase		387.7[b]	586.7[b]	1.52[a]	1307.3	2626.8	2.02
XOS		365.9[a]	561.6[a]	1.55[b]	1284.0	2570.6	2.02
	SEM	10.449	12.08	0.038	29.202	86.161	0.078
Means for main effect of inclusion level (IL)							
	Low	368.9	577.8	1.57	1278.0	2621.8	2.07
	High	384.7	570.4	1.49	1313.2	2570.6	1.97
	SEM	10.449	12.08	0.038	29.202	86.161	0.078
P values							
Additive Type (AT)		0.044	0.045	0.449	0.430	0.518	0.978
Inclusion Level (IL)		0.139	0.543	0.034	0.236	0.595	0.197
AT × IL		0.806	0.535	0.944	0.744	0.193	0.385

XOS- xylo-oligosaccharide; BWG- body weight gain; FI- feed intake; FCR; feed conversion ratio; [1]P- values for control vs. additive types – the mean for the control group was compared to all other treatments containing an additive irrespective of type or level; [ab] different superscripts with the same column indicate means that are significantly ($P < 0.05$) different.

concentration of acetic acid, n-butyric acid and total SCFA were greater ($P < 0.05$) following additive supplementation compared to the control. The concentration of acetic acid, propionic acid and total SCFA was greater ($P < 0.05$) in birds aged 28 days compared to birds aged 14 days.

NSP fraction content of ileal digesta from broiler chickens aged 29 days

The concentration of WU NSP fractions in response to xylanase or XOS supplementation is shown in Table 5. There was an ingredient type × inclusion level interaction for arabinose and galactose. WU arabinose and galactose concentration were greater when diets were supplemented with the high level of xylanase compared to the low level. When the control treatment was compared to the other treatments individually, arabinose and galactose concentration were greater ($P < 0.05$) following xylanase supplementation at a high level, compared to the control. There were significant main effects of ingredient type and inclusion levels. Rhamnose concentration was greater ($P < 0.001$) following XOS compared to xylanase supplementation. Rhamnose and fructose concentration were greater ($P < 0.05$) following supplementation at a high level compared to the low level. The concentration of rhamnose, fructose, arabinose and galactose were greater ($P < 0.05$) following supplementation compared with the control.

The concentration of WE NSP fractions in response to xylanase or XOS supplementation is shown in Table 6. There was a significant ingredient type × inclusion level interaction for fructose concentration. The WE fructose concentration was greater when the high level of xylanase or the low level of XOS were included in the diet, compared to the low level of xylanase or high level of XOS. When the control treatment was compared to the other diets individually, WE fructose concentration was greater ($P < 0.05$) following supplementation of 32,000 XU/kg xylanase or 0.25 g/kg XOS compared to the control. There were no main effects of ingredient type or inclusion level for WE NSP concentration. There was a tendency ($P = 0.061$) for greater xylose concentration in diets supplemented with XOS compared to xylanase. There was a tendency for greater galactose concentration in diets supplemented with the high compared to the low inclusion level. Galactose and total WE NSP concentration were greater ($P < 0.05$) following supplementation compared with the control.

Discussion

Xylanase is used routinely in broiler diets to improve growth performance, however, there is evidence to suggest that potentially prebiotic oligosaccharides are generated during NSP hydrolysis. The generation of *in-situ* prebiotics could provide additional benefit to the use of xylanases in broiler diets over and above the reduction in digesta viscosity.

Growth performance

The birds in the current study performed below breed standards. This could be due to a multitude of reasons, including diet form and composition. It has been well established in the literature that low protein (23–20% CP) or low energy (3000–2640 Kcal/kg) diets reduce the growth performance of broilers (Govil et al. 2017; Kamran et al. 2008; Williams et al. 2014). The current study is in agreement with this, as the birds receiving the control feed, which was lower in energy by 8% and protein by 13%, ate significantly more than those birds receiving supplementation in their diet. This was expected to an extent, as it has been suggested that birds do not eat to satisfy hunger *per se* but they consume enough feed to satisfy their energy or protein requirements (Kamran et al. 2008). In the current study, the birds receiving diets low in energy and protein were able to increase their feed intake, allowing them to maintain their growth. When xylanase or XOS was added into the diet, feed intake reduced, as expected, resulting in a reduction in FCR and an increase in efficiency.

Xylanase supplementation improved BWG, FI and FCR compared to that of XOS on d 14. This was expected as the

Table 3. Coefficients of energy and nitrogen digestibility of broilers aged 29 days and fed diets supplemented with xylanase or XOS.

Additive	Inclusion level	DM	IDE (MJ/kg)	N	Energy intake (units/bird)	N intake (units/bird)
No additive		0.713	13.77	0.858	38.44	85.37
Xylanase	Low	0.652	13.28	0.837	33.23	81.18[b]
	High	0.606	11.99	0.817	29.62	69.15[a]
XOS	Low	0.630	12.22	0.812	29.54	70.23[ab]
	High	0.624	12.23	0.821	30.26	79.64[ab]
	SEM	0.0189	0.372	0.00913	1.499	1.987
P-value of control vs additive[1]		<0.001	0.003	0.001	<0.001	0.002
Means for main effect of additive type (AT)						
Xylanase		0.629	12.63	0.827	31.39	75.71
XOS		0.627	12.23	0.816	29.94	74.39
	SEM	0.0189	0.263	0.00646	1.06	1.987
Means for main effect of inclusion level (IL)						
	Low	0.641	12.75	0.824	31.43	75.16
	High	0.615	12.11	0.819	29.90	74.94
	SEM	0.0189	0.263	0.00646	1.06	1.405
P values						
Additive Type (AT)		0.170	0.095	0.550	0.317	0.937
Inclusion Level (IL)		0.909	0.284	0.245	0.343	0.643
AT × IL		0.302	0.090	0.115	0.158	<0.001

XOS- xylo-oligosaccharide; DM- dry matter; IDE- ileal digestible energy; N- nitrogen; [1]P- values for control vs. additive types – the mean for the control group was compared to all other treatments containing an additive irrespective of type or level; [abc] different superscripts with the same column indicate means that are significantly ($P < 0.05$) different.

mechanism of action of xylanase is well established in the literature. Xylanase cleaves the arabinoxylan (AX) backbone of NSP releasing the trapped nutrients (Meng and Slominski 2005) and reduces digesta viscosity (Lentle 2005). This impacts on growth performance in two ways. Firstly, the release of trapped nutrients increases the amount available for absorption in the small intestine (Meng and Slominski 2005). Secondly, reducing digesta viscosity allows sufficient mixing of the digesta, enabling more nutrients to be absorbed (Lentle 2005). There is evidence to suggest a third mechanism, namely generation of *in-situ* prebiotic oligosaccharides. During the hydrolysis of NSP, smaller oligosaccharides such as XOS are generated which have been shown to have prebiotic-like effects (Zhang et al. 2014).

Prebiotics can improve growth performance of broilers (Al-Sultan et al. 2016; Abdel- Hafeez et al. 2017). In the current study, on d 28, XOS reduced feed intake and improved FCR but had no effect on body weight gain, which was similar to the effect of xylanase supplementation. It is thought that prebiotics improve growth performance by increasing nutrient absorption due to modulating gut microflora and increasing gut integrity (Al-Sultan et al. 2016). Beneficial gut bacteria, such as *Bifidobacterium* and *Lactobacilli spp*., ferment prebiotics, such as XOS, FOS and GOS, which encourages growth whilst discouraging the colonisation of pathogenic bacteria (Xu et al. 2003; Courtin et al. 2008; Yousaf et al. 2016). This may relate to the SCFA results, discussed below.

Nutrient digestibility

Additives such as xylanase have been shown to improve nutrient digestibility (Kiarie et al. 2014). The expectation that adding carbohydrases can improve nutrient digestibility is logical, as the enzyme should decrease digesta viscosity and allow increased absorption of nutrients (Mathlouthi et al. 2002). In the current study, DM and N digestibility were reduced when either xylanase or XOS were added to the diet, however, IDE was significantly lower when XOS was included in the diet. The diets used during this study were deficient in energy and protein, so the aim of adding such additives was to improve nutrient absorption, as this is what has been described in the literature (Cowieson et al. 2017). The addition of xylanase or XOS reduced digestible energy and N intake compared to the control treatment, which may help explain why nutrient digestibility was decreased following supplementation.

The negative effect of XOS supplementation on IDE was unexpected. The literature often reports no effect of

Table 4. The effect of feeding wheat-based diets supplemented with xylanase or XOS on SCFA concentrations (mg/kg) in the caeca on days 14 and 28.

		Day 14					Day 28				
Additive	Inclusion level	Acetic acid	Propionic acid	n-Butyric acid	n-Valeric acid	Total SCFA	Acetic acid	Propionic acid	n-Butyric acid	n-Valeric acid	Total SCFA
No additive		4749	75.70	1260.7	45.3	6242	4790	193	1046	69.1	6170
Xylanase	Low	4260	56.60	1117.8	23.1	5532	5284	198	1347	78.2	6984
	High	4036	61.0	1250.0	28.7	6438	5541	172	1563	80.8	7435
XOS	Low	4949	112.0	1348.6	40.6	6528	5374	166	1345	80.1	7056
	High	5244	136.4	1505.9	55.0	7018	5177	222	1194	82.9	6756
Pooled SEM		108.8	35.54	143.13	9.87	437.2	234.9	28.9	114.8	6.92	310.6
P-value of control vs. additive[1]		0.737	0.066	0.874	0.596	0.818	0.042	0.922	0.019	0.149	0.015
Means for main effect of additive types (AT)											
Xylanase		4148[a]	56.80[a]	1183.9	32.4[a]	5485[a]	5412	185	1455	79.5	7210
XOS		5096[b]	124.2[b]	1427.3	47.8[b]	6773[b]	5275	194	1269	81.5	6906
Pooled SEM		236.5	47.66	172.07	10.89	309.1	116.1	20.5	81.1	4.89	219.6
Means for main effect of inclusion level (IL)											
	Low	4605	82.30	1233.2	38.4	6030	5412	185	1455	79.5	7210
	High	4640	98.70	1378.0	41.9	6228	5275	194	1269	81.5	6906
Pooled SEM		236.5	11.60	102.35	2.47	309.1	116.1	20.5	81.1	4.89	219.6
P- values for main effects and interactions											
Additive type (AT)		0.007	<0.001	0.128	0.026	0.006	0.563	0.760	0.114	0.774	0.335
Inclusion level (IL)		0.917	0.265	0.335	0.759	0.654	0.899	0.612	0.778	0.699	0.808
AT × IL		0.442	0.546	0.789	0.234	0.509	0.341	0.168	0.118	0.989	0.234

XOS- xylo-oligosaccharide; [1]P- values for control vs. additive types – the mean for the control group was compared to all other treatments containing an additive irrespective of type or level; [ab] different superscripts with the same column indicate means that are significantly ($P < 0.05$) different.

Table 6. The effect of feeding wheat based diets supplemented with xylanase or XOS on the concentration (g/100g) of WE NSP fractions in the ileum of broilers.

Additive	Inclusion level	Rhamnose	Fructose	Arabinose	Xylose	Galactose	Total (g/100 g)
No additive		0.0390	0.0462	0.605	0.948	0.398	2.74
Xylanase	Low	0.0422	0.0479[a]	0.624	0.898	0.463	3.04
	High	0.0619	0.0692[b]	0.855	1.143	0.607	3.84
XOS	Low	0.0484	0.0682[b]	0.773	1.284	0.507	3.74
	High	0.0520	0.0570[a]	0.810	1.216	0.556	3.63
	Pooled SEM	0.010	0.007	0.08	0.115	0.052	0.351
P-value of control vs additive types [1]		0.278	0.083	0.081	0.162	0.030	0.051
Means for main effect of additive types (AT)							
Xylanase		0.0520	0.0586	0.739	1.02	0.535	3.44
XOS		0.0502	0.0626	0.791	1.25	0.532	3.68
	Pooled SEM	0.007	0.005	0.055	0.081	0.037	0.248
Means for main effect of inclusion level (IL)							
	Low	0.0453	0.0580	0.699	1.091	0.485	3.39
	High	0.0569	0.0631	0.832	1.179	0.581	3.73
	Pooled SEM	0.007	0.005	0.055	0.081	0.037	0.248
P- values for main effects and interactions							
Additive type (AT)		0.850	0.573	0.511	0.061	0.949	0.495
Inclusion level (IL)		0.245	0.479	0.103	0.453	0.078	0.337
AT ×IL		0.471	0.032	0.227	0.190	0.369	0.261
P- values for contrasts							
Control vs. Xylanase, low level			0.866				
Control vs. Xylanase, high level			0.032				
Control vs. XOS, low level			0.039				
Control vs. XOS high level			0.291				

XOS- xylo-oligosaccharide; GlucA2- glucuronic acid; [1]P-value for control vs. additive types- the mean for the control group was compared to all other treatments containing an additive irrespective of type or level; [ab] different superscripts within the same column indicate means that are significantly ($P < 0.05$) different.

prebiotics on ileal nutrient digestibility (Kirkpinar et al. 2004; Mountzouris et al. 2010) however improvements in total tract retention have been reported (Mountzouris et al. 2010). This is achieved when populations of beneficial microflora are encouraged, which increases nutrient digestion and adsorption (De Measschalck et al. 2015) of SCFAs produced during fibre fermentation. Decreased nutrient digestibility following prebiotic supplementation has been reported previously in pigs. The authors suggested that the reduction in nutrient digestibility was due to the introduction of indigestible fibre into the diet (Smiricky- Tjardes et al. 2003). This is an unlikely explanation for the reduction in nutrient digestibly reported in the current study, however, the inclusion level of XOS was much lower that than that used by Smiricky- Tjardes et al. (2003).

There was an effect of additives supplementation on nutrient digestibility however there was little effect on growth performance especially on day 28, implying that the improvements in nutrient digestibility were not translated into growth performance, which has been reported previously (Yang et al. 2008; Gonzalez- Ortiz et al. 2016). This could indicate that 'point in time' measurements, such as nutrient digestibility, are poor tools in predicting the long-term effects of a diet on performance parameters such as body weight gain or FCR.

Table 5. The effect of feeding wheat-based diets supplemented with xylanase or XOS on the concentration (g/100g) of WU NSP fractions in the ileum of broilers.

Additive	Inclusion level	Rhamnose	Fructose	Arabinose	Xylose	Galactose	Total (g/100 g)
No additive		0.0156	0.0184	1.647[a]	2.54	0.583[a]	8.74
Xylanase	Low	0.0129	0.0170	1.721[a]	2.65	0.572[a]	9.01
	High	0.0222	0.0374	2.431[b]	3.55	0.851[b]	12.13
XOS	Low	0.0261	0.0256	2.002[ab]	3.08	0.686[ab]	10.30
	High	0.0434	0.0450	1.950[ab]	2.90	0.688[ab]	10.35
	Pooled SEM	0.004	0.005	0.155	0.292	0.047	0.840
P-value of control vs additive types[1]		0.022	0.044	0.042	0.142	0.039	0.086
Means for main effect of additive type (AT)							
Xylanase		0.0176[a]	0.0272	2.076	3.10	0.711	10.57
XOS		0.0347[b]	0.0353	1.976	2.99	0.687	10.32
	Pooled SEM	0.003	0.004	0.110	0.206	0.033	0.594
Means for main effect of inclusion level							
	Low	0.0195[a]	0.0213[a]	1.861[a]	2.87	0.629[a]	9.65
	High	0.0328[b]	0.0412[b]	2.190[b]	3.22	0.770[b]	11.24
	Pooled SEM	0.003	0.004	0.110	0.206	0.033	0.594
P- values for main effects and interactions (IL)							
	Additive Type (AT)	<0.001	0.144	0.525	0.717	0.608	0.776
	Inclusion Level (IL)	0.002	0.001	0.047	0.235	0.007	0.074
	AT × IL	0.302	0.937	0.024	0.083	0.008	0.084
	P- Values for Contrasts						
Control vs. Xylanase, low level				0.739		0.864	
Control vs. Xylanase, high level				0.002		<0.001	
Control vs. XOS, low level				0.122		0.139	
Control vs. XOS high level				0.184		0.130	

XOS- xylo-oligosaccharide; GlucA2- glucuronic acid; [1]P-value for control vs. additive types- the mean for the control group was compared to all other treatments containing an additive irrespective of type or level; [ab] different superscripts within the same column indicate means that are significantly different.

Short chain fatty acid concentration

Bird age had a significant effect on the concentration of SCFAs in the caecum of broiler chickens. On d 28, there was a greater concentration of acetic, propionic, iso-butyric and iso-valeric acid compared to SCFA on d 14. This is in agreement with Lee et al. (2017). The authors showed that the concentration of SCFAs increased as the bird aged. The reason for this was likely to be the development of the intestinal microflora. Gong et al. (2008) demonstrated that young birds (14 d of age) had a less well-developed microflora than those aged 42 d. Not only was the microflora in 14 d old birds less well developed, it was more likely to be influenced by changes in the diet or environment. In the current study, it was possible that SCFA concentration in the caeca were lower on d 14, because the microflora were less well developed and not able to ferment carbohydrate sources in order to produce SCFA. As the bird aged, the microflora matured and established itself, enabling the microbes to more readily ferment available carbohydrates, increasing the production of SCFA, which is in agreement with Lee et al. (2017).

The differences in SCFA concentration may have been related to differences in growth recorded in the current study, especially on d 14. SCFAs can influence growth performance in different ways. Firstly, as previously mentioned, SCFA can be used as an energy source for colonic cells, which increases the nutrients available to the host for growth. Secondly, xylanase enzymes randomly cleave NSP, reducing digesta viscosity and increasing nutrient absorption resulting in a decrease in the amount of nutrients available to the micro-organisms the caeca for fermentation (Lee et al. 2017).

One of the most notable detrimental nutrients fermented by colonic bacteria is protein. Protein fermentation by colonic bacteria has been associated with the production of toxic compounds, such as ammonia, which can inhibit growth and even cause disease (Apajalahil and Vinenola 2016). To combat this, it has been recommended that xylanases should be used to increase nutrient utilisation and to create fermentable carbohydrates (Apajalahil and Vinenola 2016). The current data agreed with this, as growth performance and SCFA production increased following the xylanase supplementation, however there was no effect on nitrogen digestibility.

Pre-caecal NSP fraction concentration

NSP concentration is a way to measure its hydrolysis by giving an indication of the resulting sugars in the liquid and solid phase of the ileal digesta. In a previous study, WU NSP concentration decreased while WE NSP concentration increased following xylanase supplementation (Olukosi et al. 2015). A reduction in NSP concentration indicates greater hydrolysis, which is beneficial when xylanase is used to reduce digesta viscosity. In the current study, however, the aim was to increase the concentration of potentially prebiotic oligosaccharides and investigate their effect on growth performance. NSP from wheat contains large amount of arabinoxylan (AX).

WE NSPs are the main cause of increased digesta viscosity in broilers, which is counteracted by using carbohydrases such as xylanase (Choct 2015). The concentration of WE NSP in the ileum of broilers during the current study increased when either xylanase or XOS were supplemented in the diets. This is in agreement with previous published data, as it has been reported that the disappearance of NSP from the digestive tract of broilers was significantly affected by enzyme addition (Cowieson et al. 2016; Cozannet et al. 2017). From the data above, fructose and galactose concentration (which likely represented fructo- oligosaccharide (FOS) and galacto- oligosaccharide (GOS) or galactans) was significantly increased due to xylanase addition. In addition to this, xylose concentration tended to increase XOS addition.

This tendency for xylose to increase following XOS supplementation could be as a result of undigested XOS bypassing digestion, but stoichiometry suggested this could not have been the only source of xylan in the WE fraction as 0.1 g/kg was added to the diet, and concentration increased by 3 g/kg DM. An increase in galactose (representing galactans and pectins) concentration in the ileum of broilers following carbohydrase supplementation has been reported previously (Kocher et al. 2002). This is of interest, because FOS and GOS have been associated with prebiotic effects (Kaplan and Hutkins 2000; Boehm et al. 2005; Courtin et al. 2008).

WU NSP are generally not fermented, unlike WE NSP; however, they have been associated with gut development (Choct 2015). In the current study, the concentration of arabinose, fructose and galactose were increased in response to xylanase or XOS supplementation in the WU NSP fraction. This could suggest that xylanase may have beneficial effects on gut development which is in agreement with other studies (Jimenez- Monero et al. 2009; Liu and Kim 2017). This is certainly true for the gizzard, where increases in dietary fibre have been associated with heavier gizzards, resulting in increased retention time of digesta and smaller particle size which, in turn, increases nutrient digestibility (Jimenez-Monero et al. 2009). The increase in WU NSP concentration could improve the development of the gastrointestinal tract, contributing to improvements in growth performance.

The current trial data demonstrated similarities between the effects of xylanase and XOS supplementation on growth performance, pre-caecal NSP concentration and caecal SCFA concentration, indicating similar modes of action. Consequently, it can be suggested that improvements in growth performance were partly driven by the production of *in-situ* prebiotics following xylanase supplementation. This study suggested that supplementing broiler diets with low levels of xylanase or high levels of XOS had a positive effect on the concentration of WU NSP fractions in the ileum, however, there was a limited effect of high levels of xylanase or XOS on performance. As such, more research into increasing the inclusion level of xylanase or XOS is required.

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