

Scotland's Rural College

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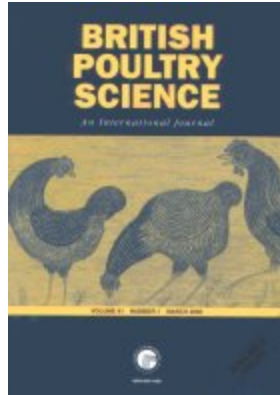
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**Xylanase and xylo- oligosaccharide prebiotic improve the growth performance and concentration of potentially prebiotic oligosaccharides in the ileum of broiler chickens**

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3 **1 Xylanase and xylo-oligosaccharide prebiotic improve the growth performance**  
4 **2 and concentration of potentially prebiotic oligosaccharides in the ileum of**  
5 **3 broiler chickens.**  
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18 **ABSTRACT**

- 19 1. The objective of this study was to investigate the effect of supplementing  
20 broiler diets with xylanase or xylo- oligosaccharide (XOS) on growth  
21 performance, the concentration of non-starch polysaccharide (NSP)  
22 hydrolysis products in the ileum and concentration of short chain fatty acids  
23 (SCFA) in the caeca of broiler chickens.
- 24 2. In total, 500 male Ross 308 broilers were used in this 29-day (d) study. The  
25 treatments were organised into a 2×2 plus 1 factorial arrangement consisting  
26 of two additives (xylanase or XOS) at two levels (low or high) plus a control  
27 treatment with no additives. This gave five treatments with 100 bird in each  
28 treatment group. The diets were slightly deficient in protein by 20 g/kg and  
29 energy by 1 MJ/kg.
- 30 3. On d 14 and 28, two birds per pen were euthanised, the caeca content  
31 collected and analysed for short chain fatty acid (SCFA) concentration. On d  
32 29, six birds per pen were euthanised and ileal digesta were collected and  
33 analysed for the concentration of NSP fractions.
- 34 4. On d 14, caecal acetic acid, iso-butyric acid, iso-valeric acid, n-valeric acid  
35 and total SCFA concentrations were significantly greater ( $P \leq 0.05$ ) when diets  
36 were supplemented with XOS compared with xylanase.
- 37 5. Ileal concentration of arabinose, galactose and glucuronic acid (GlucA2)  
38 were significantly greater ( $P \leq 0.05$ ) in the insoluble NSP fraction when diets  
39 were supplemented with a high level of xylanase, compared with the control  
40 treatment. Ileal concentration of fructose was significantly greater ( $P \leq 0.05$ )  
41 in the water soluble NSP when a high level of xylanase or low level of XOS  
42 were included in the diet compared with the control.

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3 43 6. It was concluded that xylanase and XOS had similar effects on NSP  
4  
5 44 concentration and SCFA in the caeca, although there was little effect on  
6  
7 45 performance. This observation demonstrated further benefits of xylanase  
8  
9 46 supplementation in wheat-based broiler diets beyond digesta viscosity  
10  
11 47 reduction and the release of extra nutrients.  
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16 48  
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18  
19 49 Keywords- xylanase, XOS, broilers, performance, nutrient digestibility, SCFA  
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## 50 Introduction

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

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

69 To investigate the production of potentially prebiotic oligosaccharides during  
70 xylanase activity, monosaccharides were measured in the ileal digesta and compared  
71 to monosaccharides in digesta from birds supplemented with purified XOS. The  
72 objective of the trial was to investigate similarities in profiles of monosaccharides in

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3 73 digesta from birds receiving xylanase and those receiving XOS to illustrate that NSP  
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5 74 hydrolysis products may have prebiotic-like effects similar to that of purified XOS.  
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8 75 NSP hydrolysis products are thought to be fermented by beneficial bacteria such as  
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11 76 *Bifidobacter* and *Lactobacilli spp.*, producing SCFA (Lee *et al.*, 2017). An increase  
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13 77 in the concentration of SCFAs is often associated with an increase in the population  
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15 78 of beneficial bacteria and a decrease in pathogenic bacteria (Engberg *et al.*, 2004). In  
16  
17 79 addition to this, SCFAs have been shown to influence growth performance in  
18  
19 80 broilers. Butyrate, in particular, is regarded as an available energy source, increasing  
20  
21 81 the energy available to the host for growth (Ravangard *et al.*, 2017). Supplementing  
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23 82 broiler diets with xylanase or XOS has been shown to affect the production of SCFA  
24  
25 83 in the caeca of broiler chickens (Engberg *et al.*, 2004; Lee *et al.*, 2017). This could  
26  
27 84 suggest that both ingredients have a similar mode of action. As such, the effect of  
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29 85 xylanase or XOS on SCFA concentration in the caeca of broilers was investigated in  
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31 86 the current study.  
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37 87 The objective of this experiment was to investigate the effect of supplementing  
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39 88 wheat-based diets, which were deficient in energy and protein, with xylanase or XOS  
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41 89 on growth performance, the concentration of NSP hydrolysis products in the ileum  
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43 90 and the concentration of SCFA's in the caeca of broiler chickens.  
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## 50 92 **Materials and methods**

### 51 52 53 93 *Animals and management*

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56 94 All the procedures in the experiment were approved by the SRUC Animal  
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58 95 Experiment Committee.  
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3 96 Five hundred male Ross 308, one-d-old broilers were allocated to one of five  
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5 97 treatments organised as a randomised complete block design. The birds were housed  
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7 98 10 in a pen, with ten pen replicates per treatment and provided with feed and water  
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10 99 on an *ab libitum* basis throughout the experiment (0 to 29 d). The treatments  
11  
12 100 followed a 2×2 plus 1 factorial arrangement with two different additives (xylanase or  
13  
14 101 XOS) at two inclusion levels (high and low) plus the control. The low level of  
15  
16 102 xylanase inclusion was 16, 000 XU/kg and the high was 32,000 XU/kg. The low  
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18 103 level of XOS inclusion was 0.25 g/kg and the high level was 0.1 g/kg. The lower  
19  
20 104 level of each additive was based on the standard recommendation by the  
21  
22 105 manufacturer while the higher level of each additive was chosen following a  
23  
24 106 literature search (Wang *et al.*, 2005; Zhenping *et al.*, 2013; De Measschalck *et al.*,  
25  
26 107 2015). The control diet was deficient in energy by 1 MJ/kg and protein reduced by  
27  
28 108 3%, to 20g/kg CP however; all other nutrient requirements were met to ensure that  
29  
30 109 any effects were induced by energy or protein deficiency or additive  
31  
32 110 supplementation alone. The diets were formulated to be deficient in energy and  
33  
34 111 protein to allow any improvements in growth or nutrient digestibility to become  
35  
36 112 apparent, as previous work has indicated that xylanase supplementation may be more  
37  
38 113 beneficial in nutrient-deficient diets (Francesch and Geraert, 2009). The experiment  
39  
40 114 was split into five treatments; 1) control, 2) control plus xylanase 16,000 XU/kg, 3)  
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42 115 control plus xylanase 32,000 XU/kg, 4) control plus purified XOS 0.25 g/kg and 5)  
43  
44 116 control plus purified XOS 1.0 g/kg. All of the experimental diets were provided in  
45  
46 117 mash form and birds had *ad libitum* access to feed and water throughout the feeding  
47  
48 118 trial. The xylanase used contained 160 000 U of endo- 1,4 β xylanase activity per  
49  
50 119 gram. One unit of xylanase activity was defined as the amount of enzyme required to  
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52 120 liberate 1 μmol of reducing sugars from xylan using a standardised test (Enzyme  
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3 121 Services (ESC), Hengoed, Ystrad Mynack, UK). The xylanase (Econase XT) was  
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5 122 supplied by AB Vista, Marlborough, UK. The XOS used in this study was purchased  
6  
7 123 from Shandong Lifelong Bio-technology Co., China (XOS 35A). The ingredient and  
8  
9 124 chemical composition of the control diet is shown in Table 1. Wheat bran was  
10  
11 125 included in the diet during this study to increase the amount of NSP in the diet and  
12  
13 126 maximise the potential for prebiotic oligosaccharide generation during NSP  
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15 127 hydrolysis by xylanase.  
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23 129 Table 1 here  
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29 131 ***Growth performance***  
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32 132 Feed and birds were weighed on d 0, 14 and 28. The data from feed and bird weights  
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34 133 were used to calculate body weight gain (BWG), feed intake (FI) and feed  
35  
36 134 conversion ratio (FCR).  
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40 135 ***Sample collection***  
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43 136 On d 14 and 28, two birds per pen were euthanised by cervical dislocation and used  
44  
45 137 to collect caeca content for SCFA analysis. Following euthanasia, the caeca were  
46  
47 138 removed and the content was gently squeezed into a collection tube.  
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51 139 On d 29, the remaining six birds per pen were euthanised by cervical dislocation and  
52  
53 140 ileal content was collected for nutrient digestibility and NSP analysis. Once located,  
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55 141 the distal half of the ileum was removed and the contents were flushed with water  
56  
57 142 into a collection pot. The ileal digesta from all six birds per pen was pooled and was  
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3 143 collected on day 29, as opposed to day 28, due to practical reasons relating to  
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5 144 volume of samples to be collected.  
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9 145 ***Short chain fatty acid analyses***

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11 146 Once the contents of the caeca were removed they were stored at -20°C and later  
12  
13 147 analysed for SCFA concentration as described by Khattak *et al.* (2018) using gas  
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15 148 chromatography.  
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19 149 ***NSP fraction analyses***

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22 150 The ileal digesta samples collected from six birds per pen on d 29 were dried in a  
23  
24 151 Unitherm force draft drying oven for 48 hours at 80°C. The samples were analysed  
25  
26 152 for water extractable (WE) carbohydrate components and water unextractable (WU)  
27  
28 153 NSP using HPLC, following the method of Englyst *et al.* (1994). The pre-caecal  
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30 154 NSP concentration was calculated using the equation displayed in the calculations  
31  
32 155 section.  
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37 156 ***Chemical analysis***

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40 157 The ileal digesta samples collected on d 29 were dried prior to conducting titanium  
41  
42 158 and DM analysis according to the method of Short *et al.* (1996). Dry matter (DM)  
43  
44 159 was determined using standard methods from AOAC, whereby 1g of the sample was  
45  
46 160 dried in a uniform forced drying oven (Unitherm, Russel-Lindsay Engineering Ltd,  
47  
48 161 Birmingham, England, UK) at 95°C for 24 hours. Nitrogen determination was  
49  
50 162 carried out by the combustion method (Method 934.01; AOAC). Gross energy was  
51  
52 163 determined using an isoperibol bomb calorimeter system using benzoic acid as an  
53  
54 164 internal standard (Model 6200, Parr Instruments, Moline, Illinois, USA). Ileal  
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56 165 digestibility was calculated using the index method described by Olukosi *et al.*  
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3 166 (2007). The activity of xylanase in the diet was measured using a commercial test kit  
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5 167 (Enzyme Services (ESC), Hengoed, Ystrad Mynack, UK). One BXU was defined as  
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8 168 the amount of xylanase enzyme required to liberate 1 nmol of reducing sugars per  
9  
10 169 minute from xylan at pH 5.3.

### 170 ***Calculations***

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

$$173 \quad \text{NSP Concentration} = \text{NSP conc. in digesta} \times \left( \frac{\text{Ti in diet}}{\text{Ti in digesta}} \right)$$

$$174 \quad \text{NSP Conc. In digesta (\%)} = \frac{\text{NSP Concentration}}{\text{NSP conc. in digesta}} \times 100$$

### 175 ***Statistics***

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

184

## 185 **Results**

### 186 ***Growth performance of broilers on days 14 and 28***

187 The enzyme analysis results showed that diets 1, 4 and 5 contained xylanase activity  
188 below detection threshold. Diet 2 contained 16,600 BXU/kg and diet 3 contained  
189 36,900 BXU/ kg of xylanase activity.

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

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

201

202 Table 2 here

203

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

206 Nutrient digestibility in broilers aged 29 d and fed diets supplemented with xylanase  
207 or XOS is shown in Table 3. There was a significant additive type×inclusion level  
208 interaction for nitrogen (N) intake. Birds receiving diets containing the low level of  
209 xylanase had significantly ( $P<0.01$ ) lower N intake than those receiving the high

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3 210 level of xylanase. There was no effect of additive type or inclusion level on DM or  
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5 211 N digestibility, however, there was trend for greater ileal digestible energy (IDE) in  
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7 212 birds fed diets supplemented with xylanase compared to those with XOS. The DM,  
8  
9 213 IDE and N digestibilities were lower ( $P<0.05$ ) in birds fed diets containing xylanase  
10  
11 214 or XOS compared to the control. The N and gross energy intake were lower ( $P<0.05$ )  
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13 215 in birds fed diets containing xylanase or XOS compared to the control diets.  
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21 217 Table 3 here  
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28 219 ***Short chain fatty acid concentration in the caeca of broiler chickens on days 14***  
29 220 ***and 28***

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31  
32 221 Caecal SCFA concentration in response to xylanase or XOS supplementation on d  
33  
34 222 14 is shown in Table 4. There was no significant inclusion level or additive  
35  
36 223 type $\times$ inclusion level interaction for SCFA concentration on d 14, however there was  
37  
38 224 a main effect of ingredient type. The concentration of acetic acid, propionic acid,  
39  
40 225 iso-butyric acid, iso-valeric acid and total SCFA were greater ( $P<0.05$ ) following  
41  
42 226 XOS compared with xylanase supplementation. There was no effect of ingredient  
43  
44 227 inclusion compared to the control treatment on acetic acid, n-butyric acid or n-  
45  
46 228 valeric acid concentration in broilers on d 14. However, there was a trend ( $P=0.066$ )  
47  
48 229 for xylanase to decrease propionic acid concentration and XOS to increase propionic  
49  
50 230 acid concentration.  
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56 231 Caecal SFCFA concentration in response to xylanase or XOS supplementation on d  
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58 232 28 is shown in Table 4. There was no additive type $\times$ inclusion level interaction for  
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3 233 SCFA concentration on d 28. There was no main effect of ingredient type or  
4  
5 234 inclusion level on caecal SCFA concentration on day 28. The concentration of acetic  
6  
7 235 acid, n-butyric acid and total SCFA were greater ( $P<0.05$ ) following ingredient  
8  
9 236 supplementation compared to the control. The concentration of acetic acid,  
10  
11 237 propanoic acid and total SCFA was greater ( $P<0.05$ ) in birds aged 28 days compared  
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13 238 to birds aged 14 days.  
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21 240 Table 4 here  
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27 242 ***NSP fraction content of ileal digesta from broiler chickens aged 29 days***  
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30 243 The concentration of WU NSP fractions in response to xylanase or XOS  
31  
32 244 supplementation is shown in Table 5. There was an ingredient type $\times$ inclusion level  
33  
34 245 interaction for arabinose and galactose. WU arabinose and galactose concentration  
35  
36 246 were greater when diets were supplemented with the high level of xylanase  
37  
38 247 compared to the low level. When the control treatment was compared to the other  
39  
40 248 treatments individually, arabinose and galactose concentration were greater ( $P<0.05$ )  
41  
42 249 following xylanase supplementation at a high level, compared to the control. There  
43  
44 250 were main effects of ingredient type and inclusion levels. Rhamnose concentration  
45  
46 251 was greater ( $P<0.001$ ) following XOS compared to xylanase supplementation.  
47  
48 252 Rhamnose and fructose concentration were greater ( $P<0.05$ ) following  
49  
50 253 supplementation at a high level compared to the low level. The concentration of  
51  
52 254 rhamnose, fructose, arabinose and galactose were greater ( $P<0.05$ ) following  
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54 255 supplementation compared with the control.  
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6 257 Table 5 here  
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12 259 The concentration of WE NSP fractions in response to xylanase or XOS  
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14 260 supplementation is shown in Table 6. There was a significant ingredient  
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16 261 type×inclusion level interaction for fructose concentration. The WE fructose  
17  
18 262 concentration was greater when the high level of xylanase or the low level of XOS  
19  
20 263 were included in the diet, compared to the low level of xylanase or high level of  
21  
22 264 XOS. When the control treatment was compared to the other diets individually, WE  
23  
24 265 fructose concentration was greater ( $P<0.05$ ) following supplementation of 32,000  
25  
26 266 XU/kg xylanase or 0.025% XOS compared to the control. There were no main  
27  
28 267 effects of ingredient type or inclusion level for WE NSP concentration. There was a  
29  
30 268 tendency ( $P=0.061$ ) for greater xylose concentration in diets supplemented with XOS  
31  
32 269 compared to xylanase. There was a tendency for greater galactose concentration in  
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34 270 diets supplemented with the high compared to the low inclusion level. Galactose and  
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36 271 total WE NSP concentration were greater ( $P<0.05$ ) following supplementation  
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38 272 compared with the control.  
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48 274 Table 6 here  
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54 276 **Discussion**  
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277 Xylanase is used routinely in broiler diets to improve growth performance, however,  
278 there is evidence to suggest that potentially prebiotic oligosaccharides are generated  
279 during NSP hydrolysis. The generation of *in-situ* prebiotics could provide additional  
280 benefit to the use of xylanases in broiler diets over and above the reduction in digesta  
281 viscosity.

### 282 ***Growth Performance***

283 The birds in the current study performed below breed standards. This could be due to  
284 a multitude of reasons, including diet form and composition. It has been well  
285 established in the literature that low protein (23 -20% CP) or low energy (3000-  
286 2640 Kcal/kg) diets reduce the growth performance of broilers (Govil *et al.*, 2017;  
287 Kamran *et al.*, 2008; Williams *et al.*, 2014). The current study is in agreement with  
288 this, as the birds receiving the control feed, which was lower in energy by 8% and  
289 protein by 13%, ate significantly more than those birds receiving supplementation in  
290 their diet. This was expected to an extent, as it has been suggested that birds do not  
291 eat to satisfy hunger *per se* but they consume enough feed to satisfy their energy or  
292 protein requirements (Karam *et al.*, 2008). In the current study, the birds receiving  
293 diets low in energy and protein were able to increase their feed intake, allowing them  
294 to maintain their growth. When xylanase or XOS was added into the diet, feed intake  
295 reduced, as expected, resulting in a reduction in FCR and an increase in efficiency.

296 Xylanase supplementation improved BWG, FI and FCR compared to that of XOS on  
297 d 14. This was expected as the mechanism of action of xylanase is well established  
298 in the literature. Xylanase cleaves the arabinoxylan (AX) backbone of NSP releasing  
299 the trapped nutrients (Meng *et al.*, 2005) and reduces digesta viscosity (Lentle,  
300 2005). This impacts on growth performance in two ways. Firstly, the release of



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3 301 trapped nutrients increases the amount available for absorption in the small intestine  
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5 302 (Meng *et al.*, 2005). Secondly, reducing digesta viscosity allows sufficient mixing of  
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7 303 the digesta, enabling more nutrients to be absorbed (Lentle, 2005). There is evidence  
8  
9 304 to suggest a third mechanism, namely generation of *in-situ* prebiotic  
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11 305 oligosaccharides. During the hydrolysis of NSP, smaller oligosaccharides such as  
12  
13 306 XOS are generated which have been shown to have prebiotic-like effects (Zhang *et*  
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15 307 *al.*, 2014).  
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20 308 Prebiotics can improve growth performance of broilers (Al-Sultan *et al.*, 2016;  
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22 309 Abdel- Hafeez *et al.*, 2017). In the current study, on d 28, XOS reduced feed intake  
23  
24 310 and improved FCR but had no effect on body weight gain, which was similar to the  
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26 311 effect of xylanase supplementation. It is thought that prebiotics improve growth  
27  
28 312 performance by increasing nutrient absorption due to modulating gut microflora and  
29  
30 313 increasing gut integrity (Al-Sultan *et al.*, 2016). Beneficial gut bacteria, such as  
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32 314 *Bifidobacterium* and *Lactobacilli spp.*, ferment prebiotics, such as XOS, FOS and  
33  
34 315 GOS, which encourages growth whilst discouraging the colonisation of pathogenic  
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36 316 bacteria (Xu *et al.*, 2003; Courtin *et al.*, 2008; Yousaf *et al.*, 2016). This may relate  
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38 317 to the SCFA results, discussed below.  
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#### 44 318 ***Nutrient digestibility***

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47 319 Ingredients such as xylanase have been shown to improve nutrient digestibility  
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49 320 (Kiarie *et al.*, 2014). The expectation that adding carbohydrases can improve nutrient  
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51 321 digestibility is logical, as the enzyme should decrease digesta viscosity and allow  
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53 322 increased absorption of nutrients (Mathlouthi *et al.*, 2002). In the current study, DM  
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55 323 and N digestibility were reduced when either xylanase or XOS were added to the  
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57 324 diet, however, IDE was significantly lower when XOS was included in the diet. The  
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3 325 diets used during this study were deficient in energy and protein, so the aim of  
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5 326 adding such ingredients was to improve nutrient absorption, as this is what has been  
6  
7 327 described in the literature (Cowieson *et al.*, 2017). The addition of xylanase or XOS  
8  
9 328 reduced digestible energy and N intake compared to the control treatment, which  
10  
11 329 may help explain why nutrient digestibility was decreased following  
12  
13 330 supplementation.

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18 331 The negative effect of XOS supplementation on IDE was unexpected. The literature  
19  
20 332 often reports no effect of prebiotics on ileal nutrient digestibility (Kirkpinar *et*  
21  
22 333 *al.*, 2004; Mountzouris *et al.*, 2010) however improvements in total tract retention  
23  
24 334 have been reported (Mountzouris *et al.*, 2010). This is achieved when populations of  
25  
26 335 beneficial microflora are encouraged, which increases nutrient digestion and  
27  
28 336 adsorption (De Measschalck *et al.*, 2015) of SCFAs produced during fibre  
29  
30 337 fermentation. Decreased nutrient digestibility following prebiotic supplementation  
31  
32 338 has been reported previously in pigs. The authors suggested that the reduction in  
33  
34 339 nutrient digestibility was due to the introduction of indigestible fibre into the diet  
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36 340 (Smiricky- Tjardes *et al.*, 2003). This is an unlikely explanation for the reduction in  
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38 341 nutrient digestibility reported in the current study, however, the inclusion level of XOS  
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40 342 was much lower than that used by Smiricky- Tjardes *et al.*, (2003).

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46 343 There was an effect of supplementation on nutrient digestibility however there was  
47  
48 344 little effect on growth performance especially on d 28, meaning that the  
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50 345 improvements in nutrient digestibility were not translated into growth performance,  
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52 346 which has been reported previously (Yang *et al.*, 2008; Gonzalez- Ortiz *et al.*, 2016).  
53  
54 347 This could indicate that ‘point in time’ measurements, such as nutrient digestibility,  
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56 348 are poor tools in predicting the long-term effects of a diet on performance parameters  
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58 349 such as body weight gain or FCR.

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3 350 ***Short chain fatty acid concentration***  
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6 351 Bird age had a significant effect on the concentration of SCFAs in the caecum of  
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8 352 broiler chickens. On d 28, there was a greater concentration of acetic, propionic, iso-  
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10 353 butyric and iso-valeric acid compared to SCFA on d 14. This is in agreement with  
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12 354 Lee *et al.* (2017). The authors showed that the concentration of SCFAs increased as  
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14 355 the bird aged. The reason for this was likely to be the development of the intestinal  
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16 356 microflora. Gong *et al.*, (2008) demonstrated that young birds (14 d of age) had a  
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18 357 less well-developed microflora than those aged 42 d. Not only was the microflora in  
19  
20 358 14 d old birds less well developed, it was more likely to be influenced by changes in  
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22 359 the diet or environment. In the current study, it was possible that SCFA  
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24 360 concentration in the caeca were lower on d 14, because the microflora were less well  
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26 361 developed and not able to ferment carbohydrate sources in order to produce SCFA.  
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28 362 As the bird aged, the microflora matured and established itself, enabling the  
29  
30 363 microbes to more readily ferment available carbohydrates, increasing the production  
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32 364 of SCFA, which is in agreement with Lee *et al.*, (2017).  
33  
34 365 The differences in SCFA concentration may have been related to differences in  
35  
36 366 growth recorded in the current study, especially on d 14. SCFAs can influence  
37  
38 367 growth performance in different ways. Firstly, as previously mentioned, SCFA can  
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40 368 be used as an energy source for colonic cells, which increases the nutrients available  
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42 369 to the host for growth. Secondly, xylanase enzymes randomly cleave NSP, reducing  
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44 370 digesta viscosity and increasing nutrient absorption resulting in a decrease in the  
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46 371 amount of nutrients available to the micro-organisms the caeca for fermentation (Lee  
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48 372 *et al.*, 2017).  
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3 373 One of the most notable detrimental nutrients fermented by colonic bacteria is  
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5 374 protein. Protein fermentation by colonic bacteria has been associated with the  
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8 375 production of toxic compounds, such as ammonia, which can inhibit growth and  
9  
10 376 even cause disease (Apajalahil and Vinenola, 2016). To combat this, it has been  
11  
12 377 recommended that xylanases should be used to increase nutrient utilisation and to  
13  
14 378 create fermentable carbohydrates (Apajalahil and Vinenola, 2016). The current data  
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16 379 agreed with this, as growth performance and SCFA production increased following  
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18 380 the xylanase supplementation, however there was no effect on nitrogen digestibility.

### 21 22 381 ***Pre- caecal NSP fraction concentration***

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25 382 NSP concentration is a way to measure its hydrolysis by giving an indication of the  
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27 383 resulting sugars in the liquid and solid phase of the ileal digesta. In a previous study,  
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29 384 WU NSP concentration decreased while WE NSP concentration increased following  
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31 385 xylanase supplementation (Olukosi *et al.*, 2015). A reduction in NSP concentration  
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33 386 indicates greater hydrolysis, which is beneficial when xylanase is used to reduce  
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35 387 digesta viscosity. In the current study, however, the aim was to increase the  
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37 388 concentration of potentially prebiotic oligosaccharides and investigate their effect on  
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39 389 growth performance. NSP from wheat contains large amount of arabinoxylan (AX).  
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45 390 WE NSPs are the main cause of increased digesta viscosity in broilers, which is  
46  
47 391 counteracted by using carbohydrases such as xylanase (Choct, 2015). The  
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49 392 concentration of WE NSP in the ileum of broilers during the current study increased  
50  
51 393 when either xylanase or XOS were supplemented in the diets. This is in agreement  
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53 394 with previous published data, as it has been reported that the disappearance of NSP  
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55 395 from the digestive tract of broilers was significantly affected by enzyme addition  
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57 396 (Cowieson *et al.*, 2016; Cozannet *et al.*, 2017). From the data above, fructose and  
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3 397 galactose concentration (which likely represented fructo- oligosaccharide (FOS and  
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5 398 galacto- oligosaccharide (GOS) or galactans) was significantly increased due to  
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8 399 xylanase addition. In addition to this, xylose concentration tended to increase XOS  
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10 400 addition.

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13 401 This tendency for xylose to increase following XOS supplementation could be as a  
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15 402 result of undigested XOS bypassing digestion, but stoichiometry suggested this  
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18 403 could not have been the only source of xylan in the WE fraction as 0.1 g/kg was  
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20 404 added to the diet, and concentration increased by 3 g/ kg DM. An increase in  
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22 405 galactose (representing galactans and pectins) concentration in the ileum of broilers  
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25 406 following carbohydrase supplementation has been reported previously (Kocher *et al.*,  
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27 407 2002). This is of interest, because FOS and GOS have been associated with prebiotic  
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29 408 effects (Kaplan and Hutkins, 2000; Boehm *et al.*, 2005; Courtin *et al.*, 2008).

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32 409 WU NSP are generally not fermented, unlike WE NSP; however, they have been  
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34 410 associated with gut development (Choct, 2015). In the current study, the  
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37 411 concentration of arabinose, fructose and galactose were increased in response to  
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39 412 xylanase or XOS supplementation in the WU NSP fraction. This could suggest that  
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41 413 xylanase may have beneficial effects on gut development which is in agreement with  
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43  
44 414 other studies (Jimenez –Monero *et al.*, 2009; Liu *et al.*, 2017). This is certainly true  
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46 415 for the gizzard, where increases in dietary fibre have been associated with heavier  
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48 416 gizzards, resulting in increased retention time of digesta and smaller particle size  
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51 417 which, in turn, increases nutrient digestibility (Jimenez- Monero *et al.*, 2009). The  
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53 418 increase in WU NSP concentration could improve the development of the  
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55 419 gastrointestinal tract, contributing to improvements in growth performance.

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3 420 The current trial data demonstrated similarities between the effects of xylanase and  
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5 421 XOS supplementation on growth performance, pre-caecal NSP concentration and  
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7 422 caecal SCFA concentration, indicating similar modes of action. Consequently, it can  
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9 423 be suggested that improvements in growth performance were partly driven by the  
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11 424 production of in-situ prebiotics following xylanase supplementation. This study  
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13 425 suggested that supplementing broiler diets with low levels of xylanase or high levels  
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15 426 of XOS had a positive effect on the concentration of WU NSP fractions in the ileum,  
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17 427 however, there was a limited effect of high levels of xylanase or XOS on  
18  
19 428 performance. As such, more research into increasing the inclusion level of xylanase  
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21 429 or XOS is required.  
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47  
48  
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50  
51 439 this study.  
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646 **Table 1.** The calculated and analysed nutrient content of wheat-based diets  
 647 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 <sub>2</sub> Marker	25.0
Total	1000.0
<b>Calculated content</b>	
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
<b>Analysed nutrient content</b>	
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

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649 Notes; TiO<sub>2</sub>- titanium dioxide; Na- sodium; Cl- chloride; DM- dry matter; AME-  
 650 apparent metabolizable energy; N- nitrogen

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

Additive	Inclusion Level	Day 14			Day 28		
		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 <sup>1</sup>		0.390	0.111	0.752	0.592	<0.001	0.009
Means for main effect of additive type (AT)							
Xylanase		387.7 <sup>b</sup>	586.7 <sup>b</sup>	1.52 <sup>a</sup>	1307.3	2626.8	2.02
XOS		365.9 <sup>a</sup>	561.6 <sup>a</sup>	1.55 <sup>b</sup>	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

Notes; XOS- xylo-oligosaccharide; BWG- body weight gain; FI- feed intake; FCR; feed conversion ratio; <sup>1</sup>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 ; <sup>ab</sup> different superscripts with the same column indicate means that are significantly (P<0.05) different

**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 <sup>b</sup>
	High	0.606	11.99	0.817	29.62	69.15 <sup>a</sup>
XOS	Low	0.630	12.22	0.812	29.54	70.23 <sup>ab</sup>
	High	0.624	12.23	0.821	30.26	79.64 <sup>ab</sup>
	SEM	0.0189	0.372	0.00913	1.499	1.987
P-value of control vs additive <sup>1</sup>		<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

Notes; XOS- xylo-oligosaccharide; DM- dry matter; IDE- ileal digestible energy; N- nitrogen; <sup>1</sup>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; <sup>abc</sup> different superscripts with the same column indicate means that are significantly (P<0.05) different

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

Additive	Inclusion Level	Day 14					Day 28				
		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 <sup>1</sup>		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 <sup>a</sup>	56.80 <sup>a</sup>	1183.9	32.4 <sup>a</sup>	5485 <sup>a</sup>	5412	185	1455	79.5	7210
XOS		5096 <sup>b</sup>	124.2 <sup>b</sup>	1427.3	47.8 <sup>b</sup>	6773 <sup>b</sup>	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

Notes; XOS- xylo-oligosaccharide; <sup>1</sup>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; <sup>ab</sup> different superscripts with the same column indicate means that are significantly (P<0.05) different

**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/100g)
No Additive		0.0156	0.0184	1.647 <sup>a</sup>	2.54	0.583 <sup>a</sup>	8.74
Xylanase	Low	0.0129	0.0170	1.721 <sup>a</sup>	2.65	0.572 <sup>a</sup>	9.01
	High	0.0222	0.0374	2.431 <sup>b</sup>	3.55	0.851 <sup>b</sup>	12.13
XOS	Low	0.0261	0.0256	2.002 <sup>ab</sup>	3.08	0.686 <sup>ab</sup>	10.30
	High	0.0434	0.0450	1.950 <sup>ab</sup>	2.90	0.688 <sup>ab</sup>	10.35
	Pooled SEM	0.004	0.005	0.155	0.292	0.047	0.840
P-value of control vs additive types <sup>1</sup>		0.022	0.044	0.042	0.142	0.039	0.086
Means for main effect of additive type (AT)							
Xylanase		0.0176 <sup>a</sup>	0.0272	2.076	3.10	0.711	10.57
XOS		0.0347 <sup>b</sup>	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 <sup>a</sup>	0.0213 <sup>a</sup>	1.861 <sup>a</sup>	2.87	0.629 <sup>a</sup>	9.65
High		0.0328 <sup>b</sup>	0.0412 <sup>b</sup>	2.190 <sup>b</sup>	3.22	0.770 <sup>b</sup>	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	

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3                      Control vs. XOS high level

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4 Notes; XOS- xylo-oligosaccharide; GlucA2- glucuronic acid; <sup>1</sup>P-value for control vs. additive types- the mean for the control group was  
5 compared to all other treatments containing an additive irrespective of type or level; <sup>ab</sup> different superscripts within the same column indicate  
6 means that are significantly different  
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**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/100g)
No Additive		0.0390	0.0462	0.605	0.948	0.398	2.74
Xylanase	Low	0.0422	0.0479 <sup>a</sup>	0.624	0.898	0.463	3.04
	High	0.0619	0.0692 <sup>b</sup>	0.855	1.143	0.607	3.84
XOS	Low	0.0484	0.0682 <sup>b</sup>	0.773	1.284	0.507	3.74
	High	0.0520	0.0570 <sup>a</sup>	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 <sup>1</sup>		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				

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Notes; XOS- xylo-oligosaccharide; GlucA2- glucuronic acid; <sup>1</sup>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; <sup>ab</sup> different superscripts within the same column indicate means that are significantly (P<0.05) different

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