



## Original Research Article

# Ileal profile of non-starch polysaccharides and oligosaccharides in response to exogenous enzymes in broiler chickens offered wheat- or maize-based diets under subclinical necrotic enteritis challenge



Eunjoo Kim <sup>a</sup>, Amy F. Moss <sup>a,\*</sup>, Natalie K. Morgan <sup>a</sup>, Kosar Gharib-Naseri <sup>a</sup>, Peter Ader <sup>b</sup>, Mingan Choct <sup>a</sup>

<sup>a</sup> School of Environmental and Rural Science, University of New England, Armidale, NSW, 2351, Australia

<sup>b</sup> BASF SE, 68623, Lampertheim, Germany

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## ABSTRACT

The present study evaluated the impacts of fibre-degrading enzymes on the profiles of non-starch polysaccharides (NSP) and oligosaccharides (OS) in the ileum of broiler chickens offered wheat- or maize-based diets under subclinical necrotic enteritis (NE) challenge. A  $2 \times 2 \times 4$  factorial arrangement of treatments was used. Factors were the following: NE challenge, no or yes; diet type, wheat- or maize-based; and supplemental enzymes, control (no enzyme), family 10 xylanase (XYN10), family 11 xylanase (XYN11) or  $\beta$ -mannanase (MAN). Birds in the challenged group were inoculated with *Eimeria* on d 9 and *Clostridium perfringens* on d 14 and 15. A 3-way interaction ( $P = 0.047$ ) occurred on overall (d 0 to 16) weight gain. When NE was present, all the supplemental enzymes increased weight gain in birds fed the wheat-based diet; whereas in those fed the maize-based diet supplemental XYN10 and XYN11 decreased weight gain. When NE was absent, birds fed the wheat-based diet supplemented with XYN10 or MAN presented increased weight gain compared to non-supplemented birds, but no improvements with enzyme addition were observed in birds fed the maize-based diet. A 3-way interaction ( $P = 0.002$ ) was observed on insoluble NSP level in the ileum. When NE was absent, all the supplemental enzymes reduced the ileal level of insoluble NSP, regardless of diet type. In the challenged birds, supplementing XYN10 and MAN reduced insoluble NSP level in the ileum, but only in birds fed the wheat-based diet. Ileal soluble NSP level was reduced by supplemental XYN11 and MAN, but only in birds fed the wheat-based diet, resulting in a 2-way diet type  $\times$  enzyme interaction ( $P < 0.001$ ). Ileal OS arabinose ( $P = 0.030$ ) level was highest in birds offered the wheat-based diet supplemented with XYN11. Collectively, supplementation of NSP-degrading enzymes to the wheat-based diet enhanced bird performance regardless of NE challenge, with XYN11 significantly increasing oligosaccharide release. However, enzyme addition did not improve growth performance in birds fed maize-based diet, with supplemental XYN10 and XYN11 impeding weight gain when NE was present.

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\* Corresponding author.

E-mail address: [amoss22@une.edu.au](mailto:amoss22@une.edu.au) (A.F. Moss).

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## 1. Introduction

The pathogenic strain of *Clostridium perfringens* can cause gastroenteritis and intestinal tissue necrosis, associated with necrotic enteritis (NE) in broiler chickens. NE is the most prevalent sporadic disease in poultry globally, increasing productivity losses and impairing welfare. In acute and clinical NE cases, flock mortality increases substantially by up to 10% to 40% (Mcdevitt et al., 2006), whereas subclinical NE leads to growth depression with insignificant mortality. It is believed that the subclinical form of NE

is comparatively more detrimental to the poultry industry as it is not easily diagnosed during production, delaying timely treatment (Skinner et al., 2010). Until recently, in-feed antimicrobial agents were used as the conventional method for preventing and controlling NE outbreaks; however, public health concerns about antimicrobial resistance has set the trend to phase out the use of antimicrobial drugs in livestock production worldwide.

The severity of NE can be influenced by dietary components. It has previously been established that wheat-based diets cause more severe cases of NE compared to maize-based diets in vivo and in vitro, through enhancing the growth of *C. perfringens* (Riddell and Kong, 1992; Annett et al., 2002). The main reason for this is the differences in the levels of non-starch polysaccharides (NSP) between wheat and maize. NSP can be classified into water-soluble and insoluble fractions, and the type and amounts of NSP vary widely between the ingredients (Knudsen, 2014). Wheat is generally rich in both soluble and insoluble NSP, whereas maize contains a relatively low concentration of soluble NSP but appreciable amounts of insoluble NSP (Knudsen, 1997). Each form results in a distinct impact on gut physicochemical conditions and nutrient utilisation in birds. In particular, soluble NSP has one of the greatest detrimental effects on exacerbating NE symptoms, through increasing digesta viscosity in the gut, consequently prolonging digesta transit time and providing pathogenic bacteria with nutrients to proliferate (Choct and Annison, 1992; Moore, 2016). On the other hand, the structural components of insoluble can entrap other nutrients such as starch and protein within the cell wall matrix, thereby decreasing the nutritional value of feedstuffs (Choct, 2015).

In poultry, NSP are not effectively hydrolysed by host endogenous enzymes in the small intestine but are instead, to a small extent, fermented by gut microbiota in the distal ileum and caeca. Thus, NSP-degrading enzymes are often included in poultry diets to minimise the anti-nutritive impacts of NSP. During the enzymatic hydrolysis of NSP, lower-molecular weight carbohydrates, i.e. oligosaccharides (OS), can be released, which have been shown to induce prebiotic properties in broilers. These OS are selectively fermented by beneficial gut bacteria, favourably modulating the gut environment (Kim et al., 2011). In this respect, it can be postulated that *in situ* enzymatic release of the prebiotic OS from dietary complex NSP could potentially mitigate dysbacteriosis caused by NE infection along the digestive tract (Latorre et al., 2018). Therefore, the present study measured enzyme-induced changes in concentration and composition of dietary NSP in the ileal digesta and investigated whether these changes could lead to improvements in growth performance in birds offered wheat- or maize-based diets, in the presence or absence of subclinical NE.

## 2. Materials and methods

All animal experimental procedures were reviewed and approved by the Animal Ethics Committee at the University of New England (Approval No. AEC20-005).

### 2.1. Experimental design, broiler housing and experimental diets

Day-old Cobb 500 mixed-sex broiler chicks ( $n = 1,536$ ) were obtained from Baiada hatchery in Tamworth, NSW, Australia. Upon arrival, birds were weighed and allocated to 96 floor pens (1.07 m<sup>2</sup>), ensuring there was no significant difference in initial pen weight across the experimental treatments. The present study was designed as a 2 × 2 × 4 factorial arrangement consisting of 16 treatments, 6 replicates per treatment and 16 birds per pen. Factors were NE challenge (no or yes), diet type (wheat- or maize-based diets), and enzymes (control; no enzyme, endo-β-1,4-xylanase

from family 10 and 11, and β-mannanase). Feed and water were provided ad libitum. The temperature was kept at 32 °C for the first 3 d and gradually decreased to 26 °C by 16 d of age. The lighting was maintained at 23 h per day for the first 7 d, and then 19 h per day for the remainder of the experiment.

The composition of the basal diets is shown in Tables 1 and 2. The wheat/soybean meal- and maize/soybean meal-based diets were formulated to meet the nutrient requirements for Cobb 500 broilers (Cobb-Vantress, 2018). The 2 basal diets contained either wheat or maize as the major energy grain, with a similar quantity of soybean meal as the major protein source. The use of non-conventional ingredients and not commercially used materials were avoided to make the diets industry-relevant and minimise possible anti-nutritional factors. Consequently, there were differences in crude protein and NSP levels between the wheat- and maize-based diets. Birds received the starter diets from 0 to 9 d of age and the grower from 10 to 16 d of age. Titanium dioxide (TiO<sub>2</sub>; 5 g/kg) as an indigestible marker and phytase (Natuphos E; 10,000 FTU/g) were added to all diets. Each basal diet was manufactured in one batch, which was split into 4 portions; one non-supplemented control and 3 supplemented with each of the test enzymes. The 3 enzymes used in the present study were endo-β-1,4-xylanase from glycoside hydrolase family 10 (XYN10) derived from *Aspergillus niger* and endo-β-1,4-xylanase from glycoside hydrolase family (XYN11) derived from *Pseudomonas fluorescens*, and endo-β-1,4-mannanase (MAN) derived from *Thermothelomyces thermophile*. XYN10 and XYN11 had endo-1,4-β-xylanase activities of 5,600 and 16,000 units per g, respectively, whereas MAN

**Table 1**  
Composition and nutrient levels of basal diets (%).

| Item                                    | Starter (d 0 to 9) |       | Grower (d 10 to 16) |       |
|---|--------------------|-------|---------------------|-------|
|   | Wheat              | Maize | Wheat               | Maize |
| Ingredients, as fed                     |                    |       |                     |       |
| Wheat                                   | 59.9               | —     | 65.2                | —     |
| Maize                                   | —                  | 58.7  | —                   | 64.5  |
| Soybean meal                            | 32.4               | 34.9  | 26.9                | 29.0  |
| Canola oil                              | 3.0                | 1.6   | 3.6                 | 2.00  |
| Limestone                               | 1.2                | 1.1   | 1.1                 | 1.07  |
| CaHPO <sub>4</sub> <sup>1</sup>         | 1.6                | 1.7   | 1.5                 | 1.61  |
| NaCl                                    | 0.21               | 0.21  | 0.24                | 0.23  |
| NaCO <sub>3</sub>                       | 0.21               | 0.26  | 0.10                | 0.15  |
| TiO <sub>2</sub>                        | 0.50               | 0.50  | 0.50                | 0.50  |
| Vitamin premix <sup>2</sup>             | 0.09               | 0.09  | 0.09                | 0.09  |
| Mineral premix <sup>3</sup>             | 0.11               | 0.11  | 0.11                | 0.11  |
| Choline chloride (70%)                  | 0.07               | 0.12  | 0.06                | 0.12  |
| L-Lysine•HCl                            | 0.29               | 0.28  | 0.21                | 0.22  |
| DL-Methionine                           | 0.30               | 0.32  | 0.28                | 0.27  |
| L-Threonine                             | 0.11               | 0.09  | 0.08                | 0.07  |
| Natuphos (100 g/t)                      | 0.01               | 0.01  | 0.01                | 0.01  |
| Nutrient levels <sup>4</sup> , DM basis |                    |       |                     |       |
| Metabolisable energy, kcal/kg           | 2,950              | 2,950 | 3,050               | 3,050 |
| Crude protein                           | 22.5               | 21.8  | 20.4                | 19.4  |
| Digestible lysine                       | 1.24               | 1.24  | 1.05                | 1.05  |
| Digestible methionine                   | 0.79               | 0.83  | 0.70                | 0.72  |
| Digestible methionine + Cysteine        | 0.90               | 0.90  | 0.83                | 0.80  |
| Calcium                                 | 0.90               | 0.90  | 0.85                | 0.84  |
| Available phosphorus                    | 0.45               | 0.45  | 0.43                | 0.43  |

<sup>1</sup> CaHPO<sub>4</sub> contained 18% phosphorus and 21% calcium.

<sup>2</sup> Vitamin premix provided the following per kilogram of diets: retinol 12,000 IU, cholecalciferol 5,000 IU, tocopheryl acetate 75 mg, menadione 3 mg, thiamine, 3 mg, riboflavin 8 mg, niacin 55 mg, pantothenate 13 mg, pyridoxine 5 mg, folate 2 mg, cyanocobalamin 16 µg, biotin 200 µg, cereal-based carrier 149 mg, mineral oil 2.5 mg.

<sup>3</sup> Mineral premix provided the following per kilogram of diets: Cu (sulfate) 16 mg, Fe (sulfate) 40 mg, I (iodide) 1.25 mg, Se (selenate) 0.3 mg, Mn (sulfate and oxide) 120 mg, Zn (sulfate and oxide) 100 mg, cereal-based carrier 128 mg, mineral oil 3.75 mg.

<sup>4</sup> Nutrient levels were all calculated values.

**Table 2**  
Analysed chemical composition of basal diets (% dry matter basis).<sup>1</sup>

| Item                       | Starter (d 0 to 9) |       | Grower (d 10 to 16) |       |
|----------------------------|--------------------|-------|---------------------|-------|
|                            | Wheat              | Maize | Wheat               | Maize |
| Dry matter                 | 88.9               | 87.4  | 88.6                | 88.0  |
| Gross energy, kcal/kg      | 4,013              | 3,869 | 4,013               | 3,893 |
| Crude protein              | 24.3               | 22.0  | 23.0                | 19.5  |
| Starch                     | 40.0               | 42.5  | 41.5                | 44.8  |
| Non-starch polysaccharides | 11.8               | 10.8  | 11.6                | 9.8   |
| Soluble                    | 1.29               | 0.68  | 1.14                | 0.55  |
| Rhamnose                   | 0.005              | 0.005 | 0.005               | 0.004 |
| Fucose                     | 0.004              | 0.006 | 0.004               | 0.005 |
| Ribose                     | 0.05               | 0.06  | 0.05                | 0.04  |
| Arabinose                  | 0.36               | 0.16  | 0.38                | 0.11  |
| Xylose                     | 0.42               | 0.08  | 0.43                | 0.04  |
| Mannose                    | 0.20               | 0.14  | 0.09                | 0.14  |
| Galactose                  | 0.27               | 0.22  | 0.21                | 0.17  |
| Glucose                    | 0.15               | 0.10  | 0.12                | 0.11  |
| Uronic acid                | 0.06               | 0.07  | 0.06                | 0.07  |
| Insoluble                  | 10.5               | 10.1  | 10.5                | 9.2   |
| Rhamnose                   | 0.06               | 0.09  | 0.05                | 0.06  |
| Fucose                     | 0.11               | 0.14  | 0.10                | 0.11  |
| Ribose                     | 0.01               | 0.02  | 0.02                | 0.01  |
| Arabinose                  | 2.32               | 2.00  | 2.35                | 1.94  |
| Xylose                     | 2.37               | 1.73  | 2.44                | 1.76  |
| Mannose                    | 0.25               | 0.23  | 0.22                | 0.21  |
| Galactose                  | 1.63               | 1.97  | 1.58                | 1.69  |
| Glucose                    | 2.78               | 2.32  | 3.01                | 2.22  |
| Uronic acid                | 2.19               | 2.74  | 1.97                | 2.30  |
| Oligosaccharides           | 4.82               | 5.03  | 4.27                | 4.20  |
| Rhamnose                   | 0.4                | ND    | ND                  | ND    |
| Fucose                     | ND                 | ND    | ND                  | ND    |
| Ribose                     | ND                 | ND    | ND                  | ND    |
| Arabinose                  | 0.5                | 1.0   | 0.5                 | 0.5   |
| Xylose                     | ND                 | ND    | ND                  | ND    |
| Mannose                    | 4.1                | 4.9   | 3.4                 | 3.5   |
| Galactose                  | 12.9               | 13.0  | 10.8                | 10.1  |
| Glucose                    | 30.9               | 28.3  | 27.7                | 27.9  |

ND = not detected.

<sup>1</sup> Samples analysed in quadruplicate.

contained 8,800 units of endo-1,4- $\beta$ -mannanase activity per g. Test enzymes were top-dressed and thoroughly mixed into the basal diets before pelleting. Diets were cold-pelleted at 65 °C. The starter diets were crumbled.

## 2.2. Necrotic enteritis challenge

On 9 d of age, birds in the challenged groups were gavaged with 1 mL of a suspension containing 5,000 sporulated *Eimeria cervulina* and *Eimeria maxima*, and 2,500 sporulated *Eimeria brunetti* (*Eimeria* Pty Ltd., Ringwood, Victoria, Australia). Birds in the non-challenged groups were orally administered 1 mL sterile phosphate-buffered saline. On 14 and 15 d of age, birds in the challenged group were gavaged with 1 mL of *C. perfringens* EHE-NE18 ( $10^8$  CFU/mL), whereas birds in the non-challenged group orally received 1 mL of sterile thioglycollate broth as a sham treatment.

## 2.3. Data and sample collection and chemical analyses

On 16 d of age, 4 birds per pen were selected and euthanised for digesta sample collection. The ileal contents were collected from the Meckel's diverticulum to the ileocaecal junction and pooled by gently squeezing the digesta into the containers. These samples were kept at -20 °C until further analysis.

Ileal digesta samples were lyophilised and, along with the diet samples, ground to pass through a 0.5 mm sieve. The diets and freeze-dried digesta samples were analysed for dry matter, NSP and

TiO<sub>2</sub>. Dry matter contents were measured by placing samples in a drying oven at 105 °C to a constant weight. Soluble and insoluble NSP and their constituent sugars (rhamnose, fucose, ribose, arabinose, xylose, galactose and glucose) were measured as alditol acetates by gas chromatography using the method described by Englyst et al. (1994) and Theander et al. (1995) with some modifications (Morgan et al., 2019) using Agilent 8890 GC equipped with Agilent 7693A Autosampler (Agilent Technologies Inc., Palo Alto, CA, USA). Uronic acid was measured following the method by Scott (1979) using a spectrophotometer at 450 and 400 nm (UV-1600PC, VWR, Darmstadt, Germany). The conversion factor 0.9 was used to convert constituent sugar values to polysaccharide values (Knudsen, 1997). TiO<sub>2</sub> contents were measured by a colourimetric method, as described by Short et al. (1996), using a Cary 50 Bio UV-Visible spectrophotometer equipped with a Cary 50 MPR microplate reader (Varian Inc., Palo Alto, CA USA) set at 410 nm.

## 2.4. Calculation and statistical analysis

Ileal NSP and OS concentrations (g/kg marker DM) were corrected using the TiO<sub>2</sub> marker concentration in the digesta and diet in the following equation:

$$\text{Ileal NSP or OS concentration} = \text{NSP or OS constituent sugar}_{\text{ileal digesta}} \times (\text{TiO}_2 \text{ ileal digesta} / \text{TiO}_2 \text{ diet})$$

All data were checked for normal distribution and analysed using a 3-way ANCOVA (IBM SPSS Statistics version 27, Armonk, NY). The percentage of male birds in a replicate pen did not have a significant effect on growth performance. Means were separated using Tukey's HSD test ( $P < 0.05$ ). The non-normal rhamnose, fucose and ribose levels in the soluble and insoluble NSP, and OS were analysed using a nonparametric Kruskal–Wallis test.

## 3. Results

### 3.1. Growth performance

Growth performance from 0 to 16 d of age is summarised in Table 3. During the starter phase (d 0 to 9), birds offered the wheat-based diet presented a lower feed conversion ratio ( $P = 0.033$ ), associated with greater weight gain ( $P = 0.018$ ), compared to those fed the maize-based diet. A 2-way diet type  $\times$  enzyme interaction ( $P < 0.001$ ) was found on starter feed intake. In birds offered the wheat-based diet, all the supplemental enzymes reduced the feed intake, whereas in those fed the maize-based diet supplemental XYN10 increased the feed intake compared to non-supplemented birds and birds fed supplemental MAN.

During the grower phase (d 10 to 16), challenging the birds decreased weight gain ( $P < 0.001$ ) and feed intake ( $P < 0.001$ ) and increased feed conversion ratio ( $P = 0.014$ ) compared to the non-challenged birds. A 2-way diet type  $\times$  enzyme interaction ( $P = 0.003$ ) was observed for grower weight gain. In birds offered the wheat-based diet, all the supplemental enzymes increased weight gain compared to the non-supplemented birds, whereas the opposite was true in birds fed the maize-based diet.

Overall (d 0 to 16), NE challenge decreased feed intake ( $P < 0.001$ ) and increased the feed conversion ratio ( $P = 0.003$ ). A 3-way interaction ( $P = 0.047$ ) occurred for overall weight gain. In non-challenged birds offered the wheat-based diet, supplementing XYN10 and XYN11 increased weight gain compared to non-supplemented birds. When NE was present, enzyme supplementation to the wheat-based diet increased weight gain compared to non-supplemented birds, regardless of the types of enzymes. However, enzyme supplementation did not affect weight gain in

**Table 3**  
Growth performance of birds in response to necrotic enteritis challenge, diet type and supplemental enzymes at 16 d of age.<sup>1</sup>

| Item                           |           |                       | Weight gain, g   |                   |                   | Feed intake, g    |                  |                  | Feed conversion ratio, <sup>2</sup> g/g |                    |                    |       |  |  |
|--------------------------------|-----------|-----------------------|------------------|-------------------|-------------------|-------------------|------------------|------------------|---|--------------------|--------------------|-------|--|--|
| Challenge                      | Diet type | Enzyme                | d 0-9            | d 10-16           | d 0-16            | d 0-9             | d 10-16          | d 0-16           | d 0-9                                   | d 10-16            | d 0-16             |       |  |  |
| Two-way interaction            |           |                       |                  |                   |                   |                   |                  |                  |   |                    |                    |       |  |  |
|                                | Wheat     | Control               | 225              | 401 <sup>c</sup>  | 627               | 236 <sup>b</sup>  | 403              | 638              | 1.044                                   | 1.005              | 1.018              |       |  |  |
|                                |           | XYN10                 | 223              | 419 <sup>a</sup>  | 642               | 225 <sup>a</sup>  | 413              | 639              | 1.010                                   | 0.989              | 0.996              |       |  |  |
|                                |           | XYN11                 | 223              | 415 <sup>a</sup>  | 638               | 223 <sup>a</sup>  | 418              | 641              | 1.003                                   | 1.006              | 1.004              |       |  |  |
|                                | Maize     | MAN                   | 223              | 420 <sup>a</sup>  | 642               | 227 <sup>a</sup>  | 420              | 647              | 1.021                                   | 1.004              | 1.009              |       |  |  |
|                                |           | Control               | 215              | 419 <sup>a</sup>  | 634               | 224 <sup>a</sup>  | 410              | 633              | 1.043                                   | 0.978              | 1.000              |       |  |  |
|                                |           | XYN10                 | 221              | 407 <sup>bc</sup> | 628               | 236 <sup>b</sup>  | 414              | 650              | 1.066                                   | 1.022              | 1.036              |       |  |  |
|                                |           | XYN11                 | 222              | 408 <sup>b</sup>  | 630               | 230 <sup>ab</sup> | 401              | 631              | 1.034                                   | 0.984              | 1.001              |       |  |  |
|                                |           | MAN                   | 220              | 408 <sup>bc</sup> | 628               | 226 <sup>a</sup>  | 403              | 628              | 1.025                                   | 0.989              | 1.001              |       |  |  |
|                                |           | Three-way interaction |                  |                   |                   |                   |                  |                  |   |                    |                    |       |  |  |
| No                             | Wheat     | Control               | 224              | 426               | 651 <sup>bc</sup> | 236               | 422              | 658              | 1.049                                   | 0.991              | 1.011              |       |  |  |
|                                |           | XYN10                 | 224              | 444               | 668 <sup>a</sup>  | 220               | 426              | 646              | 0.981                                   | 0.960              | 0.967              |       |  |  |
|                                |           | XYN11                 | 221              | 441               | 662 <sup>ab</sup> | 221               | 441              | 662              | 1.006                                   | 1.000              | 1.001              |       |  |  |
|                                |           | MAN                   | 225              | 440               | 665 <sup>a</sup>  | 226               | 428              | 654              | 1.009                                   | 0.971              | 0.983              |       |  |  |
|                                | Maize     | Control               | 217              | 435               | 652 <sup>bc</sup> | 222               | 434              | 656              | 1.024                                   | 0.998              | 1.006              |       |  |  |
|                                |           | XYN10                 | 218              | 428               | 651 <sup>bc</sup> | 233               | 423              | 655              | 1.067                                   | 0.977              | 1.007              |       |  |  |
|                                |           | XYN11                 | 222              | 438               | 660 <sup>bc</sup> | 230               | 421              | 652              | 1.036                                   | 0.963              | 0.988              |       |  |  |
|                                |           | MAN                   | 219              | 423               | 646 <sup>c</sup>  | 221               | 416              | 638              | 1.007                                   | 0.976              | 0.986              |       |  |  |
|                                | Yes       | Wheat                 | Control          | 226               | 377               | 603 <sup>fg</sup> | 236              | 383              | 619                                     | 1.039              | 1.018              | 1.026 |  |  |
|                                |           |                       | XYN10            | 223               | 393               | 616 <sup>de</sup> | 231              | 401              | 632                                     | 1.039              | 1.018              | 1.025 |  |  |
|                                |           |                       | XYN11            | 226               | 390               | 615 <sup>de</sup> | 226              | 394              | 619                                     | 1.001              | 1.011              | 1.006 |  |  |
|                                |           |                       | MAN              | 221               | 398               | 619 <sup>d</sup>  | 227              | 413              | 640                                     | 1.034              | 1.037              | 1.034 |  |  |
|                                |           | Maize                 | Control          | 213               | 402               | 615 <sup>de</sup> | 225              | 385              | 610                                     | 1.062              | 0.959              | 0.993 |  |  |
|                                |           |                       | XYN10            | 224               | 380               | 604 <sup>fg</sup> | 239              | 406              | 644                                     | 1.065              | 1.067              | 1.066 |  |  |
|                                |           |                       | XYN11            | 222               | 378               | 600 <sup>g</sup>  | 229              | 381              | 609                                     | 1.032              | 1.005              | 1.015 |  |  |
|                                |           |                       | MAN              | 220               | 389               | 610 <sup>ef</sup> | 229              | 389              | 619                                     | 1.043              | 1.001              | 1.016 |  |  |
|                                |           |                       | Main effects     |                   |                   |                   |                  |                  |   |                    |                    |       |  |  |
|                                |           |                       | Challenge        |                   |                   |                   |                  |                  |   |                    |                    |       |  |  |
| No                             |           |                       | 221              | 434 <sup>a</sup>  | 656               | 226               | 426 <sup>a</sup> | 653 <sup>a</sup> | 1.022                                   | 0.979 <sup>b</sup> | 0.994 <sup>b</sup> |       |  |  |
| Yes                            |           |                       | 222              | 388 <sup>b</sup>  | 610               | 230               | 394 <sup>b</sup> | 624 <sup>b</sup> | 1.039                                   | 1.014 <sup>a</sup> | 1.023 <sup>a</sup> |       |  |  |
| Diet type                      |           |                       |                  |                   |                   |                   |                  |                  |   |                    |                    |       |  |  |
| Wheat                          |           |                       | 224 <sup>a</sup> | 414               | 637               | 228               | 414              | 641              | 1.020 <sup>b</sup>                      | 1.001              | 1.007              |       |  |  |
| Maize                          |           |                       | 220 <sup>b</sup> | 409               | 629               | 228               | 407              | 636              | 1.042 <sup>a</sup>                      | 0.993              | 1.010              |       |  |  |
| Enzyme                         |           |                       |                  |                   |                   |                   |                  |                  |   |                    |                    |       |  |  |
| Control                        |           |                       | 220              | 411               | 630               | 230               | 406              | 636              | 1.043                                   | 0.992              | 1.009              |       |  |  |
| XYN10                          |           |                       | 222              | 412               | 634               | 231               | 414              | 644              | 1.038                                   | 1.005              | 1.016              |       |  |  |
| XYN11                          |           |                       | 223              | 412               | 634               | 226               | 409              | 636              | 1.019                                   | 0.995              | 1.002              |       |  |  |
| MAN                            |           |                       | 221              | 413               | 634               | 226               | 412              | 638              | 1.023                                   | 0.996              | 1.005              |       |  |  |
| SEM                            |           |                       | 0.9              | 2.9               | 2.8               | 1.0               | 3.2              | 3.4              | 0.0053                                  | 0.0066             | 0.0054             |       |  |  |
| P-value                        |           |                       |                  |                   |                   |                   |                  |                  |   |                    |                    |       |  |  |
| Challenge                      |           |                       | 0.755            | <0.001            | <0.001            | 0.030             | <0.001           | <0.001           | 0.098                                   | 0.014              | 0.003              |       |  |  |
| Diet type                      |           |                       | 0.018            | 0.090             | <0.001            | 0.811             | 0.234            | 0.364            | 0.033                                   | 0.587              | 0.770              |       |  |  |
| Enzyme                         |           |                       | 0.780            | 0.638             | 0.442             | 0.214             | 0.793            | 0.753            | 0.270                                   | 0.904              | 0.752              |       |  |  |
| Challenge × Diet type          |           |                       | 0.962            | 0.479             | 0.190             | 0.929             | 0.875            | 0.872            | 0.995                                   | 0.694              | 0.723              |       |  |  |
| Challenge × Enzyme             |           |                       | 0.784            | 0.025             | 0.082             | 0.573             | 0.246            | 0.183            | 0.601                                   | 0.228              | 0.151              |       |  |  |
| Diet type × Enzyme             |           |                       | 0.223            | <0.001            | <0.001            | <0.001            | 0.305            | 0.397            | 0.181                                   | 0.409              | 0.142              |       |  |  |
| Challenge × Diet type × Enzyme |           |                       | 0.390            | 0.080             | 0.047             | 0.512             | 0.868            | 0.991            | 0.309                                   | 0.475              | 0.775              |       |  |  |

Control = no enzyme; XYN10 = xylanase from family 10; XYN11 = xylanase from family 11; MAN = β-mannanase; SEM = standard error of the mean.

<sup>a–g</sup>Values in a column with no common superscripts differ significantly ( $P < 0.05$ ).<sup>1</sup> Mean values are based on 6 replicates per treatment, and male bird percentage of each replicate pen was used as a covariate.<sup>2</sup> Feed conversion ratio corrected for mortality.

the non-challenged birds offered the maize-based diet, but in the challenged birds fed the maize-based diet supplementing XYN10 and XYN11 decreased weight gain.

### 3.2. Ileal soluble non-starch polysaccharides constituent sugars profile

The ileal levels of soluble NSP constituent sugars (g/kg marker) are presented in Table 4. Two-way challenge × diet type interactions occurred for soluble ribose ( $P = 0.029$ ), arabinose ( $P < 0.001$ ), xylose ( $P < 0.001$ ), galactose ( $P = 0.004$ ) and total soluble NSP ( $P < 0.001$ ) levels in the ileum. In birds fed the wheat-based diet, challenging the birds reduced the levels of soluble arabinose, xylose and total soluble NSP in the ileum compared to the non-challenged birds. The challenge had no impact on ileal soluble ribose or galactose level in birds fed the wheat-based diet.

In birds fed the maize-based diet, NE challenge increased the ileal level of soluble ribose and galactose compared to the non-challenged birds, but had no impact on ileal soluble arabinose, xylose or total soluble NSP.

Two-way diet type × enzyme interactions were observed for soluble xylose ( $P < 0.001$ ), glucose ( $P = 0.046$ ) and total NSP ( $P < 0.001$ ) levels in the ileum. In birds fed the wheat-based diet, supplementing XYN11 reduced the ileal xylose level, whilst supplementing MAN reduced ileal glucose level, compared to birds fed the control wheat-based diet or wheat-based diets with other enzyme treatments. The total NSP level in the ileum was reduced by supplemental XYN11 and MAN compared to the non-supplemented birds or birds supplemented with XYN10 when the wheat-based diet was fed. The main effect of the NE challenge was observed for ileal soluble rhamnose ( $P < 0.001$ ) and fucose ( $P < 0.001$ ) levels, with challenged birds presenting higher levels of

**Table 4**

Ileal concentration (g/kg marker, dry matter basis) of soluble non-starch polysaccharides constituent sugars in response to necrotic enteritis challenge, diet type and supplemental enzymes at 16 d of age.<sup>1</sup>

| Item         |                                | Rhamnose | Fucose            | Ribose            | Arabinose         | Xylose           | Mannose          | Galactose         | Glucose           | Uronic acid       | Total             |                   |                   |
|--------------|--------------------------------|----------|-------------------|-------------------|-------------------|------------------|------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Challenge    | Diet type                      | Enzyme   |                   |                   |                   |                  |                  |                   |                   |                   |                   |                   |                   |
| No           | Wheat                          |          | 0.51              | 0.40              | 0.11 <sup>a</sup> | 3.5 <sup>a</sup> | 4.2 <sup>a</sup> | 0.56              | 2.5 <sup>ab</sup> | 0.98              | 0.26              | 11.1 <sup>a</sup> |                   |
|              | Maize                          |          | 0.63              | 0.49              | 0.06 <sup>b</sup> | 1.4 <sup>c</sup> | 0.7 <sup>c</sup> | 0.69              | 2.4 <sup>b</sup>  | 0.32              | 0.29              | 5.6 <sup>c</sup>  |                   |
| Yes          | Wheat                          |          | 0.40              | 0.31              | 0.10 <sup>a</sup> | 2.8 <sup>b</sup> | 3.4 <sup>b</sup> | 0.53              | 2.3 <sup>b</sup>  | 0.93              | 0.26              | 9.4 <sup>b</sup>  |                   |
|              | Maize                          |          | 0.52              | 0.44              | 0.09 <sup>a</sup> | 1.4 <sup>c</sup> | 0.7 <sup>c</sup> | 0.68              | 2.9 <sup>a</sup>  | 0.41              | 0.32              | 6.2 <sup>c</sup>  |                   |
|              | Wheat                          | Control  |                   | 0.43              | 0.34              | 0.12             | 3.3              | 4.1 <sup>a</sup>  | 0.58              | 2.5               | 0.98 <sup>a</sup> | 0.27              | 10.9 <sup>a</sup> |
|              |                                | XYN10    |                   | 0.46              | 0.36              | 0.11             | 3.4              | 4.5 <sup>a</sup>  | 0.58              | 2.6               | 0.99 <sup>a</sup> | 0.27              | 11.3 <sup>a</sup> |
|              |                                | XYN11    |                   | 0.51              | 0.40              | 0.11             | 2.8              | 2.7 <sup>b</sup>  | 0.55              | 2.4               | 1.08 <sup>a</sup> | 0.27              | 9.2 <sup>b</sup>  |
|              | Maize                          | MAN      |                   | 0.42              | 0.33              | 0.08             | 3.0              | 3.9 <sup>a</sup>  | 0.47              | 2.1               | 0.77 <sup>b</sup> | 0.24              | 9.7 <sup>b</sup>  |
|              |                                | Control  |                   | 0.61              | 0.43              | 0.09             | 1.3              | 0.4 <sup>c</sup>  | 0.68              | 2.4               | 0.33 <sup>c</sup> | 0.32              | 5.4 <sup>c</sup>  |
|              |                                | XYN10    |                   | 0.57              | 0.47              | 0.06             | 1.4              | 0.8 <sup>c</sup>  | 0.67              | 2.6               | 0.34 <sup>c</sup> | 0.31              | 6.0 <sup>c</sup>  |
|              |                                | XYN11    |                   | 0.54              | 0.48              | 0.09             | 1.4              | 0.9 <sup>c</sup>  | 0.69              | 2.8               | 0.40 <sup>c</sup> | 0.30              | 6.4 <sup>c</sup>  |
|              | MAN                            |          | 0.59              | 0.48              | 0.07              | 1.3              | 0.6 <sup>c</sup> | 0.70              | 2.8               | 0.38 <sup>c</sup> | 0.29              | 5.9 <sup>c</sup>  |                   |
| Main effects |                                |          |                   |                   |                   |                  |                  |                   |                   |                   |                   |                   |                   |
| Challenge    |                                |          |                   |                   |                   |                  |                  |                   |                   |                   |                   |                   |                   |
|              | No                             |          | 0.57 <sup>a</sup> | 0.44 <sup>a</sup> | 0.09              | 2.4              | 2.4              | 0.63              | 2.4               | 0.65              | 0.27              | 8.3               |                   |
|              | Yes                            |          | 0.46 <sup>b</sup> | 0.38 <sup>b</sup> | 0.09              | 2.1              | 2.0              | 0.61              | 2.6               | 0.67              | 0.29              | 7.8               |                   |
| Diet type    |                                |          |                   |                   |                   |                  |                  |                   |                   |                   |                   |                   |                   |
|              | Wheat                          |          | 0.45 <sup>b</sup> | 0.36 <sup>b</sup> | 0.10              | 3.1              | 3.8              | 0.55 <sup>b</sup> | 2.4               | 0.96              | 0.56 <sup>a</sup> | 10.3              |                   |
|              | Maize                          |          | 0.58 <sup>a</sup> | 0.46 <sup>a</sup> | 0.08              | 1.4              | 0.6              | 0.69 <sup>a</sup> | 2.7               | 0.36              | 0.30 <sup>b</sup> | 5.9               |                   |
| Enzyme       |                                |          |                   |                   |                   |                  |                  |                   |                   |                   |                   |                   |                   |
|              | Control                        |          | 0.52              | 0.39              | 0.10              | 2.3              | 2.3              | 0.63              | 2.5               | 0.66              | 0.29              | 8.2               |                   |
|              | XYN10                          |          | 0.51              | 0.41              | 0.08              | 2.4              | 2.6              | 0.63              | 2.6               | 0.67              | 0.29              | 8.6               |                   |
|              | XYN11                          |          | 0.53              | 0.44              | 1.00              | 2.1              | 1.8              | 0.62              | 2.6               | 0.74              | 0.28              | 7.8               |                   |
|              | MAN                            |          | 0.50              | 0.40              | 0.08              | 2.2              | 2.2              | 0.58              | 2.4               | 0.57              | 0.27              | 7.8               |                   |
| SEM          |                                |          | 0.015             | 0.011             | 0.005             | 0.11             | 0.19             | 0.022             | 0.07              | 0.038             | 0.006             | 0.27              |                   |
| P-value      |                                |          |                   |                   |                   |                  |                  |                   |                   |                   |                   |                   |                   |
|              | Challenge                      |          | <0.001            | <0.001            | 0.547             | <0.001           | <0.001           | 0.673             | 0.127             | 0.575             | 0.132             | 0.012             |                   |
|              | Diet type                      |          | <0.001            | <0.001            | 0.010             | <0.001           | <0.001           | 0.002             | 0.062             | <0.001            | 0.001             | <0.001            |                   |
|              | Enzyme                         |          | 0.947             | 0.266             | 0.159             | 0.153            | <0.001           | 0.861             | 0.710             | 0.037             | 0.503             | 0.017             |                   |
|              | Challenge × Diet type          |          | 0.998             | 0.312             | 0.029             | <0.001           | <0.001           | 0.888             | 0.004             | 0.095             | 0.240             | <0.001            |                   |
|              | Challenge × Enzyme             |          | 0.424             | 0.579             | 0.390             | 0.508            | 0.393            | 0.482             | 0.497             | 0.074             | 0.298             | 0.305             |                   |
|              | Diet type × Enzyme             |          | 0.127             | 0.518             | 0.628             | 0.062            | <0.001           | 0.633             | 0.157             | 0.046             | 0.957             | <0.001            |                   |
|              | Challenge × Diet type × Enzyme |          | 0.496             | 0.762             | 0.063             | 0.546            | 0.404            | 0.958             | 0.664             | 0.570             | 0.693             | 0.252             |                   |

Control = no enzyme; XYN10 = xylanase from family 10; XYN11 = xylanase from family 11; MAN = β-mannanase; SEM = standard error of the mean.

<sup>a-c</sup>Values in a column with no common superscripts differ significantly ( $P < 0.05$ ).

<sup>1</sup> Values are based on 6 replicates per treatment, and male bird percentage of each replicate pen was used as a covariate.

these sugars compared to the non-challenged birds. Birds fed the wheat-based diet presented higher ileal levels of soluble rhamnose ( $P < 0.001$ ), fucose ( $P < 0.001$ ) and mannose ( $P = 0.002$ ) compared to those fed the maize-based diet.

### 3.3. Ileal insoluble non-starch polysaccharides constituent sugars profile

Table 5 presents the ileal concentration of insoluble NSP and their constituent sugars at 16 d of age. Three-way interactions occurred for insoluble xylose ( $P < 0.001$ ) and glucose ( $P = 0.036$ ) and total insoluble NSP levels ( $P = 0.002$ ) in the ileum. In birds fed the wheat-based diet, supplementing XYN10 and XYN11 reduced the ileal xylose level when NE was absent. In challenged birds fed the wheat-based diet, all the supplemental enzymes reduced the ileal xylose level compared to non-supplemented birds. When the maize-based diet was fed, supplemental XYN10 decreased the ileal xylose level compared to the control birds, regardless of NE. Supplemental XYN11 reduced insoluble xylose level compared to the control only in the challenged birds. In the challenged birds fed the wheat-based diet, supplementing XYN11 reduced the ileal glucose level compared to non-supplemented birds, but the enzymes had no impact on the non-challenged birds. In birds fed the maize-based diet, the enzymes had no impact on insoluble glucose level in the absence of NE, but when NE was present XYN10 reduced the level in comparison to the non-supplemented birds. In the non-challenged birds, all the supplemental enzymes reduced the ileal

insoluble NSP level compared to the non-supplemented birds, regardless of the diet type. However, in the presence of NE, supplementation of XYN10 and MAN to the wheat-based diet reduced the ileal level of insoluble NSP compared to the non-supplemented birds, but enzyme supplementation had no impact on the insoluble NSP level in birds fed the maize-based diet. Ileal levels of insoluble rhamnose, fucose, ribose, arabinose, galactose and uronic acid were higher in the non-challenged birds compared to the challenged birds ( $P < 0.050$ ). Birds fed the wheat-based diet presented lower levels of ileal insoluble rhamnose, fucose, arabinose, galactose and uronic acid compared to those fed the maize-based diet ( $P < 0.001$ ). Insoluble mannose level in the ileum was lower in birds fed the diets supplemented with MAN compared to those fed the control diet or diets supplemented with XYN10 ( $P = 0.015$ ). Ileal level of insoluble uronic acid was lower in birds supplemented with XYN10 and MAN compared to those fed the control diet or diets supplemented with XYN11 ( $P < 0.001$ ).

### 3.4. Ileal oligosaccharides profile

The ileal OS levels measured at 16 d of age is presented in Table 6. A 3-way challenge × diet type × enzyme interaction ( $P = 0.030$ ) occurred for the ileal xylose in the OS fraction. In birds fed the wheat-based diet, supplementing XYN11 increased the ileal xylose level compared to non-supplemented birds, regardless of NE challenge. Supplementation of MAN to the wheat-based diet reduced the ileal xylose level compared to the non-supplemented

**Table 5**Ileal concentration (g/kg marker, dry matter basis) of insoluble non-starch polysaccharides constituent sugars in response to necrotic enteritis challenge, diet type and supplemental enzymes at 16 d of age.<sup>1</sup>

| Item         | Rhamnose                       | Fucose            | Ribose            | Arabinose        | Xylose            | Mannose            | Galactose           | Glucose            | Uronic acid          | Total                |                    |                    |                    |
|--------------|--------------------------------|-------------------|-------------------|------------------|-------------------|--------------------|---------------------|--------------------|----------------------|----------------------|--------------------|--------------------|--------------------|
| Challenge    | Diet type                      | Enzyme            |                   |                  |                   |                    |                     |                    |                      |                      |                    |                    |                    |
| No           | Wheat                          | Control           | 0.53              | 1.0              | 0.03              | 20.6               | 23.2 <sup>ab</sup>  | 1.4                | 12.7                 | 15.4 <sup>a</sup>    | 13.3               | 78.0 <sup>c</sup>  |                    |
|              |                                | XYN10             | 0.52              | 1.1              | 0.02              | 20.6               | 16.3 <sup>cde</sup> | 1.2                | 13.3                 | 13.0 <sup>abc</sup>  | 13.4               | 70.8 <sup>de</sup> |                    |
|              |                                | XYN11             | 0.55              | 1.2              | 0.03              | 21.5               | 15.9 <sup>cde</sup> | 1.2                | 14.5                 | 10.4 <sup>abcd</sup> | 15.3               | 72.4 <sup>d</sup>  |                    |
|              | Maize                          | MAN               | 0.43              | 1.0              | 0.02              | 20.4               | 22.7 <sup>ab</sup>  | 0.8                | 12.6                 | 12.9 <sup>abcd</sup> | 12.7               | 74.3 <sup>d</sup>  |                    |
|              |                                | Control           | 0.88              | 1.5              | 0.03              | 25.3               | 25.4 <sup>a</sup>   | 1.1                | 21.9                 | 10.7 <sup>abcd</sup> | 21.9               | 96.8 <sup>a</sup>  |                    |
|              |                                | XYN10             | 0.78              | 1.5              | 0.05              | 23.5               | 22.4 <sup>b</sup>   | 1.0                | 21.0                 | 8.0 <sup>cd</sup>    | 19.7               | 87.2 <sup>b</sup>  |                    |
|              | Yes                            | Wheat             | XYN11             | 0.83             | 1.5               | 0.00               | 23.6                | 22.8 <sup>ab</sup> | 0.9                  | 20.5                 | 7.3 <sup>d</sup>   | 19.6               | 86.6 <sup>b</sup>  |
|              |                                |                   | MAN               | 0.77             | 1.4               | 0.02               | 23.2                | 23.6 <sup>ab</sup> | 1.0                  | 20.0                 | 8.4 <sup>cd</sup>  | 18.5               | 86.2 <sup>b</sup>  |
|              |                                |                   | Control           | 0.47             | 0.8               | 0.00               | 17.3                | 18.0 <sup>c</sup>  | 1.2                  | 10.0                 | 15.6 <sup>a</sup>  | 12.6               | 64.3 <sup>ef</sup> |
| Maize        | XYN10                          | 0.28              | 0.6               | 0.00             | 13.0              | 14.2 <sup>e</sup>  | 1.3                 | 7.5                | 12.3 <sup>abcd</sup> | 10.1                 | 55.7 <sup>g</sup>  |                    |                    |
|              | XYN11                          | 0.43              | 1.0               | 0.00             | 16.3              | 15.1 <sup>de</sup> | 1.1                 | 11.0               | 8.9 <sup>cde</sup>   | 11.7                 | 57.3 <sup>fg</sup> |                    |                    |
|              | MAN                            | 0.35              | 0.7               | 0.00             | 13.7              | 14.8 <sup>e</sup>  | 0.9                 | 8.3                | 12.7 <sup>abcd</sup> | 10.1                 | 55.1 <sup>g</sup>  |                    |                    |
| Main effects | Challenge                      | Control           | 0.70              | 1.3              | 0.00              | 19.6               | 21.1 <sup>b</sup>   | 1.1                | 16.1                 | 14.3 <sup>ab</sup>   | 18.7               | 82.4 <sup>bc</sup> |                    |
|              |                                | XYN10             | 0.70              | 1.2              | 0.00              | 19.3               | 18.0 <sup>c</sup>   | 1.2                | 15.9                 | 9.1 <sup>cd</sup>    | 18.9               | 76.0 <sup>cd</sup> |                    |
|              |                                | XYN11             | 0.72              | 1.2              | 0.00              | 19.1               | 17.9 <sup>cd</sup>  | 1.3                | 16.0                 | 11.7 <sup>abcd</sup> | 19.0               | 78.0 <sup>c</sup>  |                    |
| SEM          | Challenge                      | MAN               | 0.68              | 1.3              | 0.00              | 21.0               | 21.3 <sup>b</sup>   | 0.9                | 16.9                 | 12.0 <sup>abcd</sup> | 19.8               | 83.6 <sup>bc</sup> |                    |
|              |                                | No                | 0.66 <sup>a</sup> | 1.3 <sup>a</sup> | 0.03 <sup>a</sup> | 22.3               | 21.5                | 1.1                | 17.1 <sup>a</sup>    | 10.8                 | 16.8               | 81.5               |                    |
|              |                                | Yes               | 0.54 <sup>b</sup> | 1.0 <sup>b</sup> | 0.00 <sup>b</sup> | 17.4               | 17.6                | 1.1                | 12.7 <sup>b</sup>    | 12.1                 | 15.1               | 69.0               |                    |
| Diet type    | Wheat                          | 0.45 <sup>b</sup> | 0.9 <sup>b</sup>  | 0.01             | 17.9              | 17.5               | 1.1                 | 11.2 <sup>b</sup>  | 12.6                 | 12.4                 | 66.0               |                    |                    |
|              | Maize                          | 0.76 <sup>a</sup> | 1.4 <sup>a</sup>  | 0.01             | 21.8              | 21.6               | 1.1                 | 18.5 <sup>a</sup>  | 10.2                 | 19.5                 | 84.6               |                    |                    |
| Enzyme       | Control                        | 0.65              | 1.2               | 0.02             | 20.5              | 21.9               | 1.2 <sup>a</sup>    | 15.2               | 14.0                 | 16.6                 | 80.4               |                    |                    |
|              | XYN10                          | 0.57              | 1.1               | 0.02             | 19.1              | 17.7               | 1.2 <sup>a</sup>    | 14.4               | 10.6                 | 15.5                 | 72.4               |                    |                    |
|              | XYN11                          | 0.63              | 1.2               | 0.01             | 20.4              | 17.9               | 1.1 <sup>ab</sup>   | 15.5               | 9.6                  | 16.4                 | 73.6               |                    |                    |
|              | MAN                            | 0.56              | 1.1               | 0.01             | 19.6              | 20.6               | 0.9 <sup>b</sup>    | 14.4               | 11.5                 | 15.3                 | 74.8               |                    |                    |
| SEM          | 0.022                          | 0.03              | 0.002             | 0.41             | 0.41              | 0.04               | 0.50                | 0.37               | 0.46                 | 1.35                 |                    |                    |                    |
| P-value      | Challenge                      | <0.001            | <0.001            | <0.001           | <0.001            | <0.001             | 0.457               | <0.001             | 0.023                | 0.002                | <0.001             |                    |                    |
|              | Diet type                      | <0.001            | <0.001            | 1.000            | <0.001            | <0.001             | 0.457               | <0.001             | <0.001               | <0.001               | <0.001             |                    |                    |
|              | Enzyme                         | 0.068             | 0.287             | 0.646            | 0.165             | <0.001             | 0.015               | 0.302              | 0.202                | <0.001               | <0.001             |                    |                    |
|              | Challenge × Diet type          | 0.882             | 0.674             | 1.000            | 0.122             | 0.948              | 0.367               | 0.574              | 0.001                | 0.093                | <0.001             |                    |                    |
|              | Challenge × Enzyme             | 0.823             | 0.543             | 0.646            | 0.683             | 0.015              | 0.490               | 0.652              | 0.750                | 0.727                | 0.495              |                    |                    |
|              | Diet type × Enzyme             | 0.844             | 0.236             | 0.350            | 0.130             | 0.017              | 0.626               | 0.225              | 0.006                | 0.521                | 0.649              |                    |                    |
|              | Challenge × Diet type × Enzyme | 0.418             | 0.570             | 0.350            | 0.112             | <0.001             | 0.558               | 0.419              | 0.036                | 0.137                | 0.002              |                    |                    |

Control = no enzyme; XYN10 = xylanase from family 10; XYN11 = xylanase from family 11; MAN = β-mannanase; SEM = standard error of the mean.

<sup>a–g</sup>Values in a column with no common superscripts differ significantly ( $P < 0.05$ ).<sup>1</sup> Values are based on 6 replicates per treatment, and male bird percentage of each replicate pen was used as a covariate.

birds only in the absence of NE. Supplementing either XYN10 or XYN11 heightened the ileal xylose level in birds fed the maize-based diet compared to non-supplemented birds with or without NE challenge. Two-way challenge × diet type interactions were found in the ileal levels of arabinose ( $P = 0.012$ ) and mannose ( $P < 0.001$ ), with NE challenge reducing the ileal levels of these sugars compared to non-challenged birds when the wheat-based diet was fed only. A 2-way diet type × enzyme interaction ( $P < 0.001$ ) occurred on the ileal arabinose level; the enzymes had no impact in birds fed the maize-based diets, but in birds fed the wheat-based diets with supplementation of XYN11 significantly increased the level. Non-challenged birds presented higher ( $P < 0.001$ ) levels of rhamnose, galactose, glucose and total OS in the ileum compared to the challenged birds. Birds fed the maize-based diet presented higher ileal levels of rhamnose ( $P < 0.001$ ), galactose ( $P < 0.001$ ) and total OS ( $P = 0.008$ ) compared to those offered the wheat-based diet. Supplementing XYN10 and XYN11 increased ( $P = 0.011$ ) the ileal rhamnose level compared to birds supplemented with MAN.

#### 4. Discussion

The present study elucidated the efficacy of NSP-degrading enzymes on ileal NSP and OS profiles in birds offered wheat- or

maize-based diets from 0 to 16 d of age. The hypothesis that enzyme-released OS would provide protection against subclinical NE was also investigated. In the present study, NE challenge decreased overall weight gain and feed intake by 7% and 4%, respectively, and impaired feed conversion ratio by 0.014-points without significant mortality rate, indicating the successful introduction of mild necrotic enteritis. In general, challenged birds presented lower ileal insoluble NSP and OS concentrations. The reason for this is unclear, which requires further elucidation. One possible explanation was that a lower feed intake in challenged birds compared to non-challenged birds may contribute to the flow of insoluble NSP and OS in the ileum. In birds fed the wheat-based diet, all the supplemental enzymes significantly increased weight gain and numerically lowered feed conversion ratio compared to the non-supplemented birds, even in the presence of NE. However, this was not the case for the maize-based diet. Enzyme supplementation numerically impaired or significantly decreased overall weight gain in birds fed the maize-based diet, regardless of NE infection, with XYN10 presenting a more pronounced negative impact on growth performance.

It is well established that endo-β-1,4-xylanases degrade D-xylose backbones present in xylans and arabinoxylans (AX), releasing low-molecular carbohydrates such as arabinoxylan-oligosaccharides (AXOS) (Morgan et al., 2017, 2019). AX is the

**Table 6**Ileal concentration (g/kg marker, dry matter basis) of oligosaccharides constituent sugars in response to necrotic enteritis challenge, diet type and supplemental enzymes at 16 d of age.<sup>1</sup>

| Item                           |              | Rhamnose | Arabinose          | Xylose            | Mannose           | Galactose         | Glucose           | Total <sup>2</sup> |                   |                   |      |
|--------------------------------|--------------|----------|--------------------|-------------------|-------------------|-------------------|-------------------|--------------------|-------------------|-------------------|------|
| Challenge                      | Diet type    | Enzyme   |                    |                   |                   |                   |                   |                    |                   |                   |      |
| No                             | Wheat        |          | 0.28               | 0.91 <sup>a</sup> | 1.38              | 2.9 <sup>a</sup>  | 7.6               | 16.5               | 29.5              |                   |      |
|                                | Maize        |          | 0.40               | 0.67 <sup>b</sup> | 0.34              | 2.2 <sup>b</sup>  | 11.0              | 17.9               | 32.5              |                   |      |
| Yes                            | Wheat        |          | 0.19               | 0.65 <sup>b</sup> | 0.95              | 1.8 <sup>c</sup>  | 5.1               | 11.1               | 19.6              |                   |      |
|                                | Maize        |          | 0.29               | 0.57 <sup>b</sup> | 0.32              | 1.9 <sup>bc</sup> | 7.6               | 12.6               | 23.2              |                   |      |
|                                | Wheat        | Control  |                    | 0.23              | 0.52 <sup>b</sup> | 0.39              | 2.2               | 6.3                | 12.8              | 22.4              |      |
|                                |              | XYN10    |                    | 0.25              | 0.50 <sup>b</sup> | 0.65              | 2.2               | 6.4                | 14.4              | 24.2              |      |
|                                |              | XYN11    |                    | 0.28              | 1.72 <sup>a</sup> | 3.51              | 2.5               | 6.8                | 12.6              | 27.2              |      |
|                                | Maize        | MAN      |                    | 0.19              | 0.40 <sup>b</sup> | 0.12              | 2.3               | 5.9                | 15.5              | 24.5              |      |
|                                |              | Control  |                    | 0.34              | 0.60 <sup>b</sup> | 0.07              | 2.0               | 9.1                | 14.9              | 27.0              |      |
|                                |              | XYN10    |                    | 0.35              | 0.63 <sup>b</sup> | 0.54              | 2.1               | 9.9                | 17.7              | 31.2              |      |
|                                | No           | Wheat    | XYN11              |                   | 0.35              | 0.62 <sup>b</sup> | 0.65              | 2.1                | 9.3               | 15.6              | 28.5 |
|                                |              |          | MAN                |                   | 0.33              | 0.63 <sup>b</sup> | 0.08              | 2.1                | 8.9               | 12.6              | 24.7 |
|                                |              |          | Control            |                   | 0.27              | 0.68              | 0.66 <sup>c</sup> | 2.7                | 7.9               | 14.8              | 27.1 |
| Maize                          |              | XYN10    |                    | 0.30              | 0.58              | 0.73 <sup>c</sup> | 2.8               | 8.0                | 17.2              | 29.5              |      |
|                                |              | XYN11    |                    | 0.32              | 1.95              | 3.99 <sup>a</sup> | 3.0               | 7.8                | 15.5              | 32.4              |      |
|                                |              | MAN      |                    | 0.23              | 0.43              | 0.15 <sup>d</sup> | 3.0               | 6.7                | 18.6              | 29.1              |      |
| Wheat                          |              | Control  |                    | 0.42              | 0.65              | 0.11 <sup>d</sup> | 2.3               | 11.3               | 19.1              | 33.9              |      |
|                                |              | XYN10    |                    | 0.42              | 0.72              | 0.55 <sup>c</sup> | 2.2               | 11.8               | 21.7              | 37.4              |      |
|                                |              | XYN11    |                    | 0.38              | 0.67              | 0.64 <sup>c</sup> | 2.2               | 10.8               | 16.2              | 30.9              |      |
| Maize                          |              | MAN      |                    | 0.37              | 0.65              | 0.08 <sup>d</sup> | 2.1               | 10.3               | 14.3              | 27.9              |      |
|                                |              | Control  |                    | 0.18              | 0.35              | 0.12 <sup>d</sup> | 1.6               | 4.7                | 10.8              | 17.7              |      |
|                                | XYN10        |          | 0.20               | 0.42              | 0.57 <sup>c</sup> | 1.7               | 4.8               | 11.5               | 19.0              |                   |      |
| Yes                            | Wheat        | XYN11    |                    | 0.23              | 1.48              | 3.02 <sup>b</sup> | 2.0               | 5.8                | 9.7               | 22.0              |      |
|                                |              | MAN      |                    | 0.15              | 0.37              | 0.10 <sup>d</sup> | 1.7               | 5.2                | 12.4              | 19.9              |      |
|                                |              | Control  |                    | 0.27              | 0.55              | 0.03 <sup>d</sup> | 1.8               | 6.9                | 10.7              | 20.2              |      |
|                                | Maize        | XYN10    |                    | 0.28              | 0.55              | 0.53 <sup>c</sup> | 1.9               | 8.1                | 13.8              | 25.0              |      |
|                                |              | XYN11    |                    | 0.32              | 0.57              | 0.66 <sup>c</sup> | 1.9               | 7.8                | 14.9              | 26.1              |      |
|                                |              | MAN      |                    | 0.28              | 0.62              | 0.08 <sup>d</sup> | 2.0               | 7.5                | 11.0              | 21.5              |      |
|                                | Main effects |          |                    |                   |                   |                   |                   |                    |                   |                   |      |
|                                | Challenge    |          |                    |                   |                   |                   |                   |                    |                   |                   |      |
|                                | No           |          |                    | 0.34 <sup>a</sup> | 0.79              | 0.86              | 2.5               | 9.3 <sup>a</sup>   | 17.2 <sup>a</sup> | 31.0 <sup>a</sup> |      |
|                                | Yes          |          |                    | 0.24 <sup>b</sup> | 0.61              | 0.64              | 1.8               | 6.3 <sup>b</sup>   | 11.8 <sup>b</sup> | 21.4 <sup>b</sup> |      |
|                                | Diet type    |          |                    |                   |                   |                   |                   |                    |                   |                   |      |
| Wheat                          |              |          | 0.24 <sup>b</sup>  | 0.78              | 1.17              | 2.3               | 6.3 <sup>b</sup>  | 13.8               | 24.6 <sup>b</sup> |                   |      |
| Maize                          |              |          | 0.34 <sup>a</sup>  | 0.62              | 0.33              | 2.1               | 9.3 <sup>a</sup>  | 15.2               | 27.8 <sup>a</sup> |                   |      |
| Enzyme                         |              |          |                    |                   |                   |                   |                   |                    |                   |                   |      |
| Control                        |              |          | 0.28 <sup>ab</sup> | 0.56              | 0.23              | 2.1               | 7.7               | 13.9               | 24.7              |                   |      |
| XYN10                          |              |          | 0.30 <sup>a</sup>  | 0.57              | 0.60              | 2.1               | 8.1               | 16.0               | 27.7              |                   |      |
| XYN11                          |              |          | 0.31 <sup>a</sup>  | 1.17              | 2.08              | 2.3               | 8.0               | 14.1               | 27.8              |                   |      |
| MAN                            |              |          | 0.26 <sup>b</sup>  | 0.52              | 0.10              | 2.2               | 7.4               | 14.1               | 24.6              |                   |      |
| SEM                            |              |          | 0.010              | 0.044             | 0.111             | 0.07              | 0.26              | 0.54               | 0.78              |                   |      |
| P-value                        |              |          |                    |                   |                   |                   |                   |                    |                   |                   |      |
| Challenge                      |              |          | <0.001             | <0.001            | <0.001            | <0.001            | <0.001            | <0.001             | <0.001            |                   |      |
| Diet type                      |              |          | <0.001             | <0.001            | <0.001            | 0.017             | <0.001            | 0.122              | 0.005             |                   |      |
| Enzyme                         |              |          | 0.011              | <0.001            | <0.001            | 0.660             | 0.313             | 0.271              | 0.065             |                   |      |
| Challenge × Diet type          |              |          | 0.381              | 0.012             | 0.001             | <0.001            | 0.094             | 0.939              | 0.818             |                   |      |
| Challenge × Enzyme             |              |          | 0.466              | 0.062             | 0.038             | 0.982             | 0.155             | 0.583              | 0.422             |                   |      |
| Diet type × Enzyme             |              |          | 0.351              | <0.001            | <0.001            | 0.755             | 0.643             | 0.060              | 0.143             |                   |      |
| Challenge × Diet type × Enzyme |              |          | 0.610              | 0.127             | 0.030             | 0.889             | 0.975             | 0.251              | 0.429             |                   |      |

Control = no enzyme; XYN10 = xylanase from family 10; XYN11 = xylanase from family 11; MAN = β-mannanase; SEM = standard error of the mean.

<sup>a–d</sup>Values in a column with no common superscripts differ significantly ( $P < 0.05$ ).<sup>1</sup> Values are based on 6 replicates per treatment, and male bird percentage of each replicate pen was used as a covariate.<sup>2</sup> Total oligosaccharides included rhamnose, fucose, ribose, arabinose, xylose, mannose, galactose and glucose.

most abundant NSP found in the cell walls of wheat and maize, with the majority being insoluble in water. AXOS and soluble AX are fermented by beneficial gut microbiota, such as *Bifidobacteria*, producing short-chain fatty acids, thereby exerting prebiotic properties (Courtin et al., 2008). Thus, the present study hypothesised that the addition of XYN10 or XYN11 to the wheat- or maize-based diet could degrade complex AX into AXOS during digestion, and potentially solubilise a proportion of the insoluble AX. The released OS were expected to be detected in the ethanol-soluble fraction of the ileal digesta.

Supplementation of XYN10 and XYN11 to the wheat-based diet increased weight gain and feed efficiency to a similar extent, regardless of NE. However, the impacts on ileal NSP concentration

were different between the 2 xylanases. Supplemental XYN11 in the wheat-based diet significantly increased degradation of both insoluble and soluble NSP, regardless of NE challenge. This subsequently increased arabinose and xylose in the OS fraction of the ileal digesta in birds fed this treatment, indicating the enzymatic degradation of dietary AX to AXOS. On the other hand, supplemental XYN10 reduced total ileal insoluble NSP level, with no effect on soluble NSP or OS levels, suggesting there was likely comparatively less *in situ* production of AXOS with this enzyme treatment. The differences in substrate affinity between the 2 xylanases likely resulted in varying extents of polymeric AX degradation, as previously seen in many studies (Choct et al., 2004; Morgan et al., 2017; Bautil et al., 2021). Thus, the disparity in ileal NSP degradation

pattern between XYN10 and XYN11 implies that *in situ* enzymatic release of OS may be more closely associated with soluble NSP degradation than solubilisation of insoluble NSP.

Although XYN10 and XYN11 presented distinct impacts on NSP degradation in birds fed the wheat-based diet, the 2 xylanases resulted in a similar improvement in weight gain. Thus, improved growth performance by XYN11 addition might be a consequence of liberation of nutrients entrapped by insoluble NSP, similar to that seen with XYN10, rather than *in situ* release of AXOS. This is partly reflected in the reduced insoluble glucose level seen in the ileum following the addition of supplemental enzymes. Although the majority of non-starch glucose is assumed to be cellulose, resistant starch can be partly measured as glucose residues during the NSP analysis (Englyst et al., 1983; Knudsen, 1997), which is hardly degraded by exogenous xylanase. Therefore, it is supposed that the reduced insoluble glucose level by either XYN10 or XYN11 supplementation to the wheat-based diet was the result of the removal of starch that was resistant to small intestinal digestion or entrapped within the cell wall matrix. This likely contributed to bird growth improvement. Hence, it is also possible that the amount of AXOS released by XYN11 was insufficient to influence animal performance. The intestinal microbiota of birds at 16 d of age may have been still in the process of adaptation to fermentable fibre because the intestinal microbiota is progressively established with advancing age (Pan and Yu, 2014). The more mature digestive system can harbour more abundant bacteria with fewer fluctuations in microbiota composition and content, which may aid with more efficient utilisation of fibrous substrates (Yang et al., 2009; Bautil et al., 2019). Thus, the benefits of the prebiotic OS released by XYN11 may be translated into animal performance at a later age. Nonetheless, the wheat-based diet supplemented with XYN10 and XYN11 were efficacious in enhancing animal performance, regardless of NE infection.

The efficacy of supplemental xylanase has been shown to be less pronounced in maize-based diets compared to wheat or barley-based diets, due to the lower concentration of soluble NSP in maize and recalcitrant characteristics of maize AX (Olukosi et al., 2007; Kiarie et al., 2014). As expected, in the present study, soluble NSP and their constituent sugar levels in the ileum were not affected by enzyme addition, due to a lower level of dietary soluble NSP in the maize-based diet. However, regardless of NE challenge, supplementation of XYN10 or XYN11 to the maize-based diet, to some extent, reduced the ileal level of insoluble glucose and xylose, leading to a reduction of total insoluble NSP. This likely indicates that the addition of XYN10 or XYN11 to the maize-based diet possibly loosened or opened up the structure of insoluble AX, thereby releasing entrapped starch or degrading resistant starch, similar to that observed in the wheat-based diet supplemented with xylanases. However, reduced insoluble NSP level in the ileum did not enhance animal performance in birds fed the maize-based diet, suggesting that this was not enough to influence physiological responses.

An explanation for the markedly impeded weight gain in the challenged birds fed the maize-based diet supplemented with either XYN10 or XYN11 is unclear in the present study. A possible assumption is that these xylanases solubilised, but did not degrade, the insoluble AX in maize, which may have increased the prevalence of solubilised NSP, thereby affecting the digesta viscosity. Previously, Pettersson and Åman (1989) demonstrated that the incubation of the rye/wheat-based diet with xylanase and  $\beta$ -glucanase reduced the insoluble AX fraction and released soluble pentoses (arabinose and xylose), subsequently increasing the viscosity of the extract of the diet-enzyme mixture. In the present study, supplementation of XYN10 or XYN11 to the maize-based diet resulted in a numerical increase in soluble NSP level in the ileum

compared to the non-supplemented birds, which may partly support the above assumption. A viscous intestinal environment is one of the major predisposing factors of NE challenge in poultry, because it slows down the digesta transit time, thereby increasing the available nutrients for the proliferation of pathogen bacteria species (Moore, 2016).

Soybean meal is the primary protein source in poultry feed, and rich in galactomannans consisting of mannose backbones with galactose as side groups (Hsiao et al., 2006). Endo- $\beta$ -1,4-mannanase is believed to randomly cleave  $\alpha$ -mannan main chains of  $\beta$ -mannan-containing polysaccharides, releasing manno-oligosaccharides (MOS) and mannose (Moreira, 2008). Although isolated MOS supplementation has been shown to have positive immunomodulatory potential (Jackson et al., 2003; Kim et al., 2011), the *in vivo* generation of MOS by  $\beta$ -mannanase has rarely been investigated in broilers. In the present study, supplementing MAN enhanced weight gain and reduced ileal insoluble and soluble NSP levels in birds fed the wheat-based diet, regardless of NE challenge. However, no changes in OS and their constituent sugars were observed upon MAN supplementation, suggesting no *in situ* release of OS during insoluble NSP hydrolysis. Thus, it is assumed that improved bird performance in the wheat-based diet supplemented with MAN was associated with reduced digesta viscosity and release of entrapped nutrients (Lee et al., 2003). The decreased insoluble NSP level in the ileum upon MAN supplementation mainly have resulted from reduced insoluble glucose level, possibly indicating degradation of resistant starch (Englyst et al., 1983), as mentioned earlier.

Similarly, MAN supplementation to the maize-based diet reduced the ileal level of insoluble NSP and insoluble glucose in the absence of NE, although this did not affect growth performance. In the presence of NE, supplemental MAN did not influence animal performance or NSP and OS profiles in the ileum. This is consistent with earlier studies where MAN supplementation to maize/soy-based diets did not improve bird performance compared to non-supplemented birds (Mehri et al., 2010; Kong et al., 2011), although it positively affected jejunal digesta viscosity. The inclusion level of MAN in maize/soy-based diets appeared to be a crucial factor that determines a significant response of birds, with higher doses than the commercially recommended dose resulting in more pronounced and consistent efficacy (Jackson et al., 2004; Arsenault et al., 2017). These may partially explain the reason for the observed lower magnitude of response to supplemental MAN in birds fed the maize-based diet in the present study. Furthermore, as the inclusion level of soybean meal was similar between the 2 diets, the disparity of bird response to MAN was probably dictated by wheat or maize.

## 5. Conclusion

The growth performance in birds fed the wheat-based diet was enhanced by the supplemental enzymes, with significant improvement in weight gain and numerical reduction in feed conversion ratio observed, regardless of the NE challenge. MAN supplementation resulted in improved growth performance when supplemented to the wheat- but not maize-based diet, regardless of NE. The distinct modes of action between XYN10 and XYN11 in the wheat-based diet were notable, with XYN11 acting on both soluble and insoluble NSP but XYN10 acting on insoluble NSP only. The supplementation of XYN11 to the wheat-based diet shows promise in releasing prebiotic oligosaccharides *in situ*, although further investigation is warranted to confirm consistent improvements in animal performance. In contrast, weight gain was not improved by supplemental enzymes in birds offered the maize-based diet. Moreover, supplementation of either XYN10 or XYN11

to the maize-based diet exacerbated the negative impact of NE on growth performance, which may stem from altered concentrations in soluble and insoluble NSP levels as a consequence of XYN10 and XYN11 addition.

### Author contributions

**Amy F. Moss:** conceptualisation, investigation, methodology, writing-review and editing. **Natalie K. Morgan:** conceptualisation, investigation, methodology, writing-review and editing. **Kosar Gharib-Naseri:** investigation, writing-review and editing. **Peter Ader:** resources, writing-review and editing. **Mingan Choct:** conceptualisation, data curation, project administration, writing-reviewing and editing, supervision, funding acquisition.

### Declaration of competing interest

All the listed authors declare that we have no affiliations with or involvement in any organisation or entity with any financial interest related to the publication of this manuscript. Authors agree the authorship criteria and have read and approved this manuscript.

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