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Original Research Article

# Non-starch polysaccharide-degrading enzymes may improve performance when included in wheat- but not maize-based diets fed to broiler chickens under subclinical necrotic enteritis challenge

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ABSTRACT

The present study investigated whether supplementing fibre-degrading enzymes can ameliorate the severity of subclinical necrotic enteritis (NE) in broiler chickens offered wheat- or maize-based diets. A total of 1,544 mixed-sex broiler chickens were assigned to 16 experimental treatments as a 2 × 2 × 4 factorial arrangement of treatments. The factors were the following: NE challenge, yes or no; diet type, wheat- or maize-based; and enzyme supplementation, control (no enzyme), family 10 xylanase (XYN10), family 11 xylanase (XYN11) or β-mannanase (MAN). Each treatment was replicated 6 times, with 16 birds per replicate pen. A three-way challenge × diet type × enzyme interaction occurred for body weight at 21 d of age ($P = 0.025$) and overall feed conversion ratio ($P = 0.001$). In the non-challenged birds fed the wheat-based diet, supplementing MAN increased d 21 body weight compared to the control. In challenged birds fed the maize-based diet, supplemental XYN11 impeded body weight and overall FCR compared to the control. Birds offered the maize-based diet presented heavier relative gizzard weights at both 16 and 21 d of age ($P < 0.001$) and reduced liveability ($P = 0.046$) compared to those fed the wheat-based diet. Enzyme supplementation reduced ileal and jejunal digesta viscosity at 16 d of age only in birds fed the wheat-based diet ($P < 0.001$). XYN11 increased ileal digesta viscosity in birds fed the maize-based diet, and MAN reduced it in birds fed the wheat-based diet at 21 d of age ($P = 0.030$). Supplementing XYN11 improved ileal soluble non-starch polysaccharides (NSP) digestibility in birds fed the wheat-based diet compared to non-supplemented birds ($P < 0.001$). Birds fed the wheat-based diet displayed a higher abundance of *Bifidobacterium, Lactobacillus* and Enterobacteriaceae and butyric acid in the caeca at 16 d of age compared to birds fed the maize-based diet ($P < 0.05$). In conclusion, supplemental XYN11 exacerbated the negative impact of NE on growth performance in birds fed the maize-based diet. Supplementing wheat-based diets with fibre-degrading enzymes ameliorates production losses induced by NE.



## 1. Introduction

Wheat, maize and soybean meal are the most commonly used feed ingredients for poultry diets globally. These vegetable feedstuffs contain substantial amounts of non-starch polysaccharides (NSP) with varying physicochemical characteristics. Based on their water solubility, NSP can be divided into soluble and insoluble fractions, which induce different impacts on the gastrointestinal tract of birds (Choct, 1997). For instance, viscous grains such as wheat and barley contain a higher concentration of soluble NSP,

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which hold a large amount of water in their matrices, leading to increased small intestinal digesta viscosity (Choct and Annison, 1992). This viscous gut environment is often detrimental to bird growth as it markedly hinders nutrient digestion and absorption, by interfering with accessibility of endogenous enzymes to their substrates (Bedford, 1997). Non-viscous grains, such as maize, have very low soluble NSP content so do not induce increased digesta viscosity. They do however still contain NSP in the insoluble form, which generally decreases the nutritional value of feed ingredients by encapsulating protein and lipids within intact cell walls, thereby acting as a nutrient diluent and physical barrier during digestion (Holland et al., 2020).

In spite of the anti-nutritional properties of NSP on nutrient digestibility and bird growth, the provision of the appropriate type and form of dietary NSP can aid the proliferation and development of beneficial microbiota in the gut (Snel et al., 2002). A moderate amount of insoluble NSP in poultry diets also appears beneficial for the physical development of the foregut (Hetland et al., 2004). However, birds are inherently incapable of digesting dietary NSP, due to a lack of endogenous enzymes for NSP hydrolysis. In the absence of exogenous enzyme inclusion to poultry diets, the utilisation of NSP can only be achieved by acidic degradation in the gizzard and microbial fermentation in the hindgut (Choct, 2015). As such, exogenous NSP-degrading enzymes are commonly supplemented in poultry diets to improve nutrient utilisation and growth performance of birds.

Another mechanism by which NSP-degrading enzymes may improve bird performance is the in situ generation of bioactive compounds from complex NSP. During the hydrolysis of NSP, NSP-degrading enzymes may release prebiotic oligosaccharides that are non-digestible but selectively utilised by beneficial gut bacteria, particularly *Bifidobacteria* and *Lactobacillus* (Svihus et al., 2013). The microbial fermentation of oligosaccharides produces volatile fatty acids (VFA) as end products. VFA can provide the gut epithelium with energy and maintain low pH along the gastrointestinal tract, which may limit the proliferation of acid-sensitive pathogens (Malbert, 1999; Merrell and Camilli, 2002). These oligosaccharides have been shown to induce prebiotic effects in poultry when supplemented into the diet (Keerqin et al., 2017; Morgan et al., 2019). Nonetheless, the in vivo generation of prebiotic oligosaccharides and their impacts on growth performance and gut health has scarcely been studied in chickens.

The prebiotic effects of oligosaccharides generated in situ by feed enzymes on animal performance may be key to maintaining and improving performance in the post-antibiotic era. There have been growing concerns worldwide about bacterial resistance to antimicrobial agents, resulting in restrictions and bans in the use of in-feed antibiotics during chicken-meat production (Castanon, 2007). The consequence of this has been increased incidences of enteric diseases, such as necrotic enteritis (NE) caused by pathogenic *Clostridium perfringens*. The clinical signs of NE include dehydration, diarrhoea, and lower weight gain and feed intake, leading to flock mortality (Shojadoost et al., 2012). Subclinical NE presents the above clinical signs with less significant mortality compared to the clinical form. However, subclinical NE may be more detrimental to broiler productivity than clinical NE, as lack of obvious signs results in delayed diagnosis, thereby increasing economic losses (Skinner et al., 2010). The pathogenesis of NE can be affected by diet composition, with the anti-nutritional impacts of NSP presenting one predisposing dietary factor that exacerbates the severity of NE in broilers (Annett et al., 2002). Thus, it is crucial to elucidate how dietary NSP and NSP-degrading enzymes relate to NE severity along the gastrointestinal tract in order to develop nutritional strategies to alleviate such health challenges, and improve overall chicken-meat production.

Therefore, the present study investigated the hypothesis that supplementing wheat- or maize-based diets with xylanase, from either family 10 or family 11, or β-mannanase, would improve growth performance, nutrient utilisation and VFA production in broiler chickens during subclinical NE challenge, via in situ production of prebiotic oligosaccharides.

## 2. Materials and methods

### 2.1. Animal ethics

All experimental procedures were approved by the Animal Ethics Committee of University of New England (AEC20-005).

### 2.2. Experimental design, housing and diets

Cobb 500 mixed-sex broiler chickens ($n = 1{,}544$) were obtained from a commercial hatchery (Baiada, Tamworth, NSW, Australia). Upon arrival, all birds were weighed and allocated to 96 floor-pens (1.07 $m^2$). Initial pen weights were checked to ensure there was no statistical differences between pens. Wood shavings were used as a bedding material (depth = 7 cm). The experimental design was completely randomised with a $2 \times 2 \times 4$ factorial arrangement of treatments, resulting in 16 treatments, 6 replicate pens per treatment and 16 birds per pen. Factors were NE challenge (yes or no), diet type (wheat- or maize-based), and enzyme treatment (control; no enzyme, xylanase from family 10 and 11, and β-mannanase). All pens were equipped with bell feeders and nipple drinkers. Feed and water were provided ad libitum. The temperature was maintained at 32 °C and gradually reduced to 22 °C by 21 d of age. Birds received 23 h of light (35 lx) for the first seven d and 19 h (15 lx) for the remainder of the experiment.

The formulated and analysed composition of the basal diets are shown in Tables 1 and 2, respectively. The diets were formulated based on wheat and soybean meal or maize and soybean meal, according to the nutrient specification for Cobb 500 broilers (Cobb-Vantress, 2018). The dry matter, crude protein and amino acid contents of wheat, maize and soybean meal were measured using near-infrared reflectance spectroscopy (Foss NIR 6500, Denmark) predicted by Evonik AMINO NIR Advanced calibration before diet formulation. Starter diets were offered from 0 to 9 d post–hatch and the grower from 10 to 21 d post–hatch. All diets contained phytase (Natuphos E; 10,000 FTU/g) and titanium dioxide (5 g/kg) as an indigestible marker. Diets were cold-pelleted at 65 °C, and the starter diet was crumbled. Each basal diet was split into 4 portions; one portion was the control (non-enzyme treatment), and the other portions were supplemented with one of the three test enzymes. Xylanase from family 10 of glycoside hydrolase (XYN10) was from *Aspergillus niger* and contained 5,600 units of endo-β-1,4-xylanase activity per gram. Xylanase from family 11 of glycoside hydrolase (XYN11) originated from *Pseudomonas fluorescens* and contained 16,000 units of endo-β-1,4-xylanase activity per gram. Beta-mannanase (MAN) from *Thermothelomyces thermophile* contained 8,800 units of endo-β-1,4-mannanase activity per gram. The enzymes were top-dressed on top of the basal formula and thoroughly homogenised into the diet before pelleting.

### 2.3. Necrotic enteritis challenge

On 9 d of age, birds in the challenged group were orally inoculated with 1 mL of a suspension containing 5,000 sporulated *Eimeria acervulina* and *Eimeria maxima*, and 2,500 sporulated oocysts of *Eimeria brunetti* (Eimeria Pty Ltd., Glenorie, NSW, Australia). Birds in the non-challenged group were gavaged with sterile phosphate buffered saline as a sham treatment. On 14 and 15 d of

**Table 1**
Basal diet composition.

| Item | Starter (d 0 - 9) | | Grower (d 10 - 21) | |
|---|---|---|---|---|
| | Wheat | Maize | Wheat | Maize |
| Ingredients, % as fed | | | | |
| Wheat (11.1% CP) | 59.94 | – | 65.19 | – |
| Maize (8.8% CP) | – | 58.70 | – | 64.51 |
| Soybean meal (47% CP) | 32.37 | 34.90 | 26.89 | 29.04 |
| Canola oil | 3.04 | 1.59 | 3.63 | 2.00 |
| Limestone | 1.18 | 1.15 | 1.13 | 1.07 |
| Dicalcium phosphate[1] | 1.58 | 1.69 | 1.48 | 1.61 |
| Salt | 0.21 | 0.21 | 0.24 | 0.23 |
| Sodium bicarbonate | 0.21 | 0.26 | 0.10 | 0.15 |
| $TiO_2$ | 0.50 | 0.50 | 0.50 | 0.50 |
| Vitamin premix[2] | 0.09 | 0.09 | 0.09 | 0.09 |
| Mineral premix[3] | 0.11 | 0.11 | 0.11 | 0.11 |
| Choline Cl (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/metric ton) | 0.01 | 0.01 | 0.01 | 0.01 |
| Calculated value, % | | | | |
| Metabolisable energy, kcal/kg | 2,950 | 2,950 | 3,050 | 3,050 |
| Crude protein | 22.5 | 21.8 | 20.4 | 19.4 |
| Crude fat | 4.9 | 4.1 | 5.5 | 4.6 |
| Digestible arginine | 1.30 | 1.29 | 1.15 | 1.13 |
| 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 |
| Sodium | 0.18 | 0.18 | 0.16 | 0.16 |
| Chloride | 0.24 | 0.24 | 0.24 | 0.24 |
| Choline | 1,700 | 1,700 | 1,600.0 | 1,600.0 |
| Linoleic 18:2 | 1.46 | 1.83 | 1.63 | 2.03 |

[1] Dicalcium phosphate contained: phosphorus, 18%; calcium, 21%.
[2] Vitamin concentrate supplied per kilogram of diet: retinol, 12,000 IU; cholecalciferol, 5,000 IU; tocopheryl acetate, 75 mg, menadione, 3 mg; thiamine, 3 mg; riboflavin, 8 mg; niacin, 55 mg; pantothenate, 13 mg; pyridoxine, 5 mg; folate, 2 mg; cyanocobalamin, 16 μg; biotin, 200 μg; cereal-based carrier, 149 mg; mineral oil, 2.5 mg.
[3] Trace mineral concentrate supplied per kilogram of diet: Cu (sulfate), 16 mg; Fe (sulfate), 40 mg; I (iodide), 1.25 mg; Se (selenate), 0.3 mg; Mn (sulfate and oxide), 120 mg; Zn (sulfate and oxide), 100 mg; cereal-based carrier, 128 mg; mineral oil, 3.75 mg.

**Table 2**
Analysed composition of experimental diets (%, DM basis).[1]

| Item | Starter (d 0 - 9) | | Grower (d 10 - 21) | |
|---|---|---|---|---|
| | Wheat | Maize | Wheat | Maize |
| Dry matter | 88.9 | 87.4 | 88.6 | 88.0 |
| Ash | 6.8 | 7.2 | 6.8 | 6.7 |
| Gross energy, MJ/kg | 16.8 | 16.2 | 16.8 | 16.3 |
| Crude protein | 24.3 | 22.0 | 22.8 | 19.5 |
| Starch | 40.0 | 42.5 | 41.5 | 44.8 |
| Oligosaccharides | 4.82 | 5.03 | 4.27 | 4.20 |
| Total NSP | 11.8 | 10.8 | 11.6 | 9.8 |
| Soluble NSP | 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 NSP | 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 |

NSP = non-starch polysaccharides.
[1] Samples analysed in quadruplicate.

age, birds in the challenged group were orally inoculated per os with 1 mL of *C. perfringens* EHE-NE18 ($10^8$ CFU/mL), while those in the non-challenged group received 1 mL of sterile thioglycolate broth as a sham treatment.

### 2.4. Data and sample collection

Performance, including body weight, feed intake and mortality, was recorded for the starter (d 0 to 9) and grower (d 10 to 21) phases. Feed conversion ratio was calculated and corrected for mortality. Mortality was recorded daily, and the liveability was calculated from 7 to 21 d of age, following *Emeria* spp. and *C. perfringens* challenge. On 16 (post–NE) and 21 d post–hatch (after recovery), 4 birds per pen were chosen for biopsy. At 16 d of age, 2 of the birds selected for sampling were orally administered with fluorescein isothiocyanate-dextran (FITC-d; 4.16 mg/kg live weight) using a crop needle. After 210 min, all 4 sampled birds (2 FITC-d treated and 2 not-treated) were stunned and decapitated for blood collection. Blood samples were collected into vacutainers containing a silicone-separator gel for serum separation. At both 16 and 21 d of age, digesta samples were collected from the jejunum and ileum of all 4 sample birds. The caecal contents were collected and pooled per replicate, mixed and then a subsample was collected into sterile 2 mL Eppendorf tubes, frozen in liquid nitrogen and then stored at −20 °C until microbial analysis. The empty gizzard weight was recorded following removal of gizzard contents. The abdominal fat pad was collected and weighed. The relative weight of the gizzard and fat pad was expressed as percentage of live body weight. The duodenum, jejunum and ileum of all sample birds were examined for intestinal lesion scores by experienced personnel, without prior knowledge of the experimental design, according to Keyburn et al. (2006). Lesions in the gut section were scored as follows: 0, no lesions; 1, thin walled or fragile intestine; 2, focal necrosis with 1 to 5 foci; 3, focal necrosis with 6 to 15 foci; 4, focal necrosis with more than 16 foci; 5, 2 to 3 cm patches of necrosis; 6, severe and extensive necrosis.

### 2.5. Chemical analysis

Digesta samples were lyophilised and ground to pass through a 0.5-mm sieve using a centrifuge mill (ZM 200, Retsch, Haan, Germany). Diet samples were also ground through a 0.5-mm sieve. Diet and digesta samples were analysed for dry matter, nitrogen, gross energy, NSP constituent sugars and $TiO_2$. Dry matter content was measured according to the standard method of AOAC (2012) (method 930.15). Nitrogen was determined by the combustion method (LECO Corp., St. Joeseph, MI), and the determined nitrogen values were multiplied by a factor of 6.25 to obtain protein values. Gross energy was determined using a bomb calorimeter (6400, Parr Instruments, Moline, IL) and standardised with benzoic acid. Gas chromatography of NSP constituent sugars as alditol acetates were performed with an Agilent 8890 GC equipped with an Agilent 7693 A Autosampler, according to the methods of Theander et al. (1995) and Englyst et al. (1994), with some modifications as described by Morgan et al. (2019). Uronic acid was quantified following the method of Scott (1979) using a spectrophotometer at 450 and 400 nm (UV–1600PC, VWR, Darmstadt, Germany). The concentration of $TiO_2$ was determined by the method described by

Short et al. (1996) using UV-spectroscopy at 410 nm (Cary 50 Bio UV–Visible spectrophotometer, Varian Inc., Palo Alto, CA). VFA composition (formic, acetic, propionic, butyric, isobutyric, valeric and isovaleric acid), along with lactic acid and succinic acid, in fresh ileal and caecal contents was determined by gas chromatography (Model CP3800, Varian Inc., Palo Alto, CA), as per the procedure described by Bach Knudsen et al. (1991) and Richardson et al. (1989), with ethylbutyric acid as an internal standard. Blood samples were kept at room temperature for 3 h and centrifuged at 3,000 × *g* at 4 °C for 10 min to separate the serum from the blood cells. Serum FITC-d levels were measured at the excitation wavelength of 485 nm and emission wavelength of 528 nm using Synergy HT, multimode microplate reader (SpectraMax M2e, Molecular Devices, CA), as outlined by Kuttappan et al. (2015). The fluorescence levels were then calculated using an equation from a previously drawn standard curve with known FITC-d concentrations ($R^2$ = 0.992). The FITC-d values obtained from FITC-d free birds were used as a blank.

### 2.6. Caecal bacterial group quantification

Caecal bacterial DNA was extracted using DNeasy PowerSoil Pro Kit (QIAGEN, Germany) according to the manufacturer's instructions. The extracted DNA was checked for quantity and purity by spectrophotometry (NanoDrop ND-8000, ThermoFisher Scientific, Waltham, MA). The extracted DNA with high purity (>1.8 at a wavelength of A260/280 nm) was diluted 20 times in nuclease-free water. qPCR was performed with a real-time PCR system (Rotor-Gene Q, Qiagen, Germany) using SensiFAST SYBR No-ROX (Bioline, Sydney, Australia) to quantify *Bacillus* spp., *Bacteroides* spp., *Bifidobacterium* spp., *Ruminococcus* spp., *Lactobacillus* spp. and Enterobacteriaceae, and SensiFAST Probe No-ROX (Bioline, Sydney, Australia) to quantify *C. perfringens*. The primers and probe used for the bacterial groups are shown in Table 3. Bacteria numbers are expressed as $\log_{10}$ genomic DNA copy number/g wet digesta.

### 2.7. Calculation and statistical analysis

Apparent nutrient digestibility (%) was calculated using the following equation:

$$\text{Digestibility}(\%) = \{[(\text{Nutrient}/\text{TiO}_2)_{\text{diet}} - (\text{Nutrient}/\text{TiO}_2)_{\text{digesta}}]/(\text{Nutrient}/\text{TiO}_2)_{\text{diet}}\} \times 100$$

All data were checked for normal distribution before statistical analysis. Normally distributed data were analysed using three-way ANCOVA (IBM SPSS Statistics 27, Armonk, NY). The performance criteria and relative organ weights were analysed with the percentage of male birds in each pen as a covariate. Means were separated using Tukey's multiple range test when $P < 0.05$. The non-normal VFA concentration, intestinal lesion score and liveability data were analysed using a nonparametric Kruskal–Wallis test.

## 3. Results

### 3.1. Growth performance and liveability

Table 4 presents the effects of experimental treatments on growth performance. At 9 d of age, birds offered the wheat-based diet exhibited a greater ($P$ = 0.012) body weight and lower feed conversion ratio ($P$ = 0.014) compared to birds fed the maize-based diet. A two-way diet type × enzyme interaction occurred for feed intake from 0 to 9 d of age, where birds offered the wheat-based diet supplemented with XYN11 presented a greater feed intake compared to the non-supplemented birds. At 21 d of age, a three-way challenge × diet type × enzyme interaction ($P$ = 0.025) was detected for body weight at 21 d of age. In the non-challenged birds offered the wheat-based diet, supplementing MAN increased body weight by 4.2% compared to those fed the control wheat-based diet. However, when NE was present, supplementing XYN11 to birds offered the maize-based diet significantly decreased body weight compared to birds fed the diet without enzymes or with MAN, by 4.0% and 4.4%, respectively at 21 d of age. When NE was absent, supplementing XYN10 and XYN11 numerically improved body weight at 21 d of age, regardless of diet type. Overall (d 0 to 21) feed conversion ratio followed a similar pattern ($P$ = 0.001). In challenged birds fed with maize-based diet, supplementing XYN11 impeded overall feed conversion ratio compared to those fed the control diet, by 0.06 point. Three-way challenge × diet type × enzyme interactions were observed for feed intake during the grower phase ($P$ = 0.004) and overall period ($P$ = 0.019). When NE was present, birds fed the maize-based diet supplemented with XYN11 exhibited a lower feed intake compared to non-supplemented birds. In the absence of NE, enzyme supplementation increased feed intake in birds fed the wheat-based diet, but reduced it in birds fed the maize-based diet.

Table 5 shows that birds offered the maize-based diet presented a lower ($P$ = 0.046) liveability from 9 to 21 d of age compared to those fed the wheat-based diet. Birds fed a diet supplemented with XYN11 presented a lower ($P$ = 0.034) liveability compared to those offered the diets supplemented with XYN10 and MAN.

**Table 3**
Details of primers used for caecal bacterial group quantification.

| Target group or organism | Primer sequence (5′ – 3′) | Amplicon length, bp | Annealing temperature, °C | Reference |
|---|---|---|---|---|
| *Bacillus* spp. | F- GCA ACG AGC GCA ACC CTT GA<br>R- TCA TCC CCA CCT TCC GGT | 92 | 60 | Han et al. (2012) |
| *Bacteroides* spp. | F- GAG AGG AAG GTC CCC CAC<br>R- CGC TAC TTG GCT GGT TCA G | 108 | 63 | Layton et al. (2006) |
| *Bifidobacterium* spp. | F- GCG TCC GCT GTG GGC<br>R- CTT CTC CGG CAT GGT GTT G | 106 | 63 | Requena et al. (2002) |
| *Clostridium perfringens* | F- GCA TAA CGT TGA AAG ATG G<br>R- CCT TGG TAG GCC GTT ACC C<br>TaqMan Probe-FAM-TCA TCA TTC AAC CAA AGG AGG AAT CC-TAMRA | 105 | 60 | Wise and Siragusa (2005) |
| Enterobacteriaceae | F- CAT TGA CGT TAC CCG CAG AAG AAG C<br>R- CTC TAC GAG ACT CAA GCT TGC | 190 | 63 | Bartosch et al. (2004) |
| *Lactobacillus* spp. | F- CGA TGA GTG CTA GGT GTT GGA<br>R- CAA GAT GTC AAG ACC TGG TAA G | 186 | 63 | Fu et al. (2006) |
| *Ruminococcus* spp. | F- GGC GGC YTR CTG GGC TTT<br>R- CCA GGT GGA TWA CTT ATT GTG TTA A | 157 | 63 | Ramirez-Farias et al. (2008) |

**Table 4**
Effects of subclinical necrotic enteritis challenge, diet type and supplemental enzymes on growth performance of broilers.[1,2]

| Challenge | Diet type | Enzyme | Body weight, g | | Feed intake, g | | | Feed conversion ratio, g/g[3] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | d 9 | d 21 | d 0 - 9 | d 10 - 21 | d 0 - 21 | d 0 - 9 | d 10 - 21 | d 0 - 21 |
| Three-way interaction | | | | | | | | | | |
| No | Wheat | Control | 265 | 1,001$^{bcd}$ | 235.9 | 1,034$^{ab}$ | 1,270$^{abcd}$ | 1.048 | 1.416$^{abc}$ | 1.327$^{abc}$ |
| | | XYN10 | 265 | 1,025$^{ab}$ | 220.2 | 1,047$^{ab}$ | 1,268$^{abcd}$ | 0.980 | 1.381$^{abcd}$ | 1.289$^{bcd}$ |
| | | XYN11 | 260 | 1,028$^{ab}$ | 220.7 | 1,060$^{a}$ | 1,281$^{abc}$ | 1.007 | 1.380$^{abcd}$ | 1.296$^{abcd}$ |
| | | MAN | 265 | 1,043$^{a}$ | 226.4 | 1,062$^{a}$ | 1,288$^{a}$ | 1.009 | 1.364$^{bcd}$ | 1.285$^{bcd}$ |
| | Maize | Control | 257 | 1,017$^{abc}$ | 222.0 | 1,061$^{a}$ | 1,283$^{ab}$ | 1.024 | 1.397$^{abcd}$ | 1.314$^{abcd}$ |
| | | XYN10 | 258 | 1,039$^{a}$ | 233.0 | 1,032$^{abc}$ | 1,265$^{abcd}$ | 1.067 | 1.324$^{cd}$ | 1.267$^{cd}$ |
| | | XYN11 | 262 | 1,049$^{a}$ | 229.7 | 1,027$^{abc}$ | 1,257$^{abcd}$ | 1.045 | 1.303$^{d}$ | 1.244$^{abcd}$ |
| | | MAN | 259 | 1,020$^{ab}$ | 221.0 | 1,048$^{ab}$ | 1,269$^{abcd}$ | 1.008 | 1.377$^{abcd}$ | 1.294$^{abcd}$ |
| Yes | Wheat | Control | 267 | 949$^{e}$ | 235.7 | 1,005$^{bcd}$ | 1,241$^{abcde}$ | 1.040 | 1.475$^{a}$ | 1.366$^{a}$ |
| | | XYN10 | 263 | 975$^{de}$ | 231.5 | 997$^{cd}$ | 1,228$^{de}$ | 1.039 | 1.402$^{abcd}$ | 1.315$^{abcd}$ |
| | | XYN11 | 267 | 972$^{de}$ | 226.2 | 1,004$^{bcd}$ | 1,230$^{de}$ | 0.999 | 1.425$^{abcd}$ | 1.322$^{abcd}$ |
| | | MAN | 260 | 978$^{de}$ | 227.4 | 1,010$^{bcd}$ | 1,237$^{bcde}$ | 1.033 | 1.406$^{abcd}$ | 1.318$^{abcd}$ |
| | Maize | Control | 253 | 985$^{cd}$ | 225.2 | 978$^{d}$ | 1,203$^{e}$ | 1.063 | 1.333$^{bcd}$ | 1.272$^{cd}$ |
| | | XYN10 | 264 | 977$^{de}$ | 238.6 | 1,024$^{abc}$ | 1,263$^{abcd}$ | 1.064 | 1.439$^{ab}$ | 1.349$^{abc}$ |
| | | XYN11 | 262 | 946$^{e}$ | 229.0 | 977$^{d}$ | 1,206$^{e}$ | 1.048 | 1.429$^{abc}$ | 1.331$^{ab}$ |
| | | MAN | 260 | 988$^{cd}$ | 229.2 | 1,003$^{bcd}$ | 1,232$^{cde}$ | 1.053 | 1.378$^{abcd}$ | 1.300$^{abcd}$ |
| Main effects | | | | | | | | | | |
| Challenge | | No | 261 | 1,028 | 226.1 | 1,046 | 1,273 | 1.023 | 1.368 | 1.290 |
| | | Yes | 262 | 971 | 230.3 | 1,000 | 1,230 | 1.042 | 1.411 | 1.321 |
| Diet type | | Wheat | 264$^{a}$ | 996 | 228.0 | 1,027 | 1,255 | 1.019$^{b}$ | 1.406 | 1.315 |
| | | Maize | 259$^{b}$ | 1,003 | 228.5 | 1,019 | 1,247 | 1.046$^{a}$ | 1.372 | 1.296 |
| Enzyme | | Control | 260 | 988 | 229.7 | 1,019 | 1,249 | 1.043 | 1.405 | 1.320 |
| | | XYN10 | 263 | 1004 | 230.8 | 1,025 | 1,256 | 1.038 | 1.386 | 1.305 |
| | | XYN11 | 263 | 999 | 226.4 | 1,017 | 1,244 | 1.025 | 1.384 | 1.298 |
| | | MAN | 261 | 1,007 | 226.0 | 1,030 | 1,256 | 1.026 | 1.381 | 1.299 |
| SEM[4] | | | 0.8 | 4.0 | 1.03 | 3.5 | 3.5 | 0.005 | 0.006 | 0.004 |
| *P*-value | | | | | | | | | | |
| Challenge | | | 0.771 | <0.001 | 0.030 | <0.001 | <0.001 | 0.078 | <0.001 | <0.001 |
| Diet type | | | 0.012 | 0.195 | 0.811 | 0.071 | 0.116 | 0.014 | 0.002 | 0.013 |
| Enzyme | | | 0.729 | 0.037 | 0.214 | 0.178 | 0.226 | 0.523 | 0.330 | 0.134 |
| Challenge × diet type | | | 0.991 | 0.879 | 0.929 | 0.953 | 0.984 | 0.842 | 0.888 | 0.878 |
| Challenge × enzyme | | | 0.605 | 0.054 | 0.573 | 0.175 | 0.087 | 0.618 | 0.014 | 0.017 |
| Diet type × enzyme | | | 0.151 | 0.094 | <0.001 | 0.038 | 0.039 | 0.177 | 0.045 | 0.023 |
| Challenge × diet type × enzyme | | | 0.301 | 0.025 | 0.512 | 0.004 | 0.019 | 0.305 | <0.001 | 0.001 |

[a-e] Within a column, means with no common superscripts differ significantly ($P < 0.05$).
[1] Means are based on 6 replicates per treatment, and male bird percentage of each replicate was used as a covariate.
[2] Control = no enzyme; XYN10 = xylanase from family 10; XYN11 = xylanase from family 11; MAN = β-mannanase.
[3] Feed conversion ratio corrected for mortality.
[4] SEM = standard error of the mean.

**Table 5**
Effects of subclinical necrotic enteritis challenge, diet type and supplemental enzymes on liveability (%) in broilers from 9 to 21 d of age.[1,2]

| Main effects | | Liveability |
|---|---|---|
| Challenge | No | 95.3 |
| | Yes | 95.3 |
| Diet type | Wheat | 96.2$^{a}$ |
| | Maize | 94.3$^{b}$ |
| Enzyme | Control | 95.2$^{ab}$ |
| | XYN10 | 96.7$^{a}$ |
| | XYN11 | 93.0$^{b}$ |
| | MAN | 96.2$^{a}$ |
| SEM[3] | | 0.48 |
| *P*-value | | |
| Challenge | | 0.957 |
| Diet type | | 0.046 |
| Enzyme | | 0.034 |
| Challenge × diet type | | 0.439 |
| Challenge × enzyme | | 0.648 |
| Diet type × enzyme | | 0.669 |
| Challenge × diet type × enzyme | | 0.500 |

[a-b] Within a column, means with no common superscripts differ significantly ($P < 0.05$).
[1] Means are based on 6 replicates per treatment, and male bird percentage of each replicate was used as a covariate.
[2] Control = no enzyme; XYN10 = xylanase from family 10; XYN11 = xylanase from family 11; MAN = β-mannanase.
[3] SEM = standard error of the mean.

### 3.2. Relative organ weights

Table 6 illustrates the effects of the experimental treatments on the relative weight of empty gizzard and abdominal fat pad. Birds fed the maize-based diet exhibited greater ($P < 0.001$) gizzard and abdominal fat pad weights compared to those fed the wheat-based diet at both 16 and 21 d of age. At 21 d of age, birds fed a diet supplemented with XYN11 presented heavier gizzard ($P = 0.011$) and abdominal fat pad ($P = 0.005$) compared to the control treatment. The NE challenge increased the relative weight of the gizzard ($P < 0.001$) and abdominal fat pad ($P < 0.001$) compared to the non-challenged birds at 21 d of age.

### 3.3. Digesta viscosity

Table 7 reports the effects of the experimental treatments on digesta viscosity. A two-way diet type × enzyme interaction ($P < 0.001$) occurred on jejunal viscosity. This showed a greater reduction in viscosity as a consequence of enzyme inclusion in birds fed the wheat-based diet compared to those fed the corn-based diets, with XYN11 resulting in a more pronounced reduction at both 16 and 21 d of age. A two-way challenge × diet type interaction ($P = 0.025$) was observed on jejunal digesta viscosity at 21 d of age. Specifically, the NE challenge heightened jejunal viscosity in birds fed the wheat-based diet, but this was not the case in birds fed the maize-based diet. Two-way diet type × enzyme interactions

**Table 6**
Effects of subclinical necrotic enteritis challenge, diet type and supplemental enzymes on relative organ weights (% of live body weight) of broilers at 16 and 21 d of age.[1,2]

| Challenge | Enzyme | Empty gizzard | | Abdominal fat pad | |
|---|---|---|---|---|---|
| | | d 16 | d 21 | d 16 | d 21 |
| Two-way interaction | | | | | |
| No | Control | 2.15 | 1.81 | 0.94$^{ab}$ | 0.77 |
| | XYN10 | 2.22 | 1.87 | 1.00$^{a}$ | 0.81 |
| | XYN11 | 2.23 | 1.93 | 0.89$^{abc}$ | 0.87 |
| | MAN | 2.27 | 1.93 | 0.93$^{abc}$ | 0.85 |
| Yes | Control | 2.20 | 1.90 | 0.77$^{c}$ | 0.74 |
| | XYN10 | 2.16 | 2.01 | 0.82$^{bc}$ | 0.79 |
| | XYN11 | 2.21 | 2.03 | 0.87$^{abc}$ | 0.80 |
| | MAN | 2.27 | 1.98 | 0.85$^{abc}$ | 0.72 |
| Main effects | | | | | |
| Challenge | No | 2.22 | 1.88$^{b}$ | 0.09 | 0.83$^{a}$ |
| | Yes | 2.21 | 1.98$^{a}$ | 0.83 | 0.76$^{b}$ |
| Diet type | Wheat | 2.11$^{b}$ | 1.85$^{b}$ | 0.81$^{b}$ | 0.71$^{b}$ |
| | Maize | 2.32$^{a}$ | 2.02$^{a}$ | 0.96$^{a}$ | 0.87$^{a}$ |
| Enzyme | Control | 2.18 | 1.85$^{b}$ | 0.85 | 0.75$^{b}$ |
| | XYN10 | 2.19 | 1.94$^{ab}$ | 0.91 | 0.80$^{ab}$ |
| | XYN11 | 2.22 | 1.98$^{a}$ | 0.88 | 0.84$^{a}$ |
| | MAN | 2.27 | 1.96$^{ab}$ | 0.88 | 0.78$^{ab}$ |
| SEM[3] | | 0.018 | 0.017 | 0.015 | 0.012 |
| *P*-value | | | | | |
| Challenge | | 0.725 | <0.001 | <0.001 | <0.001 |
| Diet type | | <0.001 | <0.001 | <0.001 | <0.001 |
| Enzyme | | 0.101 | 0.011 | 0.319 | 0.005 |
| Challenge × diet type | | 0.166 | 0.547 | 0.398 | 0.174 |
| Challenge × enzyme | | 0.597 | 0.709 | 0.045 | 0.080 |
| Diet type × enzyme | | 0.337 | 0.824 | 0.548 | 0.757 |
| Challenge × diet type × enzyme | | 0.338 | 0.853 | 0.784 | 0.316 |

[a-c] Within a column, means with no common superscripts differ significantly ($P < 0.05$).
[1] Means are based on 6 replicates per treatment with 4 birds per replicate on d 16 and 3 birds per replicate on d 21.
[2] Control = no enzyme; XYN10 = xylanase from family 10; XYN11 = xylanase from family 11; MAN = β-mannanase.
[3] SEM = standard error of the mean.

occurred on ileal digesta viscosity at both 16 and 21 d of age ($P < 0.001$ and $P = 0.030$, respectively). At 16 d of age, in birds fed the wheat-based diet supplementing enzymes reduced ileal digesta viscosity compared to the non-supplemented birds. At 21 d of age, in birds fed the maize-based diet, supplementing XYN11 increased ileal digesta viscosity compared to those fed the control diet.

### 3.4. Nutrient digestibility

The effect of the experimental treatments on apparent ileal nutrient digestibility is presented in Table 8. Birds under NE challenge exhibited a lower ileal digestibility of dry matter ($P < 0.001$), but the opposite was true for ileal digestibility of oligosaccharides ($P < 0.001$). Birds offered the maize-based diet presented a higher digestibility of dry matter ($P < 0.001$) and oligosaccharides ($P = 0.048$) in comparison with birds offered the wheat-based diet, but the reverse was true for oligosaccharides ($P = 0.048$). Ileal starch digestibility was decreased by NE challenge in birds fed the wheat-based diet, but not in birds offered the maize-based diet, resulting in a two-way challenge × diet type interaction ($P = 0.006$). Birds fed the maize-based diet exhibited ($P < 0.001$) a lower ileal digestibility of insoluble NSP than those fed the wheat-based diet. Supplementation of MAN improved ($P = 0.041$) ileal insoluble NSP digestibility compared to the control. A two-way diet type × enzyme interaction ($P < 0.001$) occurred on ileal soluble NSP digestibility, where supplementing XYN11 improved ileal soluble NSP digestibility compared to the control treatment in birds fed the wheat-based diet, but not in those fed the maize-based diet.

**Table 7**
Effects of subclinical necrotic enteritis challenge, diet type and supplemental enzymes on jejunal and ileal digesta viscosity in millipascal-seconds (mPa·s) in broilers at 16 and 21 d of age.[1,2]

| Challenge | Diet type | Enzyme | Jejunum | | Ileum | |
|---|---|---|---|---|---|---|
| | | | d 16 | d 21 | d 16 | d 21 |
| Two-way interactions | | | | | | |
| No | Wheat | | 2.90 | 2.27$^{ab}$ | 3.23 | 2.75 |
| | Maize | | 1.93 | 2.06$^{bc}$ | 2.55 | 3.05 |
| Yes | Wheat | | 2.89 | 2.41$^{a}$ | 3.00 | 2.94 |
| | Maize | | 1.70 | 1.83$^{c}$ | 2.21 | 2.81 |
| | Wheat | Control | 4.12$^{a}$ | 2.55$^{a}$ | 4.15$^{a}$ | 3.25$^{a}$ |
| | | XYN10 | 2.64$^{b}$ | 2.40$^{ab}$ | 2.90$^{b}$ | 3.01$^{ab}$ |
| | | XYN11 | 1.95$^{c}$ | 2.07$^{bcd}$ | 2.64$^{b}$ | 2.75$^{ab}$ |
| | | MAN | 2.72$^{b}$ | 2.35$^{abc}$ | 2.87$^{b}$ | 2.34$^{b}$ |
| | Maize | Control | 1.83$^{c}$ | 1.80$^{d}$ | 2.21$^{b}$ | 2.24$^{b}$ |
| | | XYN10 | 1.77$^{c}$ | 1.99$^{d}$ | 2.33$^{b}$ | 3.09$^{ab}$ |
| | | XYN11 | 1.80$^{c}$ | 2.01$^{cd}$ | 2.51$^{b}$ | 3.29$^{a}$ |
| | | MAN | 1.85$^{c}$ | 1.97$^{d}$ | 2.49$^{b}$ | 3.01$^{ab}$ |
| SEM[3] | | | 0.091 | 0.047 | 0.079 | 0.102 |
| *P*-value | | | | | | |
| Challenge | | | 0.366 | 0.582 | 0.021 | 0.881 |
| Diet type | | | <0.001 | <0.001 | <0.001 | 0.770 |
| Enzyme | | | <0.001 | 0.517 | <0.001 | 0.404 |
| Challenge × diet type | | | 0.106 | 0.025 | 0.441 | 0.387 |
| Challenge × enzyme | | | 0.070 | 0.839 | 0.146 | 0.716 |
| Diet type × enzyme | | | <0.001 | 0.038 | <0.001 | 0.030 |
| Challenge × diet type × enzyme | | | 0.267 | 0.490 | 0.180 | 0.384 |

[a-d] Within a column, means with no common superscripts differ significantly ($P < 0.05$).
[1] Means are based on 6 replicates per treatment with 4 birds per replicate on d 16 and 3 birds per replicate on d 21.
[2] Control = no enzyme; XYN10 = xylanase from family 10; XYN11 = xylanase from family 11; MAN = β-mannanase.
[3] SEM = standard error of the mean.

### 3.5. Volatile fatty acid profile

#### 3.5.1. Ileum

The profile and concentrations of VFA in the ileum at 16 and 21 d of age are shown in Tables 9 and 10, respectively. The experimental treatments had no significant impact on ileal acetic or butyric acid concentration or total VFA levels at 16 or 21 d of age ($P > 0.05$). A two-way diet type × enzyme interaction was observed for formic acid concentration at both 16 and 21 d of age, whereby XYN10 and MAN supplementation in the maize-based diet increased the level compared to the control, but the opposite was true in birds fed the wheat-based diet. The NE challenge increased ($P < 0.001$) ileal succinic acid level compared to the non-challenged birds at both 16 and 21 d of age. At 16 d of age, ileal lactic acid level was greater in the challenged birds compared to non-challenged birds, and supplementing MAN increased the level in the challenged birds, resulting in a two-way interaction between NE challenge and enzymes ($P = 0.001$). There was a two-way diet type × enzyme interaction ($P < 0.001$) on ileal lactic acid level at 16 d of age, where supplemental XYN11 increased the level only in birds offered the wheat-based diet. At 21 d of age, supplementation with XYN10 and XYN11 resulted in a lower level of ileal lactic acid compared to those supplemented with MAN and the non-supplemented birds ($P = 0.041$). At 21 d of age, challenged birds displayed ($P < 0.001$) a higher ileal level of propionic acid compared to the non-challenged birds.

#### 3.5.2. Caeca

Table 11 reports the caecal VFA profile at 16 d of age. A three-way interaction on caecal isovaleric acid level ($P = 0.026$) was observed at 16 d of age. Non-challenged birds fed the maize-based diet supplemented with MAN presented a lower isovaleric acid level in the caeca compared to corresponding birds challenged with

**Table 8**
Effects of subclinical necrotic enteritis challenge, diet type and supplemental enzymes on apparent ileal nutrient digestibility (%) in broilers at 16 d of age.[1, 2]

| Challenge | Diet type | Enzyme | Dry matter | N | Starch | Soluble NSP | Insoluble NSP | Oligo-saccharides |
|---|---|---|---|---|---|---|---|---|
| Two-way interactions | | | | | | | | |
| No | Wheat | | 63.2 | 79.2 | 95.8$^{b}$ | −64.9 | −48.7 | −16.4 |
| | Maize | | 67.1 | 78.5 | 97.1$^{b}$ | −36.9 | −75.3 | −8.3 |
| Yes | Wheat | | 55.9 | 77.5 | 91.6$^{a}$ | −74.2 | −41.1 | 6.1 |
| | Maize | | 63.0 | 77.4 | 96.2$^{b}$ | −67.6 | −73.2 | 15.3 |
| | Wheat | Control | 58.6 | 76.6 | 92.8 | −89.9$^{c}$ | −57.7 | −1.5 |
| | | XYN10 | 60.5 | 79.9 | 94.8 | −84.8$^{bc}$ | −39.7 | −4.6 |
| | | XYN11 | 61.2 | 78.1 | 95.3 | −45.3$^{ab}$ | −45.1 | −10.2 |
| | | MAN | 58.0 | 78.9 | 91.9 | −58.1$^{abc}$ | −37.1 | −4.4 |
| | Maize | Control | 65.1 | 77.4 | 96.3 | −38.9$^{a}$ | −80.9 | 9.7 |
| | | XYN10 | 65.2 | 78.5 | 96.6 | −53.7$^{abc}$ | −72.2 | −8.2 |
| | | XYN11 | 64.5 | 77.0 | 96.7 | −66.5$^{abc}$ | −66.8 | −3.5 |
| | | MAN | 65.2 | 78.9 | 97.0 | −50.0$^{abc}$ | −77.2 | 16.1 |
| Main effects | | | | | | | | |
| Challenge | | No | 65.1$^{a}$ | 78.8 | 96.5 | −50.9$^{a}$ | −62.6 | −12.3$^{b}$ |
| | | Yes | 59.5$^{b}$ | 77.5 | 93.9 | −70.9$^{b}$ | −57.2 | 10.7$^{a}$ |
| Grain type | | Wheat | 59.6$^{b}$ | 78.4 | 93.7 | −69.5 | −44.9$^{a}$ | −5.2$^{b}$ |
| | | Maize | 65.0$^{a}$ | 78.0 | 96.7 | −52.3 | −74.9$^{b}$ | 3.5$^{a}$ |
| Enzyme | | Control | 61.8 | 77.0 | 94.5 | −64.4 | −67.9$^{b}$ | 4.1 |
| | | XYN10 | 62.9 | 79.2 | 95.7 | −69.3 | −55.9$^{ab}$ | −6.4 |
| | | XYN11 | 65.9 | 77.5 | 96.0 | −55.9 | −63.3$^{ab}$ | −6.9 |
| | | MAN | 61.6 | 78.9 | 94.5 | −54.0 | −52.4$^{a}$ | 5.9 |
| SEM[3] | | | 0.07 | 0.44 | 0.36 | 3.61 | 2.58 | 2.50 |
| *P*-value | | | | | | | | |
| Challenge | | | <0.001 | 0.136 | <0.001 | 0.002 | 0.207 | <0.001 |
| Diet type | | | <0.001 | 0.650 | <0.001 | 0.006 | <0.001 | 0.048 |
| Enzyme | | | 0.817 | 0.268 | 0.151 | 0.265 | 0.047 | 0.072 |
| Challenge × diet type | | | 0.185 | 0.756 | 0.006 | 0.087 | 0.614 | 0.902 |
| Challenge × enzyme | | | 0.751 | 0.622 | 0.488 | 0.962 | 0.660 | 0.970 |
| Diet type × enzyme | | | 0.646 | 0.829 | 0.097 | <0.001 | 0.583 | 0.271 |
| Challenge × diet type × enzyme | | | 0.774 | 0.847 | 0.847 | 0.611 | 0.129 | 0.409 |

[a-c] Within a column, means with no common superscripts differ significantly ($P < 0.05$).
[1] Means are based on 6 replicates per treatment with 4 birds per replicate.
[2] Control = no enzyme; XYN10 = xylanase from family 10; XYN11 = xylanase from family 11; MAN = β-mannanase.
[3] SEM = standard error of the mean.

NE. Hence, a three-way interaction occurred on caecal succinic acid level ($P = 0.032$) at 16 d of age. In only the non-challenged birds fed the wheat-based diet, supplemental MAN increased the caecal concentration of succinic acid compared to those fed the control wheat-based diet. Two-way challenge × enzyme interactions were observed on formic ($P = 0.038$), acetic ($P = 0.004$), propionic ($P = 0.036$) and lactic acid ($P < 0.001$), and total VFA ($P = 0.003$) concentration in the caeca at 16 d of age. In birds fed the control diet, challenging the birds decreased the caecal level of formic acid compared to the non-challenged birds. Non-challenged birds offered the diets supplemented with MAN or XYN10 presented a higher caecal acetic acid level compared to the non-challenged birds fed any other treatment. Conversely, challenged birds supplemented with MAN or XYN10 presented a higher caecal concentration of propionic acid compared to the non-challenged birds fed these enzymes. Birds fed the control diet or diet supplemented with MAN presented a higher caecal concentration of lactic acid compared to those supplemented with XYN10 or XYN11 when NE was present, or the non-challenged birds fed any treatment. Caecal total VFA level was increased by MAN supplementation compared to birds fed the control diet or diet supplemented with XYN11 in the absence of the NE challenge, and in the challenged birds regardless of enzyme treatment. Two-way challenge × diet type interactions occurred ($P < 0.001$) for caecal levels of acetic acid and total VFA. In only birds fed the wheat-based diet, challenging birds decreased these levels in the caeca compared to non-challenged birds. Again, two-way diet type × enzyme interactions were observed on acetic acid ($P = 0.005$) and total VFA ($P < 0.001$) concentrations in the caeca. XYN10, XYN11 and MAN supplementation increased the caecal levels of acetic acid and total VFA only in birds fed the wheat-based diet. Other volatile fatty acids such as butyric ($P = 0.004$), isobutyric ($P < 0.001$) and valeric acid ($P < 0.001$) in the caeca were decreased following the NE challenge compared to non-challenged birds. Birds fed wheat-based diets presented a higher ($P < 0.001$) caecal level of butyric acid compared to those fed maize-based diets, but the opposite was true for isobutyric acid ($P < 0.001$). Supplemental XYN10 and MAN increased ($P = 0.023$) the caecal concentration of butyric acid compared to the control diet.

Table 12 presents the caecal VFA concentrations at 21 d of age. Two-way challenge × diet type interactions were observed for formic ($P = 0.042$), acetic ($P = 0.014$) and isobutyric acid ($P = 0.037$) levels in the caeca at 21 d of age. An increase in the caecal concentration of formic and acetic acid following the NE challenge occurred in birds fed the wheat-based diet but not in those fed the maize-based diet, whereas the opposite was true for isobutyric acid. A two-way diet type × enzyme interaction ($P = 0.019$) occurred on caecal succinic acid level at 21 d of age. In birds offered the wheat-based diet only, supplementing MAN increased the caecal succinic acid level compared to non-supplemented birds. Birds fed the wheat-based diet presented higher caecal concentrations of butyric ($P < 0.001$) and lactic acid ($P < 0.001$), and total VFA ($P < 0.001$) compared to those offered the maize-based diet, but the reverse was true for propionic ($P = 0.001$), valeric ($P < 0.001$) and isovaleric acid ($P < 0.001$) levels in the caeca.

### 3.6. Caecal bacterial quantification

The abundance of caecal microbiota groups at 16 d of age is presented in Table 13. No interactions between the factors were observed. The genomic DNA copy numbers of *C. perfringens* was higher ($P < 0.001$) in NE-challenged birds compared to non-

**Table 9**
Effects of subclinical necrotic enteritis challenge, diet type and supplemental enzymes on ileal volatile fatty acids (μmol/g) in broilers at 16 d of age.[1,2]

| Challenge | Diet type | Enzyme | Formic | Acetic | Propionic | Butyric | Total VFA[3] | Succinic | Lactic |
|---|---|---|---|---|---|---|---|---|---|
| Two-way interactions | | | | | | | | | |
| No | | Control | 0.13 | 2.7 | 0.24 | 0.11 | 3.1 | 0.09 | 13.7$^{c}$ |
| | | XYN10 | 0.05 | 2.4 | 0.23 | 0.00 | 2.7 | 0.15 | 14.4$^{c}$ |
| | | XYN11 | 0.00 | 2.7 | 0.15 | 0.00 | 2.9 | 0.17 | 20.4$^{c}$ |
| | | MAN | 0.06 | 2.6 | 0.19 | 0.14 | 3.0 | 0.10 | 14.0$^{c}$ |
| Yes | | Control | 0.06 | 3.0 | 0.16 | 0.74 | 4.0 | 0.36 | 34.0$^{b}$ |
| | | XYN10 | 0.13 | 2.6 | 0.21 | 0.14 | 3.1 | 0.35 | 33.4$^{b}$ |
| | | XYN11 | 0.05 | 3.0 | 0.19 | 0.36 | 3.6 | 0.46 | 33.1$^{b}$ |
| | | MAN | 0.09 | 2.3 | 0.60 | 0.42 | 3.6 | 0.66 | 44.4$^{a}$ |
| | Wheat | Control | 0.16$^{a}$ | 3.4 | 0.22 | 0.92 | 4.7 | 0.18 | 18.2$^{c}$ |
| | | XYN10 | 0.05$^{b}$ | 2.3 | 0.19 | 0.04 | 2.6 | 0.32 | 29.8$^{abc}$ |
| | | XYN11 | 0.02$^{b}$ | 2.9 | 0.17 | 0.18 | 3.3 | 0.34 | 35.3$^{a}$ |
| | | MAN | 0.03$^{b}$ | 2.4 | 0.16 | 0.19 | 2.8 | 0.24 | 25.2$^{abc}$ |
| | Maize | Control | 0.03$^{b}$ | 2.3 | 0.19 | 0.00 | 2.5 | 0.28 | 29.8$^{abc}$ |
| | | XYN10 | 0.13$^{a}$ | 2.7 | 0.25 | 0.11 | 3.2 | 0.18 | 18.3$^{bc}$ |
| | | XYN11 | 0.03$^{b}$ | 2.8 | 0.17 | 0.20 | 3.2 | 0.31 | 19.4$^{bc}$ |
| | | MAN | 0.11$^{a}$ | 2.5 | 0.62 | 0.37 | 3.8 | 0.52 | 32.1$^{ab}$ |
| Main effects | | | | | | | | | |
| Challenge | | No | 0.06 | 2.6 | 0.20 | 0.07 | 2.9 | 0.12$^{a}$ | 15.7 |
| | | Yes | 0.08 | 2.8 | 0.29 | 0.41 | 3.6 | 0.46$^{b}$ | 36.4 |
| Diet type | | Wheat | 0.07 | 2.8 | 0.19 | 0.31 | 3.3 | 0.26 | 26.9 |
| | | Maize | 0.08 | 2.6 | 0.31 | 0.17 | 3.2 | 0.32 | 25.2 |
| Enzyme | | Control | 0.10 | 2.8 | 0.21 | 0.43 | 3.6 | 0.22 | 23.7 |
| | | XYN10 | 0.09 | 2.5 | 0.22 | 0.07 | 2.9 | 0.25 | 23.7 |
| | | XYN11 | 0.03 | 2.8 | 0.17 | 0.18 | 3.2 | 0.31 | 27.2 |
| | | MAN | 0.07 | 2.5 | 0.39 | 0.28 | 3.3 | 0.38 | 29.6 |
| SEM[4] | | | 0.016 | 0.12 | 0.056 | 0.089 | 0.22 | 0.046 | 1.54 |
| *P*-value | | | | | | | | | |
| Challenge | | | 0.607 | 0.519 | 0.442 | 0.050 | 0.135 | <0.001 | <0.001 |
| Diet type | | | 0.841 | 0.519 | 0.282 | 0.409 | 0.771 | 0.501 | 0.307 |
| Enzyme | | | 0.361 | 0.578 | 0.501 | 0.524 | 0.751 | 0.541 | 0.033 |
| Challenge × diet type | | | 0.392 | 0.292 | 0.361 | 0.934 | 0.926 | 0.818 | 0.068 |
| Challenge × enzyme | | | 0.334 | 0.809 | 0.399 | 0.800 | 0.978 | 0.990 | 0.001 |
| Diet type × enzyme | | | 0.039 | 0.226 | 0.363 | 0.143 | 0.128 | 0.074 | <0.001 |
| Challenge × diet type × enzyme | | | 0.369 | 0.718 | 0.374 | 0.181 | 0.242 | 0.119 | 0.307 |

[a-c] Within a column, means with no common superscripts differ significantly ($P < 0.05$).
[1] Means are based on 6 replicates per treatment with 4 birds per replicate.
[2] Control = no enzyme; XYN10 = xylanase from family 10; XYN11 = xylanase from family 11; MAN = β-mannanase.
[3] Total volatile fatty acids = acetic acid + propionic acid + butyric acid + valeric acid + branched short-chain fatty acids.
[4] SEM = standard error of the mean.

challenged ones. Furthermore, NE challenge increased *Lactobacillus* ($P = 0.047$) and Enterobacteriaceae ($P = 0.011$) numbers compared to the non-challenged group, but the opposite was true for *Bifidobacterium* ($P = 0.039$). Birds fed the wheat-based diet exhibited a higher abundance of *Bifidobacterium* ($P < 0.001$), *Lactobacillus* ($P < 0.001$) and Enterobacteriaceae ($P = 0.027$) compared to those fed the maize-based diet.

### 3.7. Serum fluorescein isothiocyanate-dextran

A three-way interaction ($P = 0.008$) was found for serum FITC-d concentration (Table 14). In the absence of NE, serum FITC-d level was not affected by either the diet type or supplemental enzyme. However, in the presence of NE, supplemental enzymes reduced the serum FITC-d level in birds fed the wheat-based diet compared to non-supplemented birds, with no difference observed between the different enzyme treatments. In the challenged birds fed the maize-based diet, supplementing either XYN10 or XYN11 increased the serum level of FITC-d compared to the corresponding birds fed diets supplemented with MAN.

### 3.8. Intestinal lesion score

Table 15 illustrates the lesion scores in different parts of the small intestine at 16 d of age. A three-way interaction was found for duodenal ($P < 0.001$) and jejunal ($P = 0.008$) lesion score. In challenged birds fed the wheat-based diet, duodenal and jejunal lesion scores were significantly decreased by enzyme inclusion, with XYN10 and MAN leading to a more pronounced reduction, whereas there was no treatment effect on these lesion scores in the non-challenged birds. Birds fed the maize-based diet supplemented with XYN11 showed a higher jejunal lesion score compared to those supplemented with XYN10 and MAN, and the non-supplemented birds. Ileal lesion score was not affected by the experimental treatments.

## 4. Discussion

Subclinical NE outbreaks are difficult to detect and more economically devastating for the chicken meat industry in comparison with acute clinical NE (Shojadoost et al., 2012). In-feed antimicrobial growth promoters have been used to prevent and control NE; however, their use is now restricted in many countries due to a growing concern over the transmission of antibiotic-resistant bacteria via food consumption in public health (Castanon, 2007). Thus, a novel nutritional intervention to maintain and improve gut health was the main aim of the present study. In the present study, when compared to the non-challenged birds, the NE challenge depressed growth performance, induced necrotic lesions and increased the number of *C. perfringens* in the caecal contents without influencing mortality rates, indicating successful introduction of subclinical NE.

**Table 10**
Effects of subclinical necrotic enteritis challenge, diet type and supplemental enzymes on ileal volatile fatty acids (μmol/g) in broilers at 21 d of age.[1,2]

| Diet type | Enzyme | Formic | Acetic | Propionic | Butyric | Total VFA[3] | Succinic | Lactic |
|---|---|---|---|---|---|---|---|---|
| Two-way interaction | | | | | | | | |
| Wheat | Control | 0.5[ab] | 4.7 | 0.15 | 0.15 | 5.6 | 0.50 | 41.7 |
| | XYN10 | 0.3[b] | 4.8 | 0.27 | 0.03 | 5.4 | 0.60 | 42.9 |
| | XYN11 | 1.0[ab] | 5.4 | 0.15 | 0.12 | 6.6 | 0.48 | 34.5 |
| | MAN | 0.3[b] | 5.0 | 0.15 | 0.37 | 5.8 | 0.62 | 55.2 |
| Maize | Control | 0.3[b] | 4.5 | 0.22 | 0.01 | 5.1 | 0.79 | 69.7 |
| | XYN10 | 1.2[a] | 4.7 | 0.24 | 0.12 | 6.3 | 0.37 | 33.2 |
| | XYN11 | 0.8[ab] | 4.3 | 0.16 | 0.06 | 5.3 | 0.44 | 35.1 |
| | MAN | 0.7[ab] | 4.9 | 0.17 | 0.01 | 5.8 | 0.62 | 55.1 |
| Main effects | | | | | | | | |
| Challenge | No | 0.6 | 4.6 | 0.12[b] | 0.13 | 5.5 | 0.43[b] | 42.4 |
| | Yes | 0.7 | 5.0 | 0.26[a] | 0.09 | 6.0 | 0.68[a] | 49.7 |
| Diet type | Wheat | 0.5 | 5.0 | 0.18 | 0.18 | 5.9 | 0.55 | 43.8 |
| | Maize | 0.7 | 4.6 | 0.20 | 0.05 | 5.6 | 0.55 | 48.3 |
| Enzyme | Control | 0.4 | 4.6 | 0.19 | 0.08 | 5.3 | 0.64 | 55.7[b] |
| | XYN10 | 0.8 | 4.8 | 0.26 | 0.08 | 5.8 | 0.49 | 38.0[a] |
| | XYN11 | 0.9 | 4.9 | 0.15 | 0.10 | 6.0 | 0.46 | 35.3[a] |
| | MAN | 0.5 | 5.0 | 0.16 | 0.19 | 5.8 | 0.62 | 55.1[b] |
| SEM[4] | | 0.062 | 0.19 | 0.021 | 0.047 | 0.05 | 0.221 | 3.34 |
| *P*-value | | | | | | | | |
| Challenge | | 0.823 | 0.377 | <0.001 | 0.652 | 0.301 | 0.013 | 0.254 |
| Diet type | | 0.071 | 0.358 | 0.556 | 0.188 | 0.569 | 0.973 | 0.489 |
| Enzyme | | 0.020 | 0.926 | 0.274 | 0.812 | 0.710 | 0.441 | 0.041 |
| Challenge × diet type | | 0.378 | 0.194 | 0.590 | 0.150 | 0.245 | 0.181 | 0.053 |
| Challenge × enzyme | | 0.381 | 0.875 | 0.200 | 0.494 | 0.802 | 0.601 | 0.271 |
| Diet type × enzyme | | 0.003 | 0.755 | 0.906 | 0.424 | 0.380 | 0.274 | 0.176 |
| Challenge × diet type × enzyme | | 0.957 | 0.664 | 0.994 | 0.595 | 0.586 | 0.625 | 0.749 |

[a-b] Within a column, means with no common superscripts differ significantly ($P < 0.05$).
[1] Means are based on 6 replicates per treatment with 3 birds per replicate.
[2] Control = no enzyme; XYN10 = xylanase from family 10; XYN11 = xylanase from family 11; MAN = β-mannanase.
[3] Total volatile fatty acids = acetic acid + propionic acid + butyric acid + valeric acid + branched short-chain fatty acids.
[4] SEM = standard error of the mean.

### 4.1. Exacerbated NE symptoms by supplemental XYN11 in the maize-based diet

Enzyme type and cereal base of the diet, to an extent, affected the severity of subclinical necrotic enteritis in broiler chickens. The results from the present study show that in challenged birds fed the maize-based diet, supplementing XYN11 reduced body weight, impeded feed intake and overall feed conversion ratio. However, in the non-challenged birds fed the maize-based diet, supplemental XYN10 and XYN11 tended to increase body weight and presented little effect on feed intake when compared to the non-supplemented birds. Conversely, the growth performance of birds fed the wheat-based diet was either significantly improved or numerically increased by enzyme inclusion compared to the non-supplemented birds, regardless of the NE challenge.

This disparity between the 2 diets primarily stems from differences in digesta viscosity induced by enzyme supplementation. All the enzymes were capable of decreasing the digesta viscosity in birds fed the wheat-based diet. On the contrary, in birds fed the maize-based diet, XYN11 supplementation increased the ileal digesta viscosity due to solubilisation of insoluble NSP, thereby exacerbating the susceptibility of birds to NE. One of the major predisposing factors of NE is heightened digesta viscosity that can reduce nutrient digestibility, prolong digesta retention time and thereby increase the amount of undigested material in the intestinal lumen for pathogenic bacteria (Choct et al., 1996; Kaldhusdal and Skjerve, 1996). However, when NE was absent, increased gut viscosity induced by XYN11 inclusion likely had no impact on growth performance of birds fed the maize-based diet. Microflora dysbiosis is the major driver of NE establishment and pathogenesis (Stanley et al., 2014). Therefore, the implication is that the gastrointestinal microbiota disrupted by NE failed to adapt to the viscous gut environment upon XYN11 supplementation in birds fed the maize-based diet in the present study. Hence, in birds offered the maize-based diet, supplementing XYN11 markedly increased the serum level of leaky gut marker FITC-d and jejunal lesion score, following the NE challenge, coupled with the highest mortality observed among the treatments (11%). A higher serum leakage of FITC-d indicates impairment of gut barrier function (Latorre et al., 2018). Thus, the results from the present study emphasize that a small alteration in the gut ecology—such as that caused by dietary challenges—can considerably exacerbate the severity of NE symptoms in broilers.

### 4.2. Better growth performance and gut health by enzyme inclusions in the wheat-based diet

It should be noted that the digesta viscosity was still higher in birds fed the wheat-based diet than those fed the maize-based diet, regardless of enzyme inclusion. However, growth performance was unlikely to have been affected by this higher intestinal viscosity in birds fed the wheat-based diet, even in the presence of NE. It is possible that a higher soluble NSP content in the wheat-based diet likely acted as 'fuel' for commensal bacteria in the gut, particularly fibre-fermenting bacteria, encouraging them to establish from an early age (Lee et al., 2017; Nguyen et al., 2021). This probably boosted an adaptive change in the gastrointestinal environment, building a tolerance for intestinal viscosity prior to the NE challenge. Evidence suggested that broilers with a healthy microbiota balance presented mild NE symptoms with a less disturbance of caecal microbiota dysbiosis following *C. perfringens* challenge (Lin et al., 2017). This is reflected in the present study by higher caecal abundance of *Bifidobacterium* and Enterobacteriaceae, which are known to be beneficial bacteria, probably due to a higher level of

**Table 11**
Effects of subclinical necrotic enteritis challenge, diet type and supplemental enzymes on caecal volatile fatty acids (μmol/g) at 16 d of age.[1,2]

| Challenge | Diet type | Enzyme | Formic | Acetic | Propionic | Butyric | Isobutyric | Valeric | Isovaleric | Total VFA[3] | Succinic | Lactic |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Three-way interaction | | | | | | | | | | | | |
| No | Wheat | Control | 0.5 | 100.4 | 3.8 | 11.7 | 0.6 | 0.7 | 0.20$^{cd}$ | 117.3 | 11.6$^{bc}$ | 1.7 |
| | | XYN10 | 0.5 | 114.0 | 3.1 | 16.7 | 0.4 | 0.5 | 0.04$^{d}$ | 135.2 | 20.2$^{ab}$ | 2.5 |
| | | XYN11 | 0.4 | 108.6 | 4.4 | 18.3 | 0.6 | 0.7 | 0.16$^{cd}$ | 133.1 | 15.7$^{abc}$ | 2.3 |
| | | MAN | 0.5 | 118.7 | 3.0 | 21.7 | 0.5 | 0.5 | 0.17$^{cd}$ | 145.0 | 28.9$^{a}$ | 3.2 |
| | Maize | Control | 0.6 | 72.6 | 5.5 | 8.4 | 0.9 | 0.8 | 0.38$^{abcd}$ | 89.1 | 5.8$^{c}$ | 2.0 |
| | | XYN10 | 0.4 | 76.3 | 4.3 | 12.6 | 0.6 | 0.6 | 0.24$^{abcd}$ | 95.1 | 6.7$^{bc}$ | 1.8 |
| | | XYN11 | 0.4 | 62.5 | 4.9 | 6.8 | 0.7 | 0.6 | 0.22$^{bcd}$ | 76.2 | 10.3$^{bc}$ | 2.4 |
| | | MAN | 0.5 | 84.0 | 5.2 | 9.6 | 0.6 | 0.7 | 0.19$^{cd}$ | 100.8 | 5.8$^{c}$ | 2.2 |
| Yes | Wheat | Control | 0.2 | 70.9 | 4.9 | 18.5 | 1.0 | 1.1 | 0.53$^{bcd}$ | 95.4 | 8.4$^{bc}$ | 6.1 |
| | | XYN10 | 0.4 | 82.5 | 5.6 | 22.9 | 0.9 | 1.0 | 0.40$^{abcd}$ | 113.7 | 15.5$^{abc}$ | 3.0 |
| | | XYN11 | 0.5 | 89.5 | 4.4 | 19.7 | 0.7 | 0.8 | 0.29$^{abcd}$ | 116.0 | 17.1$^{abc}$ | 3.2 |
| | | MAN | 0.4 | 80.1 | 5.3 | 20.3 | 0.8 | 0.9 | 0.27$^{abcd}$ | 109.7 | 13.2$^{bc}$ | 8.4 |
| | Maize | Control | 0.3 | 74.5 | 5.7 | 11.8 | 1.1 | 0.8 | 0.52$^{abc}$ | 94.7 | 4.5$^{c}$ | 9.5 |
| | | XYN10 | 0.3 | 65.1 | 6.1 | 12.6 | 1.0 | 1.0 | 0.41$^{abcd}$ | 86.7 | 6.3$^{c}$ | 1.2 |
| | | XYN11 | 0.4 | 68.5 | 4.9 | 13.5 | 1.1 | 0.9 | 0.59$^{ab}$ | 89.9 | 6.9$^{bc}$ | 1.6 |
| | | MAN | 0.5 | 66.8 | 5.6 | 10.3 | 1.2 | 0.9 | 0.61$^{a}$ | 85.9 | 7.8$^{bc}$ | 4.1 |
| Two-way interactions | | | | | | | | | | | | |
| No | Wheat | | 0.44 | 110.4$^{a}$ | 3.6 | 17.0 | 0.5 | 0.6 | 0.14 | 132.6$^{a}$ | 19.1 | 2.4 |
| | Maize | | 0.49 | 73.9$^{c}$ | 5.0 | 9.3 | 0.7 | 0.7 | 0.26 | 90.3$^{c}$ | 7.2 | 2.1 |
| Yes | Wheat | | 0.37 | 80.8$^{b}$ | 5.0 | 20.3 | 0.9 | 1.0 | 0.37 | 108.7$^{b}$ | 13.5 | 5.2 |
| | Maize | | 0.37 | 68.7$^{c}$ | 5.6 | 12.1 | 1.1 | 0.9 | 0.53 | 89.3$^{c}$ | 6.4 | 4.1 |
| No | | Control | 0.54$^{a}$ | 86.5$^{abc}$ | 4.6$^{abc}$ | 9.8 | 0.8 | 0.7 | 0.29 | 103.2$^{bc}$ | 8.7 | 1.8$^{b}$ |
| | | XYN10 | 0.44$^{ab}$ | 95.1$^{ab}$ | 3.7$^{c}$ | 14.7 | 0.5 | 0.5 | 0.14 | 115.1$^{ab}$ | 13.5 | 2.2$^{b}$ |
| | | XYN11 | 0.40$^{ab}$ | 85.6$^{bc}$ | 4.7$^{abc}$ | 12.5 | 0.6 | 0.7 | 0.19 | 104.6$^{bc}$ | 13.0 | 2.4$^{b}$ |
| | | MAN | 0.48$^{ab}$ | 101.4$^{a}$ | 4.1$^{bc}$ | 15.6 | 0.6 | 0.6 | 0.18 | 122.9$^{a}$ | 17.4 | 2.7$^{b}$ |
| Yes | | Control | 0.27$^{b}$ | 72.7$^{c}$ | 5.3$^{abc}$ | 14.3 | 1.0 | 1.0 | 0.53 | 95.0$^{c}$ | 6.4 | 7.8$^{a}$ |
| | | XYN10 | 0.36$^{ab}$ | 73.8$^{c}$ | 5.9$^{a}$ | 17.8 | 1.0 | 1.0 | 0.40 | 100.2$^{bc}$ | 10.9 | 2.1$^{b}$ |
| | | XYN11 | 0.44$^{ab}$ | 79.0$^{c}$ | 4.6$^{abc}$ | 16.6 | 0.9 | 0.9 | 0.44 | 103.0$^{bc}$ | 12.0 | 2.4$^{b}$ |
| | | MAN | 0.42$^{ab}$ | 73.5$^{c}$ | 5.4$^{a}$ | 16.1 | 1.0 | 0.9 | 0.44 | 97.8$^{bc}$ | 10.5 | 6.2$^{a}$ |
| | Wheat | Control | 0.36 | 85.6$^{b}$ | 4.3 | 14.0 | 0.8 | 0.9 | 0.36 | 106.3$^{b}$ | 10.0 | 3.9 |
| | | XYN10 | 0.43 | 98.2$^{a}$ | 4.4 | 19.8 | 0.7 | 0.8 | 0.22 | 124.5$^{a}$ | 17.8 | 2.8 |
| | | XYN11 | 0.42 | 99.1$^{a}$ | 4.4 | 19.0 | 0.7 | 0.8 | 0.22 | 124.5$^{a}$ | 16.4 | 2.8 |
| | | MAN | 0.41 | 99.4$^{a}$ | 4.1 | 21.8 | 0.6 | 0.7 | 0.22 | 127.3$^{a}$ | 21.0 | 5.8 |
| | Maize | Control | 0.45 | 73.5$^{bc}$ | 5.6 | 10.1 | 1.0 | 0.8 | 0.45 | 91.9$^{c}$ | 5.1 | 5.7 |
| | | XYN10 | 0.38 | 70.7$^{c}$ | 5.2 | 12.6 | 0.8 | 0.8 | 0.33 | 90.9$^{c}$ | 6.5 | 1.5 |
| | | XYN11 | 0.41 | 65.5$^{c}$ | 4.9 | 10.2 | 0.9 | 0.8 | 0.40 | 83.0$^{c}$ | 8.6 | 2.0 |
| | | MAN | 0.49 | 75.4$^{bc}$ | 5.4 | 10.0 | 0.9 | 0.8 | 0.40 | 93.3$^{c}$ | 6.8 | 3.1 |
| Main effects | | | | | | | | | | | | |
| Challenge | | No | 0.5$^{b}$ | 92.1 | 4.3 | 13.2$^{a}$ | 0.6$^{a}$ | 0.64$^{a}$ | 0.20 | 111.5 | 13.1 | 2.3 |
| | | Yes | 0.4$^{a}$ | 74.8 | 5.3 | 16.2$^{b}$ | 1.0$^{b}$ | 0.94$^{b}$ | 0.45 | 99.0 | 10.0 | 4.7 |
| Diet type | | Wheat | 0.4 | 95.6 | 4.3$^{a}$ | 18.7$^{b}$ | 0.7$^{a}$ | 0.79 | 0.26 | 120.4 | 16.3 | 3.8 |
| | | Maize | 0.4 | 71.3 | 5.3$^{b}$ | 10.7$^{a}$ | 0.9$^{b}$ | 0.78 | 0.40 | 89.8 | 6.8 | 3.1 |
| Enzyme | | Control | 0.4 | 79.6 | 4.9 | 12.1$^{a}$ | 0.9 | 0.86 | 0.41 | 99.1 | 7.6 | 4.8 |
| | | XYN10 | 0.4 | 84.5 | 4.8 | 16.2$^{b}$ | 0.7 | 0.78 | 0.27 | 107.7 | 12.2 | 2.2 |
| | | XYN11 | 0.4 | 82.3 | 4.7 | 14.6$^{ab}$ | 0.8 | 0.77 | 0.31 | 103.8 | 12.5 | 2.4 |
| | | MAN | 0.5 | 87.4 | 4.8 | 15.9$^{b}$ | 0.8 | 0.75 | 0.31 | 110.3 | 13.9 | 4.5 |
| SEM[4] | | | 0.02 | 2.03 | 0.15 | 0.69 | 0.04 | 0.03 | 0.02 | 2.23 | 0.90 | 0.36 |
| *P*-value | | | | | | | | | | | | |
| Challenge | | | 0.019 | <0.001 | <0.001 | 0.004 | <0.001 | <0.001 | <0.001 | <0.001 | 0.022 | <0.001 |
| Diet type | | | 0.466 | <0.001 | <0.001 | <0.001 | <0.001 | 0.860 | <0.001 | <0.001 | <0.001 | 0.220 |
| Enzyme | | | 0.784 | 0.068 | 0.902 | 0.023 | 0.343 | 0.464 | 0.091 | 0.003 | 0.009 | 0.001 |
| Challenge × diet type | | | 0.493 | <0.001 | 0.112 | 0.751 | 0.813 | 0.441 | 0.546 | <0.001 | 0.080 | 0.504 |
| Challenge × enzyme | | | 0.038 | 0.004 | 0.036 | 0.530 | 0.580 | 0.337 | 0.991 | 0.003 | 0.455 | <0.001 |
| Diet type × enzyme | | | 0.470 | 0.005 | 0.706 | 0.061 | 0.850 | 0.762 | 0.780 | <0.001 | 0.086 | 0.051 |
| Challenge × diet type × enzyme | | | 0.714 | 0.789 | 0.660 | 0.275 | 0.225 | 0.203 | 0.026 | 0.489 | 0.032 | 0.254 |

[a-d] Within a column, means with no common superscripts differ significantly ($P < 0.05$).
[1] Means are based on 6 replicates per treatment with 4 birds per replicate.
[2] Control = no enzyme; XYN10 = xylanase from family 10; XYN11 = xylanase from family 11; MAN = β-mannanase.
[3] Total VFA = acetic acid + propionic acid + butyric acid + valeric acid + branched short-chain fatty acids.
[4] SEM = standard error of the mean.

fermentable fibre in the wheat-based diet. Well-established intestinal microflora can competitively eliminate pathogenic bacteria, thereby mitigating the NE symptoms.

Supplemental enzymes positively affected growth performance, digesta viscosity and gut microflora in birds receiving the wheat-based diet, which, in turn, ameliorated the negative impacts of NE on growth performance. Furthermore, the addition of enzymes to the wheat-based diet reduced serum level of FITC-d and jejunal lesion score in birds fed the wheat-based diet compared to the non-supplemented birds. Thus, better growth performance in birds fed the wheat-based diet supplemented with enzymes was likely due to improved gut health and function, mediated through a primed gut microbiota. This may indicate the enzymatic release of prebiotic oligosaccharides in situ. Reduction in digesta viscosity and increase in caecal production of VFA by enzyme inclusions in birds fed the wheat-based diet demonstrate that the enzymes hydrolysed the dietary NSP into more readily fermentable for hindgut microbiota. The anaerobic fermentation of substrates entering the caeca

**Table 12**
Effects of subclinical necrotic enteritis challenge, diet type and supplemental enzymes on caecal volatile fatty acids (μmol/g) at 21 d of age.[1,2]

| Challenge | Diet type | Enzyme | Formic | Acetic | Propionic | Butyric | Isobutyric | Valeric | Isovaleric | Total VFA[3] | Succinic | Lactic |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Two-way interactions | | | | | | | | | | | | |
| No | Wheat | | 0.30$^{b}$ | 83.6$^{b}$ | 5.3 | 20.3 | 0.8$^{c}$ | 0.8 | 0.8 | 111.4 | 13.0 | 1.8 |
| | Maize | | 0.33$^{b}$ | 68.9$^{c}$ | 6.2 | 11.8 | 1.2$^{b}$ | 0.9 | 1.2 | 89.7 | 5.8 | 0.8 |
| Yes | Wheat | | 0.76$^{a}$ | 96.1$^{a}$ | 5.3 | 18.5 | 0.9$^{c}$ | 0.8 | 0.9 | 122.7 | 9.8 | 1.8 |
| | Maize | | 0.53$^{b}$ | 69.7$^{c}$ | 6.0 | 11.3 | 1.4$^{a}$ | 1.0 | 1.4 | 90.5 | 3.1 | 0.8 |
| | Wheat | Control | 0.60 | 88.9 | 5.1 | 18.5 | 0.9 | 0.8 | 0.3 | 115.1 | 8.0$^{ab}$ | 2.2 |
| | | XYN10 | 0.50 | 90.1 | 5.1 | 17.0 | 0.9 | 0.7 | 0.3 | 114.8 | 12.5$^{a}$ | 1.7 |
| | | XYN11 | 0.49 | 91.0 | 5.5 | 19.9 | 0.8 | 0.9 | 0.3 | 118.9 | 11.2$^{a}$ | 2.0 |
| | | MAN | 0.53 | 89.5 | 5.3 | 22.2 | 0.8 | 0.9 | 0.2 | 119.4 | 14.0$^{a}$ | 1.1 |
| | Mazie | Control | 0.37 | 73.2 | 6.2 | 13.9 | 1.3 | 1.0 | 0.5 | 96.4 | 7.0$^{ab}$ | 0.8 |
| | | XYN10 | 0.44 | 72.1 | 5.9 | 11.4 | 1.4 | 1.0 | 0.5 | 92.7 | 3.8$^{b}$ | 0.9 |
| | | XYN11 | 0.40 | 65.1 | 6.0 | 11.0 | 1.2 | 0.9 | 0.4 | 85.1 | 3.6$^{b}$ | 0.6 |
| | | MAN | 0.49 | 66.7 | 6.4 | 9.8 | 1.4 | 1.0 | 0.5 | 86.2 | 3.5$^{b}$ | 0.9 |
| Main effects | | | | | | | | | | | | |
| Challenge | | No | 0.31 | 76.2 | 5.8 | 16.1 | 1.0 | 0.9 | 0.3$^{b}$ | 100.6 | 9.4$^{a}$ | 1.3 |
| | | Yes | 0.64 | 82.9 | 5.6 | 14.9 | 1.2 | 0.9 | 0.4$^{a}$ | 106.6 | 6.5$^{b}$ | 1.3 |
| Diet type | | Wheat | 0.53 | 89.9 | 5.3$^{b}$ | 19.4$^{a}$ | 0.8 | 0.8$^{b}$ | 0.3$^{b}$ | 117.1$^{a}$ | 11.4 | 1.8$^{a}$ |
| | | Maize | 0.43 | 69.3 | 6.1$^{a}$ | 11.5$^{b}$ | 1.3 | 1.0$^{a}$ | 0.5$^{a}$ | 90.1$^{b}$ | 4.5 | 0.8$^{b}$ |
| Enzyme | | Control | 0.49 | 81.1 | 5.6 | 16.2 | 1.1 | 0.9 | 0.4 | 105.8 | 7.5 | 1.5 |
| | | XYN10 | 0.47 | 81.1 | 5.5 | 14.2 | 1.2 | 0.9 | 0.4 | 103.8 | 8.2 | 1.3 |
| | | XYN11 | 0.45 | 78.1 | 5.8 | 15.5 | 1.0 | 0.9 | 0.4 | 102.0 | 7.4 | 1.3 |
| | | MAN | 0.51 | 78.1 | 5.9 | 16.0 | 1.1 | 0.9 | 0.4 | 102.8 | 8.7 | 1.0 |
| SEM[4] | | | 0.035 | 1.61 | 0.13 | 0.75 | 0.03 | 0.02 | 0.02 | 2.16 | 0.68 | 0.22 |
| *P*-value | | | | | | | | | | | | |
| Challenge | | | <0.001 | 0.005 | 0.676 | 0.376 | <0.001 | 0.373 | <0.001 | 0.076 | 0.009 | 0.988 |
| Diet type | | | 0.087 | <0.001 | 0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.033 |
| Enzyme | | | 0.904 | 0.645 | 0.818 | 0.705 | 0.063 | 0.803 | 0.194 | 0.868 | 0.814 | 0.897 |
| Challenge × diet type | | | 0.042 | 0.014 | 0.767 | 0.620 | 0.037 | 0.396 | 0.190 | 0.119 | 0.833 | 0.955 |
| Challenge × enzyme | | | 0.336 | 0.471 | 0.462 | 0.757 | 0.353 | 0.407 | 0.529 | 0.501 | 0.475 | 0.933 |
| Diet type × enzyme | | | 0.725 | 0.410 | 0.865 | 0.149 | 0.281 | 0.446 | 0.117 | 0.269 | 0.019 | 0.771 |
| Challenge × diet type × enzyme | | | 0.659 | 0.819 | 0.341 | 0.981 | 0.880 | 0.157 | 0.832 | 0.858 | 0.316 | 0.965 |

[a-c] Within a column, means with no common superscripts differ significantly ($P < 0.05$).
[1] Means are based on 6 replicates per treatment with 3 birds per replicate.
[2] Control = no enzyme; XYN10 = xylanase from family 10; XYN11 = xylanase from family 11; MAN = β-mannanase.
[3] Total VFA = acetic acid + propionic acid + butyric acid + valeric acid + branched SCFA.
[4] SEM = standard error of the mean.

**Table 13**
Effects of subclinical necrotic enteritis challenge, diet type and supplemental enzymes on abundance of caecal bacteria group at 16 d of age ($log_{10}$ genome DNA copies/g wet digesta).[1,2]

| Main effects | | *Bacillus* | *Bacteroides* | *Bifidobacterium* | *Lactobacillus* | Enterobacteriaceae | *C. perfringens* | *Ruminococcus* |
|---|---|---|---|---|---|---|---|---|
| Challenge | No | 7.80 | 5.87 | 9.93$^{a}$ | 11.03$^{b}$ | 8.18$^{b}$ | 1.83$^{b}$ | 9.51 |
| | Yes | 7.81 | 5.87 | 9.75$^{b}$ | 11.23$^{a}$ | 8.44$^{a}$ | 9.32$^{a}$ | 9.51 |
| Diet type | Wheat | 7.76 | 5.91 | 10.04$^{a}$ | 11.34$^{a}$ | 8.42$^{a}$ | 5.80 | 9.47 |
| | Maize | 7.85 | 5.83 | 9.63$^{b}$ | 10.92$^{b}$ | 8.20$^{b}$ | 5.35 | 9.55 |
| Enzyme | Control | 7.82 | 5.90 | 9.79 | 11.11 | 8.35 | 5.67 | 9.52 |
| | XYN10 | 7.82 | 5.96 | 9.70 | 11.24 | 8.24 | 4.89 | 9.53 |
| | XYN11 | 7.88 | 5.85 | 9.99 | 11.07 | 8.38 | 5.90 | 9.49 |
| | MAN | 7.70 | 5.77 | 9.86 | 11.09 | 8.26 | 5.83 | 9.49 |
| SEM[3] | | 0.048 | 0.030 | 0.048 | 0.056 | 0.052 | 0.453 | 0.018 |
| *P*-value | | | | | | | | |
| Challenge | | 0.915 | 0.937 | 0.039 | 0.047 | 0.011 | <0.001 | 0.847 |
| Diet type | | 0.333 | 0.229 | <0.001 | <0.001 | 0.027 | 0.358 | 0.055 |
| Enzyme | | 0.613 | 0.181 | 0.109 | 0.617 | 0.727 | 0.443 | 0.845 |
| Challenge × diet type | | 0.856 | 0.662 | 0.683 | 0.074 | 0.422 | 0.987 | 0.426 |
| Challenge × enzyme | | 0.098 | 0.942 | 0.605 | 0.165 | 0.381 | 0.524 | 0.421 |
| Diet type × enzyme | | 0.742 | 0.462 | 0.510 | 0.608 | 0.067 | 0.439 | 0.934 |
| Challenge × diet type × enzyme | | 0.660 | 0.332 | 0.290 | 0.560 | 0.782 | 0.540 | 0.998 |

[a, b] Within a column, means with no common superscripts differ significantly ($P < 0.05$).
[1] Means are based on 6 replicates per treatment with 4 birds per replicate.
[2] Control = no enzyme; XYN10 = xylanase from family 10; XYN11 = xylanase from family 11; MAN = β-mannanase.
[3] SEM = standard error of the mean.

produces VFA, and the fermentation pattern is highly diet-dependent (Svihus et al., 2013). Previously, Hübener et al. (2002) reported that wheat-rye diets with high soluble NSP contents produced a lot more acetate, butyrate and total VFA in the caeca compared to maize diets, similar to the outcomes from the present study. The addition of NSP-degrading enzymes to the diet can further promote the caecal production of VFA and proliferation of beneficial bacteria in birds by delivering NSP hydrolysis products to the caeca (Choct et al., 1999). The present study showed increased concentration of acetate and total VFA in the caeca due to enzyme

**Table 14**
A three-way subclinical necrotic enteritis challenge × diet type × supplemental enzymes interaction for serum fluorescein isothiocyanate-dextran (FITC-d) concentrations (mg/mL) at 16 d of age.[1,2]

| Challenge | Diet type | Enzyme | FITC-d |
|---|---|---|---|
| No | Wheat | Control | 0.10$^{e}$ |
| | | XYN10 | 0.11$^{e}$ |
| | | XYN11 | 0.12$^{e}$ |
| | | MAN | 0.10$^{e}$ |
| | Maize | Control | 0.11$^{e}$ |
| | | XYN10 | 0.11$^{e}$ |
| | | XYN11 | 0.13$^{e}$ |
| | | MAN | 0.11$^{e}$ |
| Yes | Wheat | Control | 0.30$^{abc}$ |
| | | XYN10 | 0.22$^{d}$ |
| | | XYN11 | 0.23$^{d}$ |
| | | MAN | 0.23$^{d}$ |
| | Maize | Control | 0.27$^{bcd}$ |
| | | XYN10 | 0.32$^{ab}$ |
| | | XYN11 | 0.34$^{a}$ |
| | | MAN | 0.26$^{cd}$ |
| Main effects | | | |
| Challenge | | No | 0.11 |
| | | Yes | 0.27 |
| Diet type | | Wheat | 0.18 |
| | | Maize | 0.21 |
| Enzyme | | Control | 0.20 |
| | | XYN10 | 0.19 |
| | | XYN11 | 0.21 |
| | | MAN | 0.17 |
| SEM[3] | | | 0.010 |
| *P*-value | | | |
| Challenge | | | <0.001 |
| Diet type | | | <0.001 |
| Enzyme | | | 0.070 |
| Challenge × diet type | | | 0.012 |
| Challenge × enzyme | | | 0.448 |
| Diet type × enzyme | | | 0.034 |
| Challenge × diet type × enzyme | | | 0.008 |

[a-e] Within a column, means with no common superscripts differ significantly ($P < 0.05$).
[1] Means are based on 6 replicates per treatment with 4 birds per replicate.
[2] Control = no enzyme; XYN10 = xylanase from family 10; XYN11 = xylanase from family 11; MAN = β-mannanase.
[3] SEM = standard error of the mean.

**Table 15**
A three-way subclinical necrotic enteritis challenge × diet type × supplemental enzymes interaction for intestinal lesion score at 16 d of age.[1,2]

| Challenge | Diet type | Enzyme | Duodenum | Jejunum | Ileum |
|---|---|---|---|---|---|
| No | Wheat | Control | 0.17$^{c}$ | 0.25$^{d}$ | 0.13 |
| | | XYN10 | 0.29$^{c}$ | 0.21$^{d}$ | 0.04 |
| | | XYN11 | 0.08$^{c}$ | 0.21$^{d}$ | 0.17 |
| | | MAN | 0.08$^{c}$ | 0.13$^{d}$ | 0.17 |
| | Maize | Control | 0.13$^{c}$ | 0.17$^{d}$ | 0.04 |
| | | XYN10 | 0.13$^{c}$ | 0.13$^{d}$ | 0.04 |
| | | XYN11 | 0.17$^{c}$ | 0.17$^{d}$ | 0.17 |
| | | MAN | 0.17$^{c}$ | 0.13$^{d}$ | 0.08 |
| Yes | Wheat | Control | 1.13$^{a}$ | 1.00$^{ab}$ | 0.13 |
| | | XYN10 | 0.46$^{c}$ | 0.50$^{c}$ | 0.13 |
| | | XYN11 | 0.71$^{b}$ | 0.75$^{abc}$ | 0.00 |
| | | MAN | 0.38$^{c}$ | 0.58$^{bc}$ | 0.00 |
| | Maize | Control | 0.46$^{c}$ | 0.42$^{bc}$ | 0.00 |
| | | XYN10 | 0.54$^{bc}$ | 0.29$^{cd}$ | 0.13 |
| | | XYN11 | 0.58$^{bc}$ | 1.21$^{a}$ | 0.00 |
| | | MAN | 0.54$^{bc}$ | 0.46$^{bc}$ | 0.04 |
| Main effects | | | | | |
| Challenge | | No | 0.15 | 0.17 | 0.10 |
| | | Yes | 0.60 | 0.65 | 0.05 |
| Diet type | | Wheat | 0.41 | 0.45 | 0.09 |
| | | Maize | 0.34 | 0.37 | 0.06 |
| Enzyme | | Control | 0.47 | 0.46 | 0.07 |
| | | XYN10 | 0.35 | 0.28 | 0.08 |
| | | XYN11 | 0.39 | 0.58 | 0.08 |
| | | MAN | 0.30 | 0.32 | 0.07 |
| SEM[3] | | | 0.046 | 0.054 | 0.171 |
| *P*-value | | | | | |
| Challenge | | | <0.001 | <0.001 | 0.136 |
| Diet type | | | 0.395 | 0.096 | 0.368 |
| Enzyme | | | 0.719 | 0.203 | 0.993 |
| Challenge × diet type | | | 0.465 | 0.710 | 0.764 |
| Challenge × enzyme | | | 0.837 | 0.497 | 0.069 |
| Diet type × enzyme | | | 0.464 | 0.376 | 0.673 |
| Challenge × diet type × enzyme | | | <0.001 | 0.008 | 0.579 |

[a-d] Within a column, means with no common superscripts differ significantly ($P < 0.05$).
[1] Means are based on 6 replicates per treatment with 4 birds per replicate.
[2] Control = no enzyme; XYN10 = xylanase from family 10; XYN11 = xylanase from family 11; MAN = β-mannanase.
[3] SEM = standard error of the mean.

inclusions in birds fed the wheat-based diet when compared to the non-supplemented birds. A tendency (P = 0.061) for enzyme inclusion to increase the caecal butyric acid level was also observed only in birds fed the wheat-based diet. Butyric acid is of particular interest due to its nutritional properties related to gut enterocytes, and inhibitory effects on potentially pathogenic bacteria, stimulating host immune defence system (Sunkara et al., 2011; Singh and Kim, 2021). Collectively, the development of the intestinal microbiota can be kick-started by the presence of soluble NSP, which can be further boosted by enzyme inclusions. The wheat-based diet appeared to be more advantageous than the maize-based diet following enzyme supplementation in relation to release of prebiotic oligosaccharides in situ and resulting improvements in gut health.

### 4.3. Distinct digestion dynamics between the 2 diets

In the present study, birds fed the maize-based diet presented a much higher digestibility of dry matter at the ileal level compared to those fed the wheat-based diet, whereas the opposite was true for VFA production and abundance of some bacterial groups in the caeca. This highlights the difference in nutrient digestive dynamics between the 2 diets. The likelihood is that nutrients were digested earlier in the gut with the heavier, more functional gizzards of birds offered the maize-based diets. A relatively harder particle of maize compared to wheat can enhance foregut functionality, which can aid the mixing of the digesta and exogenous and endogenous enzymes (Abdollahi et al., 2010; Rodrigues and Choct, 2018). Moss et al. (2017) has demonstrated improvements in exogenous enzyme activity, and therefore enhanced nutrient digestibility and growth performance, when gizzard weight is increased. Thus, the larger and more active gizzard of birds fed the maize-based diet in the present study likely facilitated nutrient digestion in the foregut, leading to greater nutrient utilisation. Supplemental XYN10 and XYN11 numerically improved growth performance in birds fed the maize-based diet in the absence of NE, perhaps through these enhanced gizzard functions. However, when NE was present, the earlier digestion of intact nutrients in the foregut might have provided pathogenic bacteria with more available nutrients to fuel proliferation in the small intestine.

Despite this, tangible amounts of nutrients from the wheat-based diet may also become the substrates for the resident microbiota, increasing the abundance of some bacteria groups and producing a lot more VFA in the caeca in the present study. The higher soluble NSP contents in the wheat-based diet may alter digesta retention time, enzyme efficacy and nutrient digestive dynamics. The fibre fermentation patterns of the caeca can be considerably enhanced by dietary soluble and fermentable fibre (Svihus et al., 2013). Although higher NSP contents in poultry diets are generally considered to be detrimental to nutrient utilisation and growth performance, their potential benefits on the

gastrointestinal environment should not be overlooked. For instance, the interaction of beneficial bacteria with fibre in the gut can play a major role in priming immune defence (Bao and Choct, 2010). This robust gut bacteria at an early age can have a long-lasting positive effect over time.

### 4.4. Differences in modes of action of NSP-degrading enzymes depending on the diet type

Xylanases have different substrate affinities, resulting in different impacts on digesta viscosity, NSP digestibility and intestinal microflora (Choct et al., 2004; Morgan et al., 2017). In the present study, both XYN10 and XYN11 supplementation solubilised insoluble NSP in the ileum, regardless of diet type. The addition of XYN11 to the wheat-based diet markedly improved soluble NSP digestibility in the ileum, highlighting its substrate affinity towards both soluble and insoluble NSP. Supplemental XYN10 did not affect soluble NSP digestibility in birds fed the wheat-based diet; however, it successfully reduced digesta viscosity, indicating its ability to depolymerise, to some extent, the released soluble NSP in situ. On the contrary, the addition of either XYN10 or XYN11 to the maize-based diet made digestibility of soluble NSP negative compared to the non-supplemented birds, with XYN11 markedly heightening digesta viscosity. Arabinoxylans are the most abundant insoluble NSP in both wheat and maize; however, their chemical structures and degrees of arabinose substitution vary widely between ingredients (Rose et al., 2010), which might have resulted in contrasting effects of xylanases on digesta viscosity and NSP digestibility between the 2 diets.

The response of birds to supplemental MAN was also different depending on the diet type in the present study. The supplementation of MAN was capable of improving the ileal digestibility of soluble and insoluble NSP in birds fed the wheat-based diet; however, such effects were not noted in those fed the maize-based diet. The reason for this is unclear as both diets were formulated using a similar level of soybean meal, which contained mannan-based polysaccharides as major NSP fraction (Hsiao et al., 2006). This likely indicates 2 things. Firstly, MAN may contain side activities that act on other polysaccharides, thereby presenting a differing extent of NSP degradation depending on the diet type. Secondly, the efficacy and ability of MAN may be influenced by the commensal microflora, which is primarily established based on the diet composition, as MAN supplementation markedly promoted caecal VFA production only in birds offered the wheat-based diet in the present study.

## 5. Conclusion

In conclusion, the results from the present study emphasise the complex interaction between NE challenge, diet type and supplemental enzymes. The supplementation of XYN11 in the maize-based diet markedly exacerbated the negative impacts of NE challenge, such as performance loss, leaky gut and lesion necrosis, due to elevated digesta viscosity. The wheat-based diet was notably advantaged by enzyme inclusion, presenting improved feed efficiency and VFA production, even in the presence of NE, resulting from the early establishment of robust microbiota. Collectively, wheat-based diets hold an advantage upon supplementation of NSP-degrading enzymes compared to maize-based diets in terms of in situ production of prebiotic oligosaccharides.

## Author contributions

**Eunjoo Kim:** Data curation, Formal analysis, Methodology, Investigation, Writing-Original draft preparation; **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

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

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