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In vitro degradation of non-starch polysaccharide residues in the digesta of broilers offered wheat-soy or maize-soy diets by feed enzymes

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Abstract

The present study evaluated the *in vitro* efficacy of different feed enzymes on degrading non-starch polysaccharide (NSP) residues present in digesta collected from broilers' gastrointestinal tract. Two dietary treatments, wheat-soy or maize-soy based diets, were assigned to broilers (12 replicates per treatment; 10 birds per replicate pen) from d 0 to 35. At 35 d of age, digesta was collected from the gizzard, jejunum and ileum from three birds per replicate and pooled. The digesta samples were digested *in vitro* with one of nine enzymes; GH10, GH11 and GH8 xylanase, arabinofuranosidase, a combination of GH10 xylanase and β-glucanase (XYN+BG), β-mannanase, protease, cellulase cocktail and pectate lyase. When using the gizzard digesta as substrate, GH8 xylanase reduced (P<0.005) both insoluble and soluble NSP levels compared to the control, regardless of diet type. Analysis of NSP degradation in jejunal digesta from birds fed the wheat-soy diet revealed that the three xylanases and arabinofuranosidase reduced (P<0.001) insoluble NSP level compared to the control, but the soluble NSP level was not affected by the enzyme treatments. All enzymes, except the cellulase cocktail and pectate lyase, reduced (P=0.005) the insoluble NSP level in the jejunal digesta collected from birds fed the maize-soy diet. Similarly, all enzyme preparations reduced (P<0.001) the insoluble NSP level in ileal digesta obtained from birds fed the maize-soy diet. The GH10 and GH11 xylanase, XYN+BG, cellulase cocktail and pectate lyase reduced (P<0.001) the insoluble NSP level in the ileal digesta from birds fed the wheat-soy diet compared to the control, with XYN+BG exhibiting the greatest reduction. Collectively, the present *in vitro* study indicated that the *in vitro* efficacy of enzyme preparations was dictated by the amount and type of NSP remaining undigested in various parts of the gut, which perhaps affect the *in vivo* efficacy.

Keywords: broilers, exogenous enzyme, feed additives, *in vitro* study, non-starch polysaccharides

1. Introduction

Poultry diets formulated with ingredients of plant origin contain high quantities of non-starch polysaccharides (NSP). These NSP molecules differ in physical properties, i.e. some are soluble in aqueous solutions whereas others are not. Soluble NSP exhibit anti-nutritive activities, namely increased digesta viscosity, which hinders nutrient utilisation and growth performance in birds (Smits and Annison, 1996). Insoluble NSP encapsulate nutrients within their rigid matrices, which reduces the nutritional value of feedstuffs (Hetland *et al.*, 2004). These negative impacts have led to the routine application of NSP-degrading enzymes in poultry diets to enhance the availability of dietary energy and nutrients.

When selecting appropriate enzymes for use in a diet, knowledge of target substrates and tailoring relevant enzymes to address them is of importance, as the physicochemical characteristics of NSP vary widely among feed ingredients, and even within batches of the same ingredient (Bach Knudsen, 2014). During feed

manufacturing, pelleting conditions may disrupt cell wall integrity (Abdollahi *et al.*, 2013), which likely alters the accessibility of exogenous enzymes and their target substrates. The efficacy and activity of the feed enzyme is further influenced by gastrointestinal conditions, such as pH, temperature and gut transit time (Bedford, 1995). This may influence the structure of dietary NSP during passage throughout the gastrointestinal tract of birds. Modification of the physicochemical properties of NSP can occur during normal digestive processes in birds. For instance, foregut acidification and grinding in the gizzard may break down certain glycosidic linkages and disrupt the protein bonds present in NSP (Caprita *et al.*, 2011; Mathew and Abraham, 2004), thereby increasing the permeability of cell walls. Moreover, fermentation of NSP by resident microbiota in the crop and small intestine is responsible for the modification of NSP characteristics, although this is to a lesser extent than fermentation (Jozefiak *et al.*, 2007). Thus, the target action site for the enzyme is an important consideration. Focus on the type and amount of NSP remaining undigested in various parts of the chicken gut has been, however, lacking, yielding inconsistent results for the products of NSP degradation by various enzyme preparations, both *in vivo* and *in vitro*.

The present *in vitro* study utilised the gut contents of birds as NSP substrates, with the assumption that the digesta content would have advantages over pure substrates or raw ingredients for screening various feed enzymes for their *in vivo* efficacy. The objective was to investigate the *in vitro* effects of adding feed enzymes on reducing the level of NSP remaining undigested in the gizzard, jejunal, and ileal digesta obtained from birds fed wheat-soy or maize-soy diets.

2. Materials and methods

The experimental procedures used in the present study were approved by the Animal Ethics Committee of the University of New England (AEC 18-089).

Birds, experimental diets and sample collection

A total of 240, one-day-old mixed-sex broiler chicks (Cobb 500) were sourced from a commercial hatchery (Tamworth, NSW, Australia). Upon arrival, birds were weighed and allocated to 24 pens, which were randomly assigned to one of two dietary treatments with 12 replicate pens and ten birds per pen. Birds were raised in a floor pen (120×77 m^2) with wood-shavings (depth = 7 cm). The brooding temperature was 32 °C which was gradually decreased to 22 °C by 21 d of age. Pens were equipped with a bell feeder and nipple drinkers with cups. Water and feed were provided *ad libitum*. Birds were raised with 24 h of light for the first three days and then 20 h for the rest of the study.

The two dietary treatments were a wheat-soy based diet and a maize-soy based diet that was enzyme-free and met the nutrient specifications for Cobb 500 broilers (Cobb-Vantress, 2018; Table 1). Experimental diets were provided in three phases (starter, d 0-10; grower, d 11-24 and finisher, d 25-35). All the diets were cold-pelleted at 65 °C, and the starter was crumbled.

At 35 d of age, three birds per pen, closest to the average body weight, were selected and euthanised by cervical dislocation. Digesta were collected from the gizzard, jejunum and ileum and pooled per treatment (n=36). All the samples were frozen, lyophilised and finely ground using an ultra-centrifuge mill with a 0.5 mm screen (Model ZM 200, Retsch, Hann, Germany).

Test enzymes

The commercial enzyme preparations tested included glycoside hydrolase (GH) 10 xylanase derived from *Aspergillus niger* (8,000 U/g of digesta), β-mannanase from *Termothelomyces thermophile* (8,000 U/g of digesta) and XYN+BG (a mixture of GH10 xylanase and β-glucanase from *A. niger* (512,000 U/g of digesta)). The non-commercial prototype enzyme preparations GH11 xylanase from *Pseudomonas fluorescens* (16,000 U/g of digesta), GH8 xylanase from *Escherichia coli* (<100 U/g of digesta), arabinofuranosidase from *E. coli* (<100 U/g of digesta), protease from *Bacillus* spp. (270,000 U/g of digesta), cellulase cocktail from *Trichoderma reesei* (200 U/g of digesta) and pectate lyase from *Pichia* (5,000 U/g of digesta) were evaluated. All treatments were compared against an unsupplemented control.

In vitro digestion assay

Quadruplicate samples (1 g) of gizzard, jejunal and ileal digesta were weighed into 50 ml centrifuge tubes. Samples were mixed with 9 ml of 0.1 M sodium phosphate buffer at pH 6.4 and one of the test enzymes. Tubes were sealed with a screw cap and placed vertically in a shaking water bath (200 strokes/min) at 42 °C for 2 h. The time and pH were selected top mimic small intestinal conditions in broilers, and the incubation duration was set to be 1 h shorter than the average retention time in the small intestine of broilers (Svihus and Itani, 2019). Post-incubation, the mixture was immediately placed in a water bath at 100 °C for 10 min to inactivate the enzymes. The mixture was frozen, lyophilised and ground to pass through a 0.5 mm sieve for further chemical analysis.

Determination of non-starch polysaccharides

The NSP contents in both diet and digesta samples were analysed using the method described by Englyst *et al.* (1994) with some modifications (Morgan *et al.*, 2019;

Table 1. Formulation of the experimental diets.

	Starter, d 0-12		Grower, d 13-24		Finisher, d 25-35	
Item	**Wheat**	**Maize**	**Wheat**	**Maize**	**Wheat**	**Maize**
Ingredient, %						
Wheat	64.1	-	69.5	-	71.6	-
Maize	-	60.6	-	66.3	-	67.5
Soybean meal	29.0	33.4	23.4	27.5	19.9	25.0
Canola oil	2.17	1.15	2.87	1.72	4.31	3.19
Limestone	1.17	1.13	1.12	1.08	1.06	1.02
Dicalcium phosphate	1.63	1.74	1.53	1.65	1.40	1.52
Salt	0.20	0.21	0.22	0.23	0.20	0.17
Sodium bicarbonate	0.22	0.26	0.11	0.16	0.18	0.31
TiO_2	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin-mineral premix[1]	0.18	0.18	0.18	0.18	0.18	0.18
Choline chloride	0.07	0.13	0.07	0.13	0.06	0.11
L-lysine HCl	0.31	0.28	0.24	0.22	0.26	0.22
DL-methionine	0.31	0.34	0.24	0.28	0.22	0.25
L-threonine	0.13	0.10	0.10	0.08	0.08	0.06
Analysed composition, % DM[2]						
Gross energy, kcal/kg	3,893	3,908	4,349	4,404	4,525	4,544
Crude protein	24.8	25.0	21.6	22.3	21.2	21.3
Ash	6.8	7.7	6.4	5.9	5.7	6.0
Total starch	47.2	44.4	49.6	48.1	48.9	49.2
Oligosaccharides	4.6	4.8	4.5	4.3	4.1	4.2
Total NSP	13.8	12.0	11.3	10.9	9.8	10.4
Soluble	1.42	0.79	1.39	0.68	1.72	0.86
Rhamnose	0.004	0.003	0.005	0.003	0.004	0.005
Fucose	0.006	0.006	0.005	0.005	0.003	0.005
Ribose	0.04	0.03	0.04	0.03	0.04	0.03
Arabinose	0.38	0.13	0.34	0.10	0.51	0.11
Xylose	0.46	0.04	0.41	0.04	0.64	0.06
Mannose	0.14	0.13	0.23	0.16	0.19	0.27
Galactose	0.22	0.17	0.23	0.15	0.22	0.16
Glucose	0.10	0.06	0.16	0.06	0.18	0.13
Uronic acid	0.25	0.31	0.15	0.22	0.15	0.18
Insoluble	12.33	11.21	9.94	10.22	8.11	9.53
Rhamnose	0.06	0.08	0.05	0.09	0.06	0.06
Fucose	0.11	0.13	0.10	0.12	0.08	0.10
Ribose	0.04	0.03	0.04	0.03	0.04	0.03
Arabinose	2.89	2.19	2.18	2.00	1.84	1.96
Xylose	3.06	2.09	2.54	2.03	1.91	1.90
Mannose	0.25	0.24	0.22	0.17	0.17	0.20
Galactose	1.78	2.09	1.42	1.80	1.17	1.75
Glucose	2.68	2.54	2.48	2.45	2.48	2.32
Uronic acid	2.38	3.13	2.11	2.74	1.53	2.34

[1] Trace mineral concentrate supplied per kilogram of diet: Cu (sulphate), 16 mg; Fe (sulphate), 40 mg; I (iodide), 1.25 mg; Se (selenate), 0.3 mg; Mn (sulphate and oxide), 120 mg; Zn (sulphate and oxide), 100 mg; cereal-based carrier, 128 mg; mineral oil, 3.75 mg. 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.
[2] Samples analysed in duplicates.

Theander *et al.*, 1995). The constituent sugars of the NSP were determined as alditol acetates with gas-liquid chromatography (Model CP 3800, Varian Inc., Palo Alto, CA, USA). The rhamnose, fucose, ribose, arabinose, xylose, mannose, galactose and glucose in both soluble and insoluble NSP fractions were analysed. Uronic acids were measured using the colorimetric method described by Scott (1979) in a UV-spectrophotometer at 400 and 450 nm (UV-1600PC, VWR, Darmstadt, Germany). The monosaccharide contents were converted to dry matter (DM), using the standard 930.15 method of AOAC (2012). The blank values (enzymes and enzyme carriers) were subtracted from the NSP values that were determined in the samples. The total NSP content was calculated as the sum of all constituent sugars and corrected for a polymerisation factor of 0.9. A reduction in soluble and insoluble NSP and their constituent sugars, indicated degradation of cell wall polysaccharides. Values were then expressed on a DM basis.

Statistical analysis

The male/female sex ratio of birds selected for sample collection was 0.8 and 1.3 for the birds fed the wheat-soy and the maize-soy diets, respectively. The effect of feed enzymes on NSP and their constituent sugars in the digesta was analysed for birds fed the wheat-soy and maize-soy diets, respectively. All the data were checked for normality using Shapiro-Wilk test and analysed using one-

way ANOVA (IBM SPSS Statistics 27; IBM, Armonk, NY, USA). Sex ratio of each pen did not vary significantly and all data were normally distributed. Means were separated using Tukey's test. Significant differences were assigned when $P<0.05$.

3. Results

Gizzard digesta

The NSP concentrations (g/kg DM) in the gizzard digesta upon incubation with feed enzymes are shown in Table 2. The three xylanases (GH10, GH11 and GH8), XYN+BG and protease reduced ($P<0.001$) the level of insoluble NSP in the gizzard digesta obtained from birds fed the wheat-soy diet. Only GH8 xylanase reduced both the soluble and insoluble NSP fractions in the gizzard digesta from birds fed the wheat-soy diet compared to the control treatment. All enzymes, except pectate lyase, reduced ($P<0.001$) the level of insoluble NSP compared to the digesta from birds fed the maize-soy diet. Although the soluble NSP level was generally low, GH8 xylanase reduced, and cellulase cocktail increased, soluble NSP relative to the control treatment ($P=0.003$).

Jejunal digesta

NSP degradation in the jejunal digesta following incubation with various enzymes is shown in Table 2. GH10 and GH11 xylanases and arabinofuranosidase reduced insoluble levels in the jejunal digesta from birds fed the wheat-soy diet compared to the control. No significant degradation by

Table 2. Concentration of non-starch polysaccharides (NSP) in digesta from broilers fed either a wheat-soy or maize-soy based diet following incubation with feed enzymes.[1]

	Wheat-soy diet		Maize-soy diet	
Treatment	Insoluble NSP (g/kg DM)	Soluble NSP (g/kg DM)	Insoluble NSP (g/kg DM)	Soluble NSP (g/kg DM)
Gizzard digesta				
Control	208.5^{c}	15.7^{b}	189.5^{c}	6.2^{b}
GH10 xylanase	194.5^{ab}	13.6^{ab}	175.6^{ab}	5.1^{ab}
GH11 xylanase	188.6^{a}	14.6^{ab}	175.5^{ab}	5.1^{ab}
GH8 xylanase	189.1^{ab}	11.4^{a}	165.0^{a}	4.5^{a}
Arabinofuranosidase	211.3^{c}	12.9^{ab}	171.2^{a}	5.2^{ab}
XYN+BG[2]	195.1^{ab}	13.5^{ab}	172.3^{a}	5.2^{ab}
β-Mannanase	200.7^{bc}	13.7^{ab}	175.5^{ab}	5.6^{ab}
Protease	197.0^{ab}	14.3^{ab}	166.5^{a}	5.5^{ab}
Cellulase cocktail	199.9^{abc}	14.7^{ab}	171.5^{a}	8.3^{c}
Pectate lyase	211.4^{c}	14.2^{ab}	184.3^{bc}	4.7^{ab}
SEM[3]	1.64	0.27	1.53	0.17
P-value	<0.001	0.020	0.001	0.003
Jejunal digesta				
Control	174.6^{c}	19.0	204.5^{bc}	11.0^{abc}
GH10 xylanase	157.6^{ab}	18.5	192.9^{a}	11.3^{bc}
GH11 xylanase	153.7^{a}	17.9	189.3^{a}	11.3^{bc}
GH8 xylanase	167.4^{bc}	20.8	196.1^{ab}	10.3^{abc}
Arabinofuranosidase	159.1^{ab}	21.8	186.0^{a}	10.1^{ab}
XYN+BG	172.0^{c}	18.9	190.5^{a}	11.7^{c}
β-Mannanase	174.3^{c}	19.4	193.3^{a}	9.8^{a}
Protease	173.9^{c}	21.2	191.3^{a}	10.6^{abc}
Cellulase cocktail	173.0^{c}	21.5	207.1^{c}	10.0^{ab}
Pectate lyase	174.3^{c}	20.5	195.0^{ab}	9.6^{a}
SEM	1.58	0.31	1.41	0.16
P-value	<0.001	0.053	0.005	0.004
Ileal digesta				
Control	288.7^{c}	28.5^{c}	328.5^{b}	10.9^{bcd}
GH10 xylanase	262.3^{ab}	21.1^{ab}	312.1^{a}	9.7^{ab}
GH11 xylanase	262.1^{ab}	18.8^{a}	314.8^{a}	9.6^{ab}
GH8 xylanase	275.2^{bc}	18.2^{a}	301.7^{a}	10.4^{abc}
Arabinofuranosidase	270.2^{abc}	18.4^{a}	304.0^{a}	9.1^{a}
XYN+BG	248.8^{a}	16.8^{a}	301.7^{a}	9.0^{a}
β-Mannanase	276.2^{bc}	17.9^{a}	302.0^{a}	10.0^{abc}
Protease	273.2^{bc}	27.0^{c}	308.3^{a}	12.4^{de}
Cellulase cocktail	261.4^{ab}	21.6^{ab}	304.2^{a}	12.7^{e}
Pectate lyase	256.8^{ab}	25.7^{bc}	310.2^{a}	11.6^{cde}
SEM	2.22	0.74	1.49	0.22
P-value	<0.001	<0.001	<0.001	<0.001

[1] Mean values are based on three replicates per treatment; each sample was analysed in duplicate. Values in a column with no common superscripts differ significantly ($P<0.05$).

[2] A combination of GH10 xylanase and β-glucanase.

[3] Standard error of the mean.

the enzyme preparations was observed for the soluble NSP fraction in the jejunal digesta originating from birds fed the wheat-soy diet. All enzyme preparations, except for the GH8 xylanase, pectate lyase and cellulase cocktail, decreased (P=0.005) the concentration of insoluble NSP compared to the control treatment in the jejunal digesta obtained from birds fed the maize-soy diet.

Ileal digesta

Xylanase from family GH10 and 11, XYN+BG, cellulase cocktail and pectate lyase reduced (P<0.001) the level of insoluble NSP in the ileal digesta collected from birds offered the wheat-soy diet compared to the control treatment (Table 2). For the digesta obtained from birds fed the wheat-soy diet, the level of soluble NSP was reduced (P<0.001) by the enzyme preparations compared to the control, with the exception of protease and pectate lyase. All enzyme preparations reduced the level of insoluble NSP in the ileal digesta collected from birds offered the maize-soy diet compared to the control. When using digesta from birds fed the maize-soy diet as substrate, the level of soluble NSP was decreased by XYN+BG and arabinofuranosidase, but increased by cellulase cocktail preparations, compared to the control treatment (P<0.001). The XYN+BG preparation showed a pronounced reduction in both insoluble and soluble NSP levels in the digesta obtained from birds fed both dietary treatments.

Non-starch polysaccharides constituent sugars

A detailed analysis of soluble and insoluble constituent sugar composition was conducted. The sugars that presented concentrations below 0.5 g/kg DM are not discussed. Table 3 illustrates the insoluble NSP constituent sugar profile in the digesta obtained from birds fed the wheat-soy diet following incubation with feed enzymes. The GH11 xylanase reduced (P<0.001) the level of insoluble arabinose in the gizzard digesta compared with the control treatment. When compared to the control, both GH10 and 11 xylanases reduced the recoveries of insoluble arabinose and xylose in the jejunal digesta collected from birds fed the wheat-soy diet (P<0.001). The level of insoluble uronic acid was decreased by the GH11 xylanase in the jejunal digesta (P=0.032), and by XYN+BG in the ileal digesta (P<0.001), compared to the control treatment. GH10 xylanase reduced (P<0.001) the recovery of insoluble mannose and glucose in the ileal digesta compared to the control. When compared to the control, GH11 xylanase decreased (P<0.001) the levels of arabinose, xylose, mannose and glucose in the ileal digesta collected from birds fed the wheat-soy diet. Arabinofuranosidase only reduced (P<0.001) the level of insoluble arabinose, whereas XYN+BG decreased (P<0.001) the levels of arabinose, galactose and uronic acid as part of insoluble NSP in the ileal digesta of birds fed the wheat-soy diet.

Table 4 shows the soluble NSP constituent sugar profile in digesta obtained from birds offered the wheat-soy diet upon incubation with various feed enzymes. When compared to the control, GH8 xylanase reduced the levels of xylose, mannose, galactose and glucose, whereas GH11 xylanase only reduced the level of xylose in the soluble NSP fraction of the gizzard digesta obtained from birds fed the wheat-soy diet. The XYN+BG reduced (P<0.05) the levels of soluble xylose and mannose, and arabinofuranosidase decreased (P<0.05) the recoveries of soluble glucose and uronic acid in gizzard digesta. When jejunal digesta from birds fed the wheat-soy diet acted as the substrate, protease and cellulase cocktail reduced (P<0.001) the level of soluble xylose, but increased (P<0.001) the level of uronic acid. All enzyme preparations, except protease and pectate lyase, decreased (P<0.05) the recoveries of soluble fucose, arabinose, xylose, mannose and galactose in the ileal digesta, compared to the control. The level of soluble glucose was reduced (P<0.001) by only pectate lyase compared to the control.

Table 5 shows the insoluble NSP constituent sugars in the digesta collected from birds offered the maize-soy diet. The level of galactose as part of insoluble NSP in the gizzard digesta collected from birds fed the maize-soy diet was reduced (P=0.003) by arabinofuranosidase and protease, compared to the control treatment. The concentration of insoluble glucose in the gizzard digesta obtained from birds fed the maize-soy diet was decreased (P<0.001) by both the GH10 and GH8 xylanases, protease and the cellulase cocktail, compared to the control. All enzyme preparations, except for arabinofuranosidase and β-mannanase, reduced (P<0.001) the level of insoluble uronic acids compared to the control in the jejunal digesta obtained from birds fed the maize-soy diet. Protease and cellulase cocktail reduced (P<0.001) insoluble mannose compared to the control in the jejunal digesta from birds fed the maize-soy diet. When using ileal digesta from birds fed the maize-soy diet as a substrate, the protease, cellulase cocktail and pectate lyase reduced (P<0.001) the levels of insoluble rhamnose, fucose and xylose compared to the control. The GH8 xylanase and XYN+BG led to a reduction in arabinose, xylose and uronic acid as part of the insoluble fraction, compared to the control, in the ileal digesta of birds fed the maize-soy diet (P<0.05). The GH10 and GH11 xylanase reduced (P<0.001) the level of arabinose, whereas β-mannanase reduced (P<0.05) arabinose as well as uronic acid levels in the insoluble fraction of the ileal digesta collected from birds fed the maize-soy diet, compared to the control. Arabinofuranosidase only reduced insoluble uronic acid in the ileal digesta of birds fed the maize-soy diet compared with the control.

Table 6 shows the soluble NSP constituent sugars in the digesta obtained from birds offered the maize-soy diet upon incubation with feed enzymes. Soluble mannose level in the gizzard digesta obtained from birds fed the maize-soy diet was reduced by the GH8 xylanase and XYN+BG

Table 3. The concentration of insoluble non-starch polysaccharide (NSP) constituent sugars in gizzard, jejunum and ileum digesta from broilers fed a wheat-soy based diet after incubation with feed enzymes.[1,2]

NSP (g/kg DM)	Control	GH10 xylanase	GH11 xylanase	GH8 xylanase	Arabino-furanosidase	XYN+BG[3]	β-mannanase	Protease	Cellulase cocktail	Pectate lyase	SEM[4]	*P*-value
Gizzard												
Rha	0.50^{ab}	0.49^{ab}	0.50^{ab}	0.49^{ab}	0.51^{ab}	0.56^{bc}	0.53^{abc}	0.45^{a}	0.43^{a}	0.64^{c}	0.011	<0.001
Fuc	0.74^{ab}	0.72^{ab}	0.72^{ab}	0.68^{a}	0.83^{bc}	0.75^{ab}	0.73^{ab}	0.68^{a}	0.65^{a}	0.94^{c}	0.027	<0.001
Rib	0.11^{a}	0.13^{a}	0.18^{ab}	0.13^{a}	0.31^{ab}	0.16^{ab}	0.12^{a}	0.39^{ab}	0.34^{ab}	0.47^{b}	0.565	0.002
Ara	38.0^{bc}	34.1^{ab}	31.2^{a}	33.1^{ab}	37.6^{bc}	36.2^{abc}	39.2^{bc}	35.5^{abc}	34.0^{ab}	40.4^{c}	0.70	<0.001
Xyl	75.2^{ab}	70.5^{ab}	66.9^{a}	69.8^{ab}	74.7^{ab}	73.6^{ab}	75.8^{ab}	74.3^{ab}	70.5^{ab}	76.2^{b}	0.69	0.017
Man	10.6^{a}	9.1^{a}	9.3^{a}	10.1^{a}	17.7^{bc}	10.9^{a}	10.0^{a}	17.1^{b}	14.7^{b}	21.3^{c}	0.22	<0.001
Gal	12.8^{abc}	12.2^{ab}	12.2^{ab}	12.2^{ab}	14.2^{bc}	13.2^{abc}	12.9^{abc}	12.1^{ab}	11.2^{a}	15.1^{c}	0.91	<0.001
Glu	66.9^{b}	60.9^{ab}	60.9^{ab}	61.2^{ab}	67.4^{b}	62.2^{ab}	61.0^{ab}	56.6^{a}	65.7^{b}	58.6^{ab}	0.65	<0.001
Uronic	26.9^{ab}	28.1^{b}	27.7^{a}	22.2^{ab}	21.6^{ab}	19.1^{a}	22.6^{ab}	21.7^{ab}	24.5^{ab}	21.3	1.64	0.001
Jejunum												
Rha	0.98^{c}	0.90^{abc}	0.78^{ab}	0.82^{abc}	0.86^{abc}	0.93^{bc}	0.91^{bc}	0.70^{a}	0.81^{abc}	0.77^{ab}	0.018	<0.001
Fuc	1.96^{b}	1.80^{ab}	1.80^{ab}	1.73^{ab}	1.81^{ab}	1.80^{ab}	1.82^{ab}	1.58^{a}	1.81^{ab}	1.68^{ab}	0.026	0.040
Rib	0.23	0.23	0.23	0.30	0.26	0.26	0.22	0.25	0.22	0.14	0.013	0.068
Ara	44.6^{cd}	39.8^{ab}	38.5^{a}	42.5^{abcd}	41.7^{abc}	43.6^{bcd}	44.1^{bcd}	45.1^{bcd}	44.5^{cd}	46.5^{d}	0.47	<0.001
Xyl	55.2^{bcd}	48.4^{a}	47.9^{a}	52.2^{abc}	49.6^{ab}	58.3^{d}	56.7^{cd}	54.2^{cd}	51.7^{abc}	55.5^{cd}	0.64	<0.001
Man	5.6^{abc}	5.6^{abc}	5.4^{abc}	6.4^{c}	6.0^{c}	5.8^{bc}	4.4^{a}	4.6^{a}	4.6^{ab}	4.7^{ab}	0.13	<0.001
Gal	26.4	24.4	24.4	24.3	24.5	24.6	25.1	26.9	23.5	26.3	0.27	0.077
Glu	25.2	23.1	22.3	25.2	20.2	25.6	27.3	31.7	33.8	26.0	0.93	0.081
Uronic	33.9^{b}	31.0^{ab}	29.5^{a}	32.7^{abc}	31.9^{ab}	30.1^{ab}	33.2^{ab}	28.3^{ab}	31.3^{ab}	32.1^{ab}	0.41	0.032
Ileum												
Rha	2.2^{b}	2.0^{b}	2.2^{b}	2.3^{b}	2.2^{b}	2.1^{b}	2.3^{b}	1.1^{a}	1.1^{a}	1.1^{a}	0.08	<0.001
Fuc	4.0^{c}	3.8^{bc}	4.0^{c}	3.8^{bc}	3.6^{bc}	3.5^{b}	3.9^{bc}	2.7^{a}	2.6^{a}	2.8^{a}	0.09	<0.001
Rib	0.31^{bc}	0.27^{bc}	0.31^{bc}	0.28^{bc}	0.27^{bc}	0.27^{bc}	0.38^{c}	0.19b	0.17^{b}	0.00^{a}	0.018	<0.001
Ara	80.2^{c}	72.9^{abc}	70.2^{ab}	73.6^{abc}	69.2^{ab}	67.7^{a}	75.6^{bc}	72.7^{abc}	75.7^{bc}	75.4^{bc}	0.71	<0.001
Xyl	85.6^{b}	78.0^{ab}	73.1^{a}	87.1^{b}	82.6^{ab}	77.3^{ab}	86.4^{b}	83.7^{ab}	81.0^{ab}	81.1^{ab}	0.91	<0.001
Man	7.2^{d}	5.5^{a}	6.2^{abc}	7.8^{d}	7.2^{cd}	6.8^{bcd}	5.9^{ab}	7.2^{cd}	7.5^{cd}	7.0^{cd}	0.13	<0.001
Gal	51.2^{cd}	47.1^{bcd}	51.2^{cd}	46.5^{bcd}	44.1^{bc}	42.6^{b}	47.3^{bcd}	53.1^{d}	32.2^{a}	34.0^{a}	1.16	<0.001
Glu	21.5^{cd}	14.9^{a}	15.5^{ab}	20.6^{cd}	24.4^{d}	20.4^{cd}	19.3^{bc}	20.7^{cd}	24.2^{d}	20.3^{cd}	0.60	<0.001
Uronic	68.4^{b}	67.0^{b}	68.4^{b}	63.8^{ab}	66.6^{b}	55.7^{a}	65.8^{bc}	62.1^{ab}	66.0^{b}	63.5^{ab}	0.78	<0.001

[1] Mean values are based on three replicates per treatment; each sample was analysed in duplicate. Values in a row with no common superscripts differ significantly (P<0.05).
[2] Ara = arabinose; Fuc = fucose; Gal = galactose; Glu = glucose; Man = mannose; Rha = rhamnose; Rib = ribose; Uronic = uronic acid; Xyl = xylose.
[3] A combination of GH10 xylanase and β-glucanase.
[4] Standard error of the mean.

preparations, but was increased by the cellulase cocktail (P<0.001). Compared with the control treatment, GH11 and GH8 xylanase, arabinofuranosidase and protease reduced (P<0.001) the level of soluble arabinose, whereas the cellulase cocktail and pectate lyase reduced (P<0.001) the level of soluble galactose when the substrates were the jejunal digesta collected from birds fed the maize-soy diet. The level of soluble mannose in the jejunal digesta obtained from birds fed the maize-soy diet was increased by the protease preparation. When using ileal digesta from birds fed the maize-soy diet as a substrate, soluble xylose and mannose were increased (P<0.001) by the protease, cellulase cocktail and pectate lyase, compared to the control. All the enzyme preparations, except the protease and cellulase cocktail, reduced (P<0.001) the level of soluble uronic acid in the ileal digesta collected from birds fed the maize-soy diet, compared to the control.

4. Discussion

The present *in vitro* study investigated the effects of various enzyme preparations on reducing the concentration of NSP remaining undigested in the digesta collected from various parts of the gastrointestinal tract of broiler chickens fed a common wheat-soy or maize-soy based diet. Compared to the control, most of the enzyme preparations could, to varying extents, degrade NSP residues present in the digesta across both diet types, depending on the distinct mode of action and target substrates of each enzyme preparation.

In general, the extent of NSP degradation in the gizzard and jejunal digesta was more pronounced in birds fed the maize-soy rather than the wheat-soy diet. This observation may be explained by heightened foregut digestion in birds fed the maize-soy diet, due to the hardness of the

Table 4. The concentration of soluble non-starch polysaccharide (NSP) constituent sugars in gizzard, jejunum and ileum digesta from broilers fed a wheat-soy based diet after incubation with feed enzymes.[1,2]

NSP (g/kg DM)	Control	GH10 xylanase	gh11 xylanase	GH8 xylanase	Arabino-furanosidase	XYN+BG[3]	β-mannanase	Protease	Cellulase cocktail	Pectate lyase	SEM[4]	*P*-value
Gizzard												
Rha	0.06^{c}	0.04^{bc}	0.04^{bc}	0.03^{ab}	0.02^{a}	0.05^{c}	0.04^{bc}	0.05^{bc}	0.05^{c}	0.05^{bc}	0.002	0.018
Fuc	0.09^{b}	0.08^{ab}	0.08^{ab}	0.07^{a}	0.07^{ab}	0.08^{ab}	0.08^{ab}	0.08^{ab}	0.08^{ab}	0.08^{ab}	0.002	0.418
Rib	0.28^{bc}	0.24^{ab}	0.24^{ab}	0.21^{a}	0.31^{c}	0.23^{ab}	0.23^{ab}	0.23^{ab}	0.23^{ab}	0.26^{ab}	0.006	0.007
Ara	3.7	3.7	3.7	3.4	3.6	3.4	3.6	3.3	3.3	3.6	0.05	0.054
Xyl	6.8^{c}	6.4^{bc}	5.6^{a}	6.1^{ab}	6.8^{c}	6.0^{ab}	6.8^{c}	6.5^{bc}	6.5^{bc}	7.0^{c}	0.09	0.046
Man	1.18^{b}	0.53^{ab}	0.53^{ab}	0.18^{a}	0.58^{ab}	0.31^{a}	0.69^{ab}	1.28^{b}	1.14^{b}	0.62^{ab}	0.088	0.003
Gal	2.0^{b}	1.7^{b}	1.7^{b}	1.0^{a}	1.5^{ab}	1.9^{b}	1.5^{ab}	1.8^{b}	2.0^{b}	1.6^{b}	0.06	0.005
Glu	2.8^{c}	1.8^{abc}	1.8^{abc}	1.0^{a}	1.2^{ab}	2.5^{bc}	1.9^{abc}	2.4^{bc}	2.9^{c}	2.1^{abc}	0.15	0.004
Uronic	0.74^{cd}	0.88^{d}	0.88^{d}	0.81^{cd}	0.44^{a}	0.82^{cd}	0.57^{ab}	0.51^{ab}	0.49^{ab}	0.66^{bc}	0.030	<0.001
Jejunum												
Rha	0.08^{a}	0.08^{a}	0.08^{a}	0.09^{ab}	0.10^{ab}	0.10^{ab}	0.08^{ab}	0.10^{ab}	0.11^{b}	0.08^{ab}	0.003	0.041
Fuc	0.43^{a}	0.45^{ab}	0.45^{ab}	0.48^{ab}	0.49^{ab}	0.43^{a}	0.44^{ab}	0.47^{ab}	0.45^{ab}	0.53^{b}	0.007	0.029
Rib	0.03^{a}	0.04^{a}	0.04^{a}	0.04^{a}	0.04^{a}	0.07^{a}	0.04^{a}	0.18^{b}	0.19^{b}	0.17^{b}	0.011	<0.001
Ara	6.2^{ab}	6.0^{ab}	5.6^{ab}	6.4^{ab}	6.5^{b}	5.1^{a}	6.2^{ab}	5.9^{ab}	5.7^{ab}	6.3^{ab}	0.10	0.011
Xyl	7.3^{bcd}	6.7^{ab}	6.7^{ab}	7.5^{cd}	7.6^{d}	6.7^{ab}	7.3^{bcd}	6.3^{a}	6.4^{a}	6.9^{abc}	0.08	<0.001
Man	0.67^{a}	0.70^{a}	0.70^{a}	0.95^{ab}	0.94^{a}	0.96^{ab}	0.63^{ab}	2.66^{c}	2.86^{c}	1.96^{bc}	0.143	<0.001
Gal	3.6	3.8	3.8	4.1	4.4	4.0	3.7	4.2	4.2	3.7	0.08	0.067
Glu	2.2^{ab}	2.2^{ab}	1.9^{a}	2.8^{abc}	3.4^{c}	2.9^{abc}	2.5^{abc}	3.0^{abc}	3.1^{bc}	2.3^{abc}	0.12	0.010
Uronic	0.65^{a}	0.65^{a}	0.65^{a}	0.74^{ab}	0.64^{a}	0.66^{a}	0.68^{a}	0.83^{b}	0.82^{b}	0.74^{ab}	0.015	<0.001
Ileum												
Rha	0.11^{ab}	0.13^{ab}	0.11^{ab}	0.13^{ab}	0.15^{b}	0.11^{ab}	0.09^{a}	0.10^{ab}	0.13^{ab}	0.09^{a}	0.005	0.004
Fuc	1.12^{b}	0.45^{a}	0.41^{a}	0.41^{a}	0.40^{a}	0.37^{a}	0.45^{a}	1.02^{b}	0.45^{a}	1.10^{b}	0.052	<0.001
Rib	0.15^{c}	0.06^{ab}	0.05^{a}	0.04^{a}	0.05^{a}	0.05^{a}	0.04^{a}	0.15^{c}	0.09^{b}	0.14^{c}	0.008	<0.001
Ara	10.1^{b}	5.5^{a}	5.1^{a}	4.6^{a}	4.5^{a}	4.3^{a}	5.4^{a}	9.2^{b}	5.6^{a}	9.6^{b}	0.37	<0.001
Xyl	10.4^{d}	7.6^{bc}	6.9^{abc}	6.4^{ab}	6.1^{ab}	5.6^{a}	7.3^{bc}	10.0^{d}	8.3^{c}	10.1^{d}	0.29	<0.001
Man	1.26^{c}	0.73^{ab}	0.63^{ab}	0.47^{a}	0.65^{ab}	0.68^{ab}	0.45^{a}	1.27^{c}	0.77^{ab}	0.97^{bc}	0.054	<0.001
Gal	5.5^{c}	4.0^{ab}	3.5^{a}	3.5^{a}	3.6^{a}	3.2^{a}	3.2^{a}	5.2^{bc}	4.2^{ab}	4.1^{ab}	0.17	0.017
Glu	2.3^{abc}	4.2^{d}	3.5^{bcd}	3.9^{d}	4.3^{d}	3.7^{d}	2.2^{ab}	2.3^{abc}	3.6^{cd}	1.6^{a}	0.19	<0.001
Uronic	0.82^{d}	0.77^{bcd}	0.74^{abcd}	0.68^{abc}	0.65^{ab}	0.64^{a}	0.76^{abcd}	0.79^{cd}	0.80^{cd}	0.82^{d}	0.598	0.017

[1] Mean values are based on three replicates per treatment; each sample was analysed in duplicate. Values in a row with no common superscripts differ significantly (P<0.05).
[2] Ara = arabinose; Fuc = fucose; Gal = galactose; Glu = glucose; Man = mannose; Rha = rhamnose; Rib = ribose; Uronic = uronic acid; Xyl = xylose.
[3] A combination of GH10 xylanase and β-glucanase.
[4] Standard error of the mean.

feed particles increasing physical activity of the gizzard (Dombrink-Kurtzman and Knutson, 1997; Masey-O'Neill *et al.*, 2014). The longer retention time for soaking and fermentation in the crop, coupled with the mechanical and chemical digestion by the gizzard, can reduce digesta pH, which markedly disrupts some acid-labile glycosidic linkages in the cell walls (Bedford, 1996; Caprita *et al.*, 2011; Rodrigues and Choct, 2018; Svihus, 2011), thereby influencing permeability of NSP. These foregut-induced changes in NSP structure might have enhanced response to the *in vitro* enzymatic NSP degradation in the present study. However, in ileal digesta samples, the extent of NSP degradation by the enzymes was generally greater in samples from birds fed the wheat-soy diet compared to the maize-soy diet. It is likely that the presence of soluble NSP might have primed the ileal microbiota from an early age (Nguyen *et al.*, 2021), enabling the native gut microbiota to utilise NSP more effectively. These marginal alterations in the NSP structure by microbial activity *in vivo* might have aided the *in vitro* enzymatic degradation of NSP in the present study. This was partially supported by the fact that any meaningful hydrolysis of soluble NSP upon incubation with enzymes was only observed in digesta obtained from birds fed the wheat-soy diet.

Gizzard digesta

The gizzard digesta contained substantial amounts of glucose; glucose accounted for 36% or 29% of the total insoluble NSP constituent sugars detected in the gizzard

Table 5. The concentration of insoluble non-starch polysaccharide (NSP) constituent sugars in gizzard, jejunum and ileum digesta from broilers fed a maize-soy based diet following incubation with feed enzymes.[1,2]

NSP (g/kg DM)	Control	GH10 xylanase	GH11 xylanase	GH8 xylanase	Arabino-furanosidase	XYN+BG[3]	β-mannanase	Protease	Cellulase cocktail	Pectate lyase	SEM[4]	*P*-value
Gizzard												
Rha	0.44	0.42	0.42	0.40	0.42	0.41	0.42	0.37	0.41	0.42	0.006	0.167
Fuc	0.69^{b}	0.69^{b}	0.65^{ab}	0.66^{ab}	0.61^{ab}	0.67^{ab}	0.68^{b}	0.58^{a}	0.64^{ab}	0.66^{ab}	0.008	0.008
Rib	0.21	0.20	0.20	0.20	0.33	0.16	0.32	0.28	0.21	0.28	0.020	0.118
Ara	29.9^{b}	29.9^{b}	28.7^{b}	27.9^{b}	22.2^{a}	28.7^{b}	28.8^{b}	27.1^{b}	30.1^{b}	29.8^{b}	0.41	0.029
Xyl	47.8	48.3	47.1	44.3	44.5	45.2	45.8	45.0	47.5	47.4	0.46	0.372
Man	11.8	11.9	11.9	11.2	12.5	12.5	9.9	10.6	11.0	10.8	0.29	0.207
Gal	14.4^{c}	14.3^{c}	14.5^{c}	13.4^{abc}	11.9^{a}	13.8^{bc}	14.1^{c}	12.4^{ab}	13.9^{bc}	13.6^{bc}	0.16	0.003
Glu	76.9^{c}	59.8^{ab}	62.3^{abc}	57.0^{ab}	65.0^{abc}	63.4^{abc}	64.0^{abc}	54.7^{a}	56.6^{ab}	71.0^{bc}	1.35	<0.001
Uronic	28.5^{ab}	29.5^{abc}	29.3^{abc}	28.4^{ab}	32.8^{bc}	26.6^{a}	31.0^{abc}	34.0^{c}	30.2^{abc}	30.7^{abc}	0.45	0.002
Jejunum												
Rha	1.2^{bc}	1.3^{c}	1.3^{c}	1.9^{e}	1.5^{d}	1.2^{bc}	1.6^{d}	1.0^{ab}	1.1^{ab}	1.0^{a}	0.04	<0.001
Fuc	2.5^{ab}	2.4^{ab}	2.4^{ab}	2.8^{b}	2.5^{ab}	2.4^{ab}	2.6^{ab}	2.5^{ab}	2.6^{ab}	2.3^{a}	0.03	0.017
Rib	0.20^{ab}	0.19^{ab}	0.19^{ab}	0.17^{ab}	0.15^{a}	0.19^{ab}	0.16^{a}	0.20^{ab}	0.21^{ab}	0.23^{b}	0.005	<0.001
Ara	48.4^{ab}	48.1^{ab}	46.1^{ab}	46.7^{ab}	42.8^{a}	48.4^{ab}	43.3^{a}	47.7^{ab}	51.4^{ab}	47.2^{ab}	0.57	0.003
Xyl	53.8^{ab}	53.2^{ab}	51.2^{ab}	52.4^{ab}	48.8^{a}	52.8^{ab}	50.2^{a}	51.2^{ab}	57.5^{ab}	53.9^{ab}	0.53	0.012
Man	5.7^{bc}	5.9^{c}	6.0^{c}	6.1^{c}	5.6^{bc}	5.4^{abc}	5.3^{abc}	4.6^{a}	5.2^{a}	4.8^{ab}	0.09	<0.001
Gal	36.7	36.8	36.8	36.9	34.6	36.2	35.1	35.4	36.6	34.0	0.35	0.549
Glu	28.4^{abc}	26.5^{abc}	26.5^{abc}	30.8^{c}	22.0^{a}	24.2^{abc}	28.4^{abc}	23.8^{ab}	28.5^{ab}	30.3^{bc}	0.58	0.001
Uronic	50.3^{d}	39.8^{a}	39.8^{a}	40.3^{a}	48.7^{cd}	40.8^{a}	47.9^{cd}	46.1^{bc}	47.0^{bc}	42.9^{ab}	0.66	<0.001
Ileum												
Rha	3.0^{c}	2.7^{bc}	2.7^{bc}	2.5^{b}	2.4^{b}	2.4^{b}	2.3^{b}	1.1^{a}	1.1^{a}	1.2^{a}	0.11	<0.001
Fuc	4.8^{b}	4.7^{b}	4.7^{b}	4.5^{b}	4.5^{b}	4.5^{b}	4.4^{b}	3.1^{a}	3.1^{a}	3.3^{a}	0.11	<0.001
Rib	0.24^{b}	0.23^{b}	0.25^{b}	0.26^{b}	0.25^{b}	0.24^{b}	0.24^{b}	0.04^{a}	0.04^{a}	0.04^{a}	0.016	<0.001
Ara	89.7^{b}	81.2^{a}	82.2^{a}	82.7^{a}	85.0^{ab}	83.1^{a}	82.3^{a}	86.5^{ab}	86.4^{ab}	87.0^{ab}	0.54	0.003
Xyl	94.1^{b}	91.7^{ab}	94.1^{b}	88.0^{a}	88.9^{ab}	87.9^{a}	91.3^{ab}	88.6^{a}	88.5^{a}	88.5^{a}	0.48	<0.001
Man	6.0	6.7	6.0	6.2	6.2	5.8	5.9	5.7	6.1	5.7	0.07	0.066
Gal	70.3	65.6	70.3	66.0	65.0	66.4	64.8	66.7	65.5	63.6	0.62	0.660
Glu	12.0^{ab}	13.2^{b}	12.0^{ab}	10.9^{a}	10.7^{a}	10.6^{a}	11.0^{a}	10.3^{a}	11.1^{a}	10.9^{a}	0.18	<0.001
Uronic	84.8^{b}	80.9^{ab}	77.5^{ab}	74.0^{a}	74.6^{a}	74.4^{a}	73.3^{a}	80.6^{ab}	76.2^{ab}	84.4^{b}	0.83	0.007

[1] Mean values are based on three replicates per treatment; each sample was analysed in duplicate. Values in a row with no common superscripts differ significantly (P<0.05).
[2] Ara = arabinose; Fuc = fucose; Gal = galactose; Glu = glucose; Man = mannose; Rha = rhamnose; Rib = ribose; Uronic = uronic acid; Xyl = xylose.
[3] A combination of GH10 xylanase and β-glucanase.
[4] Standard error of the mean.

digesta from birds fed the wheat-soy or maize-soy diet, respectively. The glucose component of the insoluble NSP fraction was progressively reduced during digesta transit to the small intestine. Thus, the large quantity of glucose found in the gizzard digesta suggested the presence of undigested starch. Starch granules are still tightly entrapped in NSP and protein matrices within the endosperm at the gizzard level (Rowe *et al.*, 1999), and may be erroneously detected in part as glucose. This was illustrated by its marked disappearance in gizzard digesta from birds fed the maize-soy diet as a result of enzyme application, which suggested that entrapped starch was released from the cell walls as a consequence of NSP degradation. The enzyme preparations reduced glucose, as well as arabinose and xylose to varying extents in the gizzard digesta from birds fed wheat-soy diets, which suggested better binding affinity of the enzymes in the wheat-soy diet.

In the present study, protease was included as its activity can degrade carbohydrate-protein complexes in the plant cell wall matrices, which would further contribute to cell wall disruption, as previously demonstrated in *in vivo* and *in vitro* studies (Debroas and Blanchart, 1993; Olukosi *et al.*, 2015). In the present study, it appeared that protease alone was indeed capable of degrading NSP, regardless of digesta site and dietary treatment. This was particularly evident within the gizzard digesta. It can be speculated that grinding of intact cell walls in the gizzard may expose embedded proteins to exogenous enzymes. Accordingly, the extent of NSP degradation by protease was more pronounced for

Table 6. The concentration of soluble non-starch polysaccharide (NSP) constituent sugars in gizzard, jejunum and ileum digesta from broilers fed a maize-soy based diet after incubation with feed enzymes.[1,2]

NSP (g/kg DM)	Control	GH10 xylanase	GH11 xylanase	GH8 xylanase	Arabino-furanosidase	XYN+BG[3]	β-mannanase	Protease	Cellulase cocktail	Pectate lyase	SEM[4]	*P*-value
Gizzard												
Rha	0.04^{a}	0.04^{a}	0.04^{a}	0.04^{a}	0.04^{a}	0.04^{a}	0.05^{a}	0.04^{a}	0.07^{b}	0.03^{a}	0.002	<0.001
Fuc	0.07	0.09	0.09	0.09	0.08	0.08	0.08	0.09	0.06	0.08	0.002	0.057
Rib	0.08^{a}	0.09^{a}	0.09^{a}	0.10^{a}	0.05^{a}	0.13^{a}	0.10^{a}	0.04^{a}	0.51^{b}	0.10^{a}	0.033	<0.001
Ara	1.1	1.1	1.2	1.0	1.0	1.1	1.0	1.1	0.7	1.0	0.03	0.290
Xyl	0.50	0.55	0.55	0.54	0.52	0.56	0.56	0.57	0.68	0.53	0.012	0.113
Man	1.84^{b}	1.15^{ab}	1.15^{ab}	0.59^{a}	1.19^{ab}	0.48^{a}	1.24^{ab}	1.17^{ab}	3.05^{c}	0.81^{a}	0.12	<0.001
Gal	1.6^{a}	1.7^{ab}	1.7^{ab}	1.4^{a}	1.5^{a}	1.6^{a}	1.6^{a}	1.5^{a}	2.0^{b}	1.4^{a}	0.03	0.002
Glu	1.1^{ab}	1.1^{ab}	1.1^{ab}	1.0^{ab}	1.1^{ab}	1.4^{b}	1.3^{ab}	1.2^{ab}	1.4^{b}	0.9^{a}	0.04	0.021
Uronic	0.67^{e}	0.22^{a}	0.22^{a}	0.32^{abc}	0.47^{cd}	0.41^{bcd}	0.31^{ab}	0.50^{d}	0.74^{e}	0.37^{bcd}	0.028	<0.001
Jejunum												
Rha	0.10^{bc}	0.13^{c}	0.13^{c}	0.11^{bc}	0.09^{abc}	0.12^{c}	0.09^{abc}	0.07^{ab}	0.05^{a}	0.04^{a}	0.006	<0.001
Fuc	0.61^{b}	0.59^{ab}	0.59^{ab}	0.52^{ab}	0.55^{ab}	0.59^{ab}	0.54^{ab}	0.50^{a}	0.50^{a}	0.58^{ab}	0.009	0.004
Rib	0.02^{ab}	0.02^{ab}	0.02^{ab}	0.03^{ab}	0.01^{a}	0.02^{ab}	0.04^{abc}	0.09^{d}	0.08^{cd}	0.06^{bcd}	0.005	<0.001
Ara	3.0^{c}	2.7^{abc}	2.4^{ab}	2.3^{a}	2.3^{a}	2.9^{bc}	2.6^{abc}	2.4^{ab}	2.7^{abc}	2.8^{abc}	0.05	<0.001
Xyl	1.4^{ab}	1.3^{ab}	1.4^{ab}	1.3^{ab}	1.2^{ab}	1.3^{ab}	1.2^{ab}	1.1^{a}	1.5^{b}	1.4^{ab}	0.02	0.013
Man	0.92^{a}	1.22^{a}	1.32^{a}	1.28^{a}	1.23^{a}	1.27^{a}	0.82^{a}	1.96^{b}	1.31^{a}	1.09^{a}	0.06	<0.001
Gal	4.0^{bc}	4.5^{c}	4.5^{c}	3.9^{bc}	3.6^{abc}	4.6^{c}	3.7^{abc}	3.3^{ab}	2.7^{a}	2.6^{a}	0.13	<0.001
Glu	1.4	1.5	1.5	1.4	1.4	1.5	1.2	1.6	1.4	1.4	0.03	0.564
Uronic	0.72^{ab}	0.69^{a}	0.69^{a}	0.74^{ab}	0.83^{b}	0.73^{ab}	0.77^{ab}	0.75^{ab}	0.81^{b}	0.77^{ab}	0.010	0.005
Ileum												
Rha	0.14^{a}	0.11^{a}	0.11^{a}	0.12^{a}	0.10^{a}	0.10^{a}	0.11^{a}	0.20^{b}	0.19^{b}	0.20^{b}	0.007	<0.001
Fuc	0.57^{ab}	0.54^{ab}	0.54^{ab}	0.61^{b}	0.54^{ab}	0.58^{ab}	0.56^{ab}	0.62^{b}	0.65^{b}	0.45^{a}	0.012	0.010
Rib	0.04^{a}	0.03^{a}	0.03^{a}	0.04^{a}	0.04^{a}	0.04^{a}	0.03^{a}	0.08^{b}	0.09^{b}	0.10^{b}	0.004	<0.001
Ara	2.6^{ab}	2.4^{ab}	2.4^{ab}	2.6^{ab}	2.4^{ab}	2.4^{ab}	2.8^{b}	2.5^{ab}	2.6^{ab}	2.2^{a}	0.04	0.089
Xyl	1.3^{a}	1.2^{a}	1.2^{a}	1.3^{a}	1.1^{a}	1.1^{a}	1.3^{a}	2.0^{b}	2.0^{b}	1.8^{b}	0.06	<0.001
Man	0.92^{a}	0.77^{a}	0.77^{a}	0.92^{a}	0.77^{a}	0.68^{a}	0.81^{a}	1.51^{b}	1.77^{b}	1.84^{b}	0.073	<0.001
Gal	4.1^{abcd}	3.8^{abcd}	3.8^{abcd}	4.3^{bcd}	3.6^{a}	3.7^{ab}	4.0^{abcd}	4.4^{d}	4.3^{cd}	3.7^{abc}	0.07	0.049
Glu	1.5^{bcd}	1.3^{abc}	1.3^{abc}	1.2^{abc}	1.0^{abc}	0.8^{a}	1.0^{ab}	1.5^{bcd}	1.6^{cd}	2.0^{d}	0.06	<0.001
Uronic	0.88^{c}	0.67^{b}	0.67^{b}	0.62^{ab}	0.55^{ab}	0.63^{b}	0.49^{a}	0.95^{c}	0.89^{c}	0.56^{ab}	0.025	<0.001

[1] Mean values are based on three replicates per treatment; each sample was analysed in duplicate. Values in a row with no common superscripts differ significantly (P<0.05).
[2] Ara = arabinose; Fuc = fucose; Gal = galactose; Glu = glucose; Man = mannose; Rha = rhamnose; Rib = ribose; Uronic = uronic acid; Xyl = xylose.
[3] A combination of GH10 xylanase and β-glucanase.
[4] Standard error of the mean.

the maize-soy diet with enhanced gizzard functionality, as mentioned above.

Both wheat and maize contain arabinoxylans as the main insoluble NSP fraction, albeit their degree of substitution and polymerisation can vary widely (Izydorczyk and Biliaderis, 1995; Rose *et al.*, 2010). Xylanases can degrade arabinoxylans and are classified into glycoside hydrolase families based on their protein sequence similarity, resulting in different substrate binding affinity. Thus, three xylanases from different families were selected and tested in the present study. All the xylanases exhibited hydrolytic effects on the insoluble NSP, reducing the level of arabinose, xylose and, to a lesser extent, glucose. Among the xylanases, the GH8 xylanase preparation was particularly effective in degrading both soluble and insoluble NSP fractions present in gizzard digesta, regardless of diet type. Due to the heterogeneous nature of NSP, it is difficult to assume the mode of action of xylanase from the NSP constituent sugar profiles. However, reduced recovery of uronic acid by the GH8 xylanase suggested it had side activities towards other polysaccharides.

The cellulase cocktail preparation reduced insoluble NSP level but increased soluble NSP present in the gizzard digesta from birds fed the maize-soy diet when compared to control. This most likely indicated that the cellulose cocktail solubilised the insoluble NSP fraction, but could not degrade soluble NSP when using gizzard digesta as a substrate.

Jejunal digesta

Foregut-induced changes in NSP structure can influence the efficacy of enzymes by rendering the side chains of jejunal NSP more labile to the activity of debranching enzymes. This was reflected in the present study, where arabinofuranosidase preparation notably reduced NSP level in jejunal digesta, regardless of diet type. Arabinofuranosidase is a debranching enzyme that specifically targets arabinofuranosyl side-chains attached to the xylans, facilitating the degradation of the xylan backbone (Saha, 2000). The less pronounced NSP degradation by the arabinofuranosidase preparation in the ileal digesta suggested that susceptible side chains of arabinoxylans were likely to have been taken off when the digesta passed through the jejunum, leaving little substrate for continued debranching in the ileum.

In the jejunal digesta from birds fed the wheat-soy diet, only enzymes designed to target arabinoxylans (i.e. xylanase and arabinofuranosidase) were capable of degrading NSP, when compared to the control. Arabinoxylans are responsible for increasing digesta viscosity in the gut of birds. It can therefore be postulated that the interactions between soluble NSP and water formed elongated fibre complexes *in vivo* (Chaplin, 2003), which limited the physical and chemical breakdown of NSP by other enzyme preparations *in vitro*. On the other hand, all enzyme preparations, except for the cellulase cocktail, were able to degrade NSP in digesta obtained from birds fed the maize-soy diet, where the soluble NSP level is a minor concern.

Ileal digesta

In general, the magnitude of NSP degradation by enzyme preparations was more notable when using ileal digesta in comparison to jejunal and gizzard NSP residues. There were two possible reasons for this. Firstly, absorption of digestible nutrients passing through the jejunum increases the accessibility of enzymes at the ileal level, by enabling them to reach their designated substrates more easily. Secondly, alterations in some cell wall structures may occur due to microbial activity in the ileum, thereby increasing the *in vitro* susceptibility of ileal NSP residues to enzymatic degradation. This explained the notable degradation of NSP by enzymes when substrates originated from birds fed wheat-soy diets compared to those fed maize-soy diets. A well-adapted microbiota induced by the soluble NSP content in birds fed wheat-soy diets likely contributed to the efficient disruption of cell wall integrity *in vivo*, resulting in a more pronounced *in vitro* hydrolysis of NSP following incubation with the enzymes.

Although the cellulase cocktail and pectate lyase preparations did not induce degradation of NSP in the gizzard and jejunal digesta collected from birds fed the wheat-soy diet, they markedly reduced the NSP levels in the ileal digesta. This suggested that these enzymes were incapable of degrading relatively intact cell walls present in the gizzard and jejunum. Interestingly, pectate lyase had an effect on all digesta samples obtained from birds fed the maize-soy diet, but not in those fed the wheat-soy diet. This disparity likely stemmed from foregut-induced changes in pectin structures in birds offered the maize-soy diet with a prolonged digesta retention in the gizzard, as discussed above. Hence, this may have been partially due to the higher inclusion level of soybean meal in the maize-soy diet than the wheat-soy diet. Soybean meal contains pectin as a major NSP fraction, which is the target substrate for pectate lyase (Yadav *et al.*, 2009). The above assumptions may explain why there was more pronounced degradation of NSP by β-mannanase in the digesta collected from birds fed maize-soy compared to those fed the wheat-soy diet, as soybean meal contains numerous mannan-based polysaccharides (Hsiao *et al.*, 2006).

The use of a combination of NSP-degrading enzymes is believed to be advantageous, due to multiple activities acting on complex NSP structures within the time constraints of the digestion process (Meng and Slominski, 2005). In the present study, XYN+BG, a combination of GH10 xylanase and β-glucanase, seemed to have a synergistic effect on the ileal NSP residues, compared to GH10 xylanase preparation alone. Compared to the control treatment, the highest degree of NSP degradation reached by XYN+BG was 14% for the wheat-soy diet and 8% for the maize-soy diet, when using the ileal digesta as substrates. This mainly resulted from the removal of arabinose, xylose and, to a lesser extent, glucose, which are the main products after hydrolysis of arabinoxylans and β-glucans.

5. Conclusions

The results demonstrated that the *in vitro* assay used, featuring analysis of digesta from the gizzard, jejunum and ileum, was successful in providing an assessment of the efficacy of NSP degrading enzymes. Predicting the *in vivo* efficacy of the enzymes from this *in vitro* approach is still difficult, however, it can be concluded that *in vivo* enzyme responses vary depending on the diet, section and maturity of digestive tract. Foregut functionality appears to be an important factor affecting the enzymatic degradation of NSP throughout the digestive system. Additionally, presence of soluble NSP may be another factor dictating the accessibility and efficacy of enzymes, particularly in the jejunum.

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Conflict of interest

The authors declare no conflict of interest.

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