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The effect of different wheat varieties and exogenous xylanase on bird performance and utilization of energy and nutrients

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ABSTRACT The aims of the present study were to first, determine the xylan fractions of 10 different wheat cultivar samples and their response to treatment by the same commercial xylanase enzyme preparation. Second, use information obtained to select 5 of the wheats for use within a feeding experiment to determine whether the rate of xylan release can be used to predict the feeding value of the wheats when diets have been supplemented with xylanase.

Treatment of 10 different wheat varieties by the same enzyme resulted in varying levels of hydrolysis. Soluble xylan content ranged from 7.85 to 14.40 and 3.20 to 5.13 (mg/g) when treated with and without xylanase, respectively. Oligosaccharide content ranged from 0.34 to 1.58 and 0.05 to 0.54 (mg/g) when treated with and without xylanase, respectively. Five of the 10 wheats were then selected based on the determined xylan fractions to use within a feeding experiment. A total of 360 male Ross 308 broilers were randomly allocated to 60 raised floor pens. A soybean meal (**SBM**) balancer feed was formulated to contain 12.07 MJ/kg apparent metabolizable energy (**AME**) and 392.9 g/kg crude protein (**CP**). Five diets were prepared by mixing 630 g/kg of each of the 5 experimental wheats with 370 g/kg of the balancer. Each diet was split into 2, one of which was supplemented with 100 g/MT of Econase XT (223,000 BXU/g), resulting in a total of 10 diets. The birds were fed the diets from 0 to 28 d of age. Wheat cultivar had an effect ($P = 0.044$) on feed intake (**FI**), while the addition of xylanase increased ($P < 0.05$) weight gain (**WG**) and improved feed conversion ratio (**FCR**). Various interactions were observed ($P < 0.05$) between wheat cultivars and xylanase for AME and nutrient utilization. This study suggests that wheats treated with the same xylanase, differ in their susceptibility to release soluble xylan and oligosaccharides, which may partially explain the varying performance and nutrient digestibility responses noted in the literature.

Key words: soluble xylan, oligosaccharide, nonstarch polysaccharide, broiler

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INTRODUCTION

Throughout North West Europe, wheat is the main cereal crop used in poultry feed. It can comprise up to 800 g/kg in finishing broiler diets (Wiseman and Inborr, 1990). Wheat is an important source of metabolizable energy (**ME**) and can supply up to 70% of the ME and 35% of the protein requirements for broilers (McNab, 1996). However, the nutritional profile and energy value of wheat can vary considerably between samples, thus impacting poultry performance. The main factors influencing this variability include the cultivar used, growing conditions, post-harvest handling, and storage (Choct et al., 1999; Pirgozliev et al., 2010; Smeets et al., 2016). While these are all important factors, most researchers have tried to attribute inconsistencies in the chemical composition of different wheat varieties to differences in bird performance. It is widely acknowledged that nonstarch polysaccharides (**NSP**) are one of the responsible factors for the poor feeding value of cereal grains, particularly when fed to poultry (Choct and Annison, 1990; Austin et al., 1999; Pirgozliev et al., 2015). The antinutritive effects of NSP for broilers are attributed to their physiochemical characteristics. In particular, soluble NSP which are responsible for increasing intestinal viscosity thus depressing overall digestibility of important nutrients, such as protein, starch and fat.

To alleviate the negative effects caused by NSPs in poultry feeds, it is common practice to supplement diets with NSP degrading enzymes, such as xylanase. These enzymes enable the breakdown of arabinoxylan into smaller carbohydrate fractions, and ultimately to xylo-oligosaccharides (**XOS**) which are readily fermented by bacteria, providing great prebiotic potential (Bedford,

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2000). However, different wheat cultivars vary in their susceptibility to hydrolysis by the same enzyme (Smeets et al., 2013). Therefore, the objectives of this study were to first, examine how different wheat varieties respond to treatment by the same xylanase through estimation of variance in viscosity and prebiotic potential and to second, conduct a feeding trial using information obtained from the *in vitro* work to test whether it could be replicated *in vivo*.

MATERIALS AND METHODS

In Vitro *Study*

Wheat Samples. Ten different UK produced feed wheats (Bennington, Graham, JB Diego, Siskin, Zyatt, Barrel, Dunston, Kerrin, Reflection, Shabras) were used for *in vitro* analysis to determine their content of different xylan fractions. Representative samples of each wheat were obtained from G O Davies, Westbury Ltd., Harvest House, Westbury in January 2017. The wheats were harvested in 2016.

All harvested wheat grain samples were stored in bags at ambient air temperatures in a dry store. The wheat samples were used after about 2 months of storage. Before the animal feeding experiment, wheat samples were hammer-milled using a 4 mm screen and then mixed in a horizontal mixer with the other feed ingredients. Freshly milled wheat samples were used for the feeding experiment to avoid spoilage.

Xylan Determination. Methods were designed to determine the following xylan fractions from each of the 10 wheats; soluble xylan, enzyme extractable xylan, which is defined as the total soluble xylan released when the xylanase was added to the incubation, soluble short-chain xylan, which is defined as that proportion of soluble xylan fraction that was soluble in 80% ethanol (**EtOH**) and enzyme extracted short-chain total xylan, which is defined as the total oligomeric xylan released with the addition of xylanase. Wheat samples were ground through a 0.5 mL mesh screen in a Retsch Mill. Two grams of each wheat was incubated in 10 mL of 0.05 M sodium citrate buffer (pH 5.5) at 41°C for 1 h with and without the addition of 16,000 BXU/kg Econase XT (AB Vista). Samples were centrifuged at 4,500 × *g* for 10 min. Following centrifugation, 1 mL of supernatant from each tube was collected and precipitated in 4 vol of absolute ethanol (80% final EtOH concentration). The supernatants from all incubations were acid hydrolyzed in 5 mL of 1.3 M HCl at 100°C for 1 h and neutralized once cooled, using 5 mL of 1.3 M NaOH. Xylose content was determined using a Megazyme assay procedure for D-xylose (Megazyme International, 2016).

In Vivo *Study*

Wheat Selection. The feeding experiment was designed using information obtained from the *in vitro* work on xylan determination of the different wheat cultivars. Five wheats (Bennington, Graham, JB Diego, Siskin, and Zyatt) were selected out of the 10 for use within the feeding trial. The wheats were selected based on the amount of oligosaccharides generated during the *in vitro* analysis (this was also dependent on the availability of the wheats in bulk) to test whether the *in vitro* work could be replicated *in vivo*.

Diet Preparation

A soybean meal (**SBM**) balancer feed (Table 1) was prepared, containing 12.07 MJ/kg apparent metabolizable energy (**AME**) and 392.9 g/kg crude protein (**CP**). Acid insoluble ash was used as a dietary marker and was added on top of the balancer to provide 20 g/kg of final diet (Target Feeds Ltd., Prees, Shropshire, UK). Five diets were prepared by mixing 630 g/kg of each of the 5 experimental wheats with 370 g/kg of the balancer. Each diet was then split into 2 batches, one of which was supplemented with 100 g/MT of 16,000 BXU /kg Econase XT (AB Vista, Marlborough, UK), resulting in a total of 10 diets. The diets did not contain any coccidiostat or antimicrobial growth promoters. The birds were fed the dietary treatments from the day of arrival at 0 to 28 d of age.

Proximate Analysis

Dry matter (**DM**) content of the wheats, balancer feed, and excreta was determined by drying samples in a

Table 1. Diet formulation of balancer (g/kg as fed).

Ingredient	Amount g/kg
Soybean meal (48)	700.0
Full fat soybean meal	140.0
Soya oil	70.0
Monocalcium phosphate	30.0
Limestone	30.0
NaCl	10.0
DL-methionine	10.0
Vitamin mineral premix[1]	10.8
Calculated analysis (as fed basis)	
CP	392.9
ME (MJ/kg)	12.07
Crude fat	106.2
Ca	21.4
Available P	8.5
Lysine	25.9
Methionine + cysteine	21.1
Tryptophan	4.9
Analyzed values (DM basis)	
DM	907.6
GE (MJ/kg)	17.46
CP	34.6
OIL	88.2

This balancer was fed as a part of complete diet comprised 630 g/kg of each experimental wheat and 370 g/kg of the balancer. Dietary ingredients for the balancer feed, including the marker were mixed by Target Feeds and the wheats and enzyme mixed by HAU.

[1]The vitamin and mineral premix contained vitamins and trace elements to meet the breeder's recommendations (Aviagen Ltd., Edinburgh, UK). The premix provided (units/kg) complete diet (63% wheat and 37% balancer): retinol 3600 mg, cholecalciferol 125 mg, α-tocopherol 34 mg, menadione 3 mg, thiamine 2 mg, riboflavin 7 mg, pyridoxine 5 mg, cobalamin 15 mg, nicotinic acid 50 mg, pantothenic acid 15 mg, folic acid 1 mg, biotin 200 mg, iron 80 mg, copper 10 mg, manganese 100 mg, cobalt 0.5 mg, zinc 80 mg, iodine 1 mg, selenium 0.2 mg, and molybdenum 0.5 mg.

forced air oven at 105°C until a constant weight (AOAC Official Method 934.01; AOAC (Association of Analytical Communities), 2006b). Crude protein content (N × 6.25) of the wheats, balancer feed, digesta, and excreta was determined by the combustion method (AOAC Official Method 990.03; AOAC (Association of Analytical Communities), 2006a) using Leco (FP-528 N; Leco Corp., St. Joseph, MI) with ethylenediaminetetraacetic acid (**EDTA**) as a standard (Sweeney, 1989). Oil content (as ether extract) of the wheats, balancer feed, and excreta was extracted with petroleum ether using a Soxtec Avanti 2050, Foss UK Ltd. (AOAC Official Method 945.16; AOAC (Association of Analytical Communities), 2005). Gross energy (**GE**) content of the wheats, balancer feed, and excreta was measured using an isoperibol bomb calorimeter (Parr 6200 Instrument Company, Moline, IL) (FAO (Food and Agriculture Organization of the United Nations), 2003). The acid insoluble ash (**AIA**) content of the diets, excreta, and digesta was measured after ashing the samples and treating the ash with boiling 2 M hydrochloric acid (Scott and Boldaji, 1997). Nonstarch polysaccharides and total starch contents of the wheats, balancer feed, and digesta samples were determined following the methods of Englyst et al. (1994, 2000), respectively.

Bird Husbandry

The study was approved by the Harper Adams University Research Ethics Committee. Three hundred and eighty, day-old male Ross 308 broiler chicks were obtained from a commercial hatchery (Cyril Bason Ltd., Craven Arms, UK). The birds were individually weighed and after excluding outliers, 360 of these birds were allocated using a randomized block design to 60 raised floor pens with a solid floor area of 0.60 m^2, giving 6 birds per pen. Each pen was equipped with an individual feeder and drinker. Each diet was replicated 6 times in a randomized complete block design. Feed and water were offered *ad libitum* to the birds throughout the experiment. Room temperature and lighting regime met commercial recommendations.

Data Collection

Birds were weighed on a per pen basis, at the beginning and end of the experimental study period. Total feed intake (**FI**) of each pen was determined by subtracting the residual feed at the end of the study from the feed added at the start. Feed conversion ratio (**FCR**) was calculated on a pen weight basis. The body weights of dead birds were included to calculate FCR. The AME, AMEn, and nutrient retention were determined using the total collection technique. At 24 d of age, the solid floor of each pen was replaced with a wire mesh flooring and a metal tray was inserted underneath. Excreta were then collected on a daily basis for the remaining 4 d of the study. The excreta were immediately dried at 60°C until a constant weight and then milled ready for analysis. The marker technique was used to determine nutrient digestibility from the ileal digesta. On the final day of the study, 3 birds from each pen were killed by cervical dislocation and digesta were collected from the ileum, between the Meckel's diverticulum and the cecal junction and pooled into separate pots per pen, immediately frozen at −20°C for 24 h and then freeze-dried.

Calculations

The AME of the diets was determined by measuring the GE of the diet consumed and the amount of GE excreted and calculating the difference that is retained by the bird:

$$\begin{aligned} &Dietary\ AME(MJ/kg) was\ calculated\ as: AME \\ &= ((GE\ intake\,(MJ) \\ &\quad - GE\ output\,(MJ))/(Feed\ intake\,(kg)) \end{aligned}$$

A separate calculation was applied to determine a correction for zero nitrogen (Hill and Anderson, 1958):

$$\begin{aligned} &Dietary\ AMEn\,(MJ/kg) was\ calculated\ as: AMEn \\ &= ((GE\ intake(MJ) - GE\ output(MJ)) \\ &\quad - N\ retained\,(kg) \\ &\quad * 34.39 MJ/kg))/(Feed\ intake\,(kg)) \end{aligned}$$

Dietary retention coefficients, based on excreta collection, were calculated using the following equation:

$$\begin{aligned} &Retention\ coefficients \\ &= (Nutrient\ intake \\ &\quad - Nutrient\ output)/(Nutrient\ intake) \end{aligned}$$

Digestibility coefficients, based on the marker technique, were calculated using the following equation:

$$\begin{aligned} &Digestibility\ coefficients \\ &= (N_{diet}/AIA_{diet} \\ &\quad - N_{digesta}/AIA_{digesta})/(N_{diet}/AIA_{diet}) \end{aligned}$$

where N_{diet} is the nutrient content (g/kg) of the diet; AIA_{diet} is the concentration of acid insoluble ash in the diet (g/kg); − $N_{digesta}$ is the nutrient content of the digesta (g/kg); and $AIA_{digesta}$ is the concentration of acid insoluble ash in the digesta (g/kg) (Van Keulen and Young, 1977).

Table 2. Determination of different xylan fractions (mg/g) of the 10 studied wheat samples.

Wheat	Enzyme	Soluble xylan cont.	Oligosaccharide cont.
Bennington	N	3.58	0.26
Bennington	Y	10.31	0.94
Graham	N	4.18	0.20
Graham	Y	7.85	0.74
JB Diego	N	3.49	0.34
JB Diego	Y	14.40	1.58
Siskin	N	3.96	0.44
Siskin	Y	11.87	0.48
Zyatt	N	3.65	0.54
Zyatt	Y	10.97	1.07
Barrel	N	4.55	0.42
Barrel	Y	11.60	0.34
Dunston	N	3.81	0.25
Dunston	Y	8.19	0.85
Kerrin	N	3.78	0.10
Kerrin	Y	10.33	0.90
Reflection	N	5.13	0.05
Reflection	Y	14.33	1.05
Shabras	N	3.20	0.13
Shabras	Y	9.60	0.60
St err	N	0.89	0.22
St err	Y	1.71	0.31
Effects of treatment			
Wheat variety		0.318	0.569
Enzyme		<0.0001	0.0012

Statistical Analysis

Data from the xylan determination (Table 2) were analyzed as a complete factorial with wheat and enzyme as the factors using JMP statistical software (JMP, Version 15th edition. SAS Institute Inc., Cary, NC). Broiler growth performance, AME, nutrient retention, and digestibility coefficients (Tables 5–7) were compared statistically by analysis of variance (**ANOVA**) using the Genstat statistical software package (Genstat 18th release 3.22 for Windows; IACR, Rothamstead, Hertfordshire, UK). Duncan's multiple range test was used to determine significant differences between means. Data determined by the effect of enzyme interaction with soluble xylan content and oligosaccharide content for the digestibility of DM, sNSP, iNSP, and tNSP (Table 8) were analyzed using a standard ANOVA on JMP statistical software. In all instances, differences were reported as significant at $P < 0.05$. Correlation analyses (Table 9) were also applied to determine correlations between bird performance and the chemical characteristics of the studied wheat using Genstat statistical software package. A 2-tailed test was run to determine significance at the 5% level.

RESULTS AND DISCUSSION

In Vitro *Study*

Xylan Determination The main objective of this study was to determine the variation in the susceptibility of different wheat varieties to hydrolysis by a xylanase and whether this variation could explain subsequent performance. During the *in vitro* analysis, samples were incubated with and without the xylanase and the subsequent supernatant derived was precipitated either with or withoutEtOH to determine total soluble and oligomeric xylan fractions respectively. This oligomeric fraction is of great interest, as it is the most potent prebiotic (Bedford et al., 2022). It is clear that adding the enzyme to the wheat sample not only dissolves previously insoluble arabinoxylan but breaks down polymeric soluble arabinoxylan into oligosaccharides (Table 2). Wheats that yielded significant quantities of oligomeric xylan in the presence of xylanase could represent a wheat that responds well to enzyme treatment. As shown in Table 2, the addition of enzyme significantly

Table 3. Proximate analysis of the studied wheat cultivar samples and experimental diets.

	Wheat cultivar samples					
Nutrients	Bennington	Graham	JB Diego	Siskin	Zyatt	CV
DM (g/kg)	879.9	886.5	894.7	882.7	897.5	0.009
GE (MJ/kg DM)	15.35	16.00	15.96	15.89	16.08	0.018
CP (g/kg)	10.6	11.2	11.3	11.4	11.6	0.034
Oil (g/kg)	7.0	4.9	1.7	0.1	0.5	1.054
	Experimental diets					
	Bennington	Graham	JB Diego	Siskin	Zyatt	CV
DM (g/kg)	872.3	876.8	883.1	873.6	884.0	0.006
GE (MJ/kg DM)	18.40	18.76	18.85	18.99	18.97	0.017
CP (g/kg)	19.90	19.67	21.04	21.11	21.07	0.034
Oil (g/kg)	60.9	34.4	50.8	49.3	48.4	0.194

Table 4. Polysaccharide composition of the studied wheat cultivars (g/kg).

Polysaccharides	Bennington	Graham	JB Diego	Siskin	Zyatt
sNSP					
Rhamnose	0.20	0.0	0.11	0.13	0.11
Fucose	0.04	0.44	0.23	0.23	0.26
Arabinose	6.5	7.4	7.5	7.9	7.9
Xylose	8.9	9.3	11.5	11.3	11.3
Mannose	1.5	2.6	2.4	3.0	2.7
Galactose	1.4	3.0	2.9	3.6	2.9
Glucose	4.7	4.8	4.5	5.6	5.4
Glucuronic acid	0.0	0.0	0.0	0.20	0.0
Galacturonic acid	0.0	0.0	0.0	0.0	0.0
Total sNSP	23.3	27.7	29.1	31.8	30.4
iNSP					
Rhamnose	0.27	0.0	0.0	0.0	0.0
Fucose	0.0	0.0	0.0	0.0	0.0
Arabinose	13.4	11.4	12.7	12.4	13.7
Xylose	20.0	16.4	19.8	19.3	21.0
Mannose	2.6	2.0	1.4	1.8	1.8
Galactose	0.67	0.94	0.91	1.1	1.0
Glucose	18.9	15.0	19.0	17.4	18.4
Glucuronic acid	0.0	0.0	0.0	0.0	0.0
Galacturonic acid	0.0	0.0	0.0	0.0	0.0
Total iNSP	55.8	45.8	53.8	51.9	55.9
Total NSP					
Rhamnose	0.47	0.0	0.11	0.13	0.11
Fucose	0.0	0.44	0.23	0.23	0.26
Arabinose	19.9	18.8	20.2	20.2	21.6
Xylose	28.8	25.8	31.2	30.5	32.3
Mannose	4.1	4.6	3.8	4.7	4.4
Galactose	2.1	3.9	3.8	4.6	3.9
Glucose	23.6	19.8	23.5	23.0	23.8
Glucuronic acid	0.0	0.0	0.0	0.20	0.0
Galacturonic acid	0.0	0.0	0.0	0.0	0.0
Total NSP	79.0	73.4	82.9	83.7	86.3
Starch	663.3	654.5	623.2	620.0	624.7

Abbreviations: iNSP, insoluble NSP; sNSP, soluble NSP.

Table 5. Effect of the experimental diets on daily feed intake, daily weight gain, and feed conversion ratio (FCR) from 0 to 28 d of age.

Treatment factor	Feed intake (g/b/d DM)	Weight gain (g/b/d)	FCR
Wheat cultivar			
Bennington	45.84[b]	29.46	1.564
Graham	43.84[ab]	28.87	1.520
JB Diego	45.21[ab]	29.50	1.535
Siskin	42.18[a]	27.63	1.533
Zyatt	43.68[ab]	28.05	1.568
SEM	0.877	0.729	0.029
Xylanase			
−	43.94	27.88	1.584
+	44.36	29.53	1.504
SEM	0.555	0.461	0.019
Wheat cultivar*xylanase			
Bennington − xylanase	44.59	28.21	1.589
Bennington + xylanase	47.10	30.71	1.539
Graham − xylanase	43.24	27.75	1.560
Graham + xylanase	44.44	30.00	1.480
JB Diego − xylanase	46.14	29.85	1.549
JB Diego + xylanase	44.29	29.16	1.522
Siskin − xylanase	41.98	26.74	1.578
Siskin + xylanase	42.37	28.52	1.488
Zyatt − xylanase	43.76	26.83	1.646
Zyatt + xylanase	43.60	29.27	1.490
SEM	1.240	1.031	0.042
Probabilities of statistical differences			
Wheat cultivar	0.044	0.275	0.742
Xylanase	0.596	0.015	0.004
Wheat cultivar*xylanase	0.503	0.503	0.605

[a,b]Means within a column not sharing a common superscript are significantly different.

increased soluble xylan and oligosaccharide content of all wheats ($P < 0.05$).

In Vivo *Study*

Proximate Analysis of the Studied Wheat The proximate analysis of the 5 wheats used in the feeding trial is shown in Table 3. Dry matter, gross energy, crude protein and oil content ranged from 882.7 to 897.5 (g/kg), 17.44 to 18.03 (MJ/kg DM), 12.1 to 13.1 (g/kg DM), and 0.1 to 7.9 (g/kg DM), respectively. Table 4 displays the NSP content of the studied wheats. It should be noted that the soluble xylan content is much higher than the values shown in Table 2. This disparity is likely a result of the different extraction methods used during analysis. Soluble, insoluble, and total NSP content ranged from 23.3 to 31.8 (g/kg), 45.8 to 55.9 (g/kg), and 73.4 to 86.3 (g/kg), respectively. Others have reported similar ranges (Widyaratne and Zijlstra, 2007; Azhar et al., 2019). Of the studied wheats, arabinoxylans (**AX**) represent on average 62% of the total NSP content, which is similar to figures reported by Freeman et al. (2017). According to Smeets et al. (2013), arabinoxylan was the most abundant NSP determined among 9 wheats with sNSP and iNSP accounting on average, a respective 44 and 64%.

In wheat grain, arabinoxylan is a major polymer of the cell wall and is therefore considered to be the main problem regarding its application in poultry feed (Bedford and Morgan, 1995; Izydorczyk and Biliaderis, 1995; Pirgozliev et al., 2015). Arabinoxylan consists of 2 pentose sugars, arabinose and xylose. The arabinose-to-xylose ratio (**A**/**X**) is an important parameter, as it can be used to indicate structural variations, such as the degree and pattern of substitution of the xylan backbone with

Table 6. Effect of the experimental diets on apparent metabolizable energy (AME), nitrogen corrected metabolizable energy (AMEn), dry matter retention (DMR), nitrogen retention (NR), and fat digestibility (FD) from 0 to 28 d of age.

Treatment factor	AME (MJ/kg DM)	AMEn (MJ/kg DM)	DMR	NR	FD
Wheat cultivar					
Bennington	13.95	13.47	0.758	0.648	0.831[b]
Graham	14.50	14.01	0.769	0.654	0.788[a]
JB Diego	14.19	13.71	0.752	0.632	0.801[ab]
Siskin	14.10	13.62	0.751	0.632	0.768[a]
Zyatt	14.44	13.93	0.764	0.669	0.830[ab]
SEM	0.078	0.076	0.005	0.006	0.008
Xylanase					
−	13.97	13.49	0.745	0.633	0.802
+	14.50	14.01	0.773	0.660	0.805
SEM	0.050	0.048	0.003	0.004	0.005
Wheat cultivar*xylanase					
Bennington − xylanase	13.56[a]	13.10[a]	0.733[a]	0.617[a]	0.821
Bennington + xylanase	14.34[cde]	13.84[cde]	0.784[cd]	0.680[d]	0.842
Graham − xylanase	14.16[bcd]	13.68[bcd]	0.751[ab]	0.636[abc]	0.782
Graham + xylanase	14.85[e]	14.34[e]	0.787[d]	0.671[cd]	0.794
JB Diego − xylanase	13.78[ab]	13.33[ab]	0.730[a]	0.603[a]	0.793
JB Diego + xylanase	14.59[de]	14.10[de]	0.774[bcd]	0.661[bcd]	0.809
Siskin − xylanase	14.03[abc]	13.55[abc]	0.755[abc]	0.645[abcd]	0.781
Siskin + xylanase	14.16[bcd]	13.70[bcd]	0.748[ab]	0.619[ab]	0.754
Zyatt − xylanase	14.32[cd]	13.81[bcd]	0.756[abc]	0.666[cd]	0.833
Zyatt + xylanase	14.56[de]	14.05[cde]	0.772[bcd]	0.672[cd]	0.826
SEM	0.111	0.108	0.007	0.009	0.012
Probabilities of statistical differences					
Wheat cultivar	<0.001	<0.001	0.036	<0.001	<0.001
Xylanase	<0.001	<0.001	<0.001	<0.001	0.708
Wheat cultivar*xylanase	0.006	0.011	<0.001	<0.001	0.232

[a,b,c,d,e]Means within a column not sharing a common superscript are significantly different.

Table 7. Effect of the experimental diets on the ileal digestibility of dry matter (DMD), protein (PD), starch (SD), soluble NSP (sNSPD), insoluble NSP (iNSPD), total NSP (tNSPD), and oligosaccharide content of the studied wheats (mg/g) from 0 to 28 d of age.

Treatment factor	DMD	PD	SD	sNSPD	iNSPD	tNSPD	Oligo cont.
Wheat cultivar							
Bennington	0.779	0.854	0.991	0.048	0.206	0.162	
Graham	0.771	0.854	0.970	0.065	0.186	0.147	
JB Diego	0.768	0.849	0.953	0.201	0.234	0.223	
Siskin	0.769	0.854	0.962	0.198	0.243	0.228	
Zyatt	0.773	0.866	0.965	0.193	0.249	0.231	
SEM	0.006	0.007	0.008	0.033	0.023	0.025	
Xylanase							
−	0.763	0.851	0.954	0.125	0.197	0.176	
+	0.782	0.858	0.983	0.157	0.249	0.221	
SEM	0.004	0.005	0.005	0.021	0.015	0.016	
Wheat cultivar*xylanase							
Bennington − xylanase	0.759	0.837	0.989^{de}	−0.018^{a}	0.122^{a}	0.083^{a}	0.26
Bennington + xylanase	0.800	0.872	0.994^{d}	0.230^{bc}	0.289^{b}	0.241^{ab}	0.94
Graham − xylanase	0.761	0.853	0.959^{b}	0.008^{a}	0.156^{ab}	0.109^{ab}	0.20
Graham + xylanase	0.781	0.856	0.982^{de}	0.121^{ab}	0.217^{ab}	0.186^{ab}	0.74
JB Diego − xylanase	0.753	0.848	0.926^{a}	0.172^{bc}	0.205^{ab}	0.195^{ab}	0.34
JB Diego + xylanase	0.783	0.850	0.980^{e}	0.230^{c}	0.262^{ab}	0.252^{b}	1.58
Siskin − xylanase	0.767	0.852	0.958^{b}	0.190^{bc}	0.256^{ab}	0.262^{b}	0.44
Siskin + xylanase	0.772	0.855	0.975^{bcd}	0.197^{bc}	0.229^{ab}	0.195^{ab}	0.48
Zyatt − xylanase	0.773	0.866	0.952^{bc}	0.190^{ac}	0.247^{ab}	0.230^{ab}	0.54
Zyatt + xylanase	0.773	0.856	0.978^{cde}	0.197^{ab}	0.250^{ab}	0.233^{ab}	1.07
SEM	0.009	0.010	0.006	0.047	0.033	0.035	
Probabilities of statistical differences							
Wheat cultivar	0.725	0.838	<0.001	0.001	0.284	0.044	
Xylanase	0.001	0.310	<0.001	0.285	0.015	0.044	
Wheat cultivar*xylanase	0.125	0.253	0.002	0.030	0.050	0.033	

^{a,b,c,d,e}Means within a column not sharing a common superscript are significantly different.

arabinose (Cleemput et al., 1995; Izydorczyk and Biliaderis, 1995; Faltermaier et al., 2014). However, Smeets et al. (2013) found the A/X ratio of 9 European grown wheats varied. In the present experiment, the A/X ratio of the studied wheats for soluble, insoluble and total AX fractions ranged from 0.652 to 0.796, 0.641 to 0.695, and 0.647 to 0.729, respectively.

Bird Performance The effects of the diets on bird performance are shown in Table 5. Birds fed diets containing Bennington had a higher FI ($P = 0.044$) compared with birds fed diets containing Siskin, which maintained the lowest FI. However, Bennington had lower AMEn and CP compared to Siskin, thus partially explaining the reason for the difference in FI. In addition, this may also contribute to the lack of difference in bird growth between the 2 diets. As expected, the addition of xylanase increased WG ($P = 0.015$) and improved FCR ($P = 0.004$). The improvement in bird performance by the addition of xylanase may be attributed to its ability to hydrolyze the arabinoxylan backbone, releasing arabinose and xylose into the small intestine (Choct et al., 2004).

AME and Nutrient Utilization A number of interactions can be observed for AME and nutrient utilization (Table 6) between the different treatment groups. The addition of xylanase improved the AME ($P = 0.006$), AMEn ($P = 0.011$), and dry matter retention (**DMR**) ($P < 0.001$) of diets containing Bennington, Graham and JB Diego. The addition of xylanase also improved the nitrogen retention (**NR**) of diets containing Bennington and JB Diego ($P < 0.001$). Differences were also observed between wheat cultivars for fat digestibility (**FD**), with birds fed diets containing Bennington having a higher ($P < 0.05$) FD compared with birds fed diets containing Graham and Siskin. It is expected that the addition of xylanase to wheat-based diets will improve AME and nutrient digestibility. In general, wheats that had the lowest AME responded more to xylanase addition than those that had higher AME. Given that no dietary adjustments were made or that the differences in AME between wheats or the AME contribution of the enzyme, it would be expected that the combinations yielding the highest AME would produce birds with greater fat pad than those with the lowest AME. Ideally,

Table 8. Effect of enzyme interaction with soluble xylan content and oligosaccharide content of the studied wheats for dry matter digestibility (DMD), soluble NSP digestibility (sNSPD), insoluble NSP digestibility (iNSPD), and total NSP digestibility (tNSPD).

Source	DMD *P* values	sNSPD *P* values	iNSPD *P* values	tNSPD *P* values
Enzyme	0.0009	0.3196	0.0133	0.0432
Soluble xylan cont.	0.629	0.8342	0.6648	0.617
Oligosaccharide cont.	0.6848	0.0018	0.0564	0.012
Enzyme*soluble xylan cont.	0.0791	0.1897	0.071	0.0815
Enzyme*oligosaccharide cont.	0.013	0.016	0.009	0.0061
Block	0.3662	0.0001	0.5307	0.0018

Table 9. Correlation between bird performance and the chemical characteristics of the studied wheat.

	AMEn MJ/kg DM	DMD	FCR	WG g/b/d	Soluble NSP	Insoluble NSP	Total NSP	Oligosaccharide cont.	Soluble xylan cont.
AMEn MJ/kg DM	-								
DMD	0.4565***	-							
FCR	−0.1817	0.0321	-						
WG g/b/d	0.0554	−0.0272	−0.6098***	-					
Soluble NSP	0.2051	0.4151***	0.1005	0.0838	-				
Insoluble NSP	0.2952*	0.7382***	0.1159	−0.0162	0.6224***	-			
Total NSP	0.2723*	0.6503***	0.1271	0.0344	0.8939***	0.9064***	-		
Oligosaccharide cont.	0.5701***	0.3826**	−0.1951	0.2122	0.2299	0.3665**	0.3334**	-	
Soluble xlyan cont.	0.5145***	0.3657**	−0.3267**	0.2410	0.1236	0.3055*	0.2382	0.8302***	-

*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

prediction of AME content of wheat and subsequent AME contribution by enzyme by near-infrared spectroscopy (**NIRS**) analysis would enable nutritionists to move one step closer to the precision nutrition concept, where all nutrients are delivered at exactly the required level. As previously mentioned, following *in vitro* analysis, those wheats which produced the greatest increment in oligosaccharide content with xylanase treatment were expected to perform better in AME with enzyme treatment. This can be seen with Bennington and JB Diego.

The effect of the different dietary treatments on nutrient digestibility determined on ileal digesta is presented in Table 7. The addition of xylanase improved ($P = 0.001$) dry matter digestibility (**DMD**). The dietary treatments had no effect ($P > 0.05$) on protein digestibility (**PD**). Interactions ($P < 0.05$) between the xylanase and the different wheat varieties can be observed for the digestibility of starch (**SD**), soluble NSP (**sNSPD**), insoluble NSP (**iNSPD**), and total NSP (**tNSPD**). In general, those wheats with the poorer digestibility coefficients responded most to enzyme addition. Information from Table 7 was used to perform a second analysis showing the effect of enzyme interaction with soluble xylan and oligosaccharide content of the studied wheat (Table 8) on digestibility of the different fiber fractions and dry matter. Results show significant interactions ($P < 0.05$) between enzyme and oligosaccharide content for each measured variable. This finding indicates that wheats high in oligosaccharide content have higher fiber digestibility, however adding the enzyme removes this difference. A number of significant correlations between bird performance and the chemical characteristics of the studied wheats were observed (Table 9). For AMEn both oligosaccharide and soluble xylan content correlated well with AME which suggests that much of the variation in AME is accounted for by the fermentable xylan fraction of wheat. For performance, FCR is best described by the soluble xylan content of the wheats which again suggests good FCR is associated with a higher content of soluble xylan. Both soluble and oligosaccharide xylan content can be increased by addition of the xylanase. Figure 1 shows the correlations between AME and all other analyses. Multiplying the oligosaccharide content by the soluble xylan content produces a logarithmic relationship that explains 56% of the variation of AME while taking into

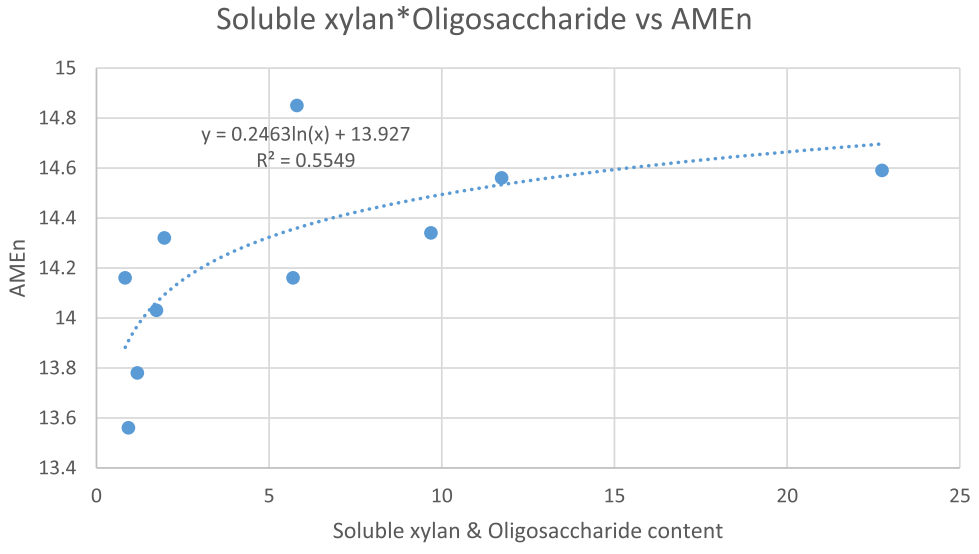

Figure 1. Correlation between soluble xylan*oligosaccharide content and AME.

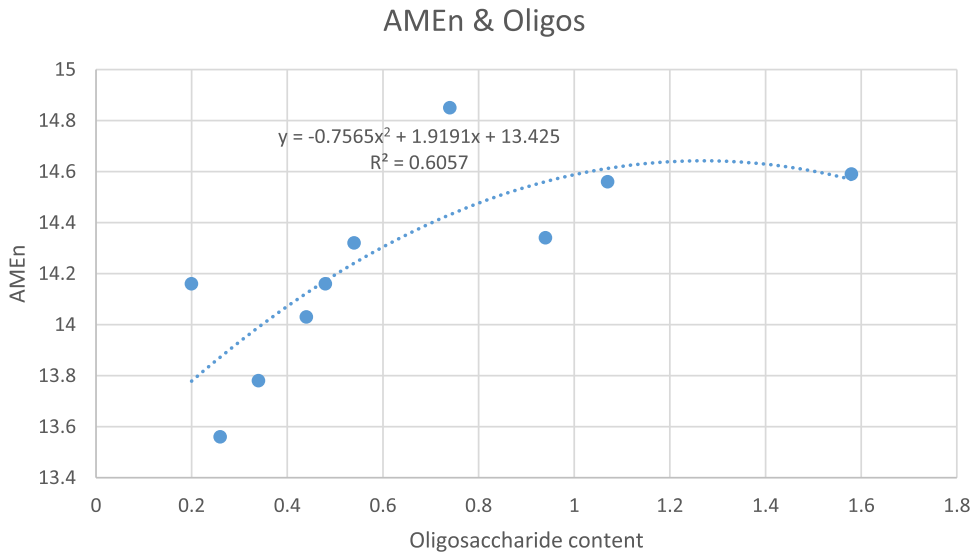

Figure 2. Correlation between AME and oligosaccharide content.

account both measures (Figure 1). Figure 2 also displays a good logarithmic relationship that describes 60% of the variation of AME. It shows that the higher the oligosaccharide content of the wheat, the better the AME. It is possible to increase the oligosaccharide content by using an enzyme, but the increment in oligosaccharide content is not always predictable.

CONCLUSIONS

Data from this study show that wheat differs in its susceptibility to release soluble xylan and oligosaccharides in the presence of a xylanase, which may explain part of the variation in the response in wheat-based diets when a xylanase is added. Wheat samples high in soluble xylan and/or oligosaccharide content predicted better dietary AMEn and reduced FCR compared to the rest of the studied variables. In general, wheats with lower AME are more responsive to the addition of xylanase addition compared with wheats with higher AME.

DISCLOSURES

The authors declare that they have no conflicts of interest to declare. All co-authors have reviewed the manuscript and agree with the contents. There is no financial interest to report. The authors confirm that the information presented in the manuscript is our own work, is novel and has not been published elsewhere, nor is it currently under consideration by any other journal.

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