



Original research article

# Evaluation of the effect of different wheats and xylanase supplementation on performance, nutrient and energy utilisation in broiler chicks

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## ABSTRACT

The aim of this study was to evaluate the performance, nutrient utilisation and energy metabolism of broiler chicks fed 8 different wheat samples, supplemented or not with xylanase. Seven-hundred sixty eight male broilers (1-day-old) were distributed to 16 experimental treatments (6 replicates per treatment). The treatments were in a factorial arrangement with 8 different wheats and 2 levels of xylanase (0 or 16,000 BXU/kg). The predicted apparent metabolisable energy (AME) of the wheat samples ranged from 13.0 to 13.9 MJ/kg and all diets were formulated to contain the same amount of wheat. Body weight gain (BWG) and feed intake (FI) were measured at 21 d, as was jejunal digesta viscosity, and feed conversion ratio (FCR) calculated. On day 24, one representative bird per pen was selected to calculate whole body energetics. At 21 d, 3 chicks per replicate were randomly allocated to metabolism cages for energy and nutrient utilisation determinations, and were continued on the experimental diets until 24-d-old. No interactions were observed for any performance response variables, ileal nutrient utilisation or digesta viscosity. Xylanase improved BWG and reduced FCR and digesta viscosity ( $P < 0.05$ ). Wheat influenced dry matter (DM) utilisation and xylanase increased ileal digestible energy ( $P = 0.04$ ). Xylanase also improved ( $P < 0.05$ ) DM and nitrogen retention. Apparent metabolisable energy and AME corrected for nitrogen (AMEn) were subject to an interaction whereby wheats 2 and 6, which returned the lowest AME and AMEn values, responded to xylanase supplementation and the remainder did not. Net energy for production and the efficiency of energy use for production were not influenced by xylanase, but were affected by wheat ( $P < 0.05$ ). Despite the significant differences between wheats with regards to their nutrient utilisation and energy metabolism in birds, xylanase removed this variance and resulted in more homogeneous performance.

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## 1. Introduction

Variation in the nutritive value of wheat samples is a reflection of genetic and environmental effects, and the economic impact of

these variations on poultry performance highlights the need for improved predictors of wheat quality (Yegani and Korver, 2012). This is a concern for plant breeders, farmers and animal nutritionists. Thus, nutritionists need to know the nutritional requirements of commercial poultry, and be able to determine or predict the nutritive value of each batch of raw material in an accurate and timely manner (van Kempen and Simmins, 1997).

The use of near-infrared spectroscopy (NIRS) provides an opportunity to determine the chemical composition of feedstuffs and their nutritive values before inclusion in the diet (Olukosi et al., 2011; Owens et al., 2009). The information from NIRS can be used to reduce or minimize nutrient imbalances in commercial rations fed to the animals. However, there are potential errors associated

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with NIRS technology such as sample-related and chosen reference method errors which can lead to high values for coefficient of variation (Yegani and Korver, 2012), and as a result care must be taken in establishing NIRS calibration to ensure it is robust, precise and accurate. Near-infrared spectroscopy calibrations now exist which can predict non-starch polysaccharide (NSP) and energy contents of wheat. In particular xylans are often considered an anti-nutrient in wheat, and as a result variation in content of this component between wheat samples may contribute to differences in nutritive value. Xylanases are the major enzymes involved in arabinoxylan degradation, hydrolysing the 1,4- $\beta$ -D-xylosidic linkage between xylose residues in the backbone in a random manner (Mendis et al., 2016). Therefore it is hypothesised that their supplementation in poultry feed may balance animal performance although differences in the nutritive value of different wheat origins. This work was undertaken to determine if such a calibration by NIRS accurately predicts animal performance, and if so whether the application of an NSP-degrading xylanase would reduce the performance differences between samples of wheat which differ in NSP content (Bedford, 2000).

## 2. Materials and methods

All the experimental procedures received prior approval from the Scotland's Rural College's Animal Experiment Committee.

### 2.1. Birds and experimental design

A total of 768 one-day old male broiler chicks (Ross 308) obtained from a commercial hatchery were used in the study for 2 experiments to determine growth performance and whole-body energy metabolism (Exp. 1) and nutrient utilisation (Exp. 2) responses. For Exp. 1 ( $n = 768$ ) and for Exp. 2 ( $n = 288$ ), birds were allocated to 16 experimental treatments in a randomized complete block design with an  $8 \times 2$  factorial arrangements of treatments (8 wheat samples and 2 levels of xylanase), having in both experiments 6 replicates per treatment. Throughout the study, feed and water were supplied *ad libitum* and animals were raised under controlled conditions of light and temperature, as breeder recommended.

#### 2.1.1. Experiment 1

Birds were reared up to day 24 in floor pens. All broiler chickens and feed were weighed on days 0 and 21 to calculate growth performance responses: body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR). On day 21, 2 chickens were randomly selected and euthanized by an overdose of sodium pentobarbital and jejunal digesta were collected for viscosity measurement. On day 24, 1 representative bird (on BW basis) per floor pen was selected and fasted prior to euthanasia to calculate whole body energetics.

#### 2.1.2. Experiment 2

On day 21, 3 chicks were randomly selected from each of the 96 floor pens and transferred to 96 metabolism cages (for energy and nutrient utilisation trial) where chickens continued to receive the corresponding diets until 24 days of age. Excreta and ileal digesta were collected on day 24 and pooled on a cage basis for calculation of nutrient utilisation.

### 2.2. Diets and wheat selection

Starter experimental diets based on wheat and soybean-meal were formulated to be marginally lower in metabolizable energy (ME) than Ross 208 requirements (Table 1). Eight wheat samples

**Table 1**  
Ingredient and calculated composition as-fed of the experimental diets.

Item	Control	+ Xylanase
Ingredient, g/kg		
Wheat – feed	585	585
Soybean meal 48	325	325
Soy oil	44.4	44.4
NaCl	3.00	3.00
Sodium bicarbonate	1.87	1.87
DL-methionine	2.99	2.99
Lysine HCl	2.46	2.46
Threonine	0.77	0.77
Limestone	7.86	7.86
Dicalcium phosphate	15.5	15.5
Vitamin premix <sup>1</sup>	4.90	4.90
Phytase <sup>2</sup>	+	+
Xylanase <sup>3</sup>	–	+
Calculated nutrient composition, %		
Crude protein	22.4	22.4
Ca	0.90	0.90
P	0.74	0.74
Available phosphorous	0.45	0.45
Fat	5.72	5.72
Fibre	2.55	2.55
Met	0.62	0.62
Cys	0.38	0.38
Met + Cys	1.00	1.00
Lys	1.35	1.35
His	0.55	0.55
Trp	0.28	0.28
Thr	0.88	0.88
Arg	1.45	1.45
Ile	0.92	0.92
Leu	1.64	1.64
Phe	1.05	1.05
Val	1.00	1.00
AME, MJ/kg	12.8	12.8

AME = apparent metabolisable energy.

<sup>1</sup> Vitamin/mineral premix supply per kilogram of diet: vitamin A, 16,000 IU; vitamin D<sub>3</sub>, 3000 IU; vitamin E, 25 IU; vitamin B<sub>1</sub>, 3 mg; vitamin B<sub>2</sub>, 10 mg; vitamin B<sub>6</sub>, 3 mg; vitamin B<sub>12</sub>, 15  $\mu$ g; nicotinic acid, 60 mg; pantothenic acid, 14.7 mg; folic acid, 1.5 mg; biotin, 125  $\mu$ g; choline chloride, 25 mg; Fe as iron sulphate, 20 mg; Cu as copper sulphate, 10 mg; Mn as manganese oxide, 100 mg; Co as cobalt oxide, 1.0 mg; Zn as zinc oxide, 82.222 mg; I as potassium iodide, 1 mg; Se as sodium selenite, 0.2 mg; and Mo as molybdenum oxide, 0.5 mg.

<sup>2</sup> Quantum Blue 5G, AB Vista, Marlborough, UK; 5000 FTU/g.

<sup>3</sup> Econase XT 25P, AB Vista, Marlborough, UK; 160,000 BXU/g.

originating from Germany and United Kingdom were obtained. Dry matter (DM), gross energy (GE), fat, nitrogen (N), calcium (Ca) and the phosphorous (P) contents of wheat samples were chemically analysed and further NIRS analyses were performed (Tables 2 and 3). A fixed amount of each wheat (58.6%) was used in the formula regardless of their chemical composition. Diets were predicted to contain 12.8 ME MJ/kg based on assumed average wheat apparent ME (AME) 58.6% came from wheat grain. Control diets were supplemented with 16,000 BXU/kg of xylanase following

**Table 2**  
Analysed nutrient composition and coefficient of variation (CV) of the wheat samples.

Item	Wheat samples								CV
	1	2	3	4	5	6	7	8	
Gross energy, MJ/kg	18.0	18.1	18.1	18.0	18.2	17.9	18.0	18.1	<1
Viscosity, cP	10.5	8.50	12.8	13.0	11.3	11.2	7.60	7.80	21
Dry matter, %	87.2	87.4	87.8	87.5	87.1	87.2	88.6	87.6	<1
Fat, %	1.49	1.37	1.48	1.37	1.26	1.15	1.24	1.94	17
Nitrogen, %	2.22	1.88	2.37	2.10	2.02	1.79	1.55	1.79	13
Calcium, %	0.03	0.03	0.03	0.03	0.03	0.02	0.02	0.05	31
Phosphorous, %	0.28	0.32	0.34	0.33	0.38	0.29	0.27	0.33	11
Phytic acid, %	0.75	0.77	0.64	0.72	0.81	0.92	0.53	0.53	19

**Table 3**  
Nutrient composition predicted by near-infrared spectroscopy (NIRS) and coefficients of variation (CV) of the wheat samples.

Item	Wheat samples								CV
	1	2	3	4	5	6	7	8	
Energy, MJ/kg and ether extract, %									
GE	16.5	16.5	16.6	16.6	16.6	16.4	16.3	16.4	<1
AME	13.4	13.4	13.2	13.0	13.3	13.6	13.9	13.4	2
Fat	2.10	2.23	2.16	2.40	2.28	2.25	1.95	2.13	6
Fibre									
NDF	15.1	16.4	16.1	19.0	17.2	16.1	14.0	16.1	9
ADF	2.44	2.82	2.94	3.94	3.15	2.99	2.11	3.02	18
Lignin	0.72	0.97	0.89	1.15	1.06	1.02	0.95	0.94	13
AX	7.66	8.10	7.97	9.19	8.48	7.83	7.30	8.02	7
Soluble AX	0.56	0.61	0.57	0.62	0.63	0.59	0.58	0.57	4
$\beta$ -glucan	1.21	1.66	1.66	2.36	1.88	1.63	1.78	1.90	18
Total insoluble NSP	10.2	11.4	11.1	13.2	12.1	11.1	10.4	11.3	8
Total soluble NSP	1.84	2.47	2.36	3.23	2.75	2.43	2.56	2.64	15
Protein, % and amino acid profile, g/100 g CP									
CP	13.4	11.44	13.6	11.7	11.9	10.6	8.36	10.2	15
Lysine	3.01	3.35	2.96	3.42	3.26	3.18	3.07	3.19	5
Methionine	1.56	1.68	1.60	1.65	1.69	1.70	1.78	1.67	4
Leucine	7.37	6.64	7.07	6.47	6.43	6.98	7.22	7.10	5
Threonine	3.30	3.42	3.25	3.42	3.34	3.41	3.41	3.42	2
Tryptophan	1.16	1.21	1.16	1.21	1.19	1.22	1.30	1.27	4
Tyrosine	3.21	3.13	3.20	3.09	3.12	3.23	3.28	3.23	2
Valine	4.75	4.91	4.76	4.96	4.86	4.90	4.99	4.95	2
Phenylalanine	4.43	4.39	4.55	4.53	4.47	4.44	4.53	4.50	1
Histidine	2.57	2.62	2.59	2.61	2.63	2.62	2.60	2.58	<1
Isoleucine	3.48	3.43	3.53	3.45	3.46	3.47	3.46	3.46	<1
Arginine	5.18	5.43	5.09	5.52	5.33	5.07	4.54	4.99	6
Alanine	4.28	3.76	4.11	3.89	3.67	4.04	4.33	4.22	6
Asparagine	6.06	6.22	5.79	6.27	5.98	6.04	5.59	6.00	4
Cysteine	2.19	2.34	2.20	2.27	2.34	2.33	2.40	2.32	3
Glutamine	24.9	23.5	26.0	23.2	24.7	24.1	24.0	23.4	4
Glycine	3.99	4.27	3.98	4.19	4.23	4.22	4.19	4.15	3
Proline	9.23	8.99	9.78	9.23	9.39	9.28	9.91	9.40	3
Serine	4.88	4.83	4.83	4.70	4.79	4.84	4.81	4.78	1

GE = gross energy; AME = apparent metabolizable energy; NDF = neutral detergent fibre; ADF = acid detergent fibre; AX = arabinoxylan; NSP = non-starch polysaccharides; CP = crude protein.

supplier recommendations (Econase XT, AB Vista, Marlborough, UK; 160,000 BXU/g), resulting in 16 experimental diets in total. All diets contained phytase supplemented at 500 FTU/kg (Quantum Blue, AB Vista, Marlborough, UK; 5,000 FTU/g). Activity of xylanase and phytase were determined using the reference method of analysis recommended by the supplier. Titanium dioxide (0.3%) was added to all the diets as an indigestible marker. Feed samples were taken at the beginning and throughout the experimental period for DM, N, fat and GE analysis.

### 2.3. Jejunal viscosity

Approximately 1.5 g (wet weight) of the fresh jejunal digesta were analysed according to Bedford et al. (1991). The viscosity (expressed as centipoise units, cP = 1/100 dyne sec/cm<sup>2</sup>) was determined using a Brookfield DV II digital viscometer.

### 2.4. Nutrient utilisation and total tract retention

Total tract retention and ileal nutrient utilisation were calculated using the index method (Olukosi et al., 2007), with titanium dioxide as the indigestible marker.

### 2.5. Net energy and nutrient accretion

Net energy for production (NEp), heat production (HP) and carcass fat and protein accretion were determined using the

comparative slaughter technique as described by Olukosi et al. (2008a, b). Briefly, 6 birds were euthanized at day 0 without feeding and kept frozen prior to processing and chemical analyses. On day 24, following euthanasia the carcasses were frozen and ground prior to freeze drying. Gross energy, N and fat contents were analysed. All the calculations for NEp, ME intake, HP as well as the efficiencies of energy for fat and protein retention (Fat-ER and CP-ER, respectively) are as described previously (Olukosi et al., 2008a). Net energy for production and HP were expressed per kilogram feed by dividing the total NEp (MJ) or HP (MJ) by kilogram of feed intake.

### 2.6. Chemical analyses

Ileal digesta and excreta were analysed for DM, N, fat and GE. Dry matter was determined by drying the samples in a drying oven (Uniterm, Russel-Lindsey Engineering Ltd., Birmingham, England, UK) at 105°C for 24 h (method 934.01; AOAC, 2006). Total N content was determined by the combustion method (method 968.06; AOAC, 2006). Gross energy was determined in an adiabatic oxygen bomb calorimeter (model 6200; Parr Instruments, Moline, IL) using benzoic acid as an internal standard. Titanium concentration in samples of diets and ileal digesta was determined using the method of Short et al. (1996).

### 2.7. Statistical analyses

Pen served as the experimental unit for FI, BWG and FCR, and cage as experimental unit for nutrient utilisation, jejunal viscosity, net energy and nutrient accretion. Data were analysed using the PROC MIXED command of SAS (SAS Inst. Inc., Cary, NC). When effects were found to be significant, treatment means were separated using Tukey's Highly Significant Difference test. Statistical significance was accepted at  $P < 0.05$  and trends were discussed at  $P < 0.10$ .

## 3. Results

### 3.1. Wheat nutritive value by NIRS and chemical analyses

The chemical analysis of the wheat samples indicates that they are all very similar in chemical composition and GE (Table 2). The predicted nutritive values by NIRS showed slightly more variability in nutrient composition between wheat varieties (Table 3), but remained close to expected average values. The predicted GE was underestimated while the predicted fat content was higher than chemically analysed. The predicted AME of wheat varieties ranged from 13.0 to 13.9 MJ/kg (CV < 2%). There was a great deal of variability (CV > 10%) in the predicted contents for crude protein, acid detergent fibre,  $\beta$ -glucan, lignin and total non-starch polysaccharides, but low variability (CV < 8%) in all other analysed chemical components, including amino acids.

### 3.2. Feed enzyme activity, growth performance and jejunal viscosity

Enzyme activities in feed samples were close to expected (16,038 BXU/kg average value analysed in all the xylanase-supplemented diets). No interactions were observed in any of the performance parameters measured (Table 4). There were no effects of wheat on performance or jejunal digesta viscosity. Nevertheless, improvements in performance were observed when xylanase was supplemented, regardless of wheat sample. Xylanase application resulted in a near significant 20 g ( $P = 0.077$ ) increase in BWG. Although FI was not influenced by xylanase, FCR was significantly improved by 4 points (1.33 vs. 1.37, xylanase vs. control, respectively;  $P = 0.003$ ). Xylanase supplementation also reduced viscosity

**Table 4**  
Animal performance and jejunal digesta viscosity.<sup>1</sup>

Item	Weight gain, g/bird	Feed intake, g/bird	Feed conversion ratio, g/g	Jejunal viscosity, cP
Wheat effect				
1	824	1105	1.341	2.81
2	816	1103	1.353	3.13
3	820	1087	1.329	2.89
4	817	1097	1.345	3.01
5	781	1087	1.394	2.77
6	791	1079	1.369	2.94
7	787	1049	1.341	2.40
8	791	1065	1.349	2.67
SEM	16	16	0.019	0.06
Xylanase effect				
0 BXU/kg	793 <sup>b</sup>	1088	1.373 <sup>a</sup>	3.32 <sup>a</sup>
16,000 BXU/kg	813 <sup>a</sup>	1079	1.331 <sup>b</sup>	2.34 <sup>b</sup>
SEM	8	8	0.010	0.03
<i>P</i> -value				
Wheat	0.465	0.192	0.367	0.380
Xylanase	0.072	0.496	0.003	<0.001
Interaction	0.951	0.950	0.894	0.845

<sup>a,b</sup> Means in the same column with different letters differ at  $P < 0.05$ .

<sup>1</sup> Mean values for 6 replicate cages with 8 broilers per replicate cage.

of jejunal digesta (3.32 vs. 2.34 cP, for control and xylanase supplemented diets, respectively;  $P < 0.001$ ). In the diets without xylanase supplementation, there were low and non-significant correlations between nutrient content of the wheats and FCR (Table 5). For the birds receiving xylanase supplemented diets, FCR was positively correlated with the analysed P and the predicted contents by NIRS of NDF, total and soluble AX as well as insoluble NSP. In addition, FCR was positively correlated with the analysed fat content.

### 3.3. Nutrient utilisation and total tract retention

No interactions between the main factors were observed for any of the ileal nutrient utilisation results (Table 6). The DM utilisation of wheat 3 was significantly lower compared with wheats 6, 7 and 8

**Table 5**  
Correlation of feed conversion ratio (FCR) with the analysed chemical composition and the predicted values by near-infrared spectroscopy (NIRS) of wheat in diets supplemented with or without xylanase.

Item	Pearson's correlation coefficients with FCR	
	Without xylanase	With xylanase
Analysed composition		
GE	-0.27	0.38
Fat	0.14	-0.26
Nitrogen	-0.47	0.07
Calcium	-0.43	0.36
Phosphorous	-0.35	0.70*
NIRS predicted composition		
CP	0.06	0.07
Fat	-0.27	0.68*
GE	-0.09	0.49
AME	0.53	-0.45
ADF	-0.34	0.63
NDF	-0.31	0.69*
Total AX	-0.36	0.73*
Soluble AX	0.25	0.85*
β-glucan	-0.34	0.55
Lignin	0.07	0.62
Total insoluble NSP	-0.26	0.74*
Total soluble NSP	-0.23	0.60

GE = gross energy; NIRS = near-infrared spectroscopy; CP = crude protein; AME = apparent metabolisable energy; ADF = acid detergent fibre; NDF = neutral detergent fibre; AX = arabinoxylan; NSP = non-starch polysaccharides.

\* $P < 0.05$ .

**Table 6**  
Ileal nutrient utilisation of nutrients.<sup>1</sup>

Item	Dry matter, %	Nitrogen, %	Energy, %	IDE, MJ/kg
Wheat effect				
1	68.0 <sup>bc</sup>	78.0	70.8 <sup>bc</sup>	13.2 <sup>b</sup>
2	66.9 <sup>bc</sup>	74.8	70.0 <sup>c</sup>	13.1 <sup>b</sup>
3	65.2 <sup>c</sup>	74.4	69.5 <sup>c</sup>	13.2 <sup>b</sup>
4	68.8 <sup>bc</sup>	78.2	72.0 <sup>abc</sup>	13.7 <sup>ab</sup>
5	66.9 <sup>bc</sup>	75.9	69.6 <sup>c</sup>	13.0 <sup>b</sup>
6	70.2 <sup>ab</sup>	78.1	73.2 <sup>abc</sup>	13.8 <sup>ab</sup>
7	70.4 <sup>ab</sup>	79.4	73.9 <sup>ab</sup>	14.0 <sup>a</sup>
8	73.0 <sup>a</sup>	79.7	76.0 <sup>a</sup>	14.4 <sup>a</sup>
SEM	1.47	1.42	1.47	0.28
Xylanase effect				
0 BXU/kg	67.8	76.9	70.9 <sup>b</sup>	13.35 <sup>b</sup>
16,000 BXU/kg	69.5	77.8	72.9 <sup>a</sup>	13.77 <sup>a</sup>
SEM	0.74	0.71	0.74	0.14
<i>P</i> -value				
Wheat	0.012	0.062	0.019	0.004
Xylanase	0.111	0.205	0.057	0.039
Interaction	0.550	0.104	0.571	0.577

IDE = ileal utilization of energy.

<sup>a-c</sup> Means in the same column with different letters differ at  $P < 0.05$ .

<sup>1</sup> Mean values for 6 replicate cages with 8 broilers per cage.

( $P < 0.05$ ), whereas wheats 1, 2, 4 and 5 had intermediate values. Wheats 7 and 8 had greater energy utilisation ( $P < 0.001$ ) compared with wheats 1, 2, 3 and 5, whereas wheats 4 and 6 were in between. Xylanase supplementation increased ileal utilisation of energy (IDE) measured as MJ/kg ( $P = 0.04$ ), regardless of wheat. Ileal N utilisation tended to be influenced by wheat ( $P = 0.06$ ), and was not influenced by xylanase supplementation (see Table 7).

There were significant interactions of the main factors for all total tract measurements ( $P < 0.001$ ). Xylanase supplementation improved the retention of DM and N as well as AME and AMEn for diets based on wheats 2 and 6. For those diets based on wheats 3, 4, 5 and 8, xylanase inclusion led to no effect or marginally lower results in total tract retention of N, AME and AMEn.

### 3.4. Net energy and nutrient accretion

There were no interactions between wheat and xylanase for any energy utilisation and efficiency responses, except for HP and Kre-protein, and no xylanase effect on any of the responses (Table 8). Net energy for production and  $K_{RE}$  were greater ( $P < 0.05$ ) for wheat

**Table 7**  
Total tract retention of nutrients.<sup>1</sup>

Wheat & Xylanase effect		Dry matter, %	Nitrogen, %	AME, MJ/kg	AMEn, MJ/kg
Wheat	Xylanase, BXU/kg				
1	0	69.4 <sup>cde</sup>	62.9 <sup>de</sup>	13.5 <sup>ef</sup>	13.0 <sup>e</sup>
1	16,000	71.2 <sup>bc</sup>	60.7 <sup>ab</sup>	14.1 <sup>cde</sup>	13.5 <sup>cd</sup>
2	0	65.4 <sup>g</sup>	57.9 <sup>f</sup>	12.9 <sup>h</sup>	12.4 <sup>g</sup>
2	16,000	73.4 <sup>a</sup>	65.7 <sup>a</sup>	14.5 <sup>ab</sup>	14.0 <sup>ab</sup>
3	0	68.6 <sup>de</sup>	58.1 <sup>cd</sup>	13.8 <sup>fg</sup>	13.2 <sup>e</sup>
3	16,000	68.4 <sup>e</sup>	57.4 <sup>cd</sup>	13.8 <sup>fg</sup>	13.2 <sup>ef</sup>
4	0	71.5 <sup>abc</sup>	63.3 <sup>abc</sup>	14.2 <sup>abc</sup>	13.8 <sup>abc</sup>
4	16,000	70.6 <sup>cd</sup>	62.7 <sup>cd</sup>	14.1 <sup>cd</sup>	13.6 <sup>cd</sup>
5	0	69.6 <sup>cde</sup>	64.6 <sup>e</sup>	13.5 <sup>def</sup>	13.0 <sup>de</sup>
5	16,000	66.1 <sup>fg</sup>	57.7 <sup>ef</sup>	13.0 <sup>gh</sup>	12.4 <sup>fg</sup>
6	0	65.2 <sup>g</sup>	55.1 <sup>ef</sup>	13.0 <sup>h</sup>	12.4 <sup>g</sup>
6	16,000	73.1 <sup>ab</sup>	66.9 <sup>abc</sup>	14.4 <sup>ab</sup>	13.9 <sup>ab</sup>
7	0	68.0 <sup>ef</sup>	62.0 <sup>ef</sup>	13.6 <sup>fg</sup>	13.1 <sup>ef</sup>
7	16,000	69.9 <sup>cde</sup>	65.3 <sup>e</sup>	14.1 <sup>ef</sup>	13.7 <sup>e</sup>
8	0	72.8 <sup>ab</sup>	65.8 <sup>abc</sup>	14.5 <sup>a</sup>	14.0 <sup>a</sup>
8	16,000	71.4 <sup>abc</sup>	64.1 <sup>abc</sup>	14.2 <sup>bc</sup>	13.7 <sup>bc</sup>
Pooled SEM		0.73	0.79	0.12	0.12
P-value					
Wheat		<0.001	<0.001	<0.001	<0.001
Xylanase		<0.001	<0.001	<0.001	<0.001
Interaction		<0.001	<0.001	<0.001	<0.001

AME = apparent metabolizable energy; AMEn = AME corrected for nitrogen.

<sup>a–h</sup> Different letters mean significant differences between treatments, highlighting the statistical interaction between main factors wheat × xylanase ( $P < 0.05$ ).<sup>1</sup> Mean values for 6 replicate cages with 3 broilers per replicate cage.**Table 8**  
Energy utilisation, energy retained and efficiencies of energy use.<sup>1</sup>

Item	Energy utilisation, MJ/kg		Energy retained, MJ/kg		Efficiencies of energy use for energy, protein and fat retention accretion		
	Nep <sup>2</sup>	HP <sup>3</sup>	Protein-ER <sup>4</sup>	Fat-ER <sup>5</sup>	K <sub>RE</sub> <sup>6</sup>	Kre-Protein <sup>7</sup>	Kre-Fat <sup>8</sup>
Wheat effect							
1	5.59 <sup>abc</sup>	6.84 <sup>bc</sup>	3.92 <sup>ab</sup>	2.20 <sup>bc</sup>	0.45 <sup>bc</sup>	0.275 <sup>ab</sup>	0.154 <sup>abcd</sup>
2	5.94 <sup>a</sup>	6.35 <sup>d</sup>	3.89 <sup>ab</sup>	2.48 <sup>a</sup>	0.48 <sup>a</sup>	0.275 <sup>ab</sup>	0.175 <sup>a</sup>
3	5.53 <sup>abc</sup>	6.54 <sup>cd</sup>	3.94 <sup>a</sup>	2.04 <sup>c</sup>	0.46 <sup>ab</sup>	0.289 <sup>a</sup>	0.149 <sup>bcd</sup>
4	5.53 <sup>c</sup>	7.24 <sup>a</sup>	3.95 <sup>a</sup>	2.04 <sup>c</sup>	0.43 <sup>c</sup>	0.269 <sup>bc</sup>	0.139 <sup>d</sup>
5	5.32 <sup>c</sup>	6.74 <sup>bc</sup>	3.96 <sup>a</sup>	2.05 <sup>c</sup>	0.44 <sup>bc</sup>	0.277 <sup>ab</sup>	0.143 <sup>cd</sup>
6	5.64 <sup>abc</sup>	6.60 <sup>bcd</sup>	3.89 <sup>ab</sup>	2.24 <sup>abc</sup>	0.46 <sup>ab</sup>	0.275 <sup>ab</sup>	0.158 <sup>abcd</sup>
7	5.72 <sup>bc</sup>	6.44 <sup>bcd</sup>	3.83 <sup>b</sup>	2.25 <sup>abc</sup>	0.47 <sup>b</sup>	0.278 <sup>ab</sup>	0.163 <sup>ab</sup>
8	5.77 <sup>ab</sup>	7.22 <sup>ab</sup>	3.84 <sup>b</sup>	2.41 <sup>ab</sup>	0.44 <sup>bc</sup>	0.258 <sup>c</sup>	0.162 <sup>abc</sup>
SEM	0.135	0.155	0.031	0.098	0.011	0.0050	0.0078
Xylanase effect							
0 BXU/kg	5.54	6.68	3.92	2.18	0.454	0.275	0.153
16,000 BXU/kg	5.72	6.81	3.89	2.24	0.457	0.274	0.158
SEM	0.068	0.077	0.016	0.049	0.005	0.0025	0.0039
P-value							
Wheat	0.029	0.001	0.032	0.008	0.012	0.017	0.029
Xylanase	0.950	0.060	0.135	0.373	0.327	0.869	0.354
Interaction	0.198	0.018	0.429	0.922	0.284	0.006	0.984

<sup>a–d</sup> Means in the same column with different letters differ at  $P < 0.05$ .<sup>1</sup> Mean values for 6 replicate cages with 1 broiler per replicate cage.<sup>2</sup> NEp – net energy for production.<sup>3</sup> HP – heat production.<sup>4</sup> Protein-ER – energy retained as protein.<sup>5</sup> Fat ER – energy retained as fat.<sup>6</sup> K<sub>RE</sub> – efficiency of energy use for production.<sup>7</sup> Kre-Protein – efficiency of energy use for protein accretion.<sup>8</sup> Kre-Fat – efficiency of energy use for fat accretion.

2 compared with wheats 4, 5 and 7, but similar, although numerically higher, than the other wheats. Energy retained as protein was greater ( $P < 0.05$ ) for wheats 3, 4 and 5 compared with wheats 7 and 8. Energy retained as fat and Kre-fat was greater ( $P < 0.05$ ) for wheat 2 than wheats 1, 3, 4 and 5. The interaction observed for HP ( $P = 0.02$ ) was explained by xylanase supplementation increasing HP when birds were fed wheats 2 and 6 (data not shown), but decreased HP for wheat 8, with no effect observed for the remaining wheats. The interaction noted for Kre-protein ( $P = 0.006$ ; data not

shown) was due to xylanase addition resulting in birds fed wheats 3 and 8 being more efficient in protein accretion, whereas it was reduced for wheats 2 and 6, with no effect on the remaining wheats.

#### 4. Discussion

It is well known that wheats, even of the same variety, can vary in both chemical composition and nutritive value (Theander et al., 1989). The current study investigated the effect of wheat sample

and xylanase supplementation on the performance of broilers fed starter diets. In spite of the variability found between wheats in both the analysed chemical composition and that predicted by NIRS, performance was not affected. Nevertheless, supplementation with xylanase improved BWG, FCR and reduced digesta viscosity, as has been shown in numerous studies (Olukosi et al., 2007; Wu et al., 2004; Zyla et al., 1999). Arabinoxylan is the main NSP in cereals, representing about 60%–70% of the cell walls of the endosperm and aleurone layer. Although AX from different sources differs in their substitution along the xylan backbone, a general structure can be assigned for AX: a backbone of  $\beta$ -(1,4)-linked xylose residues, which are substituted with arabinose residues on the C(O)-2 and/or C(O)-3 position and phenolic acids can be linked on the C(O)-5 position of arabinose. The structure of AX leads to high water holding capacity in the gastrointestinal tract resulting in high viscosity, and as a consequence production is less efficient. Xylanases cleave AX by internally hydrolysing the  $\beta$ -1,4- $\beta$ -D-xylosidic linkage between xylose residues yielding fragments of oligosaccharides with a high or low degree of substitution (Mendis et al., 2016). The use of xylanase with wheats varying in the level and content of soluble NSP mitigates much of the negative effects of arabinoxylan (AX) in monogastrics (Bedford, 2000).

Feed conversion ratio was not correlated with any of the analysed or predicted composition values of wheat without xylanase, but those supplemented with the enzyme had an unexpected positive correlation with the predicted contents of fat and fibre components (NDF, AX, soluble AX and total insoluble NSP). These findings are puzzling and suggest that the presence of more fibre (substrate) when the enzyme is present resulted in poorer performance but that the presence of this fibre in the absence of the enzyme was, if anything, marginally beneficial. Scott et al. (1999) found a significant relationship between predicted AME and FCR in wheat based diets with enzymes ( $r = -0.46$ ), which is in agreement with this study ( $r = -0.45$ ).

Non-starch polysaccharide degrading enzymes reduce digesta viscosity in the animal by shortening the molecular weight of NSP and also partly remove the nutrient encapsulation effect of the cell wall components and, as a consequence, nutrient absorption is promoted and performance maximized (Masey O'Neill et al., 2012, 2014a, b; Persia et al., 2002). In this study the measured intestinal viscosity of all samples was extremely low in comparison with the literature, which suggests that the wheat samples employed were not particularly challenging from a viscosity viewpoint. In a similar study, xylanase supplementation improved performance and homogeneity of broilers fed different Chinese maize samples varying in chemical composition (Masey O'Neill et al., 2012).

Some studies have reported improved performance and energy utilisation when NSP-enzymes are used in diets based on wheat, rye, barley (Bedford and Morgan, 1996; Bedford and Schulze, 1998) or maize (Masey O'Neill et al., 2012), but other studies have only shown improvements in animal performance without changes in nutrient utilisation (Hong et al., 2002; Wu et al., 2004). Wheat sample influenced ileal DM, N, energy utilisation and IDE. In particular, wheats 6, 7 and 8 were particularly good samples and this coincided with wheats 7 and 8 having the lowest viscosity. On the other hand, wheats 6, 7 and 8 also had lower contents of N (and predicted crude protein), acid detergent fiber, AX and NSP compared with the other wheats. Xylanase use increased energy utilisation and AME. Aside from the effect of reducing viscosity, there may be an additional benefit of increasing the permeability of the aleurone layer. This may enhance contact with digestive enzymes and their substrates, for better nutrient utilisation (Parkkonen et al., 1997).

The interaction of the main factors for all total tract measures of nutrient utilisation was significant. This was mostly due to the large responses of wheats 2 and 6 to xylanase addition due to their comparatively low nutrient utilisation in the absence of enzyme. Feed conversion ratio and measured AME significantly correlated in both wheats, suggesting the added benefit ( $r = -0.65$ ,  $P = 0.023$  and  $r = -0.49$ ,  $P = 0.11$ , respectively; data not shown). This observation implies that xylanase may have greater effects in poorer quality samples, elevating their nutritive value and thus reducing the variability between samples. Nonetheless, none of the results from the chemical analysis or NIRS predictions suggested that these two samples may have had a poorer nutritive value than the others. In this regard, it is noteworthy the low correlation between the predicted AME and the measured AME ( $r = -0.16$ ;  $P = 0.13$ ) suggesting the limited capacity of NIR to predict animal performance (data not shown). Wheats 3, 4, 5 and 8 had higher nutrient utilisation in the absence of enzyme, which may be due to the presence of endoxylanase in the outer layer of wheat (Cleemput et al., 1997) being responsible for part of the degradation of AX (Dornez et al., 2006), or lesser content of xylanase inhibitors or both.

The response of broilers to dietary intervention in general and enzyme supplementation in particular is usually measured using performance responses or ileal nutrient utilisation and total tract nutrient retention. These can be adequate for measuring gross efficiency of nutrient utilisation, but to further characterize the efficiency of nutrient utilisation it is useful to delineate the weight gain into the composition of gain, i.e., protein or fat, especially because of the differences in the efficiency with which these nutrients are deposited (Olukosi and Adeola, 2008). Net energy for production can be used as a more sensitive measure of energy utilisation by chickens receiving exogenous enzymes because it takes into account the efficiency of utilisation of ME for growth (Bhuiyan and Iji, 2015; Pirgozliev and Rose, 1999; Olukosi and Adeola, 2008; Olukosi et al., 2008a). Net energy for production is not only dependent on body weight but also on the amount of energy deposited in the carcass, which is an indication of how effectively the enzyme used facilitated energy utilisation. Net energy for production and  $K_{RE}$  were not influenced by xylanase supplementation but were influenced by wheat sample. Wheat samples 2, 7 and 8 presented better indices of energy utilisation, which may be related to the fact that they have the lowest viscosities compared with the other wheats. Heat production and  $K_{RE}$ -Protein varied depending on wheat and xylanase inclusion.

Interestingly, xylanase supplementation of wheats 2 and 6 increased total tract AME retention,  $Nep$  and  $HP$  but reduced  $K_{RE}$ ,  $K_{RE}$ -CP and  $K_{RE}$ -Fat and the efficiency of energy use for protein and fat accretion, as has been demonstrated previously (Bhuiyan and Iji, 2015; Daskiran et al., 2004; Olukosi and Adeola, 2008; Olukosi et al., 2008a). In the current study, the comparison between animal performance and energy utilisation must be considered with caution as only one bird from each replicate was selected. The extrapolation of the performance data derived from 8 animals per replicate may thus have some mis-alignment with the energy utilisation results obtained from one individual bird.

The utilisation of ME was more efficient for energy deposition and less for protein and fat. Nevertheless the efficiency of protein accretion was almost two-fold that of fat accretion, which was similarly shown by previous authors (Olukosi and Adeola, 2008; Olukosi et al., 2008b). The genetics and age of birds are important factors (Leeson and Summers, 1997; Lopez and Leeson, 2005). The higher proportion and retention of protein than fat is likely because the young broiler chicks were still actively growing and have not reached the stage at which fat deposition can overtake protein deposition (Bregendahl et al., 2002; Sanz et al., 2000).

## 5. Conclusion

Under the current experimental conditions xylanase supplementation may compensate for the poorer nutritive value of some wheats, enabling more homogenous broiler chick performance. Unfortunately the predicted nutrient composition by NIR did not accurately predict animal performance, and moreover taken together the predicted nutrient and chemically determined contents of the wheats used in this study did not allow for accurate ranking of the samples prior to feeding, which may relate to the very low viscosity of the wheat samples employed. In this regard, the use of the xylanase as an insurance policy is justified.

## Conflict of interests

All authors declare no conflict of interests.

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