Original scientific paper
DOI: /10.5513/JCEA01/27.3.5048
Journal of Central European Agriculture, 2026, 27(3), p.619-626

Energy and nutrient utilisation of laying hen diets containing graded levels of supplementary xylanase

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Received: October 8, 2025; accepted: November 26, 2025

ABSTRACT

The effect of graded levels of exogenous xylanase (XYL) at 0, 1500, 2000 or 2500 xylanase units (XU/kg) in laying hen diet containing 150 g/kg wheat distillers dried grains with solubles (DDGS) was examined. A total of 72, twenty-two-week-old, Hy-Line Brown laying hens were allocated to 24 cages, three birds in a cage. Each diet was fed for nine days to six cages following randomisation. Overall, supplementary XYL increased ($P < 0.05$) dietary apparent metabolizable energy (AME), nitrogen corrected AME (AMEn) and nitrogen retention coefficient. For every 100 XU, dietary AME and AMEn increased by 0.020 MJ and 0.018 MJ, respectively. As expected, there were no differences in bird growth or egg production variables. Further research is warranted to study the impact of graded levels of XYL in laying hen diets formulated to contain greater DDGS inclusion.

Keywords: wheat DDGS, graded levels xylanase, metabolisable energy, laying hens

INTRODUCTION

The global demand for eggs has steadily increased due to their affordability, high nutritional value, and versatility in human diets (Yang et al., 2018; Johnson et al., 2020). In recent years, the inclusion of high-fibre ingredients, such as distillers dried grains with solubles (DDGS) in poultry/ layer diets has gained attention due to their potential benefits on gut health, satiety, and sustainable feed formulation (Whiting et al., 2017; Watts et al., 2020). The DDGS is a by-product of ethanol production from corn, wheat, or other grains, as maize DDGS was the most studied one (Świątkiewicz and Koreleski, 2008; Whiting et al., 2017). During fermentation, starch within the grain is converted into ethyl alcohol and CO_2 (Lumpkins et al., 2005). Following this conversion, residual components are increased by approximately three-fold. As a result, DDGS is high in important nutrients such as protein, fat, vitamins, and minerals, making it a valuable, cost-effective component in poultry nutrition (Świątkiewicz and Koreleski, 2008). It should be noted that wheat DDGS and maize DDGS differ mainly in nutrient composition and feeding value. Wheat DDGS generally contains higher crude protein, fibre, and phosphorus but lower fat and energy compared to maize DDGS (Lumpkins et a., 2005). The higher fibre, or non-starch polysaccharides (NSP) content in wheat DDGS can reduce digestibility in monogastric animals like poultry and pigs. Maize DDGS, on the other hand, provides more metabolizable energy due to its higher fat and starch residue, making it more suitable for energy-dense diets (Świątkiewicz and Koreleski, 2008). Ruminants can utilize both effectively, but maize DDGS is often preferred for non-ruminant feed due to better energy efficiency. However, the high NSP content in wheat DDGS possesses anti-nutritional properties when fed to poultry, re-

ducing dietary energy availability. The viscous nature of NSP causes dietary bulk, resulting in reduced feed intake and nutrient utilisation (Yang et al., 2020; Whiting et al., 2023). Fibre-degrading enzymes, e.g. xylanase (XYL), have been used in poultry diets to alleviate the negative effects caused by NSP (Whiting et al., 2019; Pirgozliev et al., 2023). The effect of exogenous XYL on performance, energy and nutrient availability was primarily studied with broiler chickens (Liu and Kim, 2017; Van Hoeck et al., 2021; Morgan et al., 2022), although information is scarce for laying hens, particularly when fed diets containing wheat DDGS (Whiting et al., 2017, 2019). Work with broilers suggested that there may be advantages to feeding higher doses of XYL (Oduguwa et al., 2007; Nusairat and Wang, 2021; Singh et al., 2021), but again, there is a lack of information regarding laying hens; thus, further research is warranted in this field. Therefore, the present experiment aimed to examine the effect of graded levels of XYL (0, 1500, 2000 and 2500 U/kg) when supplemented into a diet containing 150 g/kg wheat DDGS. It has been hypothesised that increasing the dose of dietary XYL in the diet would increase dietary metabolizable energy (ME) and nutrient digestibility.

MATERIALS AND METHODS

Diet formulation

A diet containing 596.2 g/kg wheat, 150 g/kg wheat DDGS and 60.8 g/kg soya bean meal (SBM) as main feed ingredients, 11.74 MJ/kg ME and 165 g/kg crude protein (CP) (Table 1) was formulated to meet breeders' recommendations (Hy-Line International, 2018).

The diet was then split into four parts, which were supplemented with either 0, 1500, 2000 or 2500 XYL units (XU/kg), resulting in four experimental diets in total. Danisco Xylanase (EC 3.2.1.8) in powder form was used in this study. Danisco Xylanase was developed by Danisco Animal Nutrition (DuPont Industrial Biosciences, Marlborough, UK) and is a preparation of endo-1,4-ß-xylanase produced from a species of fungus called *Trichoderma reesei*. The diets did not contain any coccidiostat, antimicrobial growth promoters, prophylactic or other similar additives. Birds were fed a commercial feed only until 22 weeks of age, when approximately 90% egg production was reached. Experimental diets were administered for nine days with six replicates per treatment.

Table 1. Ingredient composition of experimental feed (g/kg)

Ingredient	g/kg
Wheat	596.2
Soya bean meal (48)	60.8
Distillers dried grains with solubles (wheat)	150.0
Veg oil	42.5
Dicalcium phosphate	12.8
Limestone	123.3
NaCl	1.2
Lysine	4.6
Methionine	2.6
Tryptophan	4.8
Vitamin and trace mineral premix[1]	1.2
Calculated composition (as fed basis)	
Metabolisable energy (MJ/kg)	11.74
Crude protein	165.0
Crude fat	54.5
Ca	48.3
Available P	4.1
Analysed values (DM basis)	
Dry matter (g/kg)	913
Gross energy (MJ/kg)	16.88
Crude protein	168.7
Crude fat	59.2
Neutral detergent fibres	154.3

[1] Vitamin and mineral premix provided (units per kg/feed): retinol, 2160 µg; cholecalciferol, 75 µg; α-tocopherol, 25 mg; menadione, 1.5 mg; riboflavin, 5 mg; pantotenic acid, 8 mg; cyanocobalamin, 0.01 mg; pyridoxine, 1.5 mg; thiamine, 1.5 mg; folic acid, 0.5 mg; niacin, 30 mg; biotin, 0.06 mg; iodine, 0.8 mg; copper, 10 mg; iron, 80 mg; selenium, 0.3 mg; manganese, 80 mg; and zinc, 80 mg.

Chemical analyses

Analyses for feed and excreta were carried out in duplicate. Dry matter (DM) of the diets and excreta was determined by drying samples in a forced air oven at 105 °C, until a constant weight (AOAC, 2006). Crude protein (CP) (N x 6.25) of the samples was determined by the combustion method using Leco (FP-528 N; Leco Corp., St. Joseph, MI) with EDTA as a standard (Sweeney, 1989). Oil (as ether extract) was extracted with petroleum ether using a Soxtec Avanti 2050, Foss UK Ltd (AOAC, 2006). Gross energy of the diets and excreta was measured using an isoperibol bomb calorimeter (Parr 6200 Instrument Company, Moline, IL, 61265, USA) as described elsewhere (Pirgozliev et al., 2006). Neutral detergent fibre (NDF) of the feed and excreta was analysed according to Van Soest et al. (1991) using an FT 122 Fibertec™ hot extraction unit (200-230V). Starch and NSP in the DDGS sample were determined following standard techniques (Englyst et al., 1994, 2000). The XYL activity in the feed samples was measured at the DuPont Nutrition Biosciences Innovation Laboratories (Brabrand, Denmark). One XYL unit was defined as the amount of enzyme that releases 0.48 µmol of reducing sugar as xylose from wheat arabino xylan per minute at pH 4.2 and 50 °C. All samples were analysed in technical duplicates.

Bird husbandry

The study was approved by the Animal Experimental Committee (project number 10-201502) of Harper Adams University. A total of 72 sixteen-week-old Hy-Line Brown laying hens were obtained from a commercial supplier (Country Fresh Pullets Ltd, Oswestry, Shropshire). The birds were randomly allocated to 24 enriched cages (Hellmann Poultry GmbH & Co. KG), over two tiers) in groups of three. Each cage was equipped with a separate feeder at the front and two nipple drinkers inside, perches, a nest and a bathing area with no bedding material on the floor of the cage.

Temperature was maintained at 21 °C, and relative humidity was between 50 and 70%. The birds had *ad libitum* access to feed and water. Lighting was set to 10 hours a day at 16 weeks of age and was gradually increased to achieve 16 hours of light at approximately 30 weeks (Hy-Line International, 2018). The nine-day study started when the birds were 22 weeks old (body weight 1.737 kg, STDEV ± 0.064). Each diet was fed to 6 cages following randomisation. During the final 96 hours of the study excreta were collected daily and dried immediately at 60 °C, until a constant weight and was used to calculate dietary ME (Hill and Anderson, 1958; apparent metabolisable energy (AME) and nitrogen corrected AME (AMEn)) and nutrient retention coefficients including dry matter (DMR), nitrogen (NR), fat (FR) retention and neutral detergent fibres digestibility (NDFD). Hen's productive performance was also recorded as a control measure to ensure no abnormalities were caused by feeding the dietary treatments.

Statistical analysis

Data for this study were analysed as a randomised block design by analysis of variance (ANOVA) using GenStat 23rd edition (IACR Rothamsted, Hertfordshire, UK). Pre-planned comparison and a polynomial contrast test were performed to test for overall difference between all XYL-supplemented diets versus the control diet and for linear (L) responses to XYL supplementation across diets. In addition, a regression analysis was employed to test the relationship between XYL activity and dietary AME and AMEn.

RESULTS

During the study period, no adverse effects of the different dietary treatments were observed for hen performance, and all birds remained healthy throughout the study period.

The determined XYL activity is presented in Table 2.

Table 2. Analysis of XYL in the experimental diets

Diets	XYL expected	XYL analysed
0 XU	0	0
1500 XU	1500	1576
2000 XU	2000	1950
2500 XU	2500	2630

Diets consisted of 4 experimental treatments: XYL = xylanase; 0 XU (XYL units) = the experimental feed without XYL supplementation; 1500 XU = the experimental feed supplemented with 1500 XU; 2000 XU = the experimental feed supplemented with 2000 XU; 2500 XU = the experimental feed supplemented with 2500 XU. Data analysed in technical duplicates.

The analysed chemical composition in the diet and DDGS is presented in Tables 1 and 3 and was within the expected range.

Table 3. Proximate analysis and carbohydrate composition (g/kg DM) in different sugar fractions of the experimental wheat DDGS

	Wheat DDGS
Dry matter	896.0
Gross energy (MJ/kg DM)	21.78
Crude protein	354.0
Starch	41.5
Crude fat	49.8
Neutral detergent fibres	493.3
Ash	54.9
Soluble non-starch polysaccharides (sNSP)	
Xylose	15.2
Arabinose	11.1
Mannose	5.7
Galactose	2.4
Glucose	7.2
Galacturonic acid	4.1
Total sNSP	45.7
Insoluble non-starch polysaccharides (iNSP)	
Xylose	66.8
Arabinose	43.2
Mannose	6.8
Galactose	8.6
Glucose	63.4
Galacturonic acid	0.0
Total iNSP	188.8
Total non-starch polysaccharides (NSP)	
Xylose	82.0
Arabinose	54.3
Mannose	12.5
Galactose	11.0
Glucose	70.5
Galacturonic acid	4.1
Total NSP	234.5

DDGS = wheat distillers' dried grains with solubles; data analysed in technical duplicates.

The effect of XYL levels supplementation in diets on energy utilisation and nutrient retention coefficients is presented in Table 4. Although there was a linear pattern (L<0.05) of XYL to increase AME, AMEn and NR, there was no significant difference (P>0.05) between diets. Overall, XYL-supplemented diets had greater AME, AMEn and NR compared to the unsupplemented diet via contrast test (P<0.05). There were no differences (P>0.05) in DMR, FR and NDFD between dietary treatments.

Table 5 displays the relationship between XYL activity and dietary ME. For every 100 XU, dietary AME and AMEn increased by 0.020 MJ and 0.018 MJ (P<0.05), respectively.

There were no differences (P>0.05) in performance data (Table 6).

Table 4. The effect of graded levels of xylanase supplementation on AME, AMEn, DMR, NR, FR and NDFD coefficients, determined on excreta when fed to laying hens from 22 to 23 weeks of age (data based on 4 days collection period)

Xylanase dose	AME (MJ/kg DM)	AMEn (MJ/kg DM)	DMR	NR	FR	NDFD
0 XU	12.28	11.99	0.671	0.312	0.821	0.380
1500 XU	12.58	12.23	0.678	0.373	0.836	0.420
2000 XU	12.68	12.33	0.664	0.378	0.836	0.410
2500 XU	12.75	12.40	0.677	0.370	0.846	0.417
SEM	0.136	0.119	0.0059	0.0211	0.0123	0.0288
P	0.132	0.133	0.396	0.144	0.580	0.758
L	0.023	0.024	0.793	0.040	0.191	0.357
Contrast	0.031	0.034	0.772	0.026	0.225	0.311

AME = apparent metabolizable energy; AMEn = nitrogen corrected AME; DMR = dry matter retention coefficient; NR = nitrogen retention coefficient; FR = fat retention coefficient; NDFD = neutral detergent fibre digestibility; XU = xylanase unit; SEM = pooled standard error of means; P = Fisher's probability; L = orthogonal polynomial contrast for linear response; Contrast = significance between the 0XU versus all xylanase-supplemented diets determined by comparisons contrast tests; There is a statistically significant difference when $P<0.05$.

Table 5. Relationship between xylanase activity and AME and AMEn

Dependent variates	Constant	Xylanase activity	r^2	Residual standard deviation
AME (MJ/kg DM)	12.27 (±0.154)	0.0002024 (±0.0000883)	0.20	0.367*
AMEn (MJ/kg DM)	11.98 (±0.136)	0.0001757 (±0.0000778)	0.19	0.323*

AME = apparent metabolizable energy; AMEn = nitrogen corrected AME; Statistical significance of regression equation: *$P<0.05$.

Table 6. The effect of graded levels of xylanase supplementation on daily feed intake, weight gain, average egg weight, eggs per hen day, daily egg mass output and feed conversion ratio for egg production when fed to laying hens from 22 to 23 weeks of age (data based on a 9-day feeding period)

Xylanase dose	Feed intake (g/b/d)	Weight gain (g/b/d)	Egg weight (g)	Eggs (hen/d)	Egg mass output (g/b/d)	Feed conversion ratio
0 XU	124.8	7.9	59.5	0.95	56.6	2.217
1500 XU	125.4	7.3	59.2	0.97	57.4	2.188
2000 XU	121.4	6.2	59.2	0.95	56.2	2.164
2500 XU	124.5	5.4	58.2	0.99	57.9	2.146
SEM	3.16	1.82	1.0	0.016	1.17	0.0552
P	0.816	0.778	0.795	0.201	0.735	0.819
Contrast	0.782	0.467	0.611	0.295	0.700	0.445

XU = xylanase unit; SEM = pooled standard error of means; P = Fisher's probability; Contrast = significance between the 0XU versus all xylanase-supplemented diets determined by contrast tests. There is a statistically significant difference when $P<0.05$.

DISCUSSION

The benefits of supplementing broiler feeds with NSP-degrading enzymes have been associated with the enzyme's ability to reduce intestinal viscosity, degrade the cell wall of NSP and release nutrients that have been encapsulated in the gut (Olukosi and Bedford, 2019; Pirgozliev et al., 2023). In comparison with broilers, older birds, such as laying hens, may be better at coping with this higher gut viscosity and therefore their response to XYL supplementation may be to a smaller degree when compared with younger birds (Olukosi and Bedford, 2019).

However, digesta viscosity was not measured in this experiment. Several studies have confirmed that the use of an NSP hydrolysing enzyme such as XYL has improved ME and nutrient digestibility for laying hens (Pan et al., 1998; Mathlouthi et al., 2003; Pirgozliev et al., 2010). It was therefore expected in this experiment that the addition of XYL would improve nutrient availability. Although dietary XYL increased ME and NR, there was no effect on DMR, FR and NDFD. Compared to broilers, laying hens may be better at managing with dietary fibres (Bedford, 2018), thus an inclusion of 150 g/kg wheat DDGS may not have a significant impact on the efficiency of digestion. In this study, dietary XYL may have hydrolysed some fibres from the wheat and SBM, which have less NDF compared to DDGS, thus explaining the lack of overall impact on NDFD. Fat digestibility is an important aspect of poultry nutrition, particularly for laying hens, as fat provides essential fatty acids and energy. At moderate levels (typically 4-8% of the total diet), hens can digest fat efficiently (Ravindran et al., 2016), thus suggesting an explanation for the lack of differences in FR between treatments. The DMR represents the overall dietary nutrient retention; the lack of response to fats and fibres may be the reason for the lack.

Feeding graded levels of XYL to broiler chickens does not always follow the same response. Some authors (Liu and Kim, 2017; Van Hoeck et al., 2021; Nusairat and Wang, 2021; Singh et al., 2021) observed an increase in performance, ME and nutrient availability with dietary XYL increase; others (Olukosi et al., 2007; Rabello et al., 2021) saw no benefit of increasing dietary XYL in broiler diets. Morgan et al. (2022) reported that overall, a double dose of XYL had no effect on bird performance, meat yield, ileal viscosity, ileal starch, energy or DM digestibility or digesta DM content. However, birds fed a diet with double dose of XYL had an increased caecal concentration of butyric, propionic, valeric and lactic acid and *Bifidobacteria* and *Enterobacteria* spp. (Morgan et al., 2022). Thus, suggesting that the results obtained in the current study are not unexpected. In addition, the benefits of dietary XYL may be more obvious on a physiological level, e.g. gastrointestinal tract development and caecal fermentation, and may require a prolonged feeding period to become obvious (Lin et al., 2023).

The NDF content in the experimental diet was 154 g/kg DM and similar to the average dietary level in laying hen diets (Patt et all., 2022). Patt et al. (2022) reported that even a greater than 154 g/kg level of dietary NDF did not have a negative impact on performance but reduced the risk for the occurrence of feather pecking in laying hens, i.e., improved well-being. The increase in ME with XYL supplementation would depend on substrate availability and units of XYL. Xylanase is an enzyme that breaks down xylan, a type of hemicellulose found in plant cell walls, into simpler sugars. Xylan is a major fibre component in many plant materials, including DDGS (Whiting et al., 2019), thus supplementing DDGS-containing poultry diets with XYL may increase energy and nutrient availability. However, the DDGS takes a relatively small part of the dietary formulation in this study, which can suggest an explanation for the lack of pronounced ME increase with greater XYL inclusion in diets.

The performance data are shown as baseline performance metrics. The performance results were used to assess the comparability of the study, i.e., FI, growth and egg production, as differences in performance were not expected for a feeding period of nine days. Overall, the performance of the birds was within breeders' recommendations (Hy-Line International, 2018).

CONCLUSION

Results from the present study conclude that overall, dietary XYL increased dietary metabolizable energy and nitrogen retention. However, there was no difference in response between dietary XYL levels, thus suggesting that dietary fibres were not really exceeding the biologically acceptable value for the hens and did not provide additional substrate for the higher dosage of the enzyme. However, formulating a diet with greater DDGS content may respond more visibly to higher xylanase levels. Overall, xylanase supplementation improved dietary metabolisable energy and nitrogen retention; thus, the use of fibre-degrading enzymes should be considered when formulating layer diets which contain a high inclusion of wheat DDGS.

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