

## **Energy and nutrient utilisation of laying hens fed fibre rich diet containing graded levels of supplementary fungal xylanase**

**Isobel M. Whiting<sup>1</sup>, Stephen P. Rose<sup>1</sup>, Alexander M. Mackenzie<sup>1</sup>,  
Ahmed M. Amerah<sup>2</sup>, Vasil R. Pirgozliev<sup>1\*</sup>**

<sup>1</sup> The National Institute of Poultry Husbandry, Harper Adams University, Shropshire, UK

<sup>2</sup> Danisco Animal Nutrition, Marlborough, Wiltshire, UK

*(Accepted November 18, 2025)*

The effect of graded levels of exogenous xylanase (XYL) at 0, 1500, 2000 or 2500 xylanase units (XU/kg) in laying hen diet containing 300 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. Supplementing diets with 2500 XU, the highest XYL level, increased ( $p < 0.05$ ) dietary apparent metabolisable energy (AME) and nitrogen corrected AME (AMEN) in a linear pattern ( $L < 0.05$ ), and nitrogen retention coefficient ( $p < 0.05$ ) following quadratic response ( $Q < 0.05$ ). For every 100 XU, dietary AME and AMEN increased by 0.013 MJ and 0.012 MJ, respectively. There were no differences observed in bird growth or egg production variables. Further research is warranted to study the impact of greater XYL activities in laying hen diets formulated to contain greater DDGS inclusion rates.

**KEY WORDS:** wheat distillers dried grains with solubles (DDGS) / xylanase / metabolisable energy / nutrient retention coefficients / laying hens

---

\*Corresponding author: [vpirgozliev@harper-adams.ac.uk](mailto:vpirgozliev@harper-adams.ac.uk)

Eggs are a globally important food source, valued for their high nutritional content, economic affordability, and versatility in culinary and industrial applications [Yang *et al.* 2018]. As a result, consumer and industry demand for eggs continues to grow [Adejola *et al.* 2025]. The efficiency and productivity of laying hens are highly influenced by the nutritional quality and composition of their diets [Macelline *et al.* 2021]. Achieving optimal egg production, egg weight, and feed conversion requires precise formulation of energy and protein sources, with special attention to amino acid balance [Zhang *et al.* 2025]. In recent years, the poultry industry has increasingly explored the use of alternative feed ingredients to reduce dependency on conventional protein sources, such as soybean meal [Krawczyk *et al.* 2024]. One such ingredient is wheat distillers dried grains with solubles (DDGS), a by-product of wheat-based ethanol production that provides substantial amounts of protein, fibre, residual oil, and highly available phosphorus [Whiting *et al.* 2019]. While wheat DDGS can partially replace conventional protein sources in layer diets, its inclusion is limited by factors such as high fibre and non-starch polysaccharide (NSP) content, variable amino acid digestibility, and potential heat damage to lysine during processing. Typically, inclusion levels up to 10-15% are considered safe without negatively affecting laying performance [Świątkiewicz and Koreleski 2008], but higher levels may compromise feed efficiency and energy utilisation if not adequately balanced with supplemental amino acids and enzymes.

Fibre-degrading enzymes, such as xylanase (XYL), have been incorporated into poultry diets to mitigate the adverse effects associated with high fibres [Whiting *et al.* 2019, Pirgozliev *et al.* 2023a]. Xylanase, a non-starch polysaccharide-degrading enzyme, enhances nutrient utilisation and gut function in poultry by hydrolysing arabinoxylans into fermentable oligosaccharides [Morgan *et al.* 2020]. This action reduces intestinal viscosity, improves nutrient absorption, and modulates the gut microbiota, promoting short-chain fatty acid production that supports intestinal integrity and immune competence [Kubiś *et al.* 2020, Wiśniewska *et al.* 2023]. In healthy birds, XYL optimises energy and amino acid metabolism, improving feed efficiency [Liu and Kim 2017, Van Hoeck *et al.* 2021, Yang *et al.* 2020, Morgan *et al.* 2022]. Under disease or stress conditions, XYL mitigates metabolic disturbances by lowering oxidative stress and inflammation, stabilising lipid and glucose metabolism, and enhancing antioxidant capacity, thereby supporting resilience and maintaining physiological homeostasis in the gastrointestinal and hepatic systems [Pirgozliev *et al.* 2010, Pirgozliev *et al.* 2023b, Amerah and Ravindran 2015, Lin *et al.* 2023]. However, there is limited information regarding its use in laying hen feed, particularly when diets are supplemented with wheat DDGS [Whiting *et al.* 2017, 2019]. Studies in broilers have suggested potential benefits from feeding higher doses of XYL, with improvements observed in nutrient utilisation and performance [Pirgozliev *et al.* 2016, Nusairat and Wang 2021, Singh *et al.* 2021]. However, there remains a lack of information regarding the response of laying hens to varying levels of XYL supplementation, particularly when diets include a high amount of wheat DDGS.

Therefore, the aim of the present experiment was to investigate the effects of graded levels of XYL (0, 1500, 2000, and 2500 U/kg) on performance, energy utilisation, and nutrient digestibility in laying hens fed a diet containing 300 g/kg wheat DDGS. It was hypothesised that increasing dietary XYL inclusion would enhance the metabolisable energy (ME) content of the diet and improve nutrient digestibility.

## **Material and methods**

The experiment was performed at the National Institute of Poultry Husbandry (NIPH), Harper Adams University, UK. The study was approved by the Animal Experimental Committee of Harper Adams University (Project No. 10-201502).

### **Diet formulation**

A diet containing 491 g/kg wheat, 300 g/kg wheat DDGS and 50 g/kg soya bean meal (SBM) as main feed ingredients, 11.52 MJ/kg ME and 198.4 g/kg crude protein (CP) (Table 1) was formulated to meet breeders' recommendations [Hy-Line Management Guide, 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 a 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 *Trichoderma reesei* endo-1,4- $\beta$ -xylanase. 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.

### **Chemical analyses**

Proximate analyses of DDGS, feed, and excreta samples were performed using standard methods [AOAC, 2007]. Dry matter (DM) content of diets and DDGS was determined by oven-drying samples at 105°C until a constant weight was achieved. Excreta were dried at 60°C until a constant weight. Crude protein (CP) was measured using the combustion method (Leco FP-528 N; Leco Corp., St. Joseph, MI), with EDTA as a calibration standard, as described by Sweeney [1989]. Oil content (ether extract) was determined using petroleum ether in a Soxtec Avanti 2050 system (Foss UK Ltd) as demonstrated elsewhere [Pirgozliev and Bedford 2012]. Gross energy of feed and excreta was analysed with an adiabatic bomb calorimeter (Parr 6200; Parr Instrument Company, Moline, IL), as described by Pirgozliev *et al.* [2006]. Neutral detergent fibre (NDF) was measured according to Van Soest *et al.* [1991] using an FT 122 Fibertec™ hot extraction unit (200 - 230 V). Starch and non-starch polysaccharides (NSP) in DDGS were determined following the procedures of Englyst *et al.* [1994, 2000]. Retention coefficients were calculated as described elsewhere. Xylanase activity in feed samples was assessed at the DuPont Nutrition Biosciences

Innovation Laboratories (Brabrand, Denmark). One XYL unit was defined as the amount of enzyme required to release 0.48  $\mu\text{mol}$  of reducing sugar (as xylose) from wheat arabinoxylan per minute at pH 4.2 and 50°C. All analyses were conducted in technical duplicates.

#### **Bird husbandry**

Seventy-two 20-week-old Hy-Line Brown laying hens were sourced from a commercial supplier (Country Fresh Pullets Ltd, Oswestry, Shropshire) and allocated to 24 enriched cages (Hellmann Poultry GmbH & Co. KG), with three birds per cage. The trial followed a randomised block design, consisting of six blocks distributed across two tier levels. Each block included four cages, each assigned to a different dietary treatment. The housing environment was maintained in accordance with industry standards [Hy-Line Management Guide, 2018]. The study commenced when the birds reached 22 weeks of age (average body weight  $1.736 \pm 0.068$  kg) and lasted for nine days. Feed intake was recorded throughout the experimental period. During the final four days, excreta were collected daily and immediately dried. These samples were used to determine dietary ME values, including apparent metabolisable energy (AME) and nitrogen-corrected AME (AMEn) [Hill and Anderson 1958], as well as nutrient retention coefficients, including dry matter retention (DMR), nitrogen retention (NR), fat retention (FR), and neutral detergent fibre digestibility (NDFD) as described elsewhere [Pirgozliev *et al.* 2010]. Hen productive performance was monitored throughout the study to ensure that no adverse effects were associated with the dietary treatments.

#### **Statistical analysis**

Statistical analyses were performed using GenStat (23<sup>rd</sup> edition) statistical software (IACR Rothamsted, Hertfordshire, UK). Pre-planned polynomial contrast tests were performed to test for linear (L) and quadratic (Q) 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 and discussion**

No adverse effects of the dietary treatments on hen performance were observed during the study, and all birds remained healthy and survived the experimental period.

The analysed chemical composition of the diets and DDGS is presented in Tables 1 and 3 and was within the expected range. The measured XYL activity levels are shown in Table 2. The effects of XYL supplementation on energy utilisation and nutrient retention coefficients are summarised in Table 4. A linear pattern ( $L < 0.05$ ) was observed for increasing levels of XYL supplementation, with diets containing 2500 XU exhibiting greater ( $p < 0.05$ ) AME and AMEn values compared to the unsupplemented control, and the diet supplemented with 1500 XU. A similar pattern

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

Ingredient	g/kg
Wheat	491.0
Soya bean meal (48)	50.0
DDGS	300.0
Veg oil	35.0
Dicalcium phosphate	10.5
Limestone	101.5
NaCl	1.0
Lysine	3.8
Methionine	2.2
Tryptophan	4.0
Vitamin and trace mineral premix*	1.0
Calculated composition (as fed basis)	
ME (MJ/kg)	11.52
CP	198.4
Fat	48.7
Ca	40.0
Available P	4.3
Analysed values (DM basis)	
DM (g/kg)	910
GE (MJ/kg)	17.75
CP	201.4
Fat	57.6
NDF	214.1

\*Vitamin and mineral premix provided (units per kg/feed): retinol, 2160 µg; cholecalciferol, 75 µg;  $\alpha$ -tocopherol, 25 mg; menadione, 1.5 mg; riboflavin, 5 mg; pantothenic 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.

**Table 2.** Analysis of xylanase (XYL) in the experimental diets

Diets	XYL expected	XYL analysed
0 XU	0	0
1500 XU	1500	1610
2000 XU	2000	2054
2500 XU	2500	2598

Diets consisted in 4 experimental treatments: 0 XU (xylanase units) – the experimental feed without xylanase (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.

was observed for NR coefficients. Dietary DMR showed a tendency to improve ( $p=0.077$ ), although this was not statistically significant. No significant differences ( $p>0.05$ ) were detected among dietary treatments for FR or NDFD.

**Table 3.** Proximate analysis and carbohydrates composition (g/kg DM) in different sugar fractions of the experimental wheat distillers dried grains with solubles (DDGS)\*

Item	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
<i>Soluble non-starch polysaccharides (sNSP)</i>	
xylose	15.2
arabinose	11.1
mannose	5.7
galactose	2.4
glucose	7.2
galacturonic acid	4.1
total sNSP	45.7
<i>Insoluble non-starch polysaccharides (iNSP)</i>	
xylose	66.8
arabinose	43.2
mannose	6.8
galactose	8.6
glucose	63.4
galacturonic acid	0.0
total iNSP	188.8
<i>Total non-starch polysaccharides (NSP)</i>	
xylose	82.0
arabinose	54.3
mannose	12.5
galactose	11.0
glucose	70.5
galacturonic acid	4.1
total NSP	234.5

\*Data analysed in technical duplicates.

Table 5 illustrates the relationship between XYL activity and dietary ME. For every 100 XU increase in XYL activity, AME and AMEn increased by 0.013 MJ and 0.012 MJ, respectively.

The performance data are shown as baseline performance metrics (Table 6). No significant differences ( $p > 0.05$ ) were observed in hen performance parameters across dietary treatments. 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. The data were used to ensure there were no adverse effects of the diets.

The supplementation of broiler diets with NSP degrading enzymes, such as XYL, has been widely associated with reduced intestinal viscosity, breakdown of NSP cell walls, and the release of encapsulated nutrients in the gut [Olukosi and Bedford 2019, Pirgozliev *et al.* 2023]. Compared to broilers, older birds like laying hens may be

**Table 4.** The effect of graded levels xylanase supplementation on apparent metabolisable energy (AME), nitrogen corrected AME (AMEn), total tract dry matter (DMD), nitrogen (ND), fat (FD) and neutral detergent fibres (NDFD) digestibility coefficients, determined on excreta when fed to laying hens from 22 to 23 weeks of age (data based on 3 days collection period)

Xylanase dose	AME (MJ/kg DM)	AMEn (MJ/kg DM)	DMR	NR	FR	NDFD
0 XU	12.56 <sup>a</sup>	12.18 <sup>a</sup>	0.656	0.344 <sup>a</sup>	0.815	0.466
1500 XU	12.58 <sup>a</sup>	12.22 <sup>a</sup>	0.658	0.329 <sup>a</sup>	0.831	0.462
2000 XU	12.78 <sup>ab</sup>	12.38 <sup>ab</sup>	0.651	0.361 <sup>ab</sup>	0.844	0.473
2500 XU	12.96 <sup>b</sup>	12.55 <sup>b</sup>	0.678	0.377 <sup>b</sup>	0.841	0.491
SEM	0.086	0.080	0.007	0.0103	0.0083	0.0131
<i>p</i> -value	0.021	0.028	0.077	0.034	0.124	0.424
L	0.008	0.009	0.151	0.051	0.027	0.256
Q	0.068	0.098	0.079	0.026	0.793	0.232
Deviation	0.692	0.778	0.120	0.316	0.453	0.983

XU – xylanase unit; SEM – pooled standard error of means; *p* – Fisher’s probability; L – orthogonal polynomial contrast for linear response; Q – orthogonal polynomial contrast for quadratic response; deviation – orthogonal polynomial contrast for deviation from linearity.

<sup>ab</sup>Means in a row bearing different superscript differ significantly at *p*<0.05.

**Table 5.** Relationship between xylanase activity and apparent metabolisable energy (AME) and nitrogen corrected AME (AMEn)

Dependent variates	Constant	Xylanase activity	r <sup>2</sup>	Residual standard deviation
AME (MJ/kg DM)	12.54 (±0.0865)	0.0001274 (±0.0000498)	0.23	0.204*
AMEn (MJ/kg DM)	12.17 (±0.0776)	0.0001182 (±0.0000447)	0.25	0.183*

Statistical significance of regression equation: \**p*<0.05.

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

Xylanase dose	FI (g/b/d)	WG (g/b/d)	Egg weight (g)	Eggs (b/d)	Egg mass output (g/b/d)	FCR
0 XU	118.9	5.8	58.5	0.97	57.1	2.094
1500 XU	120.3	5.8	57.2	1.00	57.4	2.087
2000 XU	122.9	7.7	60.0	0.85	50.5	2.523
2500 XU	122.9	7.7	59.3	0.98	57.8	2.109
SEM	2.48	1.27	1.38	0.045	2.31	0.1527
<i>p</i> -value	0.602	0.557	0.552	0.123	0.139	0.179
L	0.221	0.261	0.576	0.488	0.558	0.438
Q	0.840	0.596	0.487	0.793	0.459	0.575
Deviation	0.653	0.505	0.269	0.026	0.034	0.049

XU – xylanase unit; SEM – pooled standard error of means; *p* – Fisher’s probability; L – orthogonal polynomial contrast for linear response; Q – orthogonal polynomial contrast for quadratic response; deviation – orthogonal polynomial contrast for deviation from linearity.

better adapted to cope with increased gut viscosity and therefore may exhibit a less pronounced response to XYL supplementation [Olukosi and Bedford 2019]. Although digesta viscosity was not measured in the present study, the lack of dirty eggs (data not in tables) supports the assumption that viscosity was not a problem in the recent study [Chesson 2001]. Research has demonstrated that supplementation with XYL, can enhance ME and nutrient digestibility in laying hens [Pan *et al.* 1998, Mathlouthi *et al.* 2003]. Based on these findings, it was anticipated that XYL addition would improve nutrient availability in the present study. While a high dosage of dietary XYL did lead to increases in ME and NR, it had no significant effect on the rest of retention coefficients studied. Laying hens have more developed gastrointestinal tract and may be more capable of handling higher dietary fibre levels than broilers [Ricke and Rothrock 2020]. Therefore, the inclusion of 300 g/kg wheat DDGS in a balanced diet may not have significantly affected digestive efficiency. In addition, an increase dietary fibre level led to reduced feather pecking [Patt *et al.* 2022], a common welfare issue, especially in free-range hens [Pettersson *et al.* 2016]. One key area of research and management focuses on dietary fibre as a tool to mitigate feather pecking.

In the present study, dietary XYL may have primarily hydrolysed fibre fractions from wheat and soybean meal, ingredients with lower NDF content than DDGS, potentially explaining the lack of a significant effect on overall NDFD. Fat is a critical component of poultry nutrition, particularly for laying hens, as dietary fat supplies both energy and essential fatty acids. At moderate inclusion levels (typically 4 - 8% of the total diet), hens are generally able to digest fat efficiently [Ravindran *et al.* 2016], which may explain the absence of significant differences in FR between dietary treatments. Dietary DMR reflects the overall efficiency of nutrient utilisation; therefore, the lack of response in FR and NDFD may account for the absence of a significant effect on DMR.

Feeding high XYL to broiler chickens has not consistently produced uniform responses. While some studies have reported improvements in bird performance, ME and nutrient availability with increasing dietary XYL inclusion [Liu and Kim 2017, Van Hoeck *et al.* 2021, Nusairat and Wang 2021, Singh *et al.* 2021], others have found no significant benefits [Olukosi *et al.* 2007, Rabello *et al.* 2021]. Morgan *et al.* [2022] reported that a double dose of XYL had no effect on growth performance, ME or nutrient digestibility but led to an increased caecal population of *Bifidobacteria* and *Enterobacteria* spp and increased concentration of butyric, and propionic acids. These findings support the view that the outcomes observed in the current study are not unexpected. Moreover, the benefits of dietary XYL supplementation may be more pronounced at the physiological level, such as promoting gastrointestinal tract development and enhancing caecal fermentation. Such effects may require a prolonged feeding period to manifest fully [Lin *et al.* 2023].

## Conclusions

Results from the present study indicate that the highest xylanase dose tested (2500 XU/kg) produced the greatest improvement in energy utilisation, suggesting that this level may be adequate for diets containing 300 g/kg wheat DDGS. However, as 2500 U/kg was the maximum xylanase inclusion level evaluated, it remains uncertain whether higher doses could yield additional benefits, although the use of higher enzyme doses may not be economically sustainable. Overall, xylanase supplementation improved dietary metabolisable energy, indicating that the inclusion of fibre-degrading enzymes should be considered when formulating layer diets with high levels of wheat DDGS.

## Disclosures

The authors declare no conflicts of interest.

## REFERENCE

1. ADEJOLA Y.A., SIBANDA T.Z., RUHNKE I., BOSHOFF J., POKHREL S., WELCH, M., 2025 - Analyzing the risk of short-term losses in free-range egg production using commercial data. *Agriculture* 15, 743.
2. AMERAH A.M., RAVINDRAN V., 2015 - Effect of coccidia challenge and natural betaine supplementation on performance, nutrient utilization, and intestinal lesion scores of broiler chickens fed suboptimal level of dietary methionine. *Poultry Science* 94(4), 673-680.
3. AOAC, 2007 - Official Methods of Analysis of the AOAC, 18th ed.; Association of Official Analytical Chemists: Arlington, VA, USA.
4. CHESSON A., 2001 - Non-starch polysaccharide degrading enzymes in poultry diets: influence of ingredients on the selection of activities. *World's Poultry Science Journal* 57, 251-263.
5. ENGLYST H.N., QUIGLEY M.E., HUDSON G.J., 1994 - Determination of dietary fibre as non-starch polysaccharides with gas-liquid chromatographic, high-performance liquid chromatographic or spectrophotometric measurement of constituent sugars. *Analyst* 119, 1497-1509.
6. ENGLYST K.N., HUDSON G.J., ENGLYST H.N., 2000 - Starch analysis in food. In Encyclopaedia of analytical chemistry; Meyers RA, editor; Chichester: John Wiley and Sons; p. 4246-4262.
7. HILL F.W., ANDERSON D.L., 1958 - Comparison of metabolizable energy and productive energy determinations with growing chicks. *Journal of Nutrition* 64, 587-603.
8. HY LINE MANAGEMENT GUIDE, 2018 - 1592407567-bm\_com\_eng.pdf (accessed on 20 June 2025).
9. KRAWCZYK J., LEWKO L., ŚWIĄTKIEWICZ S., 2024 - Effect of feeding selected strains of conserved breed hens with diets containing legumes on egg quality and content and activity of lysozyme. *Annals of Animal Science* 24, 201-209.
10. KUBIŚ M., KOŁODZIEJSKI P., PRUSZYŃSKA-OSZMAŁEK E., SASSEK M., KONIECZKA P., GÓRKA P., FLAGA J., KATARZYŃSKA-BANASIK D., HEJDYSZ M., WIŚNIEWSKA Z., KACZMAREK S.A., 2020 - Emulsifier and xylanase can modulate the gut microbiota activity of broiler chickens. *Animals* 10, 2197.
11. LIN Y., LOURENCO J.M., OLUKOSI O.A., 2023 - Effects of xylanase, protease, and xylo-oligosaccharides on growth performance, nutrient utilization, short chain fatty acids, and microbiota in Eimeria-challenged broiler chickens fed high fiber diet. *Animal Nutrition* 15, 430-442.
12. LIU W.-C., KIM I.-H., 2017 - Effects of dietary xylanase supplementation on performance and functional digestive parameters in broilers fed wheat-based diets. *Poultry Science* 96, 566-573.

13. MACELLINE S.P., TOGHYANI M., CHRYSYAL P.V., SELLE P.H., LIU S.Y., 2021 - Amino acid requirements for laying hens: a comprehensive review. *Poultry Science* 100, 101036.
14. MATHLOUTHI N., MOHAMED M.A., LARBIER M., 2003 - Effect of enzyme preparation containing xylanase and  $\beta$ -glucanase on performance of laying hens fed wheat/barley- or maize/soybean meal-based diets. *British Poultry Science* 44, 60-66.
15. MORGAN N.K., WALLACE A., BEDFORD M.R., HAWKING K.L., RODRIGUES I., HILLIAR M., CHOCT M., 2020 - In vitro versus in situ evaluation of xylan hydrolysis into xylo-oligosaccharides in broiler chicken gastrointestinal tract. *Carbohydrate polymers* 230, 115645.
16. MORGAN N., BHUIYAN M.M., WALLACE A., HOPCROFT R., 2022 - Comparing a single dose of xylanase to a double dose or cocktail of non-starch polysaccharide-degrading enzymes in broiler chicken diets. *Journal of Applied Animal Nutrition* 10, 91-102.
17. NUSAIRAT B., WANG J-J., 2021 - The effect of a modified GH11 xylanase on live performance, gut health, and clostridium perfringens excretion of broilers fed corn-soy diets. *Frontiers in Veterinary Medicine* 8, 523.
18. OLUKOSI O.A., BEDFORD M.R., 2019 - Comparative effects of wheat varieties and xylanase supplementation on growth performance, nutrient utilization, net energy, and whole-body energy and nutrient partitioning in broilers at different ages. *Poultry Science* 98, 2179-2188.
19. OLUKOSI O.A., BEDFORD M.R., ADEOLA O., 2007 - Xylanase in diets for growing pigs and broiler chicks. *Canadian Journal of Animal Science* 87, 227-235.
20. PAN C.F., IGBASAN F.A., GUENTER W., MARQUARDT R.R., 1998 - The effect of enzyme and inorganic phosphorus supplements in wheat and rye-based diets on laying hen performance, energy and phosphorus availability. *Poultry Science* 77, 83-89.
21. PATT A., HALLE I., DUDDE A., OLBRICH A., SIEBURG-ROCKEL J., KRAUSE E.T., 2022 - Influence of different dietary fibre contents in the diet on feather pecking, locomotor activity and performance of laying hens. *British Poultry Science* 63, 571-580.
22. PETTERSSON L.C., FREIRE R., NICOL C. J., 2016 - Factors affecting ranging behaviour in commercial free-range hens. *Worlds Poultry Science Journal* 72, 137-149.
23. PIRGOZLIEV V.R., ROSE S.P., KETTLEWELL P.S., 2006 - Effect of ambient storage of wheat samples on their nutritive value for chickens. *British Poultry Science* 47, 342-349.
24. PIRGOZLIEV V., KARADAS F., PAPPAS A., ACAMOVIC T., BEDFORD M.R., 2010 - The effect on performance, energy metabolism and hepatic carotenoid content when phytase supplemented diets were fed to broiler chickens. *Research in Veterinary Science* 89, 203-205.
25. PIRGOZLIEV V., BEDFORD M.R., 2012 - Energy utilisation and growth performance of chicken fed diets containing graded levels of supplementary bacterial phytase. *British Journal of Nutrition* 109(2), 248-253.
26. PIRGOZLIEV V., WHITING I., ROSE S.P., IVANOVA S.G., STAYKOVA G., AMERAH A.M., 2016 - Variability between wheat dry distillers grains with solubles samples influence the effectiveness of exogenous enzymes when fed to broiler chickens. *Veterinary Medicine Animal Studies* 6, 61-69.
27. PIRGOZLIEV V.R., MANSBRIDGE S.C., WHITING I.M., ABDULLA J.M., ROSE S.P., KLJAK K., JOHNSON A., DRIJFHOUT F., ATANASOV A.G., 2023a - The benefits of exogenous xylanase in wheat-soy based broiler chicken diets, consisting of different soluble non-starch polysaccharides content. *Poultry* 2, 123-133.
28. PIRGOZLIEV V.R., MANSBRIDGE S.C., WHITING I.M., KLJAK K., JOZWIK A., ROLLINGER J.M., ATANASOV A.G., ROSE, S.P., 2023b - Feeding black pepper (*Piper nigrum*) or exogenous xylanase improves the blood lipid profile of broiler chickens fed wheat-based diets. *Veterinary Sciences* 10(9), 587.

29. RABELLO C.B., COSTA M.J., NOGUEIRA W.C., BARBOSA J.G., RIOS-ALVA J.C., WYATT C.L., YORK T.W., SERRANO M.P., OVIEDO-RONDÓN E., 2021 - Effects of graded levels of exogenous xylanase in corn-soy diets with two amino acid density and fat levels postpellet in broiler chickens: live performance, energy utilization, digestibility, and carcass characteristics. *Poultry Science* 100, 820-834.
30. RAVINDRAN V., TANCHAROENRAT P., ZAEFARIAN F., RAVINDRAN G., 2016 - Fats in poultry nutrition: Digestive physiology and factors influencing their utilisation. *Animal Feed Science and Technology* 213, 1-21.
31. RICKE S.C., ROTHROCK JR, M.J., 2020 - Gastrointestinal microbiomes of broilers and layer hens in alternative production systems. *Poultry Science* 99, 660-669.
32. SINGH A.K., MISHRA B., BEDFORD M.R., JHA R., 2021 - Effects of supplemental xylanase and xylooligosaccharides on production performance and gut health variables of broiler chickens. *Journal of Animal Science and Biotechnology* 12, 98.
33. SWEENEY R.A., 1989 - Generic combustion method for determination of crude protein in feeds: collaborative study. *Journal of the Association of Official Analytical Chemists* 72, 770-774.
34. ŚWIĄTKIEWICZ S., KORELESKI J., 2008 - The use of distillers dried grains with solubles (DDGS) in poultry nutrition. *World's Poultry Science Journal* 64, 257-265.
35. VAN HOECK V., WUD., SOMERS I., WEALLEANS A., VASANTHAKUMARI B.L., GONZALEZ SANCHEZ A.L., MORISSET D., 2021 - Xylanase impact beyond performance: a prebiotic approach in broiler chickens. *Journal of Applied Poultry Science* 30, 100193.
36. VAN SOEST P.J., ROBERTSON J.B., LEWIS B.A., 1991 - Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* 74, 3583-3597.
37. WHITING I.M., PIRGOZLIEV V., ROSE S.P., WILSON J., AMERAH A.M., IVANOVA S.G., STAYKOVA G.P., OLUWATOSIN O.O., OSO A.O., 2017 - Nutrient availability of different batches of wheat distillers dried grains with solubles with and without exogenous enzymes for broiler chickens. *Poultry Science* 96, 574-580.
38. WHITING I.M., ROSE S.P., MACKENZIE A.M., AMERAH A.M., PIRGOZLIEV V.R., 2019 - Effect of wheat distillers dried grains with solubles and exogenous xylanase on laying hen performance and egg quality. *Poultry Science* 98, 3756-3762.
39. WIŚNIEWSKA Z., KOŁODZIEJSKI P., PRUSZYŃSKA-OSZMAŁEK E., KONIECZKA P., KINSNER M., GÓRKA P., FLAGA J., KOWALIK K., HEJDYSZ M., KUBIŚ M., JAROSZ Ł.S., 2023 - Combination of emulsifier and xylanase in triticale-based broiler chickens diets. *Archives of Animal Nutrition* 77, 187-204.
40. YANG Z., PIRGOZLIEV V.R., ROSE S.P., WOODS S., YANG H.M., WANG Z.Y., BEDFORD M.R., 2020 - Effect of age on the relationship between metabolizable energy and digestible energy for broiler chickens. *Poultry Science* 99, 320-330.
41. YANG Z., ROSE S.P., YANG H.M., PIRGOZLIEV V., WANG Z.Y., 2018 - Egg production in China. *World's Poultry Science Journal* 74, 417-426.
42. ZHANG H., XUAN Y., GUO D., ZENG Q., BAI S., LIU Y., DING X., ZHANG K., WANG J., 2025 - Effects of dietary low protein levels and amino acid patterns on production performance, egg quality and intestinal function in laying hens. *Poultry Science* 104, 105578.

