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ORIGINAL PAPER

Supplementary carbohydrase enzymes increase energy and nutrient availability of diets containing stinging nettle (*Urtica dioica*) when fed to laying hens

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ABSTRACT. The experiment evaluated the impact of graded levels (0, 2.5, 5%) of dried stinging nettle (SN) leaves on dietary apparent metabolizable energy (AME), n-corrected AME (AMEn), dry matter (DMR), nitrogen (NR), fat (FR) retentions and neutral detergent fibres digestibility (NDFD), without and with exogenous carbohydrase enzymes (XYL). Six experimental diets were formulated and fed to seventy-two Hy-Line Brown hens from 50 to 52 weeks of age. Each diet was fed to six enriched laying hen cages, two birds in a cage, following randomisation. The SN contained 351 g/kg neutral detergent fibre (NDF), and its inclusion linearly decreased ($P < 0.05$) the AMEn and FR ($P < 0.001$) and tended ($P < 0.01$) to reduce AME and DMR in diets. Exogenous XYL supplementation increased AME, AMEn, DMR, NR and NDFD ($P < 0.05$) and FR ($P < 0.001$) in diets. Each g of SN was predicted to reduce dietary AME and AMEn by 6.7 and 6.5 J, respectively, although XYL supplementation increased them by 0.36 and 0.30 MJ ($P < 0.05$), respectively. Therefore, supplementing SN enriched diets (especially over 2.5%) with XYL may be a strategy to mitigate the reduction in available energy due to higher dietary fibre content, and to increase nutrient availability in laying hen diets.

Introduction

Stinging nettle (*Urtica dioica* L.; SN) is an herbaceous plant that is rich in carotenoids, including lutein, lutein isomers, β-carotene and β-carotene isomers as the major carotenoid group (Tadesse et al., 2023). Originating from the colder regions of Northern Europe and Asia, today SN is spread all over the world and has a long history as a one among numerous plants used as feed and medication (Loetscher et al., 2013), and even as a potential source of textile fibre (Bacci et al., 2009). Although hens avoid eating SN in the hen yard, because of the stinging or burning effect of the fresh plant (Hammershoj et al., 2010), the SN can still be incorporated in diets – to increase the

yellowness and carotenoids content in egg yolks (Loetscher et al., 2013; Pirgozliev et al., 2024) and to potentially improve antioxidant status of poultry (Pirgozliev et al., 2021; Gunjević et al., 2023). However, the SN is rich not only in carotenoids, but also in fibres (Bacci et al., 2009), thus high dietary inclusion rates may compromise energy and nutrient availability of the diets when fed long term. Studies have reported that the supplementation of fibre degrading enzymes (carbohydrase; XYL), e.g., xylanase and glucanase, is an effective way to improve the nutritional quality of high fibre diets for poultry (Whiting et al., 2019; Pirgozliev et al., 2023; Šimić et al., 2023). Although there are some studies reporting the impact of high fibre plants, e.g., grass (Jonsson and McNab, 1983), on bird performance and dietary metabolizable energy (ME; presented as apparent ME (AME) or n-corrected AME (AMEn)), there is limited information on the impact of SN on dietary ME and nutrient retention coefficients. Using *in vitro* technique, Andualem et al. (2016) measured the impact of SN on gas production, ME and organic matter digestibility. However, ME and nutrient availability of whole and morphological fractions (leaf, flower and stem) of SN were evaluated through *in vitro* tests and there is no *in vivo* information on the direct impact of XYL on the ME of SN-based diets. The variation in ME content in diets and dietary components provides essential information allowing to formulate diet that meets the appropriate energy needs of the respective category of poultry.

This experiment aimed to examine the impact of the level of SN and XYL supplementation on dietary ME, dry matter (DMR), nitrogen (NR), fat (FR) retentions and neutral detergent fibres (NDFD) digestibility coefficients. In addition, some hen performance variables including feed intake (FI), egg production (eggs per hen day), egg mass and feed conversion ratio (FCR) for egg production were recorded as control measures of baseline performance metrics. It has been hypothesised that increasing dietary SN will decrease ME and retention coefficients in diets, although XYL supplementation will increase them.

Material and methods

The experiment was conducted at the National Institute of Poultry Husbandry (NIPH), Harper Adams University (UK) and the study protocol was approved (0150–202102- STAFF) by the University's Research Ethics Committee. This manuscript complies with the ARRIVE 2.0 guidelines (Percie du Sert et al., 2020).

Animals and experimental design

In the experiment, seventy-two 50 to 52-week-old Hy-Line Brown (Cyril Bason, Craven Arms, Shropshire, SY7 9NG, UK) laying hens were allocated in pairs to 36 enriched layer cages. The rearing conditions followed industry recommendations (Hy-Line, 2025) as previously described (Whiting et al., 2022).

Dietary formulation

Two weeks before the start of the study all birds were fed a diet containing 11.56 MJ/kg AME and 172 g/kg crude protein (Table 1). The same diet was used as a basis for the experimental diets. Six diets in total were produced: control diet (C); diet based on the C diluted with 2.5% dried and milled SN (C+2.5% SN); diet based on the C diluted with 5.0% dried and milled SN (C+5.0% SN); diet prepared with supplementing C diet with 12 g/kg non-starch polysaccharides (NSP) with exogenous carbohydrase enzymes (XYL) (RONOZYME® MultiGrain; DSM, Kaiseraugst, Switzerland); diet made with supplementing C+2.5% SN diet with 12 g/kg XYL;

Table 1. Formulation of laying hen experimental basal diet

Ingredients, %	Basal diet
Barley	10.00
Wheat	53.50
Soya meal	17.50
Full fat soya	5.00
L-lysine	0.05
DL methionine	0.15
Soya oil	2.00
Limestone	10.00
Monocalcium phosphate	0.80
Salt	0.25
Sodium bicarbonate	0.15
Layer Vit-Min Premix[1]	0.10
Titanium dioxide	0.50
Calculated provisions	
apparent metabolizable energy, MJ/kg	11.56
crude protein, g/kg	172.0
oil, g/kg	43.0
available lysine, g/kg	8.26
methionine + cysteine, g/kg	0.664
Ca, g/kg	41.7
available P, g/kg	3.1

[1] vitamin and mineral premix contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units/kg diet) the following: IE: vit. A (retinyl acetate) 10 000; vit. D_3 (cholecalciferol) 2 000; mg: vit. E (dl-a-tocopherol) 25; vit. K_3 (menadione) 1.5; vit. B_1 (thiamin) 1.0; vit. B_2 (riboflavin) 3.5; vit. B_6 (pyridoxine-HCl) 1.0; niacin 30; D-pantothenic acid 12; choline chloride 350; folic acid 0.8; biotin 0.1; iron 50; copper 10; manganese 60; zinc 54; iodine 0.7; selenium 0.1; µg: vit. B_{12} (cyanocobalamin) 15

diet made with supplementing C+5.0% SN diet with 12 g/kg XYL. Dried and milled dietary SN leaves were obtained from the market (Target Feeds, Whitchurch SY13 3LT, UK). The enzyme is a unique combination of endo-1,4-β-glucanase with minimum activity of 800 U/g, endo-1,3(4)-β-glucanase with minimum activity of 700 U/g and endo-1,4-β-xylanase with minimum activity of 2700 U/g. The enzymes can hydrolyse the complex cell-wall NSP structures found in poultry diets, thus improving the release of entrapped energy and nutrients, as well as reducing intestinal viscosity. Each diet was fed to 6 cages following randomisation. The diets were fed to assess the impact of the enzyme on AME and retention coefficients when grade levels of SN were fed. The experimental diets were fed in a meal form for two weeks, when birds were 50 and 51 weeks of age and did not contain any coccidiostat, antimicrobial growth promoters or other similar additives.

Egg production and determination of egg quality

The hens were individually weighed at the beginning of the study, at 50 week of age. Feed intake of each cage was recorded and presented on a cage basis for the entire two-week study period. Egg numbers, egg weights, feed conversion ratio for egg production and egg production were measured following previous reports (Whiting et al., 2019; Pirgozliev et al., 2023).

Determination of ME and nutrient retention coefficients

All hens were individually weighed at the beginning of the study, at 50 week of age and randomly allocated to 36 enriched cages. The birds had *ad libitum* access to feed and water. To ensure all hens were healthy and environmental conditions were adequate, birds were observed at least twice a day. The birds were offered the experimental diets on arrival to allow them to acclimatize to the diets prior to excreta collection. The study began when the birds were 50 weeks old with an average body weight of 1.963 kg (SD ± 0.190). The birds received the experimental diets for 14 days, as the first 10 days were given to birds to adjust to the feed and conditions. During the final 4 days of the experiment, at 52 week of age, excreta were collected daily and dried promptly at 60 °C, until a constant weight and stored for further processing and analyses.

Dietary AME, AMEn and nutrient retention coefficients of the experimental diets were determined following standard techniques (Hill and Anderson 1958; Pirgozliev et al., 2014).

Analysis of control diet, SN and excreta samples

The excreta, as well as C diet and SN samples were ground to pass through a 0.75 mm screen. Dry matter (DM) in feed, SN and excreta samples was determined by drying of samples in a forced draft oven at 105 °C to a constant weight (AOAC International, 2000; method 934.01). Crude protein (6.25 × N) in samples was determined by the combustion method (AOAC, 2000; method 990.03) employing a LECO FP-528 N (Leco Corp., St. Joseph, MI, USA). Oil (as ether extract) was extracted with diethyl ether by the ether extraction method (AOAC International, 2000; method 945.16) using a Soxtec system (Foss Ltd., Warrington, UK). Dietary Ca and P were determined as described elsewhere (Lim et al., 2021; Whiting et al., 2022). Gross energy in feed and excreta were determined with bomb calorimeter as described by Pirgozliev et al. (2006). The neutral detergent fibre (NDF) in RSM, C and excreta were determined using a Fibertec™ apparatus (FOSS FT 122 Fibertec, Foss Analytical, Hilleroed, Denmark) following Van Soest et al. (1991) procedure. The NSP content in C and SN was determined by the method of Englyst et al. (1994). Titanium dioxide in feed and excreta was determined following the method of Short et al. (1996).

Calculations

The AME and AMEn values of the experimental diets were determined following the method of Hill and Anderson (1958):

$$\text{AME} = \text{GE Diet} - \frac{(\text{GE Excreta} \times \text{Ti Diet})}{\text{Ti Excreta}},$$

where: AME – apparent metabolizable energy content of diet, MJ/kg; GE Diet and GE Excreta – gross energy of diet and excreta, respectively, MJ/kg; Ti Diet and Ti Excreta – titanium dioxide in diet and excreta, respectively, %.

$$\text{AMEn} = \text{GE Diet} - \frac{(\text{GE Excreta} \times \text{Ti Diet})}{\text{Ti Excreta}} - 34.39 \times \text{N Retained},$$

where: AMEn – N-corrected apparent metabolizable energy content of the diet MJ/kg; GE Diet and GE Excreta – gross energy of diet and excreta, respectively, MJ/kg; Ti Diet and Ti Excreta – titanium dioxide in diet and excreta, respectively, %; 34.39 – energy value of uric acid, MJ/kg; and N Retained – N retained by birds per kg of diet consumed, g/kg. The retained N was calculated as:

$$\text{N Retained} = \text{N Diet} - \frac{\text{N Excreta} \times \text{Ti Diet}}{\text{Ti Excreta}},$$

where: N Diet and N Excreta – N contents of diet and excreta, respectively, %; Ti Diet and Ti Excreta – titanium dioxide in diet and excreta, respectively, %.

Total tract nutrient retention coefficients were calculated using the following equation:

$$\text{Nutrient retention} = \frac{(\text{N/Ti})\text{Diet} - (\text{N/Ti})\text{Digesta}}{(\text{N/Ti})\text{Diet}},$$

where: (N/Ti)Diet – ratio of the dry matter (DM), fat, nitrogen or neutral detergent fibre (NDF) to TiO_2 in diet, and (N/Ti)Digesta – ratio of DM, fat, nitrogen or NDF to TiO_2 in excreta.

$$\text{FCR egg production} = \frac{\text{Feed intake}}{\text{Egg mass}}.$$

The feed conversion ratio (FCR) for egg production was calculated by dividing the feed intake per hen over the study period by the egg mass for the same period.

Statistical analysis

The observational unit was the enriched cage with two birds. Experimental data were analysed using Genstat (23rd Edition) statistical software package (IACR Rothamstead, Hertfordshire, UK). Comparisons among studied variables were performed by two-way ANOVA using a 3 × 2 factorial design (SN levels × enzyme), followed by orthogonal polynomials contrasts to test for linear (L) response to SN level. Linear regression analysis was used to assess the relationship between SN levels and supplemental XYL activity and dietary ME. Data are expressed as means and their pooled standard errors of means (SEM). In all instances, differences were reported as significant at $P < 0.05$.

Results and discussion

The analysed chemical composition of the C, SN, C+2.5% SN and C+5.0% SN samples is presented in Table 2. The SN had less GE and crude fat, but more crude protein, and four times greater NDF content compared to C diet. Detailed information on the carbohydrates in different sugar fractions of the C and SN samples is presented in Table 3. The main soluble NSP sugar fraction in C diet was xylose although in SN it was the galactose. Glucose was the main carbohydrate constituent determined in the insoluble and in the total fractions. The SN sample had over twice greater NSP content.

Table 2. Determined chemical composition of the laying hen basal diet and stinging nettle[1] (SN) and calculated chemical composition of diets with different additions of SN

Item	control	SN	control + 25 g/kg SN	control + 50 g/kg SN
Dry matter, g/kg	923.0	907.0	922.6	922.2
Gross energy, MJ/kg	15.44	13.86	15.40	15.36
Crude protein, N * 6.25, g/kg	169	226	170.4	171.9
Crude fat, g/kg	48.0	24.0	47.4	46.8
Neutral detergent fibres, g/kg	83.0	351.0	89.7	96.4
Ca, g/kg	30.7	n.d.	29.9	29.2
P, g/kg	5.4	n.d.	5.3	5.1

[1] analysed in technical duplicate; n.d. – not determined

Table 3. Carbohydrates in different sugar fractions in control diets (C) and stinging nettle (SN), g/100 g[1]

Carbohydrates	rha	fuc	ara	xyl	man	gal	glu	GlcA	GalA	g/100g
Soluble NSP (C)	0.1	0.0	0.4	0.7	0.2	0.3	0.5	0.0	0.0	2.1
Insoluble NSP (C)	0.0	0.1	1.6	2.4	0.2	1.0	2.5	0.0	0.4	8.1
Total NSP (C)	0.1	0.1	2.0	3.1	0.4	1.2	3.0	0.0	0.4	10.2
Soluble NSP (SN)	0.5	0.0	0.7	0.0	0.3	0.9	0.0	0.1	3.3	5.8
Insoluble NSP (SN)	0.4	0.1	0.8	2.2	0.4	1.0	9.7	0.0	3.0	17.7
Total NSP (SN)	0.9	0.1	1.5	2.2	0.7	1.9	9.7	0.1	6.3	23.5

all data are the results of a chemical analysis conducted in duplicate; NSP – non-starch polysaccharides, rha – rhamnose, fuc – fucose, ara – arabinose, xyl – xylose, man – mannose, gal – galactose, glu – glucose, GlcA – glucuronic acid, GalA – galacturonic acid; [1] analysed in technical duplicates

Chemical composition of SN depends on the part of the plant, i.e. leaf, flower, stem (Andualem et al., 2016), season and processing procedure (Rutto et al., 2013) and geographical location (Loetscher et al., 2013). The CP of the reported SN leaf sample was lower than the leaf value shown by Andualem et al. (2016) but very similar to the CP reported by the same authors in SN flower. The NDF content was high and stayed between NDF in flower and whole SN plant as reported by Andualem et al. (2016). Results reported by Loetscher et al. (2013) and Rutto et al. (2013) showed lower CP and NDF values of the two batches of SN. However, those authors published data from the whole plant, obtained during different seasons and from different locations, which can explain the observed variations.

The effects of dietary SN and XYL are presented in Table 4. As expected, no statistically significant differences ($P > 0.05$) were observed in any of the studied variables. Two weeks is a very short period to show an impact on egg production results, however this information was used as control measure of baseline performance metrics. There were no unexpected variations within studied variables and the results agreed with breeders' recommendations (Hy-Line, 2025).

Table 4. Effect of stinging nettle (SN) and carbohydrase (XYL) supplementation on egg production variables in laying hens

Treatment	FI, g/hen/day	Egg, hen/day	Egg mass, g/hen/day	Average egg weight, g	FCR, g:g
SN, g/kg					
0	120.4	0.952	57.4	63.3	2.002
25	116.3	0.902	59.0	63.3	2.075
50	114.6	0.893	56.9	62.4	2.114
SEM	2.30	0.0331	2.15	0.84	0.0904
XYL					
no	117.1	0.895	58.3	63.0	2.126
yes	117.2	0.937	57.2	62.9	2.001
SEM	1.88	0.0271	1.76	0.69	0.0738
Probabilities					
SN	0.213	0.405	0.771	0.680	0.679
L	0.090	0.216	0.870	0.452	0.390
deviation	0.673	0.612	0.487	0.660	0.879
XYL	0.968	0.287	0.673	0.878	0.245
SN * XYL	0.257	0.733	0.126	0.370	0.831

FI – daily feed intake; FCR – feed conversion ratio; SEM – pooled standard error of the mean; P – probability of differences; L – linear response; deviation – orthogonal polynomial contrast for deviation from linearity

The results on dietary AME, AMEn and dietary nutrient retention coefficients are shown in Table 5. Inclusion of 5% SN tended to reduced AME (P = 0.087) and DMR (P = 0.053), and reduced AMEn (P = 0.049), as SN inclusion led to a linear response (L < 0.05). Fat retention coefficient was reduced in both diets containing SN (P = 0.001) following more pronounced linear fashion (L < 0.001) with the increase of SN inclusion. The NR and NDFD coefficients were not affected by SN dietary inclusion (P > 0.05). However, dietary supplementation with the NSP degrading enzyme improved/increased all studied variables. There was no SN level by enzyme supplementation interaction observed (P > 0.05). Linear regression analysis indicated that for every increase of 1 g SN in diets, there was a reduction of dietary AME and AMEn by 6.7 and 6.5 J, respectively (Table 6) without XYL. However, supplementation with XYL increased dietary AME and AMEn, respectively by 0.36 and 0.30 MJ (Table 6). Thus, suggesting XYL may be important in diets containing SN, especially more than 2.5%.

Table 5. Effect of stinging nettle (SN) and carbohydrase (XYL) supplementation on dietary available energy and nutrient retention coefficients in laying hens

Treatment	AME, MJ/kg	AMEn, MJ/kg	DMR	FR	NR	NDFD
SN, g/kg						
0	11.49	11.14^{b}	0.696	0.826^{b}	0.532	0.362
25	11.45	11.12^{b}	0.696	0.802^{a}	0.508	0.370
50	11.16	10.82^{a}	0.675	0.789^{a}	0.523	0.336
SEM	0.109	0.096	0.0065	0.0059	0.0178	0.0245
XYL						
no	11.19	10.88	0.676	0.783	0.496	0.326
yes	11.55	11.17	0.702	0.829	0.546	0.385
SEM	0.089	0.079	0.0053	0.0048	0.0145	0.0200
Probabilities						
SN	0.087	0.049	0.053	0.001	0.623	0.600
L	0.043	0.027	0.038	<0.001	0.724	0.468
deviation	0.366	0.253	0.185	0.485	0.370	0.487
XYL	0.011	0.015	0.002	<0.001	0.025	0.049
SN * XYL	0.918	0.897	0.156	0.288	0.899	0.631

AME – apparent metabolizable energy; AMEn – N-corrected apparent metabolizable energy; DMR – dry matter retention; FR – fat retention; NR – nitrogen retention; NDFD – neutral detergent fibres digestibility; SEM – pooled standard error of the mean; P – probability of differences; L – linear response; deviationorthogonal polynomial contrast for deviation from linearity

Table 6. The relationship between the dietary apparent metabolizable energy (AME) and N-corrected AME (AMEn), and the stinging nettle (SN) content, with and without carbohydrase (XYL) supplementation when fed to laying hens, MJ/kg

Dependent variate	Explanatory variates Constant	SN, g	XYL effect (yes)	r^2	Residual standard deviation[1]
AME	11.36 (± 0.069)	0.0067 (± 0.00180)	0.355 (± 0.0735)	0.88	0.0900**
AMEn	11.04 (± 0.076)	0.0060 (± 0.00200)	0.296 (± 0.0816)	0.81	0.0999**

[1] statistical significance of regression equation; **P < 0.05

Metabolizable energy in poultry feed closely relates to dietary composition as high fibre content reduces dietary ME (Karkelanov et al., 2021; Chobanova et al., 2024). In our study, dietary NDF increases with the inclusion of dietary SN, thus providing a logical explanation on ME reduction. Fibre content reduced, although increased XYL inclusion, the performance, nutrient retention and ME in poultry diets (Whiting et al., 2018), which further confirms our results. Xylanase could degrade the complex cell wall of NSP, alleviating the nutrient-encapsulating effect of the cell wall, providing more starch and protein to the birds (Pirgozliev et al., 2023). Galactose was the main soluble NSP sugar fraction in SN (9 g/kg) and second total NSP fraction (19 g/kg) in this study. Research by Douglas et al. (2003) showed that supplementation of galactose to diets reduces ME when fed to broilers. Poultry does not produce digestive enzymes exhibiting α-galactosidase activity, thus cannot digest galactosides, i.e. they are considered anti-nutritional factors (Gitzelmann and Auriccio, 1965; Coon et al., 1990). Research by Liu et al. (2021) suggested that α-galactosidase could transform indigestible oligosaccharides into available sugars, improving the feeding value of diets. A combination of α-galactosidase and XYL

supplementation resulted in increased digestibility of nutrients and energy contents of diets (Liu et al., 2021), thus suggesting that a combination of the two enzymes may bring better results in the study.

A recent investigation on the egg production variables and egg quality of SN fed laying hens (Pirgozliev et al., 2024) has suggested that 50 g/kg SN may be incorporated in diets for at least 4 weeks without significant reduction in egg production but improved carotenoid content in egg yolks. This suggest that feeding SN may also improve hepatic antioxidant status of birds (Pirgozliev et al., 2022), thus potentially providing further health benefits. The increased ME and nutrient digestibility when feeding XYL in this study suggest that the use of fibre degrading enzymes may also benefit egg production in laying hens. However, enzyme supplementation in experiments involving high fibre diets may provide more meaningful information when performed over longer period.

Conclusions

Dietary metabolizable energy was linearly reduced by stinging nettle (SN) inclusion but increased by exogenous xylanase supplementation. Dietary chemical composition, including all non-starch polysaccharide fractions, should be considered when choosing to use fibre degrading enzymes. Longer feeding experiments may provide further practical information for poultry producers, enhancing strategies to increase energy and nutrient availability in SN enriched laying hen diets.

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Conflict of interest

The Authors declare that there is no conflict of interest.

References

Andualem D., Negesse T., Tolera A., 2016. Methane concentration, organic matter digestibility, metabolisable energy and short chain fatty acid production of morphological fractions of stinging nettle (*Urtica simensis*) measured through an in vitro gas test. Glob. Vet. 16, 276–284, https://doi.org/10.5829/idosi.gv.2016.16.03.102186

AOAC International, 2000. Official methods of analysis of AOAC International. 17th edition. Washington, DC (USA)

Bacci L., Baronti S., Predieri S., di Virgilio N., 2009. Fiber yield and quality of fiber nettle (*Urtica dioica* L.) cultivated in Italy. Ind. Crops Prod. 29, 480-484, https://doi.org/10.1016/j.indcrop.2008.09.005

Chobanova S., Karkelanov N., Mansbridge S.C., Whiting I.M., Tukša M., Rose S.P., Pirgozliev V.R., 2024. Metabolizable energy value of fat and meals obtained from black soldier fly larvae (*Hermetia illucens*) for broiler chickens. Poultry 3, 298–306, https://doi.org/10.3390/poultry3030022

Gitzelmann R., Auriccio S., 1965. The handling of soya alpha-galactosides by a normal and a galactosemic child. Pediatrics 36, 231–235, https://doi.org/10.1542/peds.36.2.231

Coon C.N., Leske K., Akavanichan O., Cheng T., 1990. Effect of oligosaccharide-free soybean meal on true metabolizable energy and fiber digestion in adult roosters. Poult. Sci. 69, 787–793, https://doi.org/10.3382/ps.0690787

Douglas M.W., Persia M., Parsons C.M., 2003. Impact of galactose, lactose, and Grobiotic-B70 on growth performance and energy utilization when fed to broiler chicks. Poult. Sci. 82, 1596–1601, https://doi.org/10.1093/ps/82.10.1596

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, https://doi.org/10.1039/AN9941901497

Gunjević V., Grbeša D., Zurak D., Kiš G., Janječić Z., Svečnjak Z., Bedeković D., Duvnjak M., Pirgozliev V., Kljak K., 2023. Effect of maize hybrid in complete feed on the production performance and economic considerations in laying hens. Sustainability 15, 15748, https://doi.org/10.3390/su152215748

Hammershøj M., Kidmose U., Steenfeldt S., 2010. Deposition of carotenoids in egg yolk by short-term supplement of coloured carrot (*Daucus carota*) varieties as forage material for egg-laying hens. J. Sci. Food Agric. 90, 1163–1171, https://doi.org/10.1002/jsfa.3937

Hy-Line, 2025. Available online: https://hyline.com/varieties/brown/2025/ (accessed on 27 March 2025).

Hill F.W., Anderson D.C., 1958. Comparison of the metabolizable energy and production energy determination with growing chicks. J. Nutr. 64, 587–603, https://doi.org/10.1093/jn/64.4.587

Jonsson G., McNab J.M., 1983. A comparison of methods for estimating the metabolisable energy of a sample of grass meal. Brit. Poult. Sci. 24, 349–359, https://doi.org/10.1080/00071668308416749

Karkelanov N., Chobanova S., Whiting I.M., Dimitrova K., Rose S.P., Pirgozliev V., 2021. Pelleting increases the metabolizable energy of de-hulled sunflower seed meal for broilers. S. Afr. J. Anim. Sci. 51, 290–295, https://doi.org/10.4314/sajas.v51i3.2

Liu X., Xing K., Ning R., Carné S., Wu X., Nie W., 2021. Impact of combined α-galactosidase and xylanase enzymes on growth performance, nutrients digestibility, chyme viscosity, and enzymes activity of broilers fed corn-soybean diets. J. Anim. Sci. 99, 1–11, https://doi.org/10.1093/jas/skab088

Loetscher Y., Kreuzer M., Messikommer R.E., 2013. Utility of nettle (*Urtica dioica*) in layer diets as a natural yellow colorant for egg yolk. Anim. Feed Sci. Technol. 186, 158–168, https://doi.org/10.1016/j.anifeedsci.2013.10.006

Lim C., Poaty Ditengou J., Ryu K., Ku J., Park M., Whiting I.M., Pirgozliev V., 2021. Effect of maize replacement with different triticale levels on layers production performance, egg quality, yolk fatty acid profile and blood parameters. J. Anim. Feed Sci. 30, 360–366, https://doi.org/10.22358/jafs/144848/2021

Percie du Sert N.P., Ahluwalia A., Alam S. et al., 2020. Reporting animal research: Explanation and elaboration for the ARRIVE Guidelines 2.0. PLOS Biology 18, e3000411, https://doi.org/10.1371/journal.pbio.3000411

Pirgozliev V.R., Rose S.P., Kettlewell P.S., 2006. Effect of ambient storage of wheat samples on their nutritive value for chickens. Brit. Poult. Sci. 47, 342–349, https://doi.org/10.1080/00071660600741750

Pirgozliev V., Bravo D., Rose S.P., 2014. Rearing conditions influence nutrient availability of plant ex-tracts supplemented diets when fed to broiler chickens. J. Anim. Phys. Anim. Nutr. 98 (4), 667–671, https://doi.org/10.1111/jpn.12119

Pirgozliev V., Kljak K., Whiting I.M., Rose S.P., Mansbridge S.C., Enchev S., Atanasov A., Stringhini J.H., 2021. Feeding dry stevia leaf (*Stevia rebaudiana*) or xylanase improves the hepatic antioxidative status of broiler chickens. Res. Vet. Sci. 136, 227–229, https://doi.org/10.1016/j.rvsc.2021.03.001

Pirgozliev V.R., Whiting I.M., Kljak K., Mansbridge S.C., Atanasov A.G., Rose S.P., Enchev S.B., 2022. Stevia (*Stevia rebaudiana*) improves carotenoid content in eggs when fed to laying hens. Foods 11, 1418, https://doi.org/10.3390/foods11101418

Pirgozliev V.R., Mansbridge S.C., Whiting I.M., Abdulla J.M., Rose S.P., Kljak K., Johnson A., Drijfhout F., Atanasov A.G., 2023. The benefits of exogenous xylanase in wheat–soy-based broiler chicken diets, consisting of different soluble non-starch polysaccharides content. Poultry 2(2), 123–133, https://doi.org/10.3390/poultry2020012

Pirgozliev V.R., Kljak K., Whiting I.M., Mansbridge S.C., Atanasov A.G., Enchev S.B., Tukša M.., Rose S.P., 2024. Dietary stinging nettle (*Urtica dioica*) improves carotenoids content in laying hen egg yolk. Brit. Poult. Sci. 1–6, https://doi.org/10.1080/00071668.2024.2406335

Rutto L.K., Xu Y., Ramirez E., Brandt M., 2013. Mineral properties and dietary value of raw and processed stinging nettle (*Urtica dioica* L.). Int. J. Food Sci. 1, 857120, https://doi.org/10.1155/2013/857120

Short F.J., Gorton P., Wiseman J., Boorman K.N., 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Technol. 59, 215–221, https://doi.org/10.1016/0377-8401(95)00916-7

Šimić A., González-Ortiz G., Mansbridge S.C., Rose S.P., Bedford M.R., Yovchev D., Pirgozliev V.R., 2023. Broiler chicken response to xylanase and fermentable xylooligosaccharide supplementation. Poult. Sci. 102 (11), 103000, https://doi.org/10.1016/j.psj.2023.103000

Tadesse T.Y., Zeleke M.M., Dagnew S.B., Addis G.T., 2023. A systematic review of ethnobotanical, phytochemical, and ethnopharmacological studies of *Urtica simensis* (Stinging Nettle). J. Exp. Pharmacol. 15, 177–186, https://doi.org/10.2147/JEP.S404506

Van Soest P.V., Robertson J.B., Lewis B.A., 1991. Methods for dietary fibre, neutral detergent fibre, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597, https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Whiting I.M., Pirgozliev V., Rose S.P., Karadas F., Mirza M.W., Sharpe A., 2018. The temperature of storage of a batch of wheat distillers dried grains with solubles samples on their nutritive value for broilers. Brit. Poult. Sci. 59, 76–80, https://doi.org/10.1080/00071668.2017.1380297

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. Poult. Sci. 9, 3756–3762, https://doi.org/10.3382/ps/pez063

Whiting I.M., Pirgozliev V., Kljak K., Orczewska-Dudek S., Mansbridge S.C., Rose S.P., Atanasov A.G., 2022. Feeding dihydroquercetin in wheat-based diets to laying hens: impact on egg production and quality of fresh and stored eggs. Brit. Poult. Sci. 63, 735–741, https://doi.org/10.1080/00071668.2022.2090229