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Original Research Article

Dietary soluble non-starch polysaccharide level and xylanase supplementation influence performance, egg quality and nutrient utilization in laying hens fed wheat-based diets

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ABSTRACT

The aim of this study was to evaluate the effects of dietary soluble non-starch polysaccharide (sNSP) content and xylanase supplementation on production performance, egg quality parameters, and nutrient digestibility in Hy-line Brown layers from 25 to 32 wk of age. A total of 144 Hy-line Brown laying hens (25 wk of age) were randomly allocated to 1 of 4 wheat-based dietary treatments in a 2 × 2 factorial experimental design, with 36 replicates of individual hens per treatment. The diets were formulated to contain either a high or low sNSP level (at 13.3 or 10.8 g/kg) and were supplemented with either 0 or 12,000 BXU/kg exogenous xylanase. Birds were fed these treatment diets for an 8-wk period, and hen production performance, including daily egg production, average egg weight, daily egg mass, feed conversion ratio and proportion of dirty and abnormal eggs were measured at bird age 25 to 28 wk and 29 to 32 wk. An interaction between sNSP content of the diet and xylanase supplementation was observed on daily egg production from 25 to 28 wk of age ($P = 0.018$); birds fed the high sNSP diet without xylanase had lower egg production than those fed any other treatment. An interaction between the 2 dietary factors was also observed on hen weight gain at 29 to 32 wk of age ($P = 0.014$), with birds fed the low sNSP diet with 12,000 BXU/kg xylanase presenting greater weight gain compared to those fed the high sNSP diet with 12,000 BXU/kg xylanase. Feed intake at 29 to 32 wk of age was reduced by xylanase supplementation ($P = 0.047$). Xylanase supplementation also increased yolk colour score at both 28 and 32 wk of age, and decreased yolk weight at 32 wk of age ($P = 0.014$, 0.037 and 0.013, respectively). Birds fed the low sNSP diet presented lower protein digestibility ($P = 0.024$) than those fed the high sNSP diet. Additionally, birds fed high sNSP presented higher shell reflectivity at both 28 and 32 wk of age ($P = 0.05$ and 0.036, respectively). The influence of duration of feeding the treatment diets on egg quality was also determined. It was observed that egg weight, yolk weight and yolk colour score consistently increased over time, regardless of experimental treatment effects. In contrast, Haugh Unit and albumen height significantly decreased throughout the study period in all treatments, although this was less pronounced in hens fed the treatment with high sNSP and no supplemental xylanase. A reduction in shell breaking strength over time was observed only in hens fed the treatments without xylanase addition, and shell thickness was improved over time only in birds fed the low sNSP diet with xylanase. The impacts of the dietary treatments were largely inconsistent in this study, so a solid conclusion cannot be drawn. However, these findings do indicate that dietary NSP level influences layer production performance, and thus should be considered when formulating laying hen diets. It also proved that further research is warranted into how to optimize the benefits of xylanase application in laying hens.

1. Introduction

To date, fibre, or more specifically non-starch polysaccharide (NSP), has been largely ignored by nutritionists during feed formulation, despite extensive evidence highlighting its vital role and benefits in the bird, namely on digestive function, microbiota composition and gut health (Shakouri et al., 2008; Kalmendal et al., 2011; Jha et al., 2019; Adebowale et al., 2019). Wheat as

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the primary grain source is widely practiced in least-cost feed formulations for poultry, due to its relatively high nutrient concentration in comparison with other ingredients such as barley, rye, triticale and sorghum (NRC, 1994; Black et al., 2005; Cufadar et al., 2010). However, wheat contains a relatively high level of NSP, and poultry lack endogenous enzymes in the intestinal tract that can digest NSP. Intact NSP present in the gastrointestinal tract (GIT) of poultry hampers digestion processes and disrupts intestinal balance, inducing wet-litter related issues and intestinal diseases (De Keyser et al., 2018), thus impairing productivity and economic efficiency (Józefiak et al., 2006; Latorre et al., 2015; Hashemipour et al., 2016).

The behaviour of NSP in the GIT is dictated by its solubility in the gastrointestinal environment. NSP constitutes around 10% to 12% of the dry matter content of wheat, in which approximately 19% to 21% is soluble and the remaining is insoluble in water (Bach Knudsen, 1997; Rodehutscord et al., 2016). Soluble NSP (sNSP) exerts anti-nutritional effects, primarily through increasing viscosity in the intestinal environment, thus reducing nutrient digestion and absorption (Choct and Annison, 1992; Latorre et al., 2015), increasing small intestinal fermentation and prolonging feed passage rate, resulting in impaired performance and intestinal health (Choct et al., 1996). On the other hand, there has recently been considerable interest in the additional prebiotic effects of NSP; the smaller chain oligosaccharides derived from NSP in the presence of NSP-degrading enzymes can be selectively fermented by beneficial microbiota in the GIT, inducing positive effects in the bird and producing short chain fatty acids. This fermentative capacity is almost exclusively to the sNSP fraction, in which pectic polysaccharides are the most favorable fibre component for intestinal microbiota to ferment (Morgan, 2019). There have been few studies conducted examining the impact of dietary NSP on layer performance, and those findings obtained are contrasting and were primarily conducted at least a decade ago, so they do not reflect modern birds and ingredients (Lázaro et al., 2003; Safaa et al., 2009; Kim et al., 1976). This highlights that further research is warranted into how laying hens utilize dietary fibre.

One approach to overcome the detrimental impact of inclusion of wheat in poultry diets is to supplement wheat-based diets with endo-β-1, 4-xylanases to breakdown xylan, the major NSP in wheat, into small-chain xylo-oligomers, to improve nutrient utilization and performance consistency (Roberts and Choct, 2006; Senkoylu et al., 2009; Sousa et al., 2019). However, the effects of supplementing laying hen diets with xylanase on production performance and egg quality are inconsistent among studies. Senkoylu et al. (2009) and Bobeck et al. (2014) observed that xylanase supplementation enhanced FCR, feed efficiency, egg mass and egg production in laying hens. Similarly, Mirzaie et al. (2012) reported that the negative effects of high NSP wheat cultivar on hens' performance reduced, or even disappeared, following xylanase supplementation. In contrast, Pirgozliev et al. (2010) observed no improvements in production performance or impact on egg quality with xylanase supplementation, except for enhanced yolk colour score. Furthermore, Cufadar et al. (2010) and Lei et al. (2018) showed no impact of xylanase supplementation on feed intake, feed conversion ratio, egg production or egg weight, and egg mass. A possible explanation for this lack of consistency is a dearth in understanding about the specific composition and solubility of the NSP being fed, and the impact this is on the accessibility and susceptibility of the xylan in the feed ingredients. The hypothesis of this study was that sNSP level would dictate response of laying hens to xylanase, with a greater response seen in diets with a lower sNSP concentration. This will likely influence how fibre is considered by nutritionists when formulating laying hen diets. In order to test this, the effects of feeding diets formulated to contain high and low levels of dietary sNSP to commercial laying hens, with and without xylanase supplementation, on production performance, egg quality and nutrient digestibility were examined.

2. Materials and methods

2.1. Experimental design

The experiment was conducted at Laureldale Research station at the University of New England and was approved by the Animal Ethics Committee of the University of New England (AEC 19-015). All the procedures and animal care were conducted in accordance with the Australian code of practice for the care and use of animals for scientific purposes (NHMRC, 2013).

A total of 144 Hy-line Brown laying hens (25 wk of age) were randomly allocated to 1 of 4 dietary treatments, arranged in a 2 × 2 factorial experimental design with 36 replicates of individual hens per treatment. The dietary factors were sNSP level (high or low) and xylanase supplementation (0 or 12,000 BXU/kg of feed). The difference in sNSP content between high and low sNSP diets was achieved by altering the quantity of the feed ingredients in the formulation, whilst still maintaining wheat as the primary cereal. The xylanase used in this study was endo-β-1, 4-xylanase (Econase XT 5P, 800,000 BXU/g, AB Vista, Marlborough, Wiltshire, UK) at the supplemental dose of 0 or 15 g per ton of feed, to achieve supplemental levels of 0 or 12,000 BXU/kg of feed.

Birds were fed the dietary treatments for 8 wk, from 25 to 32 wk of age. The composition of the basal high or low sNSP diets is shown in Table 1; diets were formulated according to the Hy-line Brown nutrient management guidelines (Hy-line, 2018). The soluble and insoluble NSP content of the experimental diets were analysed based on the method described by Englyst and Hudson (1993), in which the constituent sugars were measured by gas–liquid chromatography.

Hens were housed individually in conventional cages (50 cm wide × 54 cm long × 45 cm high, 2,700 cm^2/hen), equipped with a feed trough and nipple drinkers. Natural light and artificial lighting were managed to provide 16 h continuous light daily (from 05:00 to 21:00). Feed, fed as mash, and water were provided ad libitum. The shed had open-air ventilation.

2.2. Data collection

2.2.1. Production performance and hen weight change

Hen weight of individual birds was recorded on arrival at 25 wk of age and after 4 and 8 wk on the experimental diets (at bird age 28 and 32 wk, respectively). Individual egg production and egg weight were recorded daily and feed intake was recorded weekly to calculate production performance. Feed conversion ratio was calculated as grams of total feed intake per total egg mass on an individual bird basis. Egg production was calculated as average hen-day production. Egg mass was determined as the egg weight multiplied by egg production. The number of dirty and abnormal eggs, including eggs weighing less than 50 g or more than 70 g, broken eggs, cracked eggs or shell-less eggs were also recorded.

2.2.2. Egg quality parameters

Internal and external egg quality was analyzed in eggs when the birds arrived and then at wk 4 and 8 of the experimental feeding period, at the same time on each collection day. An egg was collected from each of the 144 birds on the sampling day, eliminating any abnormal or damaged eggs. The quality measurements included egg weight, shell colour by reflectivity, egg shell breaking strength (using quasi-static compression), shell deformation to

Table 1
Ingredients and nutrient content of basal diets (%, as-fed basis).

Item	High sNSP	Low sNSP
Ingredients		
Wheat	37.15	36.00
Corn	3.34	15.00
Barley	15.00	3.00
Millrun	8.00	8.00
Soybean meal	12.77	17.78
Canola solvent	8.00	4.13
Meat and bone meal	0	0.455
Canola oil	4.65	3.97
Limestone	8.99	9.38
Di-calcium phosphate	0.85	1.07
Phytase[1]	0.010	0.010
Salt	0.171	0.186
Sodium bicarbonate	0.118	0.132
TiO_2	0.500	0.500
Premix[2]	0.100	0.100
Choline chloride	0.040	0.042
L-Lysine HCl	0.118	0.049
DL-Methionine	0.166	0.161
L-Threonine	0.016	0
Jabiru red	0.004	0.004
Jabiru yellow	0.003	0.003
Calculated nutrient content		
Dry matter	92.73	92.66
Metabolizable energy, kcal/kg	2,792	2,800
Crude fat	6.42	5.84
Crude fibre	2.65	2.43
Calcium	3.89	4.10
Available phosphorus	0.40	0.45
Digestible lysine	0.732	0.732
Digestible methionine + cysteine	0.665	0.665
Digestible threonine	0.511	0.523
Digestible tryptophan	0.213	0.213
Assayed nutrient content		
Dry matter	91.98	92.35
Crude protein	17.23	17.63
Ash	11.43	13.49
sNSP	1.34	1.12
iNSP	8.03	7.89
Total NSP	9.37	9.01

sNSP = soluble non-starch polysaccharide; iNSP = insoluble non-starch polysaccharide.
[1] Equivalent to the supplemental dose of 500 FTU/kg of feed (Quantum blue 5G, AB Vista, UK).
[2] Provided the following per kilogram of diet: vitamin A, 10.00 MIU; vitamin D, 3 MIU; vitamin E, 20 mg; vitamin K, 3 mg; nicotinic acid, 35 mg; pantothenic acid, 12 mg; folic acid, 1.0 mg; riboflavin, 6.0 mg; vitamin B_{12}, 0.02 g; biotin, 0.1 g; pyridoxine B_6, 5 g; thiamine B_1, 2 g; Cu, 8 g; Co, 0.2 g; Mo, 0.5 g; I, 1.0 g; Se, 0.3 g; Fe, 60 g; Zn, 60 g; Mn, 90 g; antioxidant, 20 g.

breaking point, albumen height and Haugh unit (Haugh, 1937). All measurements were conducted using equipment from Technical Services and Supplies, Dunnington, York, UK. Egg yolk colour was measured on a range from 1 to 15, from palest to darkest, based on the Roche scale (Roberts, 2005). The egg shell was separated from the albumen and yolk and dried overnight at room temperature, allowing for determination of shell weight and analysis of shell thickness using a Mitutoyo Dial Comparator gauge (Model 2109-10, Kawasaki, Japan).

2.2.3. Apparent total tract digestibility

Titanium dioxide (TiO_2) was added at 0.5% into the experimental diets as an indigestible marker, to determine the apparent total tract digestibility of dry matter, crude protein and ash. During the last 3 d of the 8-wk experimental period, fresh excreta samples were collected from individual hens, mixed thoroughly on an individual basis, and then a subsample was collected and stored at −20 °C. These subsamples were then freeze dried to constant weight. All diet and excreta samples were ground to pass through a 0.5-mm screen. These samples were dried overnight at 105 °C for dry matter determination and ashed at 600 °C for 2 h for analysis of ash content. Nitrogen content of diets and freeze-dried excreta samples was analyzed according to the Dumas combustion method, using a Leco automatic analyzer (Leco model FP-2000N analyzer, Leco Corp., MI, USA), and the crude protein content determined by multiplying the nitrogen value by a factor of 6.25. TiO_2 concentration was measured by UV-spectroscopy according to the method described by Short et al. (1996). Total tract dry matter, ash and protein digestibility was then calculated using the below equation:

$$\text{Digestibility of nutrient (\%)} = [1 - (TiO_{2\ \text{feed}}/TiO_{2\ \text{excreta}}) \times (\text{Nutrient}_{\text{excreta}}/\text{Nutrient}_{\text{feed}})] \times 100.$$

2.3. Statistical analysis

Individual bird represented the replicate unit for statistical analysis. All data were tested for normal distribution using Anderson-Darling test prior to analysis. Data were subjected to analysis of variance based on the 2 × 2 factorial design, using the GLM procedure of Minitab 18 (Minitab Inc., State College, Pennsylvania, USA), followed by Tukey multiple comparison test to separate means when significant effects ($P < 0.05$) were detected. There were no interactions between bird age and either dietary sNSP content or xylanase application, thus bird age was not considered in the statistical model. Bird age was considered as a main effect on egg quality. Hen weight was used as a covariate in the analysis of average daily feed intake and egg weight parameters, and egg weight was used as a covariate in the analysis of yolk weight and egg shell weight measurements, due to their significant contribution to these parameters. Data not following a normal distribution were subjected to the Kruskal–Wallis test using the same software to determine significant differences.

3. Results

3.1. Influence of dietary treatments on production performance in laying hens

There were no mortalities or sick birds during the experiment. Table 2 illustrates the effects of sNSP content and xylanase supplementation on production performance. A significant interaction ($P = 0.018$) between sNSP and xylanase supplementation on daily egg production was observed during the first 4 wk of the experimental period, from 25 to 28 wk of age; birds fed the diet with high sNSP and xylanase supplementation presented greater egg production compared to those fed any other dietary treatments. Feed intake from 29 to 32 wk of age was significantly lower in hens fed the diets with xylanase (121.6 vs. 124.5 g, $P = 0.047$).

The effects of the experimental diets on hen weight gain and prevalence of dirty and abnormal eggs are presented in Table 3. An interaction between sNSP and xylanase was observed for weight gain from 29 to 32 wk of age ($P = 0.014$), showing that feeding the high sNSP diet resulted in lower body weight gain compared to feeding the low sNSP diet, only in birds fed diets supplemented with xylanase (78.3 vs. 100.3 g). On the other hand, the percentage of either dirty or abnormal eggs was not influenced by treatment diets.

3.2. Effect of dietary sNSP level and xylanase supplementation on nutrient digestibility

Table 4 presents the impact of the dietary treatment on apparent total tract digestibility of dry matter, crude protein and

Table 2
Effect of dietary soluble non-starch polysaccharide (sNSP) level and xylanase supplementation on hen performance., measured from 0 to 28 d on the dietary treatments (25 to 28 wk of age) or from 29 to 56 d on the dietary treatments (29 to 32 wk of age).

Item	Daily egg production, %		Average egg weight, g		Daily egg mass, g		Feed intake, g		FCR (feed-to-egg mass ratio)	
	25 to 28 wk	29 to 32 wk	25 to 28 wk	29 to 32 wk	25 to 28 wk	29 to 32 wk	25 to 28 wk	29 to 32 wk	25 to 28 wk	29 to 32 wk
sNSP										
High	98.32	98.91	60.16	62.00	59.63	61.64	121.0	123.0	2.048	1.998
Low	99.10	98.91	60.27	61.87	59.93	61.79	120.8	123.1	2.019	1.988
Xylanase										
-	98.32	99.07	60.55	62.31	59.63	61.09	121.4	124.5[a]	2.041	2.007
+	99.11	98.75	59.88	61.87	59.56	61.34	120.4	121.6[b]	2.027	1.979
Interaction										
High-	97.54[b]	98.94	60.42	61.83	58.94	61.56	121.3	124.2	2.067	2.019
High +	99.12[a]	98.87	59.91	62.17	59.59	61.73	120.8	121.9	2.029	1.975
Low -	99.11[a]	99.20	60.69	62.78	60.32	62.61	121.5	124.9	2.014	1.994
Low +	99.10[a]	98.62	59.85	61.56	59.54	60.96	120.1	121.4	2.024	1.983
SEM	1.38	1.40	1.84	1.66	2.16	2.24	3.71	4.26	0.075	0.075
P-value										
sNSP level	0.068	0.188	0.862	0.755	0.359	0.849	0.868	0.945	0.251	0.731
Xylanase	0.170	0.821	0.277	0.438	0.928	0.330	0.441	0.047	0.584	0.291
Interaction	0.018	0.610	0.786	0.173	0.330	0.238	0.737	0.688	0.349	0.533

[a, b] Means with no common superscript within a column differ significantly ($P < 0.05$).

Table 3
Effect of dietary soluble non-starch polysaccharide (sNSP) level and xylanase supplementation on hen performance and prevalence of dirty and abnormal eggs, measured from 0 to 28 d on the dietary treatments (25 to 28 wk of age) or from 29 to 56 d on the dietary treatments (29 to 32 wk of age).

Item	Hen weight gain, g		Dirty egg, %		Abnormal eggs, %	
	25 to 28 wk	29 to 32 wk	25 to 28 wk	29 to 32 wk	25 to 28 wk	29 to 32 wk
sNSP						
High	125.6	86.3	1.72	2.78	6.67	7.61
Low	136.0	90.2	1.84	2.42	4.48	5.72
Xylanase						
-	125.2	87.2	1.56	2.89	6.23	8.78
+	136.4	89.3	2.0	2.31	4.92	4.56
Interaction						
High -	121.8	94.3[ab]	1.63	3.41	8.44	10.93
High +	129.4	78.3[b]	1.81	2.15	4.90	4.30
Low -	128.6	80.1[ab]	1.49	2.36	4.02	6.63
Low +	143.4	100.3[a]	2.19	2.48	4.94	4.82
SEM	33.34	21.47	1.45	1.95	6.56	9.35
P-value						
sNSP level	0.661	0.590	0.820	0.761	0.989	0.980
Xylanase	0.235	0.776	0.694	0.710	0.300	0.189
Interaction	0.629	0.014	0.706	0.960	0.648	0.621

[a,b] Means with no common superscript within a column differ significantly ($P < 0.05$).

crude ash, as measured after feeding the diets for 56 d (at 32 wk of age). A comparative reduction in crude protein digestibility was observed when feeding the lower level of sNSP (39.26% vs. 40.98%, $P = 0.024$). Xylanase addition tended to decrease total tract dry matter digestibility ($P = 0.074$). No interactions between the 2 dietary factors were observed.

3.3. Effect of dietary sNSP level and xylanase supplementation on egg quality

Tables 5 and 6 show the effects of the experimental diets on egg quality measurements conducted after feeding the treatment diets for 28 and 56 d, determined at 28 and 32 wk of age, respectively. At both 28 and 32 wk of age, xylanase supplementation increased yolk colour ($P = 0.014$ and $P = 0.037$, respectively), and hens fed the high sNSP diet produced eggs with higher shell reflectivity compared to those fed the low sNSP diet ($P = 0.050$ and $P = 0.036$, respectively). At 32 wk of age, xylanase supplementation also decreased yolk weight (15.57 vs. 15.05 g, $P = 0.013$). No interactions between the 2 dietary factors were observed.

Changes in egg quality measurements as affected by feeding period duration are shown in Tables 7 and 8, with Table 7 illustrating effects in birds fed the high sNSP diets and Table 8 presenting the low sNSP diets. In all 4 treatment groups, the average egg weight was consistently higher after 56 d on the dietary treatments compared to the starting egg weight ($P < 0.05$). A similar trend was also observed for yolk weight ($P < 0.001$). Interestingly, shell breaking strength was lower at 56 d compared to 0 d on the dietary treatment in the birds fed diets without xylanase supplementation ($P < 0.05$), but in the presence of xylanase there was no effect of bird age on shell breaking strength. Haugh unit was consistently lower at 56 d compared to 0 d for all treatments ($P < 0.05$), as was albumen height in birds fed the diets with xylanase or the low sNSP level, but not in those fed high sNSP without xylanase ($P < 0.05$). Additionally, yolk colour score was consistently higher in birds fed the treatments for either 28 or 56 d compared to those not yet fed the dietary treatments ($P < 0.001$). Furthermore, in birds fed the low sNSP diet with xylanase, shells were thicker after 28 and 56 d compared to 0 d on the dietary treatments ($P = 0.001$).

Table 4
Effect of dietary soluble non-starch polysaccharide (sNSP) level and xylanase supplementation on apparent total tract digestibility of nutrients, as determined in the excreta of hens fed the treatments for 56 d, at 32 wk of age (%).

Item	Dry matter	Crude protein	Crude ash
sNSP			
High	66.05	40.98[a]	25.38
Low	65.77	39.26[b]	26.38
Xylanase			
-	66.17	40.51	25.81
+	65.65	39.73	25.95
SEM	0.88	2.24	3.79
P-value[1]			
sNSP	0.346	0.024	0.435
Xylanase	0.077	0.299	0.915
Interaction	0.377	0.074	0.061

[a,b] Means with no common superscript within a column differ significantly ($P < 0.05$).
[1] No significant 2-way interactions were observed ($P > 0.05$), so separated means are not presented.

4. Discussion

4.1. The effect of dietary treatments on productive performance and nutrient digestibility of laying hen

In this study, the productive performance of laying hens fed different sNSP levels with and without xylanase supplementation was investigated. Although it is well established, and reaffirmed in this study, that xylanase has a positive effect of on laying hen performance, there has been a lack of consistency between studies in this field. The exact reasons behind this variation in response to xylanase are unknown, but are likely due to differences in ingredient combinations and batches used in feed formulations, nutrient regimes, levels, types and susceptibility of the soluble fibres present and the source and level of supplemental enzymes, as well as the age and strain of hens. This was examined in part in this study, by determining the efficacy of xylanase in hens when hens were fed wheat-based diets differing in sNSP content across an 8-wk time period.

4.1.1. Hen performance

Results from this study presented that xylanase reduced feed intake during the second half of the trial period, from 28 to 56 d on the treatment diets. Theoretically, feeding viscous feed ingredients to chickens should delay feed passage rate, leading to a reduction in feed intake (Bedford and Classen, 1992), and feeding enzymes should increase digesta passage rate, increasing feed consumption. Furthermore, it is hypothesized that the older birds, those in the second half of the trial period, would have microbiota that was better adapted to handle and utilize fibre compared to when the birds were younger (Bedford, 2018). However, as highlighted above, the opposite was found to be true in this study, with reduced feed intake seen as a result of xylanase application. A possible explanation is that the lifted level of sNSP following xylanase supplementation, as a result of the xylanase hydrolyzing insoluble NSP into sNSP, depressed feed intake. An alternative theory is that satiety and nutrient needs were already met by the diets without xylanase, and there was in fact little negative impact on viscosity, so xylanase application simply enhanced nutrient availability, meaning the amount the birds needed to consume to meet their needs was reduced. Supplementing hen diets with xylanase improves their metabolism, nutrient digestibility and absorption, as xylanase increases permeability of the aleurone layer and increases contact of digestive or exogenous enzymes with their substrates in the intestinal tract (Parkkonen et al., 1997). For example, xylanase can result in a significant increase in true metabolizable energy (Choct et al., 1995; Mathlouthi et al., 2002). In this study, energy requirements were likely higher during the second time period, from 29 to 32 wk of age, due to heightened egg production, meaning xylanase had a greater influence at this time because it helped increase the amount of energy available from the feed ingredients, meaning the birds needed to consume comparatively less feed to meet their energy requirements.

In the current study, average feed consumption of hens from all treatments was approximately 120 g/hen per day, slightly higher than the results obtained in previous studies (102 g/hen per d) at this facility with the same breed of bird (Whiting et al. 2019). Pirgozliev et al. (2010) and Mirzaie et al. (2012) noted that feed consumption of hens fed wheat-based diets in their studies were approximately 111 and 88 g/hen per day, respectively. However, the values in this study were lower than those presented by Taylor et al. (2018), who noted consumption of 124 to 126 g/hen per day when feeding xylanase in wheat-based diets, although FCR and hen-day-production were comparatively better (8% and 12%, respectively) in the current study. Accordingly, this illustrates that the feed used in the current study was well-balanced and satisfied the hens' energy and other nutrient requirements, regardless of the levels of sNSP present.

Aside from feed intake, there was a lack of response to the dietary treatments observed for bird performance in this study. This could be attributed the fact all the dietary treatments satisfied the birds nutritional needs, as shown by good performance results for both basal treatments, so xylanase was not able to instigate a notable impact. Another explanation is that the difference in sNSP

Table 5
Effect of dietary soluble non-starch polysaccharide (sNSP) level and xylanase supplementation on egg quality, measured after hens were fed the dietary treatments for 28 d, at 28 wk of age.

Item	sNSP		Xylanase		SEM	*P*-value[1]		
	High	Low	−	+		sNSP	Xylanase	Interaction
Egg weight, g	61.18	61.23	61.45	60.95	2.34	0.950	0.535	0.573
Breaking strength, N	49.42	49.08	48.34	50.15	3.01	0.742	0.081	0.207
Shell deformation, nm	0.296	0.296	0.294	0.298	0.01	0.662	0.444	0.687
Albumen height, mm	10.79	10.91	10.83	10.87	0.38	0.668	0.920	0.973
Haugh unit	102.3	102.8	102.4	102.7	1.63	0.626	0.581	0.834
Yolk colour score	11.50	11.56	11.38[b]	11.67[a]	0.36	0.662	0.014	0.098
Yolk weight, g	14.51	14.59	14.64	14.46	0.53	0.666	0.324	0.122
Shell weight, g	6.039	5.999	5.951	6.087	0.21	0.580	0.059	0.108
Shell thickness, mm	0.435	0.438	0.436	0.438	0.01	0.430	0.519	0.600
Shell reflectivity, %	24.12[a]	23.21[b]	23.85	23.48	1.45	0.050	0.514	0.101

[a,b] Means with no common superscript within a row differ significantly ($P < 0.05$).
[1] No significant 2-way interactions were observed ($P > 0.05$), so separated means are not presented.

Table 6
Effect of dietary soluble non-starch polysaccharide (sNSP) level and xylanase supplementation on egg quality, measured after hens were fed the dietary treatments for 56 d, at 32 wk of age.

Item	sNSP		Xylanase		SEM	*P*-value		
	High	Low	−	+		sNSP	Xylanase	Interaction
Egg weight, g	62.00	62.61	62.79	61.82	2.06	0.409	0.192	0.961
Breaking strength, N	46.78	47.45	46.53	47.71	3.18	0.493	0.237	0.311
Shell deformation, nm	0.293	0.290	0.289	0.294	0.02	0.978	0.389	0.778
Albumen height, mm	10.18	10.42	10.29	10.31	0.58	0.444	0.893	0.777
Haugh unit	99.7	100.4	99.7	100.3	2.49	0.605	0.475	0.641
Yolk colour score	12.00	11.93	11.82[b]	12.11[a]	0.38	0.707	0.037	0.201
Yolk weight, g	15.34	15.28	15.57[a]	15.05[b]	0.57	0.781	0.013	0.850
Shell weight, g	6.058	6.134	6.049	6.142	0.18	0.258	0.167	0.664
Shell thickness, mm	0.428	0.434	0.429	0.432	0.01	0.124	0.474	0.958
Shell reflectivity, %	24.31[a]	23.25[b]	23.95	23.6	1.66	0.036	0.901	0.207

[a,b] Means with no common superscript within a row differ significantly ($P < 0.05$).

Table 7
Effect of high dietary soluble non-starch polysaccharide (sNSP) level and xylanase supplementation on egg quality, measured after hens were fed the dietary treatments for 0, 28 or 56 d.

Item	High sNSP without xylanase			SEM	*P*-value	High sNSP with xylanase			SEM	*P*-value
	0 d	28 d	56 d			0 d	28 d	56 d		
Egg weight, g	58.22[b]	61.65[a]	62.46[a]	2.32	0.001	58.85[b]	60.70[ab]	61.53[a]	1.95	0.021
Breaking strength, N	50.02[a]	49.16[ab]	46.24[b]	2.48	0.011	50.06	49.67	47.32	3.65	0.274
Shell deformation, nm	0.289	0.296	0.288	0.01	0.277	0.296	0.295	0.298	0.01	0.957
Albumen height, mm	10.89	10.70	10.13	0.48	0.075	10.95[a]	10.84[ab]	10.39[b]	0.44	0.037
Haugh unit	103.2[a]	101.9[a]	99.4[b]	2.02	0.004	103.4[a]	102.7[ab]	100.8[b]	1.83	0.022
Yolk colour score	9.90[b]	11.34[a]	11.84[a]	0.49	<0.001	9.53[b]	11.66[a]	12.16[a]	0.54	<0.001
Yolk weight, g	13.16[c]	14.75[b]	15.50[a]	0.52	<0.001	13.39[b]	14.13[a]	14.68[a]	0.54	<0.001
Shell weight, g	6.047	6.001	5.904	0.18	0.331	6.027	5.989	5.958	0.0.17	0.748
Shell thickness, mm	0.427	0.435	0.426	0.01	0.094	0.424	0.435	0.428	0.01	0.107
Shell reflectivity, %	23.68	24.00	24.36	1.45	0.836	23.12	24.24	24.27	1.58	0.254

[a,b] Means with no common superscript within a row and within a section differ significantly ($P < 0.05$).

Table 8
Effect of low dietary soluble non-starch polysaccharide (sNSP) level and xylanase supplementation on egg quality, measured after hens were fed the dietary treatments for 0, 28 or 56 d.

Item	Low sNSP without xylanase			SEM	*P*-value	Low sNSP with xylanase			SEM	*P*-value
	0 d	28 d	56 d			0 d	28 d	56 d		
Egg weight, g	59.57[b]	61.25[ab]	63.11[a]	2.38	0.016	57.48[b]	61.21[a]	62.11[a]	2.02	<0.001
Breaking strength, N	50.90[a]	47.51[ab]	46.81[b]	3.27	0.042	50.21	50.64	48.10	2.84	0.154
Shell deformation, nm	0.294	0.292	0.289	0.01	0.724	0.302	0.301	0.290	0.01	0.233
Albumen height, mm	11.05[a]	10.91[ab]	10.48[b]	0.43	0.014	10.98[a]	10.90[a]	10.36[b]	0.43	0.011
Haugh unit	104.1[a]	102.8[ab]	100.9[b]	1.69	<0.001	103.8[a]	102.8[a]	100.6[b]	1.72	<0.001
Yolk colour score	9.61[b]	11.42[a]	11.80[a]	0.52	<0.001	9.53[b]	11.87[a]	12.06[a]	0.49	<0.001
Yolk weight, g	13.36[c]	14.56[b]	15.36[a]	0.55	<0.001	12.87[b]	14.54[a]	14.77[a]	0.52	<0.001
Shell weight, g	6.112	5.953	6.034	0.20	0.288	6.052	6.063	6.026	0.15	0.884
Shell thickness, mm	0.443	0.436	0.430	0.01	0.348	0.422[b]	0.440[a]	0.436[a]	0.01	0.001
Shell reflectivity, %	22.67	23.70	23.18	1.40	0.332	22.66	22.72	22.94	1.39	0.912

[a,b] Means with no common superscript within a row and within a section differ significantly ($P < 0.05$).

content between the 2 diets was not extreme enough. Furthermore, the soluble NSP level may also not have been high enough; previous studies have demonstrated a lack of efficiency of xylanase addition in wheat-based diets as a result of low sNSP level present in the tested diets (Silversides et al., 2006; Pirgozliev et al., 2010; Bach Knudsen, 2014). The sNSP levels in the diets used in this study were lower than those presented in previous studies, largely because the aim was to keep the diets as commercially applicable as possible. Additionally, the wheat and barley used in the rations in this study had a soluble NSP content of approximately just 1.25% and 2.8%, respectively. The sNSP levels of those 2 ingredients, which were the main source of sNSP in the formulated diets, were much lower than the levels reported in the literature (Bach Knudsen, 2014; Holtekjølen et al., 2014; Rodehutscord et al., 2016). These authors highlighted that the analyzed levels of sNSP for wheat and barley ranged from 2% to 21% and from 4.5% to 26%, respectively, although it must be noted that these samples were of a different genotype and grown in a different region. It has been well-established that wheat NSP levels dictate its nutritive values, meaning that high NSP content yield higher endo-β-1, 4- xylanase responses with regards to animal performance parameters (Dusel et al., 1998; Bedford, 2000, 2006; Cardoso et al., 2018). Thus, the lack of performance responses seen in the current study is not entirely surprising, given the relatively low levels of sNSP present in diets.

4.1.2. Egg production

Despite the lack of profound responses in performance parameters, an interaction between xylanase and sNSP content of the diet on daily egg production was detected in the first 4 wk of the

experimental period, from 25 to 28 wk of age. In birds fed the high sNSP diet, egg production was hampered in the absence of xylanase, but in the presence of xylanase egg production was equivalent to that seen in birds fed the low sNSP, irrespective of enzyme presence. This could be attributed to inadequate nutrient level consumption in the presence of sNSP, because NSP encapsulate nutrients and reduce the ability of nutrients to be absorbed through the gastrointestinal lining, by increasing viscosity and thickness of the rate-limiting unstirred water layer of the mucosa. During this early laying phase, hen requirements for energy and other nutrients is heightened, as they require sufficient quantities for both egg production and continuous growth development. This suggests the birds at this age were probably very responsive to the negative relationship between sNSP content and utilization efficiency (Choct and Annison, 1990; Bedford, 2000). However, in the current study, reduction in daily egg production did not impact other performance measurements, such as feed efficiency or egg mass production. This suggests that the influence of the viscous cereals was minimal, and only able to be detected by sensitive hen-day production indices, but not by other less sensitive measurements.

Prevalence of dirty eggs was determined as an indicator of the impact of the dietary treatments on viscosity. In the present study, neither sNSP nor xylanase had any statistical influence on the percentage of dirty eggs. This finding is in contrast with that reported by Roberts and Choct (2006), who indicated that xylanase supplementation to wheat-based diets for Hy-line CB laying hens, from 44 to 49 wk of age, caused a 6.5% and 2.4% decrease in occurrence of broken and dirty egg, respectively. The lack of response in the present study might again illustrate that the sNSP content of the diets were too low and not extreme enough for xylanase to induce a notable impact on the excreta composition. Another attributing factor to this lack of response may be the rearing conditions used in this study; hens were housed individually in cages, as opposed to a floor-rearing system, and were provided with a large space of up to 0.27 m^2/hen, which probably reduced the chance of eggs being exposed to excreta.

4.1.3. Bird age

This study illustrates that hen age and laying stage directly influenced the extent of impact of exogenous enzyme supplementation on layer performance, with heightened responses observed in younger birds that are both growing and producing eggs, when demand for nutrients is greater. This could partly explain why responses in body weight as a result of dietary NSP presence were lesser in birds from 29 to 32 wk of age compared to 25 to 28 wk of age. However, the time period of this trial was relatively short, at 8 wk, suggesting further research is warranted into responses to dietary NSP and xylanase in older birds at the end of lay, compared to birds at the beginning or during peak lay. This was also shown by Lázaro et al. (2003), who observed that feeding graded xylanase supplementation levels into wheat- or rye-based diets fed to Hy-line W77 hens did not alter production performance from 24 to 28 wk old, but had a significant impact in younger hens at 20 to 24 wk of age.

4.1.4. Nutrient digestibility

Together with impairing nutrient digestion and absorption due to viscosity, increasing retention time of feed in the GIT can also stimulate proliferation of gut microflora in the foregut. This is particularly the case given that sNSP provides a rich source of available fermentation substrates, and high viscosity means there is heightened presence of undigested nutrients in the lumen than can fuel bacteria (Choct et al., 1996; Smits et al., 1998). The consequence of this over-proliferation of bacterial population condition in the gut results in the bacteria competing with the host for nutrients, as well as inducing a change in microbial composition and imbalance (Acamovic, 2001; Pirgozliev et al., 2010; Yaghobfar and Kalantar, 2017). Thus, this overgrowth of bacteria provides another potential explanation for reduced performance observed with heightened presence of viscous grains in a diet.

It is interesting to note that in this study xylanase inclusion tended ($P = 0.077$) to reduce apparent dry matter digestibility, which appears to be in contrast with the predicted capacity for xylanase to reduce digesta viscosity, thus improving nutrient digestibility. Previous studies have shown that xylanase inclusion improves the coefficient of digestibility of dry matter in broilers (Wang et al., 2005; Luo et al., 2017). Xylanase can hydrolyze long-chain arabinoxylans into small-chain xylo-oligomers to improve nutrient digestibility, but it has been proven that xylanase has a quadratic correlation with digesta viscosity. In this study the supplemental dose was high compared with the low level of sNSP and xylan present in the diet, meaning that the xylanase likely degraded the insoluble NSP into sNSP, uplifting the digesta water holding capacity, therefore slightly reducing dry matter digestibility (Taylor et al., 2018).

In the current trial, the apparent total tract crude protein digestibility in hens fed high sNSP content was higher than those fed the low sNSP diets. This was a surprising response compared to those recorded in the literature, in which high dietary sNSP level was shown to inhibit nutrient digestibility in chickens (Choct, 1999). Angkanaporn et al. (1994) indicated that feeding sNSP increases endogenous loss of amino acids, reducing protein digestibility. Eggum (1995) stated sNSP increase faecal nitrogen concentration due to increased excretion of microbial nitrogen. Therefore, the lower level of excreted protein observed in this study in birds fed high sNSP can possibly be attributed to the variation in fecal bacteria protein. There have been numerous studies conducted investigating the influence of fibre component on microbiota composition and enumeration in the chicken gut, including the ileum and ceca, but there has been limited research examining the impact of fibre on faecal microbial characteristics (Redman et al., 2007; Bedford and Cowieson, 2012; Walugembe et al., 2015). Therefore, it is difficult to conclude if the differences in excreted crude protein observed between the high and low sNSP fed birds is attributable to the concentration of microbial population present in the excreta, which is most likely associated with the degree of bacterial fermentation and digesta passage rate.

4.2. The effect of dietary treatments on egg quality parameters of laying hen

There is a scant information currently available in the literature about the influence of sNSP separately or in combination with xylanase supplementation on egg quality. Most reports indicate that enzyme supplementation has very little effect on egg quality (Ciftci et al., 2003; Cufadar et al., 2010; Lei et al., 2018). However, in the current study, xylanase and dietary sNSP had an impact on yolk colour score and shell reflectivity, respectively. Yolk colour is dictated by the ability of yellow-orange pigments available in the feed ingredients to be deposited in the egg yolk through co-migrating with fat (Pirgozliev et al., 2010). This suggests that the observed increase in yolk colour as a result of xylanase inclusion could be attributable to the ability of xylanase to reduce bile salt deconjugation, thus enhancing fat digestion, and the reduction in digesta viscosity improving nutrient digestion and releasing carotenoids (Ciftci et al., 2003; Liu et al., 2017). These results are in agreement with Pirgozliev et al. (2010), who showed that supplementing xylanase (at 1,200, and 1,600 XU/kg) to wheat-soybean meal-rye-based diet, from 28 to 32 wk of age, in Lohmann Brown

layers improved the yolk colour index. In contrast, Roberts and Choct (2006) did not detect any effects of xylanase supplementation on yolk colour in hens fed wheat-based diets.

In this study, xylanase was found to decrease yolk weight at 32 wk of age, but not at the earlier laying period. This suggests there was potentially a reduction in unsaturated fatty acid absorption in the older birds, which is a main component of egg yolk. Furthermore, it is interesting to note that feeding high sNSP increased egg shell reflectivity compared to those fed the low sNSP diets. To the best of the author knowledge, there has been no previous reports of fibre influencing shell reflectivity, and thus further investigation is required in order to understand the mechanisms behind this observation. No enzyme effects on shell colour were observed in this study. This is in contrast to the study conducted by Roberts and Choct (2006), in which shell reflectivity was found to be darker in hens fed wheat-based diets with a high supplemental dose of xylanase than the group supplemented with lower dose. Additionally, Roberts (2004) suggested that enzymes can cause egg shell colour to become lighter in brown laying hens.

Haugh unit and albumen height are the major factors used to evaluate freshness of eggs. In this study, these 2 values were decreased over the experimental period, with the highest values obtained at 25 wk of age and lowest at 32 wk of age. This is similar to findings in previous reports of Mirzaie et al. (2012) and Samiullah et al. (2017). More precisely, Mirzaie et al. (2012) recognized that Haugh units reduced as flock age increased, with this index being higher at 33 wk of age than at 47 wk of age. This is consistent with the present study, although in this study the measured Haugh unit and albumen height were comparatively much higher. These improvements may be due to the genetics of the specific strain of hen used. It is recognized that these egg quality parameters are highest in eggs produced by Hyline Brown and lowest in those produced by HiSex (Silversides and Scott, 2001; Roberts and Ball, 2004; Roberts and Choct, 2006). Regarding the impact of xylanase supplementation on Haugh unit and albumen height, Roberts and Choct (2006) showed that hens from 44 to 49 wk of age fed wheat–based diets with xylanase had lower albumen height and Haugh units than those fed wheat-based diets without xylanase supplementation. However, in the current study, the effects of hen age on these 2 parameters were more profound and significant than the impact of either xylanase supplementation or sNSP, which did not statistically alter Haugh unit or albumen height.

Egg shell quality can be assessed through measuring shell weight, thickness, breaking strength and deformation. Importantly, xylanase was shown to have beneficial impacts on improving egg shell quality. In particular, the shell breaking strength was found to be reduced in hens fed diets without xylanase as hen age increased, but in the group fed xylanase, shell breaking strength remained stable throughout the experimental period, regardless of the sNSP level. This improvement was also indicated by the increasing shell thickness in hens fed low sNSP from 0 to 28 and 56 d of the experimental period. This improvement likely indicates the enhancement in mineral digestion and absorption in hens due to xylanase supplementation.

5. Conclusions

Overall, the findings of this study revealed the influences of dietary sNSP and xylanase supplementation on production performance, nutrient digestibility and egg quality in laying hens even though the response of the hens to the experimental factors were largely inconsistent. Xylanase was shown to induce beneficial impact on improving hen quality and hen-day production, but also increased moisture content of the excreta. Also, the unexpected impacts of sNSP level on crude protein digestibility and shell reflectivity warrant further investigation. It appears that the sNSP levels in the diets were not extreme enough to derive strong conclusions about the impact of dietary NSP on layer production performance, thus future studies in this field should focus on feeding more extreme levels with differing polymer compositions. Also, additional studies are required to obtain a deeper understanding of how to achieve the optimum benefits from feeding xylanase to laying hens.

Author contributions

Xa H. Nguyen: investigation, formal analysis, writing-original draft; **Hong T. Nguyen**: validation, methodology, investigation, project administration, writing-review and editing; **Natalie K. Morgan**: conceptualisation, validation, methodology, funding acquisition, supervision, project administration, writing-review and editing.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that might inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper

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