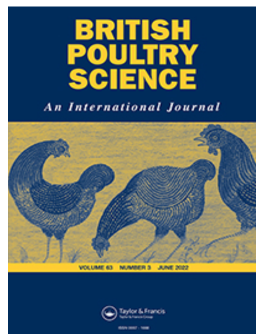

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Dietary soluble non-starch polysaccharide level and xylanase influence the gastrointestinal environment and nutrient utilisation in laying hens

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ABSTRACT

1. The objective of this study was to examine the influence of dietary soluble non-starch polysaccharide (sNSP) level and xylanase supplementation on productive performance, viscosity and pH along the gastrointestinal tract in laying hens. Excreta moisture content, ileal and caecal microbiota and short-chain fatty acid (SCFA) composition and apparent total tract nutrient utilisation were measured.
2. Hyline Brown laying hens (n = 144) were housed individually at 25 weeks of age and allocated to one of the four wheat-based dietary treatments in a 2 × 2 factorial arrangement, consisting of two levels of sNSP (High 13.40 g/kg or Low 11.22 g/kg), with or without xylanase (0 or 12,000 BXU/kg). Birds were fed the dietary treatments for 56 d.
3. Increasing dietary sNSP increased jejunum viscosity, degradability of total NSP, total tract flow of insoluble arabinose, and succinic acid concentration in the caeca ($P < 0.05$). Feeding high sNSP decreased excreta moisture content, total tract energy retention and free oligosaccharide, total tract flow of soluble and insoluble galactose and insoluble rhamnose and fucose, and ileal acetic and lactic acid concentrations ($P < 0.05$), and tended to reduce egg production ($P = 0.058$).
4. Supplementation with xylanase resulted in reduced jejunum and ileum viscosity, caecal pH, excreta moisture, flow of soluble arabinose and glucose and insoluble arabinose and xylose, caecal concentration of *Lactobacillus* sp. and isobutyric and succinic acid, and ileal concentration of *Bacillus* sp. and total anaerobic bacteria ($P < 0.05$). Xylanase application also increased energy retention and insoluble and total NSP degradation, and caecal abundance of *Bifidobacteria* sp. and valeric acid ($P < 0.05$).
5. These results reiterated the ability of xylanase to improve nutrient digestibility and reduce excreta moisture content in laying hens, and highlighted the importance of considering dietary sNSP level in laying hen diets.

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Bacteria; laying hen; viscosity; pH; xylanase

Introduction

Feed comprises approximately 70% of the cost of poultry meat and eggs. The majority of poultry diets are now plant-based, and, on average, contain approximately 10% fibre, primarily in the form of non-starch polysaccharides (NSP; Choct 2015). Dietary NSP level and composition are of concern, because of its anti-nutritive effects, namely increased digesta viscosity induced by the soluble NSP (sNSP) fraction. Heightened digesta viscosity reduces digestion and absorption of nutrients, thus impairing bird performance. Reduced passage rate induced by heightened viscosity can also result in proliferation of pathogenic bacteria earlier in the digestive tract. This can result in competition between pathogenic bacteria and the host for expensive, undigested nutrients. This can lead to the onset of infectious enteric diseases, such as necrotic enteritis (Van Immerseel et al. 2004; Bedford and Cowieson 2012). These anti-nutritive effects limit the ability of poultry nutritionists to use alternative, cheaper sNSP-rich ingredients, such as barley and rye.

A key area of concern when feeding fibre-rich, viscous feed ingredients to laying hens is the occurrence of wet and sticky excreta, which can cause poor litter quality and increase the incidence of dirty eggs, which has direct economic and environmental implications. Birds do not possess endogenous enzymes that breakdown NSP in the gastrointestinal tract so they are dependent on the ability of intestinal microbiota to ferment soluble NSP, and *via* supplementation with exogenous enzymes. Consequently, xylanase application is ubiquitous in commercial laying hen diets as a tool to enhance depolymerisation of NSP, reduce digesta viscosity and modulate bird intestinal health, especially in an era with reduced reliance on antibiotics (Bedford and Schulze 1998; Adeola and Cowieson 2011; Mahmood and Guo. 2020). However, there is still a deficit of studies and conflicting results when examining xylanase efficacy in laying hens. Of particular interest is the influence that sNSP composition and content have on xylanase efficacy. The effect of xylanase addition is well known in broilers (Mathlouthi et al. 2002; Liu et al. 2012) but is much less investigated in laying hens. This is of concern given that laying hens possess a very different gastrointestinal environment, due to their longer lifespan and thus more mature microbiota (Bederska-Łojewska et al. 2017). The hypothesis for the following study was that the microbiota in laying hens can be manipulated to be more adept at utilising dietary NSP, through feeding higher levels of sNSP coupled with exogenous xylanase supplementation (Nguyen et al. 2021). The theory is that this will stimulate xylan-degrading bacteria, increasing the ability of the bird to digest dietary NSP.

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A deeper understanding of the sNSP content of laying hen diets would allow for diets and enzyme application to be tailored to optimise laying hen productive performance. Thus, the focus of this study was to examine the influence that dietary sNSP level has on xylanase efficacy in laying hens.

Materials and methods

All experimental procedures were approved by the University of New England Animal Ethics Committee, Australia (Approval number: AEC19-015), in accordance with the Australian code of practice for the care and use of animals for scientific purposes (NHMRC 2013).

A total of 144 Hyline Brown laying hens (25 weeks of age) were randomly allocated to one of the four dietary treatments, arranged in a 2 × 2 factorial experimental design, with 36 hen replicates per treatment. There was no significant difference in initial hen starting weight between the dietary groups, with weight ranging from 1899 to 1915 g (P = 0.938). The experimental diets were wheat-based and formulated according to Hyline-Line Brown nutrient management guidelines (Hy-line 2018). The dietary factors were soluble non-starch polysaccharide level (High 13.40 g/kg or Low 11.22 g/kg) and xylanase supplementation (0 or 12,000 BXU/kg of feed). The xylanase used was a beta 1–4, endo-xylanase (Econase XT 5P 800,000 BXU/g, AB Vista, Marlborough, Wiltshire, UK). The recovered xylanase activity was below 2000 BXU/kg for the diets without xylanase, and 10,800 and 10,400 BXU/kg in the High and Low sNSP diets with supplemental xylanase, respectively. The ingredient content of the basal diets is shown in Table 1 and Table 2.

Birds were housed individually in conventional wire mesh 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 implemented to provide 16 h continuous light daily (from 0500 to 2100), and the experiment ran from April to June. Feed, as mash, and water were provided *ad libitum*. The shed had open-air ventilation. Birds were fed a common commercial laying hen diet for 7 d to adapt to the conditions in the facility, prior to being fed the experimental treatments. They were then fed the experimental treatments for 8 weeks, from 25 to 32 weeks of age.

Performance

During the 8-week experimental period, individual egg production and egg weights were recorded daily and feed intake was recorded weekly, to calculate production performance. Feed conversion ratio (FCR) was calculated from total feed intake per total egg mass, on an individual bird basis. No mortality was observed during the experimental period. The average hen-day production was calculated by dividing the total number of eggs produced by 56 (the total number of hen days per replicate cage for the whole experimental period), on an individual hen basis. Egg mass was determined as the egg weight multiplied by hen-day production. The number of eggs contaminated with faeces (dirty eggs) was recorded. Hen body weights were determined individually at the beginning and end of the experiment.

Table 1. Ingredient content of basal diets (g/100 g as fed basis).

Item	High sNSP	Low sNSP
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	-	0.45
Canola oil	4.65	3.97
Limestone	8.99	9.38
Di-calcium Phosphate	0.85	1.07
Phytase (Quantum blue 5G)[1]	0.01	0.01
Salt	0.17	0.18
Sodium bicarbonate	0.12	0.13
TiO2	0.50	0.50
UNE VM layer premix[2]	0.10	0.10
Choline Cl	0.04	0.04
L-lysine HCl	0.12	0.05
DL-methionine	0.17	0.16
L-threonine	0.02	-
Jabiru Red	0.004	0.004
Jabiru Yellow	0.003	0.003
Calculated nutrient content, %		
Dry matter	92.73	92.66
Metabolisable energy, kcal/kg	2792	2800
Crude fat	6.42	5.84
Crude fibre	2.65	2.43
Calcium	3.90	4.10
Available phosphorus	0.40	0.45
Digestible lysine	0.73	0.73
Digestible methionine + cysteine	0.66	0.66
Digestible threonine	0.51	0.52
Digestible tryptophan	0.21	0.21

sNSP = soluble non-starch polysaccharide; iNSP = insoluble non-starch polysaccharide.
[1]Equivalent to the supplemental dose of 500 FTU/kg of feed.
[2]Provided per kilogram of diet: retinol, 3000 mg; cholecalciferol, 75 mg; tocopherol, 20 mg; menadione, 3 mg; nicotinic acid B3, 35 mg; pantothenic acid B5, 12 mg; folic acid, 1.0 mg; riboflavin B2, 6.0 mg; vitamin B12, 0.02 g; biotin, 0.1 g; pyridoxine B6, 5 g; thiamine B1, 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.

Table 2. Analysed chemical composition of basal diets (as-fed basis).

Item	High sNSP	Low sNSP
Dry matter, %	91.98	92.35
Gross energy, MJ/kg	16.41	16.31
Crude protein, %	17.23	17.63
Calcium, %	3.84	4.10
Total phosphorus, %	0.63	0.66
Ash, %	11.43	13.49
Starch, %	37.99	36.83
Total NSP, g/kg	93.67	90.14
Soluble NSP, g/kg	13.40	11.22
Rhamnose	0.05	0.05
Fucose	0.03	0.03
Ribose	0.44	0.47
Arabinose	3.35	3.12
Xylose	4.03	3.87
Mannose	1.26	1.36
Galactose	1.71	1.76
Glucose	4.18	1.97
Insoluble NSP, g/kg	80.27	78.92
Rhamnose	0.39	0.47
Fucose	0.60	0.69
Ribose	0.19	0.17
Arabinose	22.00	21.88
Xylose	26.51	26.31
Mannose	1.42	1.39
Galactose	8.96	10.95
Glucose	30.20	26.91
sNSP:iNSP	0.167	0.142
Free oligosaccharides, %	3.56	3.78

Excreta collection and moisture content determination

On d 26–28 and d 54–56 (bird age 28 and 32 weeks), excreta samples were collected from each individual hen by placing a clean tray under the cage. For each cage, fresh excreta

samples were collected daily over 3 days, and then homogenised. Excreta moisture content was determined in a subsample of approximately 8–10 g fresh excreta, by weighing fresh sample into a pre-weighed porcelain crucible, oven drying at 105°C to constant weight, and then re-weighing the dried sample and crucible. The remaining excreta sample was frozen at −20°C and then freeze-dried to a constant weight for further chemical analysis.

Measurements of gastrointestinal tract pH and collection of digesta samples

On the final day of the trial, d56, each bird was weighed individually and euthanised by cervical dislocation, and tissues were harvested. The 18 replicates hens per treatment were used to measure the pH of the crop, and gizzard, duodenum, jejunum, ileum and caeca were measured twice by inserting a spear tip electrode (Sensorex Garden Grove, CA), connected to a pH metre (Mettler-Toledo, UK), directly into the digesta, ensuring that the probe did not touch the intestinal wall. The pH measurement was taken from the middle of the crop, ileum, jejunum and duodenum, and was measured in both pouches of the caeca. The pH probe was rinsed with ultra-pure water between samples.

Following the determination of pH, duodenum, jejunum, ileum and caeca digesta contents were collected from the same 18 birds, on an individual basis. The sample was homogenised in a clean 60 ml pot. A subsample of homogenised ileum and caeca digesta was collected into 2 ml tubes, which were immediately placed into liquid nitrogen and then frozen at −20°C for analysis of microbiota composition. For viscosity analysis, subsamples of homogenised duodenum, jejunum and ileum digesta were put into two 2 ml tubes per sample, which were placed into liquid nitrogen and then frozen at −20°C. The remaining caecal and ileal samples were placed onto the ice during collection and then frozen at −20°C for analysis of SCFA concentration.

Measurement of digesta viscosity

Duodenal, jejunal and ileal digesta viscosity were measured in duplicate for each individual hen. The two 2 ml tubes of digesta collected per hen were centrifuged at 10 000 × *g* for 10 min at room temperature. Viscosity was measured in a 0.5 ml supernatant, using a Brookfield DV3T Rheometer (Brookfield Ametek, Instrumentation & Speciality Controls Division, Middleboro, MA 02346 USA), with a CPA-40Z spindle at 35°C. Viscosity measurements were expressed in centipoise (cPs) unit (1 cPs = 1/100 dyne sec/cm2 = 1 mPa.s).

Chemical analysis for nutrient contents and xylanase activity

Feed ingredients, feed samples and freeze-dried excreta samples were ground through a 0.5 mm screen prior to chemical analysis, which was conducted in duplicate. Total dry matter content was determined in the diets and freeze-dried excreta by weighing approximately 1.5 g of sample into a porcelain crucible and oven-drying it at 105°C to constant weight. Gross energy of the diets and excreta samples (from the 18 replicate hens) was determined using an adiabatic bomb calorimeter (Model 6400, Parr Instruments, Moline, IL), with benzoic acid as the calibration standard. Nitrogen in the diet was determined using a combustion analyser (Leco model FP-2000 N analyser, Leco Corp., St. Joseph, MI, USA), with EDTA as the calibration standard. The nitrogen value was multiplied by 6.25 to calculate the crude protein content. Dietary total starch was measured in the diets and excreta samples using the Megazyme total starch assay (Megazyme International Ireland Ltd, Wicklow, Ireland). Titanium dioxide (TiO_2) content was quantified using UV-spectroscopy, based on the method described by Short et al. (1996). Xylanase activity of the diets was measured using a standardised ELISA, courtesy of Enzyme Services and Consultancy, UK.

Soluble and insoluble NSP and free-oligosaccharide content were determined in the feed ingredients prior to feed formulation, as well as in the feed and excreta samples with 14 individual replicate hens per treatment. This was conducted by measuring the constituent sugar components as alditol acetates using gas–liquid chromatography (Model CP3800, Varian Inc., Palo Alto, CA), following the procedure of Englyst et al. (1994) with some modifications as described by Theander et al. (1995) and Morgan et al. (2019). Briefly, samples were fat extracted using hexane, and free oligosaccharides were extracted by heating the sample at 80°C with 80% ethanol. The starch in the resulting residue was gelatinised using acetate buffer (pH 5), and α-amylase and amyloglucosidase was added at 95°C and 55°C, respectively, to remove the starch. The prepared sample was then incubated and centrifuged at 2,000 × *g* for 10 min and the resulting supernatant and residue were used for the analysis of soluble and insoluble NSP, respectively. For the soluble NSP analysis, the sugars released by the xylanases were removed using ethanol at 4°C, the residue was dried and then 2 M trifluoroacetic acid added and heated at 125°C.

For the insoluble NSP analysis, the glucose released from starch digestion was removed with water and acetone, and the resulting supernatant was removed and the residue was dried. Following this, 12M H_2SO_4 was added and the sample was heated to 35°C, and then water was added and the sample was heated to 100°C, cooled and then centrifuged at 3,000 × *g* for 15 min to sediment the insoluble materials. For the free sugar analysis, the extracted sample was dried, hydrolysed with 1 M H_2SO_4 at 100°C and centrifuged to sediment the insoluble material. Ammonium (28%) was added to an aliquot of the resulting supernatant from the insoluble NSP and free oligosaccharide samples. For the resulting samples, an internal standard was added (allose, 4 mg/ml) and the sample was evaporated to dryness and then re-dissolved in water with slight alkalinity. Freshly prepared $NaBH_4$ was added, the sample was incubated, and any excess $NaBH_4$ was decomposed with glacial acetic acid. 1-methylimidazole and acetic anhydride were added followed by water. Following this step, dichloromethane was added, the sample was centrifuged and the bottom layer collected and dried. Finally, ethyl acetate and water were added, the sample was centrifuged, and the supernatant was analysed by gas chromatography (GC; Model CP3800, Varian Inc., Palo Alto, CA, USA). The NSP and TiO_2 concentrations were determined on a dry matter basis.

Analysis of ileal and caecal short-chain fatty acid composition

Short-chain fatty acid (SCFA) and lactic acid concentrations were measured in duplicate for the ileal and caecal digesta, using the method described by Jensen et al.

(1995), with some modifications. Briefly, approximately 1.5 g and 0.8 g of fresh homogenised ileal and caecal digesta, respectively, maintained at approximately 5°C, was homogenised with 1 ml of internal standard (0.01 mol/l ethylbutyric acid). The sample was centrifuged at 3900 × *g* for 20 min at 5°C, and 1 ml of the supernatant was mixed with 0.5 ml concentrated HCl (36%) and 2.5 ml of ether. The sample was centrifuged at 2000 × *g* for 15 min at 5°C, and 400 µl of the supernatant was transferred into a GC vial. Following this, 40 µl of N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) was added to the vial, and the sample was heated at 80°C for 20 min and then maintained at room temperature for 48 h prior to analysis on a Varian CP3400 CX gas chromatograph (Varian Analytical Instruments, Palo Alto, CA, USA). The SCFA concentration in the samples was expressed as µmol/g digesta.

Analysis of ileal and caecal microbial abundance

Analysis of microbiota composition was determined in duplicate for both the ileal and caecal digesta samples. DNA extraction from both digesta samples was performed using an Isolate II Plant DNA Kit (Bioline, Alexandria, NSW, Australia) and QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) with slight modifications, as described by Keerqin et al. (2017) and Kheravii et al. (2017). The purity of the extracted DNA was assessed by a Nano-Drop ND-8000 UV spectrophotometer (Thermo Fisher Scientific, Waltham, USA). Only DNA elution that emitted ratios from 1.8 and above at a wavelength of 260/280 nm were used for PCR analysis. Following a 20x dilution with sterilised water, the extracted DNA was analysed for total anaerobic bacteria, *Bacillus, Bacteriodes, Bifidobacterium, Ruminococcus, Lactobacillus and Enterobacteria* sp. by quantitative real-time PCR analysis, using a Rotorgene 6500 real-time PCR machine, and quantification was determined using Rotorgene 6000 series software 1.7 (Corbett, Sydney, Australia). The determination of *Campylobacter and Salmonella* sp. from the caecal samples were firstly determined in a pilot analysis with four replicate hens per treatment. However; these were only detected at trace-level and were removed from further analysis. In the ileal digesta samples, *Bacteriodes, Enterobacteria and Rumminococcus* sp. were detected in negligible amount, thus were not reported. A threshold cycle averaged from the duplicate samples was used for quantification analysis. The number of target DNA copies was calculated using a standard curve constructed with plasmid DNA cloned with the amplicons. Copy numbers of plasmid DNA were calculated according to its mass, taking into account the size of the plasmid with the amplicon insert. The resulting values were expressed as $\log_{10}$ (genomic DNA copy number)/g digesta. The species-specific 16 rRNA primers utilised are described in detail by Kheravii et al. (2017).

Calculation of coefficient of nutrient digestibility, NSP flow and moisture content

The following equation was used to calculate coefficient of total tract nutrient digestibility:

$$\text{Coefficient of total tract nutrient digestibility (\%)} = 1 - [(\text{TiO}_{2\%\ \text{diet}}/\text{TiO}_{2\%\ \text{digesta or excreta}}) \times (\text{Nutrient}_{\%\ \text{digesta or excreta}} / \text{Nutrient}_{\%\ \text{diet}})]$$

where nutrient refers to either starch, energy, NSP or free-oligosaccharides (FO)

NSP total tract flow was calculated as g/kg DM intake using the equation:

$$\text{NSP flow}_{\text{excreta}} = \text{NSP}_{\text{excreta}} \times (\text{TiO}_{2\text{diet}} / \text{TiO}_{2\text{excreta}})$$

Moisture content of fresh excreta samples was determined using the following equation:

Moisture content (%) = 100 × (1 – (oven-dried matter sample weight/fresh sample weight))

Statistical analysis

All data were analysed using Minitab (Minitab 19. 2020.1, Minitab Inc., US). Data were tested for normality using the Anderson–Darling test and Kolmogorov–Smirnov test, and then analysed as a 2 × 2 factorial arrangement using General Linear Model (GLM) for normally distributed data. Tukey's multiple range test was used to determine the differences between individual treatment means when interactions were observed. Pen served as the experimental unit. Significant differences between the mean values were declared at $P < 0.05$.

Results

Performance

The data (Table 3) showed that the dietary treatments had no significant effects on hen production performance, hen weight gain and number of dirty eggs at 25–32 weeks of

Table 3. Effect of dietary soluble non-starch polysaccharide level (sNSP) and xylanase supplementation on production performance in laying hens from 25 to 32 weeks of age (56 d on dietary treatments).

Treatment	Hen-day production, %	Egg weight, g	Egg mass, g/hen/d	Feed intake, g	FCR, g/g	Hen weight gain, g	Dirty egg, %
sNSP							
High	98.6	60.9	60.6	121.9	2.02	214.5	2.24
Low	99.2	61.2	60.9	122.1	2.01	231.9	2.12
Xylanase							
No	98.7	61.2	60.8	122.6	2.02	212.4	2.20
Yes	99.1	60.9	60.5	121.5	2.01	234.0	2.16
SEM	0.28	0.63	0.77	1.75	0.027	14.53	0.462
P-value							
sNSP	0.058	0.581	0.605	0.913	0.633	0.236	0.587
Xylanase	0.223	0.628	0.635	0.536	0.813	0.142	0.953
sNSP × Xylanase	0.072	0.869	0.784	0.438	0.184	0.093	0.834

age or after 56 d on the dietary treatments (P > 0.05). There was a tendency for reduced hen-day production as a consequence of feeding the High sNSP diet (P = 0.058).

Digesta viscosity and excreta moisture content

Table 4 presents the data that showed that birds fed the diets with high sNSP level had higher jejunum viscosity (P = 0.012) and lower excreta moisture content (P = 0.010) after 56 d on the dietary treatments, compared to those fed the diets with low sNSP. Birds fed the diets supplemented with xylanase had lower jejunum and ileum viscosity (P = 0.004 and P < 0.001, respectively) after 56 d on the dietary treatments, and less excreta moisture after 28 and 56 d on the dietary treatments (P = 0.002 for both), compared to birds fed the diets without supplemental xylanase. No interactions between sNSP level and xylanase supplementation were observed for any parameters measured.

Gastrointestinal tract pH

As shown in Table 5, sNSP level had no impact on digesta pH in any section of the gastrointestinal tract. However, xylanase supplementation reduced the caecal digesta pH (P = 0.016). No interactions between sNSP level and xylanase supplementation were observed for pH in any of the gastrointestinal tract sections measured.

Utilisation of nutrient, non-starch polysaccharides and free-oligosaccharides

Table 6 shows the influence of sNSP level and xylanase supplementation on total tract digestibility of starch, energy retention and degradability of NSP and FO. Coefficient of total tract energy retention was greater in birds fed the low, compared to the high, sNSP diets (P < 0.001), and was improved by the presence of xylanase (P = 0.004). No interactions between sNSP level and xylanase were observed for utilisation of starch and energy (P > 0.05).

An interaction between sNSP level and xylanase supplementation was observed for fermentability of sNSP (P = 0.039). Xylanase supplementation resulted in increased sNSP digestibility in both diets, but the response was greater in birds fed the high sNSP compared to the low sNSP diet (21% *vs.* 13% improvement in sNSP fermentation, respectively). The addition of xylanase to the low sNSP diet resulted in similar digestibility compared to that observed in birds fed the high sNSP without enzymes. Birds fed high sNSP had higher total NSP (tNSP) degradation (P = 0.004) and lower free oligosaccharides (FO) digestibility (P < 0.001) compared to birds fed the low sNSP diet. Degradations of iNSP and tNSP were higher in birds fed the diets supplemented with xylanase compared to those fed the diets without xylanase (P < 0.001 for both), by 7% and 9%, respectively. Birds fed the high sNSP diet tended to have a lower excreta sNSP to insoluble NSP ratio (sNSP: iNSP; P = 0.062).

Tables 7 and 8 present the influence of the dietary treatments on the apparent total tract flow of sNSP and iNSP constituent sugars, respectively. Apparent total tract flow of soluble galactose was lower in birds fed the high sNSP level compared to those fed the low sNSP level (P < 0.001). Xylanase supplementation reduced the apparent total tract flow of soluble arabinose (P = 0.001) and glucose (P = 0.005). Apparent total tract flow of insoluble rhamnose, fucose and galactose was greater in birds fed diets with low, compared to high, sNSP level (P = 0.002; P < 0.001; P < 0.001, respectively).

Table 4. Effect of dietary soluble non-starch polysaccharide level (sNSP) and xylanase supplementation on duodenum, jejunum and ileum viscosity (cP) after 56 d on the dietary treatments, and excreta moisture content (%) after 28 and 56 d on the dietary treatments.

		Viscosity			Excreta moisture	
sNSP	Xylanase	Duodenum	Jejunum	Ileum	d28	d56
sNSP						
High		2.28	5.41	4.56	74.2	74.5
Low		2.24	4.19	4.54	74.7	75.1
Xylanase						
No		2.37	5.50	5.49	74.9	75.2
Yes		2.15	4.10	3.62	73.9	74.4
SEM		0.119	0.447	0.378	0.32	0.26
P-value						
sNSP		0.767	0.012	0.967	0.124	0.010
Xylanase		0.098	0.004	< 0.001	0.002	0.002
sNSP × Xylanase		0.400	0.151	0.815	0.290	0.952

Table 5. Effect of dietary soluble non-starch polysaccharide level (sNSP) and xylanase supplementation on crop, gizzard, duodenum, jejunum, ileal and caecal pH after 56 d on the dietary treatments.

sNSP	Xylanase	Crop	Gizzard	Duodenum	Jejunum	Ileum	Caeca
sNSP							
High		5.37	4.19	6.25	6.26	6.17	6.21
Low		5.29	4.20	6.31	6.32	6.19	6.18
Xylanase							
No		5.27	4.12	6.30	6.29	6.18	6.32
Yes		5.39	4.27	6.25	6.29	6.18	6.08
SEM		0.061	0.101	0.033	0.037	0.054	0.099
P-value							
sNSP		0.198	0.970	0.107	0.138	0.649	0.890
Xylanase		0.072	0.150	0.133	0.986	0.915	0.016
sNSP × Xylanase		0.362	0.107	0.174	0.448	0.871	0.947

Table 6. Effect of dietary soluble non-starch polysaccharide level (sNSP) and xylanase supplementation on total tract coefficient of digestibility of starch and energy retention, degradability of non-starch polysaccharides and free oligosaccharides (FO) (%), and ratio between sNSP and iNSP in the excreta, in laying hens after 56 d on the dietary treatments.

sNSP	Xylanase	Starch	Energy	sNSP	iNSP	tNSP	sNSP:iNSP	FO
High	No	0.988	0.743	0.631[b]	0.247	0.301	0.082	0.818
	Yes	0.987	0.749	0.761[a]	0.318	0.401	0.080	0.819
Low	No	0.988	0.752	0.548[c]	0.228	0.268	0.084	0.866
	Yes	0.988	0.764	0.619[b]	0.294	0.341	0.091	0.894
SEM		0.0021	0.0031	0.0143	0.0173	0.0156	0.0032	0.0109
sNSP								
High		0.988	0.746	0.696	0.282	0.351	0.081	0.819
Low		0.988	0.758	0.584	0.261	0.304	0.087	0.880
Xylanase								
No		0.988	0.747	0.589	0.237	0.285	0.083	0.842
Yes		0.988	0.757	0.690	0.306	0.371	0.086	0.857
P-value								
sNSP		0.822	< 0.001	< 0.001	0.217	0.004	0.062	< 0.001
Xylanase		0.743	0.004	< 0.001	< 0.001	< 0.001	0.393	0.167
sNSP × Xylanase		0.966	0.312	0.039	0.882	0.402	0.137	0.216

[a-b]Means within the same column with no common subscript differ significantly (P ≤ 0.05).

Table 7. Effect of dietary soluble non-starch polysaccharide level (sNSP) and xylanase supplementation on apparent total tract flow (g/kg DM intake) of soluble NSP in laying hens after 56d on the dietary treatments.

Treatment	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose
sNSP								
High	0.10	0.11	0.16	1.12	1.75	0.54	1.03	0.84
Low	0.10	0.10	0.15	1.08	1.73	0.63	1.38	0.89
Xylanase								
No	0.10	0.11	0.16	1.19	1.74	0.61	1.19	0.99
Yes	0.10	0.10	0.16	1.01	1.74	0.56	1.22	0.75
SEM	0.008	0.008	0.007	0.043	0.068	0.054	0.079	0.082
P-value								
sNSP	0.840	0.094	0.571	0.558	0.691	0.094	<0.001	0.537
Xylanase	0.406	0.171	0.884	0.001	0.956	0.361	0.734	0.005
sNSP × Xylanase	0.387	0.194	0.441	0.857	0.738	0.253	0.062	0.144

Table 8. Effect of dietary soluble non-starch polysaccharide level (sNSP) and xylanase supplementation on apparent total tract flow of insoluble NSP (g/kg DM intake) in laying hens after 56 d on the dietary treatments.

Treatment	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose
sNSP								
High	0.51	0.70	0.18	20.77	24.41	1.19	7.87	14.60
Low	0.60	0.85	0.19	19.82	23.07	1.22	8.98	15.85
Xylanase								
No	0.56	0.78	10.80	20.90	26.38	1.18	8.37	16.11
Yes	0.55	0.78	10.85	19.70	21.10	1.24	8.47	14.34
SEM	0.026	0.024	0.0141	0.366	0.404	0.079	0.209	1.316
P-value								
sNSP	0.002	< 0.001	0.433	0.012	0.075	0.748	< 0.001	0.343
Xylanase	0.567	0.806	0.606	0.002	< 0.001	0.552	0.650	0.180
sNSP × Xylanase	0.066	0.104	0.763	0.538	0.552	0.404	0.138	0.950

The opposite was true for insoluble arabinose total tract flow, which was comparatively higher in birds fed the high sNSP level (P = 0.012). Xylanase supplementation resulted in reduced apparent total tract flow of insoluble arabinose and xylose (P = 0.002 and P < 0.001, respectively). No interactions between dietary sNSP level and xylanase supplementation were observed for the apparent total tract flow of any NSP constituents.

Ileal and caecal short-chain fatty acid concentration

Tables 9 and 10 illustrate the effect of dietary sNSP level and xylanase supplementation on SCFA and lactic acid profiles in ileal and caecal digesta. Hens fed the high sNSP diets had significantly reduced acetic and lactic acid concentrations in the ileum (P = 0.036 and P < 0.001, respectively). Xylanase supplementation had no impact on ileal SCFA or lactic acid concentration, and no interactions between sNSP level and xylanase were observed.

An interaction between sNSP and xylanase supplementation was detected for caecal valeric acid concentration (P = 0.003), whereby supplementation with xylanase increased caecal valeric acid content by 40% in birds fed the high sNSP diet, but had no impact in birds fed the low sNSP diet. Furthermore, birds fed the high sNSP diet had higher levels of succinic acid content in the caeca compared to those fed low sNSP (P = 0.018). Hens fed the diets supplemented with xylanase presented lower concentrations of caecal isobutyric acid and succinic acid compared with those fed the diets without xylanase (P = 0.009 and P < 0.001, respectively).

Table 9. Effect of dietary soluble non-starch polysaccharide level (sNSP) and xylanase supplementation on short-chain fatty acid and lactic acid profiles in the ileum after 56 d on the dietary treatments (µmol/g fresh sample).

Treatment	Formic	Acetic	Propionic	Lactic	Succinic
sNSP					
High	2.93	2.54	0.24	4.12	0.46
Low	3.11	2.95	0.24	10.27	0.35
Xylanase					
No	2.99	2.59	0.23	7.00	0.44
Yes	3.05	2.90	0.25	7.39	0.37
SEM	0.156	0.181	0.012	1.925	0.052
P-value					
sNSP	0.381	0.036	0.656	0.001	0.222
Xylanase	0.749	0.115	0.167	0.832	0.445
sNSP × Xylanase	0.052	0.462	0.785	0.904	0.500

Table 10. Effect of dietary soluble non-starch polysaccharide level (sNSP) and xylanase supplementation on short-chain fatty acid and lactic acid profiles in the caeca after 56 d on the dietary treatments (µmol/g fresh sample).

sNSP	Xylanase	Formic	Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Valeric	Lactic	Succinic
High	No	4.39	145.29	52.06	1.03	27.13	0.40	1.77^{b}	3.30	2.37
	Yes	5.63	114.97	44.95	0.91	21.46	0.43	2.48^{a}	2.56	1.50
Low	No	4.34	119.23	51.42	1.02	23.96	0.38	1.53^{b}	2.32	1.86
	Yes	4.11	120.56	50.31	0.72	24.13	0.29	1.42^{b}	2.40	1.18
SEM		0.502	9.082	3.005	0.077	2.029	0.040	0.127	0.316	0.167
sNSP										
High		5.01	130.13	48.50	0.97	24.30	0.41	2.12	2.93	1.93
Low		4.23	119.89	50.86	0.87	24.04	0.33	1.48	2.36	1.52
Xylanase										
No		4.37	132.26	51.74	1.03	25.54	0.39	1.65	2.81	2.12
Yes		4.87	117.76	47.63	0.81	22.80	0.36	1.95	2.48	1.34
P-value										
sNSP		0.131	0.274	0.445	0.217	0.902	0.076	< 0.001	0.085	0.018
Xylanase		0.328	0.123	0.186	0.009	0.187	0.513	0.025	0.313	< 0.001
sNSP × Xylanase		0.158	0.093	0.331	0.223	0.161	0.177	0.003	0.213	0.570

[a-b]Means within the same column with no common subscript differ significantly (P ≤ 0.05).

Ileal and caecal microbial abundance

The impact of dietary treatments on the abundance of some dominant bacterial genera in the ileum and caeca are presented in Tables 11 and 12. Xylanase supplementation decreased the number of genome DNA copies of *Bacillus* sp. and total anaerobic bacteria in the ileum (P = 0.044 and P = 0.038, respectively). Xylanase supplementation resulted in a 2.4% reduction in the abundance of *Lactobacillus* sp. and a 2.1% increase in *Bifidobacteria* sp. content in the caeca (P = 0.016 and P = 0.010, respectively). Dietary sNSP level had no impact on ileum or caeca microbiota composition, and no interactions between sNSP level and xylanase were observed.

Discussion

The physiochemical properties and composition of dietary NSP, as opposed to just the total quantity, warranted consideration in laying hen diets. Of particular interest was the water-soluble fraction, due to its impact on digesta viscosity and as a substrate for intestinal bacteria fermentation, thus influencing the microbiota profile and gastrointestinal environment (Choct et al. 1996; Choct 1997; Tellez et al. 2014). The hypothesis of this study was that feeding higher sNSP would heighten the hen's response to exogenous xylanase, through increasing the abundance of substrates. It was predicted that this would result in increased production of prebiotic xylo-oligosaccharides (XOS), due to the high ara-

Table 11. Effect of dietary soluble non-starch polysaccharide level (sNSP) and xylanase supplementation on microbiota profiles in the ileum 56 d on the dietary treatments (log_{10} GDC (genome DNA copies)/g fresh sample).

Treatment	*Lactobacillus*	*Bifidobacteria*	*Bacillus*	Total anaerobic bacteria
sNSP				
High	8.18	5.41	6.53	8.61
Low	8.24	5.26	6.54	8.49
Xylanase				
No	8.35	5.34	6.69	8.71
Yes	8.08	5.32	6.37	8.39
SEM	0.180	0.168	0.154	0.158
P-value				
sNSP	0.738	0.360	0.948	0.443
Xylanase	0.152	0.904	0.044	0.038
sNSP × Xylanase	0.377	0.439	0.310	0.337

Table 12. Effect of dietary soluble non-starch polysaccharide level (sNSP) and xylanase supplementation on microbiota profiles in the caeca after 56 d on the dietary treatments ($\log_{10}$ GDC (genome DNA copies)/g fresh sample).

sNSP	Xylanase	*Lactobacillus*	*Bifidobacteria*	*Bacillus*	*Ruminococcus*	*Enterobacteriaceae*	*Bacteriodes*	Total anaerobic bacteria
sNSP								
High		10.96	10.50	7.28	8.74	5.82	10.01	12.11
Low		10.87	10.40	7.18	8.75	6.56	10.04	12.12
Xylanase								
No		11.04	10.34	7.32	8.75	6.20	10.03	12.12
Yes		10.79	10.56	7.14	8.74	6.18	10.02	12.11
SEM		0.101	0.082	0.119	0.062	0.463	0.064	0.045
P-value								
sNSP		0.415	0.211	0.404	0.946	0.122	0.629	0.825
Xylanase		0.016	0.010	0.146	0.967	0.967	0.985	0.933
sNSP × Xylanase		0.498	0.723	0.802	0.801	0.796	0.119	0.207

binoxylan content of the diets, coupled with reduced digesta viscosity and heightened release of entrapped nutrients (Taylor et al. 2018). This was expected to improve nutrient utilisation and productive performance. This hypothesis could be accepted in part, in that birds fed the high sNSP diet with xylanase presented the greatest utilisation of sNSP. The lack of interaction between sNSP and xylanase on performance and the microbiota and SCFA composition was likely due to the difference between the high and low sNSP levels being not very extreme in this study, at just 2 g/kg, and was primarily composed of glucans, not arabinoxylan. However, effects of sNSP level were observed in this study, signalling the level of influence of dietary sNSP on the gastrointestinal environment.

Exogenous endo-1,4-β-xylanase application is now largely ubiquitous in commercial laying hen feed as a tool to ameliorate the anti-nutritive effects posed by dietary NSP. However, inconsistencies have been reported between studies on laying hen responses to supplemental NSPases (Mathlouthi et al. 2002; Roberts and Choct 2006; Mirzaie et al. 2012; Bederska-Łojewska et al. 2019; Abdollahi et al. 2021). Results from the current study clearly demonstrated the ability of xylanase to hydrolyse dietary NSP and solubilise insoluble NSP. This was illustrated by the degradation of dietary insoluble and total NSP by 29% and 30%, respectively, and the reduction in jejunum and ileum viscosity. This was reaffirmed by a significant reduction in insoluble arabinoxylan (AXE) in the excreta of birds fed diets containing xylanase. Moreover, the heightened energy digestibility and degradability of NSP observed with xylanase application demonstrated its ability to both release entrapped nutrients and generate fermentable substrates that provide substrates for beneficial probiotic bacteria, which, in turn, improved the nutritive value of feed (Zhang et al. 2014).

The lack of effect of the dietary treatments on starch digestibility suggested that the energy effects were mainly attributed to NSP degradation. The lack of effect of xylanase on bird performance in this study was in contrast with a number of studies (Silversides et al. 2006; Senkoylu et al. 2009; Olgun et al. 2018). A possible explanation for this was the short duration of time in which the dietary treatments were fed for only 8 weeks, although similar time periods have been used in previous studies, such as those by Sun and Kim (2019) and Selim and Hussein (2020). Other potential explanations included the low sNSP levels in the test diets, and the fact the measured xylanase activity was lower than anticipated. Furthermore, starch digestibility in all treatment groups was relatively high and consistent, indicating high quality and digestibility of the ingredients used in the diets. Nonetheless, these findings were in agreement with Sousa et al. (2019) and Bederska-Łojewska et al. (2019), who reported a lack of performance response to xylanase in birds fed fibre-rich diets, despite physiological effects being observed.

In the current study, the presence of xylanase resulted in reduced caecal pH, which can induce an environment that is unfavourable for pathogens, enabling beneficial bacteria to flourish, resulting in heightened caecal fermentation and endogenous enzyme activity in the caeca (Van der Wielen et al. 2001; Sousa et al. 2019). This was illustrated by the reduced abundance of *Bacillus* sp. and total anaerobic bacteria in the ileum, which may have reflected the low availability of nutrients to feed the commensal microbes in the upper gut (Silva and Smithard. 2002). The higher caecal pH and greater quantities of branched SCFA in birds fed diets without xylanase may have been due to the production of toxic compounds from protein fermentation, as opposed to fibre fermentation, due to a lack of oligosaccharides present to fuel the microbiota (Qaisrani et al. 2015; Gilbert et al. 2018). This demonstrated the ability of xylanase to reduce reliance on expensive dietary protein sources as substrates for microbiota, and manipulate conditions in the gut towards fermentation of carbohydrate sources (Rehman et al. 2008). This was manifested as heightened caecal *Bifidobacteria* sp. concentrations for the xylanase diet group. Broekaert et al. (2011) reported a correlation between stimulation of *Bifidobacteria* sp. fermentation activity and reduction of protein fermentation. High proliferation of bacteria in the small intestine results in competition with the host for nutrients, which can impair nutrient utilisation and productive performance (Choct et al. 1996). Xylanase reduces this by depressing fermentation intensity in the small intestine and stimulating fermentation in the caeca (Choct et al. 1996, 1999b; Bedford and Apajalahti 2001). However, it must be noted that the concentration of SCFA did not necessarily reflect rates of production by the bacteria, as absorption from the intestine into the blood stream is rapid. In addition, reverse peristalsis may cause variable concentrations of different SCFA, hence the interpretation of SCFA data may be questionable (González-Ortiz et al. 2020).

The findings from this study were in agreement with Lee et al. (2017) and Józefiak et al. (2010), who showed that xylanase supplementation in birds fed wheat- and rye-based diets had increased *Bifidobacteria* but reduced *Lactobacillus* sp. abundance in the caeca. In contrast, Singh et al. (2021) reported a greater abundance of *Lactobacillus* sp. as a consequence of xylanase supplementation in birds fed corn-soybean-based diets. Thus, it appears that the

abundance of *Lactobacillus* sp. responds closely to the types of substrates provided by the basal cereals. *Lactobacillus* sp. is generally considered to be beneficial for intestinal development, through production of lactic and acetic acid and bacteriocins that can inhibit pathogenic bacteria proliferation (Dunkley et al. 2007; Józefiak et al. 2010). However, Inagaki and Sakata (2001) observed that increasing lactic and succinic acid may be disadvantageous, as high accumulation in the gastrointestinal tract can have negative impacts on absorption of water and solutes, inducing diarrhoea. In a well-balanced caecum, lactic acid content often remains at a low level (Lee et al. 2017). This suggests that, in the absence of xylanase, there may be too much stimulation of *Lactobacillus* sp. growth, and xylanase can rectify this. Furthermore, this illustrates that the high sNSP level may have been advantageous in its ability to reduce ileal lactic and acetic acid levels.

It is interesting to note that xylanase reduces the apparent total tract flow of soluble arabinose, but not soluble xylose. This implied less flow of branched AXE in the tract in birds fed xylanase, which suggested there may have been high volumes of arabinose and arabinose-substituted XOS generated as a consequence of enzyme application. As indicated by Bautil et al. (2019), the microbiota has a higher preference to ferment arabinose over xylose. This may have resulted in generating microbiota that were more adept at producing endogenous α-arabinofuranosidases, causing heightened utilisation of arabinose (Bautil et al. 2019). Furthermore, more caecal *Bifidobacteria* sp. were recorded in the birds fed xylanase; and these bacteria can readily degrade AXOS and AXE, leading to more arabinose production (Mäkeläinen et al. 2010; Broekaert et al. 2011; Rivière et al. 2014). Consequently, the reduced jejunum and ileum viscosity and excreta moisture observed in this study may have been associated with debranching AXE. Nevertheless, the effects of excreta moisture content and viscosity did not translate into fewer dirty eggs, which was in contrast to the studies of Chesson (2001) and Lázaro et al. (2003). However, the proportion of dirty eggs was exceptionally low, at approximately 2%, which is half of that seen by Choct et al. (1999a), and most likely due to the individual housing of the birds.

Soluble NSP flow represents the balance between sNSP generation from iNSP and sNSP fermentation, whereas iNSP flow is a direct indicator of how much insoluble NSP has been dissolved. Data for these parameters indicated that total tract flow of insoluble arabinose and xylose was reduced significantly with the use of xylanase, but its effect on xylose was greater compared with arabinose. Thus, the transit from ileum to faeces suggested that hind gut fermentation used a lot more xylose than arabinose. The sNSP present as glucans/cellulose showed considerable reductions in flow with xylanase addition, indicating that the enzyme encouraged bacterial fermentation and dissolution of NSP, which was unrelated to the xylan target of this enzyme

In broilers, the efficacy of NSP-degrading enzymes has been linked to NSP composition of the diet (Musigwa et al. 2021), but this has yet to be fully elucidated in laying hens. Furthermore, supplemental xylanase efficacy is influenced by the ingredient endogenous endo-1,4-β-xylanase activity and extract viscosity, particularly in wheat-based diets (Cardoso et al. 2018). This reiterates the importance of quantifying sNSP content, composition and chemical structure in feed ingredients for poultry diets. In the current study, feeding high sNSP resulted in a 16% increase in total NSP degradability and greater sNSP utilisation. This was likely due to the development of a microbiota that was successful at fermenting and utilising dietary sNSP substrates. It was notable that digestibility of FO was higher in birds fed the low compared to high sNSP level. Hence, there was increased reliance on FO as an energy source in birds fed the sNSP-poor diet. This was probably because the lack of available substrate caused the microbiota to be poorer at exploiting dietary NSP. The deficit of available data regarding NSP in laying hen diets makes it difficult to establish suitable levels that will yield beneficial nutritional and productive performance responses, especially as a consequence of xylanase application. The results from this study highlighted the importance of using feed ingredients as substrates to evaluate xylanase efficacy, as opposed to using purified or semi-purified diets that cannot truly reflect laying hen responses in commercial settings (Meng et al. 2005). Further studies, using more variable ranges of sNSP and different doses of supplemental xylanase, are recommended to elucidate such interactions.

In conclusion, application of xylanase in laying hen diets directly influenced gut viscosity, caecal pH, total tract flow and degradation of dietary NSP and ileal and caecal microbiota composition. The consequence of this was enhanced energy utilisation and lower excreta moisture content. Dietary sNSP level appeared to directly influence the efficacy of xylanase, mainly its ability to ferment and utilise NSP to produce SCFA. Further studies testing more extreme sNSP levels in feed are warranted to fully elucidate its relationship with xylanase efficacy in laying hens.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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