Impact of fermentable fiber, xylo-oligosaccharides and xylanase on laying hen productive performance and nutrient utilization

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ABSTRACT This study evaluated the impact of feeding xylo-oligosaccharides (**XOS**), fermentable fiber in the form of wheat bran (**WB**), and xylanase (**XYL**) on laying hen productive performance and nutrient digestibility. The hypothesis was that the WB would provide the microbiota in the hindgut with fermentable dietary xylan, and the XOS and XYL would further upregulate xylan fermentation pathways, resulting in improved nutrient utilization. Isa Brown hens (n = 96) were obtained at 39 wk of age. They were fed 12 dietary treatments, 8 hens per treatment, for 56 d. A commercial laying hen ration was fed, and for half of the treatments 10% of this ration was directly replaced with WB. The diets were then supplemented with either 1) no supplements; 2) XOS 50 g/t; 3) XOS 2000 g/t; 4) XYL (16,000 BXU/kg); 5) XYL + XOS 50 g/t, or 6) XYL + XOS 2,000 g/t. Hen performance and egg quality were measured every 14 d. On d56, ileum digesta samples were collected for determination of starch, nonstarch polysaccharide (**NSP**), XOS, protein, energy, and starch digestibility. Ceca digesta samples were also collected for analysis of XOS, short chain fatty acid (**SCFA**), xylanase and cellulase activity and microbial counts. Feeding 2,000 g/t XOS increased ileal protein digestibility. Combined 2,000 g/t XOS and XYL increased cecal *Bifidobacteria* concentration. This combination also increased cecal xylanase activity in birds fed the control diet. Cecal cellulase activity was improved by feeding WB, XYL, and 2,000 g/t XOS. XYL increased cecal lactate production. Feeding 2,000 g/t XOS with WB increased insoluble NSP degradability and shell breaking strength at d56. In summary, supplementing laying hen diets with fermentable fiber, XYL and XOS increases utilization of dietary xylan, improving nutrient utilization, performance, and gastrointestinal health.

Key words: nutrition, enzyme, prebiotic, nonstarch polysaccharides, fiber

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INTRODUCTION

Xylanase is a nonstarch polysaccharide (**NSP**) degrading enzyme that cleaves the internal β-xylosidic glycosidic linkages of xylan into xylo-oligosaccharides (**XOS**). Xylanase application in laying hen diets is common practice, with the aim of alleviating the adverse effects of dietary xylan on gastrointestinal tract viscosity and nutrient encapsulation (Cowieson and Bedford, 2009). However, laying hen response to supplemental xylanase is variable and inconsistent. Some studies have observed that xylanase has no impact (Pirgozliev et al. 2010; Bigge et al., 2018; Sousa et al., 2019; Nguyen et al. 2021a) or a negative effect (Novak et al., 2008) on laying hen performance and nutrient utilization, whereas other studies have identified positive effects induced by xylanase application in laying hen diets (Mathlouthi et al., 2002; Mirzae et al., 2012; Nguyen et al. 2021b; Souza et al., 2012; Bobeck et al., 2014; Taylor et al., 2018). This variability in findings suggests it may not always be economically beneficial to supplement xylanase into laying hen diets. The deficit of data available presenting laying hen responses to xylanase is a concern.

Recently, there has been heightened interest in the prebiotic effects of XOS in poultry. XOS fuel beneficial microbiota, resulting in improvements in SCFA production, as well as mineral and energy utilization, immune stimulation, and increased villus length in the ileum (Jommuengbout et al., 2009; Kim et al., 2011; Morgan et al., 2018). XOS can also stimulate xylan

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digestion and development of a xylan-fermenting microbiota in young birds (Bautil et al., 2020), increasing the ability of adult birds to utilize dietary xylan. This suggests there may be benefits to supplementing laying hen diets directly with XOS, as opposed to relying on in situ generation of XOS in the gastrointestinal tract in the presence of xylanase. Furthermore, XOS has notable effects at low doses, has no toxicity and is stable at acidic pH (Carvalho et al., 2013). Van Hoeck et al. (2021) and Nguyen et al. (2021a) observed that supplementing xylanase to wheat-based diets stimulated growth of several beneficial bacteria species and reduced abundance of pathogenic bacteria, which resulted in improved performance in laying hens. This suggests even greater benefits could be achieved by feeding XOS alongside xylanase, through increasing the availability of XOS as fuel for beneficial microbiota species.

Nonstarch polysaccharides (**NSP**) are considered to be antinutrients, because they reduce accessibility and absorption of nutrients by increasing digesta viscosity and acting as a physical barrier to enzymes. However, feeding broiler chickens moderate levels of dietary NSP has shown to have advantageous effects on growth performance (González-Alvarado et al., 2010), through improved nutrient digestibility (Amerah et al., 2009; Bao and Choct, 2010) and gastrointestinal health (Montagne et al., 2003; Yadav and Jha, 2019). This is likely a consequence of oligosaccharides and soluble NSP fractions being selectively fermented by beneficial bacteria, increasing production of SCFAs to be used by the bird as a source of energy and reducing the prevalence of pathogenic bacteria species (Shakouri et al., 2006). This suggests that dietary NSP could be used as a tool to manipulate the microbiota, thus enhancing bird performance. This has yet to be investigated in laying hens, which will likely respond differently due to their longer life span and more mature gastrointestinal microbiota.

The hypothesis of this study was that supplementing laying hen diets with XOS would upregulate xylan fermentation pathways and stimulate proliferation of xylan-degrading bacteria. Feeding a source of fermentable xylan provides substrates for these bacteria to utilize, and presence of xylanase ensures the xylan is soluble and fermentable, and thus able to be consumed by the bacteria. The predicted outcome was enhanced utilization of dietary xylan, resulting in improved nutrient utilization and egg quality.

MATERIALS AND METHODS

Birds and Husbandry

Isa Brown laying hens ($n = 96$) were obtained at 38 wk of age. The hens were housed individually in conventional wire mesh cages (50 cm width × 54 cm length × 45 cm height, 2,700 cm^2/hen) equipped with a feed trough and nipple drinkers. All birds were fed commercial laying hen ration for 7 d prior to being fed the experimental treatments, to adapt to the conditions in the facility. After these 7 d, the hens were randomly allocated to one of 12 dietary treatments, with 8 replicates of individual hens per treatment. They were fed the dietary treatments for 56 d, from 39 to 47 wk of age. Cage allocation was randomized across the room. Natural light and artificial lighting were implemented to provide 16 h continuous light daily (from 0500 to 2100). Feed, fed as mash, and water were provided ad libitum. The shed had open-air ventilation. Institutional and national guidelines for the care and use of animals were followed, and all experimental procedures involving animals were approved by the Animal Ethics Committee at University of New England, New South Wales, Australia (AEC21-029).

Dietary Treatments

There were 12 dietary treatments. The ingredient composition of the commercial basal ration is presented in Table 1. For half of the treatments ($n = 6$), 10% of this basal diet was replaced with wheat bran (**WB**), as a source of fermentable NSP; Table 2 presents the analyzed nutrient composition of the resulting diets. The diets were then supplemented with or without xylanase (XYL; 16,000 BXU Econase XT 5P, AB Vista, Marlborough, UK) and XOS (AB Vista, Marlborough, UK) at either 0, 50, or 2,000 g/t.

Performance and Egg Quality

During the 56-d experimental period, individual egg production was recorded daily and feed intake and hen weight were recorded every 14 d, to calculate production performance. Egg production was averaged across the 56-d period. Egg mass was calculated as the egg weight multiplied by egg production. Feed conversion ratio (**FCR**) was calculated as grams of total feed intake per total egg mass, on an individual bird basis. On d56, every bird was individually weighed and then

Table 1. Ingredient composition of the basal diet.

Ingredient	Control
Wheat	33.64
Corn	20.68
Sorghum	17.23
Faba Beans	1.48
Canola Oil	0.98
Canola Meal	2.46
Soybean Meal	7.38
Molasses	2.22
Limestone	9.84
Monodicalcium Phosphate	2.46
Vitamin/Mineral Premix[1]	0.20
Salt	0.25
Bentonite	0.98
Jabiru Red	0.11
Jabiru Yellow	0.09

[1]Provided per kilogram of diet: vitamin A, 10.00 MIU; vitamin D, 3MIU; vitamin E, 20 mg; vitamin K, 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. Analyzed nutrient composition of the control and wheat bran basal diets.

Analyzed Composition	Control	Wheat Bran
Dry Matter (g/100g)	88.36	88.64
Protein (g/100g DM)	16.46	16.50
Energy (MJ/kg DM)	16.10	16.16
Starch (g/100g DM)	61.84	63.94
Soluble NSP (g/kg DM)	7.94	10.21
Insoluble NSP (g/kg DM)	53.65	61.75
Free Oligosaccharides (g/kg DM)	35.77	36.18

euthanized, and ileum and ceca samples were collected on an individual bird basis.

Every 14 d an egg was collected from each individual hen, eliminating any abnormal or damaged eggs, for egg quality analysis. The measurements taken were egg weight, height and breadth, albumen height, yolk color, height, diameter and index, eggshell weight, reflectivity and thickness, and Haugh unit (Haugh, 1937). All measurements were conducted using equipment from Technical Services and Supplies (Dunnington, York, UK). Egg yolk color was measured on a range from 1 to 15, from palest to darkest, based on the Roche scale (Roberts, 2005). The eggshells were dried overnight at room temperature and then weighed, and shell thickness was analyzed using a Mitutoyo Dial Comparator gauge (Model 2109-10, Kawasaki, Japan).

Ileal Nutrient Digestibility

For determination of ileal digestibility, dry matter (%) content was determined in the diets and ileum digesta samples by weighing a subsample into duplicate crucibles and oven drying at 105°C to a constant weight, and then reweighing. Protein content of the diets and ileum digesta samples was determined by measuring nitrogen using the combustion method (LECO Corp., St. Joeseph, MI), with EDTA as a calibration standard, and multiplying the nitrogen value by a factor of 6.25. Diet and ileum digesta gross energy content was determined using an adiabatic bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA), standardized with benzoic acid. Starch was measured in the diets and ileum samples using the Megazyme total starch assay (Megazyme International Inreland Ltd, Wicklow, Ireland). TiO_2 marker was quantified in the diets and ileum samples by UV-spectroscopy at 410 nm (Cary 50 Bio UV-Visible spectrophotometer equipped with a Cary 50 MPR microplate reader, Varian Inc., Palo Alto, CA), as illustrated by Short et al. (1996).

The constituent sugar components of the diet and ileum digesta NSP was determined as alditol acetates using gas 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. (2018). Briefly, the sample was fat extracted using hexane and then free oligosaccharides were extracted by heating the sample at 80°C with 80% ethanol. The starch in the resulting residue was gelatinized 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 x g for 10 min and the resulting supernatant and residue was used for the analysis of soluble and insoluble NSP, respectively. For the soluble NSP analysis, the sugars released by the enzymes were removed using ethanol at 4°C, the residue was dried and then 2M 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 x g for 15 min to sediment the insoluble materials. For the free sugar analysis, the extracted sample was dried, hydrolyzed with 1M 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 all 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 then added, the sample was incubated, and any excess $NaBH_4$ was decomposed with glacial acetic acid. Next, 1-methylimidazole and 5 mL of $C_4H_6O_3$ was added followed by water, and then dichloromethane was added, the sample was centrifuged, and the bottom layer collected and dried. Finally, ethyl acetate and water was added, the sample was centrifuged, and the supernatant was analyzed by gas chromatography (Model CP3800, Varian Inc., Palo Alto, CA).

Ileal digestibility of protein, starch, energy and free oligosaccharides, and degradability of soluble and insoluble NSP were calculated using the following equation:

$$\text{Digestibility or Degradability (\%)} = [100-(\text{Nutrient } \textit{digesta} \times \text{TiO}_2 \text{ diet})/(\text{TiO}_2 \text{ digesta} \times \text{Nutrient diet})] \times 100$$

Cecal Xylanase and Cellulase Activity

Xylanase concentration in the ceca and diets was analyzed by Megazyme endo-xylanase assay kit (K-XylX6), and cellulase activity in the ceca and diets was analyzed by Megazyme endo-cellulase kit (K-CELLG3) (Megazyme, Wicklow, Ireland, UK), using a UV-spectroscopy at 510 nm (Cary 50 Bio UV-Visible spectrophotometer equipped with a Cary 50 MPR microplate reader, Varian Inc., Palo Alto, CA). Xylanase activity in the diets was determined to be <2,000 BXU/kg in the diets not supplemented with xylanase, and ranged from 15,700 to 16,800 BXU/kg in the diets supplemented with xylanase.

Cecal Short-Chain Fatty Acid Concentration

Short chain fatty acids (**SCFA**) in the ceca were measured as silyl esters by GCMS. Briefly, 400 to 500 mg of

ceca digesta was accurately weighed into a 2 mL centrifuge tube and combined with 1000 uL of 0.2M NaOH containing 10 mM of ethyl butyric acid (Internal Standard IS). The suspension was mixed thoroughly, centrifuged, and then 100 uL of supernatant was added to a new 2 mL centrifuge tube, followed by 1,500 uL of diethyl ether and 50 μL of 0.5M HCl. The solution was mixed and the bottom aqueous layer was removed. The remaining diethyl ether layer was dried by adding an excess of anhydrous sodium sulfate. The dried solution was centrifuged and 480 μL was transferred to a 2 mL GC vial. 20 uL of MTBSFA was added and was allowed to react for a minimum of 2 h prior to analysis on an Agilent 7890A GC and 5975C MSD fitted with an Agilent HP-5MS column (30 m × 0.25 mm). Six standards between 0.1 and 5 mM were prepared using the same method. Both standards and samples were analyzed within 15 h of preparation, to avoid degradation of the silyl esters. The SCFA concentration in the samples was expressed as μmol/g digesta.

Cecal Microbiota Composition

Analysis of microbiota composition was determined in duplicate in the d 56 cecal digesta samples. DNA extraction from the 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 modification, as described by Keerqin et al. (2017) and Kheravii et al. (2017). The purity of the extracted DNA was assessed by a NanoDrop ND-8000 UV spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Only DNA elution that emitted ratios of 1.8 and above at a wavelength of 260/280 nm were used for PCR analysis. Following a 20 × dilution with sterilized water, the extracted DNA was analyzed for total anaerobic bacteria, *Bacillus* spp., *Bacteriodes* spp., *Bifidobacterium* spp., *Ruminococcus* spp., *Lactobacillus* spp., and *Enterobacteriaceae* spp. 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). 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 amplicon insert. The resulting values were expressed as log10 (genomic DNA copy number)/g digesta. The species-specific 16 rRNA primers utilized are described in detail by Kheravii et al. (2017).

Ileal and Cecal XOS Concentration

The single sugars arabinose and xylose, and xylo-oligosaccharides xylobiose (X_2), xylotriose (X_3), xylotetraose (X_4), xylopentaose (X_5), and xylohexaose (X_6) were extracted from the samples using a multi-step solid phase extraction. Extracted XOS were derivatized using 1-phenyl-3-methyl-5-pyrazolone (**PMP**). Analysis of the PMP-XOS was carried out on an Agilent Single Quad LCMS equipped with Agilent ZORBAX SB-C18 column (3.0 × 150 mm, 1.8 μ) and separated using mobile phases -A: 0.1% formic acid in H_2O and B: 0.1% formic acid in acetonitrile, as described by Morgan et al. (2020). The quantity of each XOS fraction was then calculated as mg/g of TiO_2 marker. Table 3 presents the XOS concentration in the dietary treatments.

Statistical Analysis

All data were analyzed using IBM SPSS statistics version 27. Individual bird represented the replicate unit for statistical analysis. After Kolmogorov-Smirnov testing to confirm normality, univariate analysis was used to evaluate the contribution of WB, XYL and XOS on the measured parameter. Treatment means were separated using Tukey post-hoc test where appropriate. Statistical significance was declared at $P < 0.05$.

RESULTS

Performance

Table 4 presents the impact of the dietary treatments on body weight gain (**BWG**) and FCR every 14 d over

Table 3. Analyzed xylo-oligosaccharide (XOS) concentration in the dietary treatments containing wheat bran (WB), XOS, and xylanase (XYL) (mg/g TiO_2).

WB (%)	XOS (g/t)	XYL (BXU/kg)	Xylobiose (X_2)	Xylotriose (X_3)	Xylotetraoase (X_4)	Xylopentaose (X_5)	Xylohexaose (X_6)	XOS Total (X_2–X_6)
10	0	0	1.89	1.50	0.74	0.40	0.00	4.53
10	50	0	6.10	5.46	3.04	1.98	1.30	17.88
10	2000	0	51.75	25.72	8.45	3.48	1.73	91.13
10	0	16,000	45.34	34.51	8.55	0.96	0.00	89.36
10	50	16,000	41.98	32.96	9.47	1.05	0.00	85.46
10	2000	16,000	111.91	56.51	10.50	1.01	0.27	180.20
0	0	0	4.58	3.89	1.65	0.95	0.55	11.62
0	50	0	5.02	4.41	2.15	1.16	0.70	13.44
0	2000	0	81.70	41.78	13.15	3.99	0.65	141.27
0	0	16,000	42.56	31.16	8.90	1.35	0.00	83.97
0	50	16,000	5.34	4.32	1.92	1.06	0.61	13.25
0	2000	16,000	109.41	56.18	11.83	1.33	0.00	178.75

Table 4. Effect of wheat bran (WB), xylanase (XYL), and xylo-oligosaccharides (XOS) on overall egg production and feed conversion ratio (FCR) and body weight gain (BWG) every 14 days in laying hens fed the dietary treatments for 56 d (39−47 wk of age).

				d0−14		d14−28		d28−42		d42−56	
WB (%)	XOS (g/t)	XYL (BXU/kg)	Daily egg production (%)	FCR[1]	BWG (g)	FCR[1]	BWG (g)	FCR[1]	BWG (g)	FCR[1]	BWG (g)
10	0		97.92	2.33^{a}	49.38^{ab}	2.37	27.75	2.61	2.13^{c}	2.64	4.00
10	50		98.44	1.90^{ab}	41.88^{ab}	2.39	16.19	2.35	11.94^{bc}	2.81	9.19
10	2000		98.44	2.27^{ab}	47.88^{ab}	2.58	16.88	2.58	29.51^{b}	2.72	12.38
0	0		97.92	1.95^{ab}	19.25^{ab}	2.34	2.75	2.75	47.69^{a}	2.87	15.94
0	50		98.96	2.03^{ab}	81.63^{a}	2.38	10.69	2.58	27.82^{b}	2.57	30.44
0	2000		99.31	1.83^{b}	7.75^{b}	2.43	6.75	2.59	14.76^{b}	2.76	26.69
10		0	98.26	2.12	43.75	2.38^{b}	10.96	2.43	12.84	2.77	3.04
10		16,000	98.84	2.20	49.00	2.51^{a}	29.59	2.59	16.21	2.67	14.00
0		0	98.26	2.02	55.34	2.40^{b}	1.54	2.70	27.46	2.70	14.92
0		16,000	98.61	1.86	17.09	2.36^{b}	11.92	2.57	32.71	2.76	33.79
WB (%)	10		98.55	2.16	46.38	2.45	20.27	2.51	14.52	2.72	8.52
	0		98.44	1.94	36.21	2.38	6.73	2.64	30.09	2.73	24.36
XOS (g/t)	0		97.92	2.14	34.32	2.36	15.25	2.68	24.91	2.75	9.97
	50		98.70	1.97	61.75	2.38	13.44	2.46	19.88	2.69	19.82
	2000		98.87	2.05	27.81	2.50	11.81	2.58	22.13	2.74	19.54
XYL (BXU/kg)	0		98.26	2.07	49.54	2.39	6.25	2.57	20.15	2.74	8.98
	16,000		98.73	2.03	33.04	2.44	20.75	2.58	24.46	2.72	23.90
SEM			0.206	0.023	3.496	0.014	2.753	0.032	2.052	0.026	2.464
P-value											
WB			0.576	0.015	0.469	0.639	0.222	0.338	0.061	0.938	0.112
XYL			0.889	0.666	0.241	0.185	0.191	0.919	0.601	0.864	0.134
XOS			0.604	0.285	0.116	0.052	0.968	0.368	0.882	0.869	0.651
WB x XOS			0.911	0.020	0.045	0.341	0.752	0.764	0.014	0.174	0.923
WB x XYL			0.889	0.202	0.124	0.047	0.709	0.253	0.909	0.450	0.689
XYL x XOS			0.859	0.284	0.378	0.277	0.709	0.988	0.383	0.243	0.452
WB x XYL x XOS			0.966	0.531	0.233	0.699	0.591	0.636	0.321	0.833	0.658

[1]FCR calculated as feed intake per egg mass on individual bird basis.

the 56-d trial period, and overall daily egg production. The dietary treatments had no impact on daily egg production ($P > 0.050$).

At d0 to 14 on the experimental diets, FCR was found to be lower in birds fed the control diet supplemented with 2,000 g/t XOS compared to those fed the WB diet without supplemental XOS ($P = 0.020$). XOS supplementation had no impact on BWG in birds fed the diets with WB, but birds fed the control diet presented higher BWG when the diet was supplemented with 50 g/t XOS compared to 2,000 g/t XOS ($P = 0.045$).

In the presence of WB, FCR at d14 to 28 on the experimental diets was increased by XYL supplementation, but XYL supplementation had no impact on FCR in birds fed the control diet ($P = 0.047$). The dietary treatments had no impact on BWG at d14 to 28 on the treatments ($P > 0.05$).

In birds fed the diets with WB, supplementation with 2,000 g/t XOS resulted in increased BWG at d28 to 42 on the dietary treatments compared to birds fed no XOS. However, the opposite was true in birds fed the control diet, with a reduction in BWG observed when feeding either 50 or 2,000 g/t XOS ($P = 0.014$). The dietary treatments had no impact on FCR at d28 to 42 on the dietary treatments, or on FCR or BWG at d42 to 56 on the dietary treatments ($P > 0.05$).

Egg quality

Table 5 presents that there was an interaction between WB, XYL, and XOS on egg weight, Haugh unit and breadth in eggs collected after 14 d on the dietary treatments ($P = 0.025$, $P = 0.032$, and $P = 0.017$, respectively). In birds fed the diets containing WB, XOS had no impact on egg weight in the presence of XYL, but in the absence of XYL eggs were heavier when feeding 2,000 g/t XOS compared to no XOS. In birds fed the control diet, XOS had no impact on egg weight in the absence of XYL, but when XYL was present egg weight was increased by supplementation of 2,000 g/t XOS compared to no XOS. Haugh unit and egg breadth was greater in birds fed the diet containing WB, 2,000 g/t XOS and no XYL compared to those fed the control diet with XYL and no XOS. Albumen height was increased by the presence of XYL ($P < 0.001$). Yolk color was reduced by feeding WB ($P = 0.005$). Yolk diameter was higher when feeding 2,000 g/t XOS compared to feeding 50 g/t XOS ($P = 0.042$).

An interaction between WB and XYL was observed on albumen height, Haugh unit and shell reflectivity in eggs collected after 28 d on the dietary treatments ($P = 0.010$, $P = 0.014$, and $P = 0.037$, respectively), as shown in Table 6. XYL had no impact on albumen height, Haugh unit or shell reflectivity in birds fed the diets with WB, but in birds fed the control diet presence of XYL resulted in increased albumen height and Haugh unit and lower shell reflectivity (shells were darker in color). XYL supplementation increased yolk color ($P = 0.022$).

Table 7 presents that there was an interaction between WB and XYL on egg weight and shell reflectivity at d42 ($P = 0.033$ and $P = 0.034$, respectively). XYL supplementation increased egg weight and decreased shell reflectivity in birds fed the control diet, but XYL had no impact on these parameters in birds fed the diets

Table 5. Effect of wheat bran (WB), xylanase (XYL) and xylo-oligosaccharides (XOS) on egg quality parameters in laying hens fed the dietary treatments for 14 d (at 41 wk of age).

WB (%)	XOS (g/t)	XYL (BXU/kg)	d14 Egg Weight (g)	d14 Albumen height (mm)	d14 Yolk color	d14 Haugh unit	d14 Egg breadth (mm)	d14 Yolk diameter (mm)
10	0	0	60.10^{b}	7.43	8.88	87.15^{ab}	43.51^{ab}	43.17
10	50	0	61.99^{ab}	8.19	9.13	89.59^{ab}	43.96^{ab}	41.20
10	2000	0	65.01^{a}	7.54	9.13	95.38^{a}	44.70^{a}	43.20
10	0	16,000	61.30^{ab}	9.48	9.13	87.14^{ab}	43.51^{ab}	42.76
10	50	16,000	61.63^{ab}	8.74	8.38	88.43^{ab}	43.91^{ab}	42.13
10	2000	16,000	59.85^{b}	9.36	9.00	84.78^{ab}	43.63^{ab}	45.09
0	0	0	61.58^{ab}	8.33	9.75	88.75^{ab}	44.12^{ab}	42.50
0	50	0	62.19^{ab}	9.36	9.63	90.99^{ab}	43.39^{ab}	40.75
0	2000	0	60.96^{ab}	8.33	9.88	88.17^{ab}	43.81^{ab}	42.54
0	0	16,000	58.30^{b}	9.74	10.13	81.77^{b}	42.82^{b}	40.49
0	50	16,000	61.88^{ab}	8.90	9.50	88.69^{ab}	43.91^{ab}	40.43
0	2000	16,000	63.36^{a}	10.06	9.00	90.05^{ab}	44.35^{ab}	43.50
WB (%)	10		61.65	8.46	8.94^{b}	88.75	43.87	42.93
	0		61.38	9.12	9.65^{a}	88.07	43.73	41.70
XOS (g/t)	0		60.32	8.75	9.47	86.20	43.49	42.23^{ab}
	50		61.92	8.80	9.16	89.43	43.79	41.13^{b}
	2000		62.30	8.82	9.25	89.60	44.12	43.58^{a}
XYL (BXU/kg)	0		61.97	8.20^{b}	9.40	90.01	43.92	42.23
	16,000		61.05	9.38^{a}	9.19	86.81	43.69	42.40
SEM			0.223	0.080	0.061	0.381	0.051	0.196
P-value								
WB			0.764	0.506	0.005	0.661	0.512	0.121
XYL			0.307	<0.001	0.397	0.039	0.274	0.828
XOS			0.165	0.365	0.566	0.129	0.048	0.042
WB x XOS			0.903	0.346	0.616	0.762	0.872	0.974
WB x XYL			0.560	0.619	0.997	0.634	0.470	0.424
XYL x XOS			0.889	0.548	0.325	0.774	0.214	0.389
WB x XYL x XOS			0.025	0.498	0.512	0.032	0.017	0.985

[a-b]Means within the same column, within the same parameter, with no common subscript, differ significantly ($P < 0.05$)

with WB. An interaction between XOS and XYL on eggshell reflectivity was also observed ($P = 0.008$), showing that in the absence of XYL reflectivity was increased by XOS application, but in the presence of XYL the opposite was true, with XOS inducing darker shell color. Yolk color was reduced by feeding WB ($P = 0.020$).

As shown in Table 8, yolk color was reduced by the presence of WB ($P < 0.001$) and XYL ($P = 0.009$) in eggs obtained after 56 d on the dietary treatments. An interaction between WB and XOS was observed on shell breaking strength ($P = 0.046$). This showed that shell strength was increased by supplementation with 2,000 g/t XOS in birds fed the diets with WB, but in birds fed the control diet shell strength was improved with 50 g/t XOS, but not 2,000 g/t XOS. XYL increased yolk height in birds fed the control diet but had a

Table 6. Effect of wheat bran (WB), xylanase (XYL) and xylo-oligosaccharides (XOS) on egg quality parameters in laying hens fed the dietary treatments for 28 d (at 43 wk of age).

WB (%)	XYL (BXU/kg)	d28 Albumen height (mm)	d28 Yolk Color	d28 Haugh Unit	d28 Shell reflectivity (%)
10	0	9.10^{ab}	8.83	94.40^{ab}	24.41^{ab}
10	16,000	8.87^{ab}	9.38	93.71^{ab}	25.44^{ab}
0	0	8.34^{b}	9.00	90.96^{b}	26.22^{a}
0	16,000	9.52^{a}	9.50	96.53^{a}	24.68^{b}
WB (%)	10	8.99	9.11	93.89	24.93
	0	8.94	9.25	93.75	25.45
XOS (g/t)	0	9.02	9.29	94.30	24.83
	50	8.78	9.16	92.85	25.77
	2000	9.08	9.10	94.31	24.96
XYL (BXU/kg)	0	8.73	8.92^{b}	92.68	25.32
	16,000	9.20	9.44^{a}	94.95	25.06
SEM		0.038	0.032	0.188	0.089
P-value					
WB		0.863	0.514	0.917	0.390
XYL		0.082	0.022	0.089	0.677
XOS		0.618	0.783	0.583	0.395
WB x XOS		0.556	0.636	0.469	0.106
WB x XYL		0.010	0.926	0.014	0.037
XYL x XOS		0.693	0.168	0.859	0.174
WB x XYL x XOS		0.393	0.636	0.283	0.661

[a-b]Means within the same column, within the same parameter, with no common subscript, differ significantly ($P < 0.05$)

Table 7. Effect of wheat bran (WB), xylanase (XYL) and xylo-oligosaccharides (XOS) on egg quality parameters in laying hens fed the dietary treatments for 42 d (at 45 wk of age).

WB (%)	XOS (g/t)	XYL (BXU/kg)	d42 Egg weight (g)	d42 Yolk color	d42 Shell reflectivity (%)
10		0	61.50^{ab}	8.12	23.81^{ab}
10		16,000	60.71^{ab}	8.31	24.12^{ab}
0		0	59.05^{b}	9.00	25.27^{a}
0		16,000	62.15^{a}	8.88	23.44^{b}
	0	0	60.31	8.69	23.44^{b}
	50	0	60.26	8.41	25.24^{a}
	2000	0	60.26	8.59	24.94^{a}
	0	16,000	60.72	8.13	24.86^{a}
	50	16,000	61.11	9.34	23.61^{b}
	2000	16,000	62.47	8.32	22.87^{b}
WB (%)		10	61.11	8.21^{b}	23.97
		0	60.65	8.94^{a}	24.35
XOS (g/t)		0	60.59	8.41	24.15
		50	60.68	8.87	24.42
		2000	61.37	8.45	23.90
XYL (BXU/kg)		0	60.28	8.56	24.54
		16,000	61.48	8.60	23.78
SEM			0.385	0.066	0.159
P-value					
WB			0.576	0.020	0.437
XYL			0.201	0.915	0.127
XOS			0.715	0.401	0.695
WB x XOS			0.898	0.344	0.061
WB x XYL			0.033	0.622	0.034
XYL x XOS			0.696	0.121	0.008
WB x XYL x XOS			0.739	0.079	0.341

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

negative impact on yolk height in birds fed the diets with WB ($P = 0.031$). In birds fed the diets with WB, shell reflectivity was greater with supplementation of 50 g/t XOS compared to 2,000 g/t XOS, but the opposite was true in birds fed the control diet ($P = 0.028$). Shell thickness was greater in birds fed 50 g/t XOS compared to no XOS ($P = 0.045$).

Ileal Nutrient Digestibility

Table 9 presents that the dietary treatments had no impact on ileal energy or starch digestibility ($P > 0.05$). Protein digestibility was greater in birds fed 2,000 g/t XOS compared to those fed no XOS ($P = 0.038$).

Table 8. Effect of wheat bran (WB), xylanase (XYL) and xylo-oligosaccharides (XOS) on egg quality parameters in laying hens fed the dietary treatments for 56 d (at 47 wk of age).

WB (%)	XOS (g/t)	XYL (BXU/kg)	d56 Yolk color	d56 Shell breaking strength (kgf)	d56 Yolk height (mm)	d56 Shell reflectivity (%)	d56 Shell thickness (mm)
10	0		7.44	4.17^{b}	20.63	24.87^{ab}	0.43
10	50		8.51	4.10^{b}	22.19	25.69^{a}	0.43
10	2000		8.82	4.56^{a}	22.20	24.07^{b}	0.43
0	0		9.44	4.10^{b}	21.60	25.64^{ab}	0.42
0	50		8.82	4.62^{a}	22.05	23.29^{b}	0.45
0	2000		9.01	3.99^{b}	20.60	27.48^{a}	0.43
10		0	8.59	4.24	22.34^{a}	25.09	0.43
10		16,000	7.92	4.31	21.00^{b}	24.65	0.43
0		0	9.34	4.28	20.64^{b}	25.51	0.43
0		16,000	8.84	4.19	22.18^{a}	25.42	0.43
WB (%)	10		8.25^{b}	4.28	21.67	24.87	0.43
	0		9.09^{a}	4.23	21.41	25.47	0.43
XOS (g/t)	0		8.44	4.14	21.11	25.25	0.42^{b}
	50		8.66	4.36	22.12	24.49	0.44^{a}
	2000		8.91	4.27	21.40	25.77	0.43^{ab}
XYL (BXU/kg)	0		8.96^{a}	4.26	21.49	25.30	0.43
	16,000		8.38^{b}	4.25	21.59	25.04	0.43
SEM			0.062	0.038	0.121	0.171	0.002
P-value							
WB			<0.001	0.819	0.304	0.764	0.778
XYL			0.009	0.983	0.542	0.617	0.518
XOS			0.584	0.594	0.544	0.759	0.045
WB x XOS			0.067	0.046	0.817	0.028	0.099
WB x XYL			0.247	0.644	0.031	0.637	0.469
XYL x XOS			0.350	0.577	0.749	0.741	0.249
WB x XYL x XOS			0.244	0.973	0.358	0.720	0.753

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

Table 9. Effect of wheat bran (WB), xylanase (XYL) and xylo-oligosaccharides (XOS) on ileal energy, protein, starch digestibility (%) in laying hens fed the dietary treatments for 56 d (at 47 wk of age).

		Ileal Digestibility (%)		
		Energy	Protein	Starch
WB (%)	10	65.33	59.56	99.20
	0	67.57	56.96	99.36
XOS (g/t)	0	67.40	53.98[b]	99.39
	50	64.10	58.79[ab]	99.30
	2000	67.85	62.02[a]	99.14
XYL (BXU/kg)	0	66.60	58.28	99.34
	16,000	66.30	58.24	99.22
SEM		0.637	0.915	0.038
P-value				
WB		0.875	0.298	0.310
XYL		0.875	0.984	0.441
XOS		0.214	0.038	0.405
WB x XOS		0.484	0.611	0.330
WB x XYL		0.546	0.152	0.886
XYL x XOS		0.381	0.840	0.786

The dietary treatments had no impact on ileal soluble NSP degradability ($P > 0.05$, data not shown). As highlighted in Figure 1, supplementation with 2,000 g/t induced increased insoluble NSP degradability in birds fed the diet with WB, but XOS supplementation had no impact in birds fed the control diet ($P = 0.043$). An interaction between WB, XOS, and XYL was observed on free oligosaccharide digestibility ($P < 0.001$). In birds fed WB, XOS had no impact in the absence of XYL, but when XYL was present free oligosaccharide digestibility was greater when feeding 50 g/t XOS compared to 0 or 2,000 g/t XOS. In the absence of WB, when XYL was present feeding 50 g/t XOS resulted in higher free oligosaccharide digestibility compared to feeding no XOS, but XOS had no impact on free oligosaccharide digestibility in the absence of XYL.

Cecal Xylanase and Cellulase Activity

Interactions between WB, XYL and XOS were observed on cecal xylanase and cellulase activity after 56 d on the dietary treatments ($P < 0.001$ for both), as shown in Table 10. When XYL was present, supplementation with 50 g/t XOS reduced xylanase activity compared to 0 or 2,000 g/t XOS, in birds fed both the control and WB diets. When XYL was absent, feeding 50 g/t XOS increased cecal xylanase activity compared to no XOS supplementation in birds fed both the control and WB diet, and induced higher xylanase activity compared to feeding 2,000 g/t XOS in birds fed the control diet.

In the of absence of XYL, feeding 50 g/t XOS resulted in higher cecal cellulase activity compared to feeding 0 or 2,000 g/t XOS, regardless of WB presence. In the presence of XYL, cecal cellulase activity was greater with 2,000 g/t XOS compared to no XOS when WB was fed but was higher with 50 g/t XOS compared to 0 and 2,000 g/t XOS in birds fed the control diet.

Cecal Short-Chain Fatty Acid Concentration

The dietary treatments had no impact on concentration of acetic, propionic, butyric, valeric, or succinic acid in the ceca after 56 d on the dietary treatments ($P > 0.005$), as illustrated in Table 11. Iso-butyric and iso-valeric acid concentration in the ceca were increased because of feeding WB ($P = 0.004$ and $P = 0.001$, respectively). Supplementation with XYL induced increased lactate concentration in the ceca ($P = 0.021$).

Cecal Microbiota Composition

Table 12 presents that the dietary treatments had no impact on total bacteria content in the ceca, or on cecal counts of *Bacillus, Bacteriodes, Ruminococcus*, or *lactobacillus,* after 56 d on the dietary treatments ($P > 0.05$). An interaction between XYL and XOS was observed on cecal *Bifidobacteria* concentration ($P = 0.006$), showing that XOS had no impact on *Bifidobacteria* content when XYL was fed, but in the absence of XYL *Bifidobacterium* concentration was lower when feeding 2,000 g/t XOS compared to feeding no XOS. Cecal *Enterobacteria* concentration was reduced by the presence of XYL ($P = 0.001$).

Ileal and Cecal XOS Concentration

As predicted, there was consistently substantially more XOS in the diets supplemented with 2,000 g/t XOS compared to 0 or 50 g/t, and XOS concentration was higher in the diets supplemented with 50 g/t XOS compared to 0 g/t, as presented in Table 3.

As illustrated in Table 13, XYL supplementation resulted in increased concentration of xylobiose ($P < 0.001$), xylotriose ($P < 0.001$), xylotetraose ($P < 0.001$), xylopentaose ($P = 0.010$) and total XOS (X_2–X_6) ($P < 0.001$) in the ileum in birds fed the diet with WB, but XYL had no impact on ileal XOS concentration in birds fed the control diet. Xylohexaose concentration in the ileum was increased by the presence of WB ($P < 0.001$) and XYL ($P = 0.009$).

No xylopentaose or xylohexaose was detected in the ceca. Table 14 presents that WB presence increased cecal xylobiose and total XOS (X_2–X_4) concentration. XYL supplementation increased cecal xylotriose concentration in birds fed the diets with WB but had no impact in birds fed the control diet ($P = 0.039$). Supplementation with 50 or 2,000 g/t XOS increased the concentration of xylotriose in the ceca of birds fed the diets supplemented with XYL, but XOS supplementation had no impact in the absence of XYL ($P = 0.020$). The dietary treatments had no impact on xylotetroase concentration in the

DISCUSSION

The aim of this study was to examine if it is possible to accelerate nutrient digestion, and thus increase productive performance, in laying hens by establishing xylan-degrading bacteria in the bird's microbiota, through supplementing the diets with XOS, XYL and WB. The diets with WB contained approximately 6 g/kg soluble

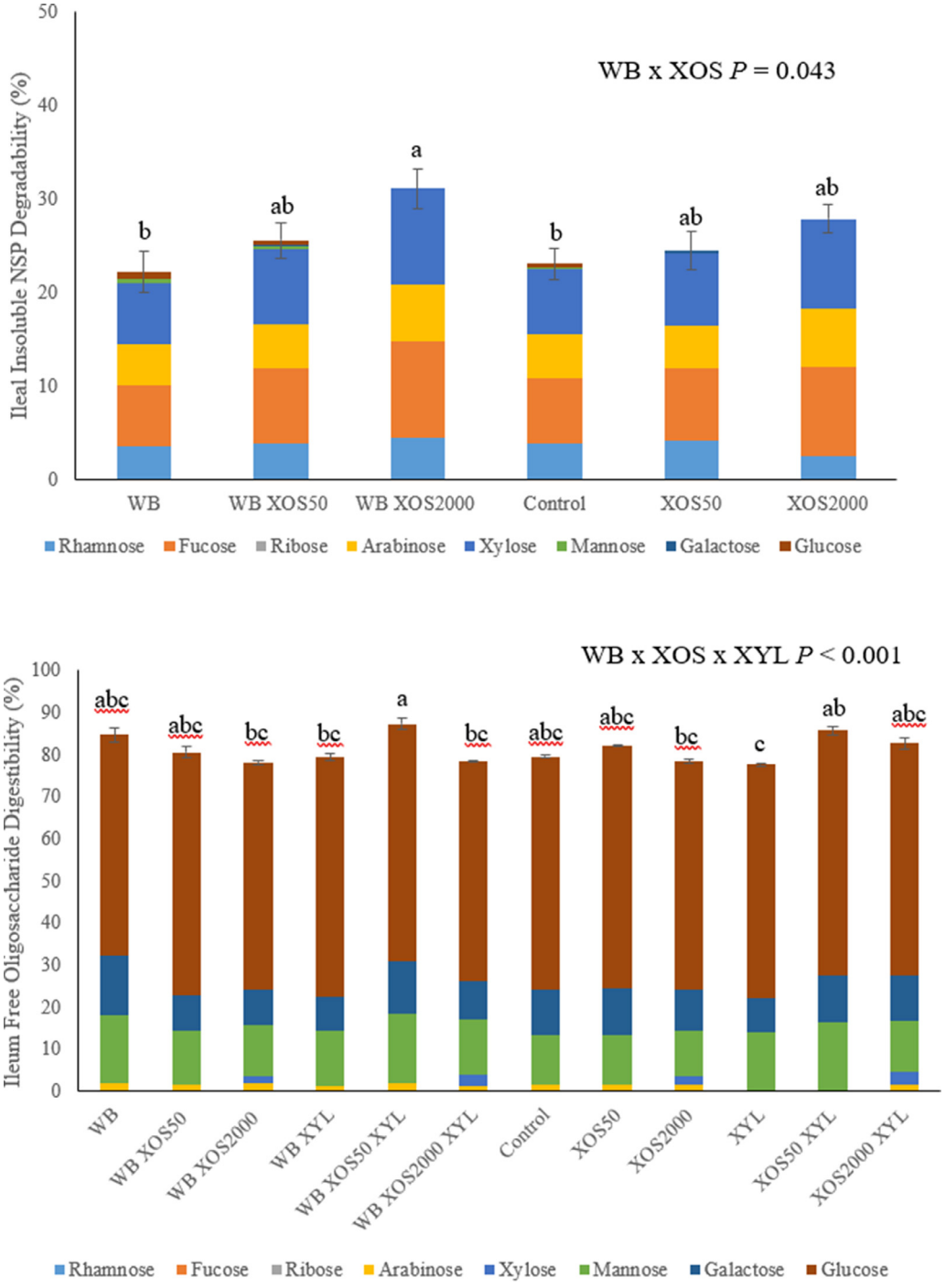

Figure 1. Effect of wheat bran (WB), xylanase (XYL) and xylo-oligosaccharides (XOS) on ileal insoluble nonstarch polysaccharide (NSP) degradability (%) and free oligosaccharide digestibility (%) in laying hens fed the dietary treatments for 56 d (at 47 wk of age).

arabinoxylan (**AX**) and 40 g/kg insoluble AX, compared to the control diet, which contained approximately 3 g/kg soluble AX and 30 g/kg insoluble AX. This confirms that the WB increased the concentration of xylan in the diet.

Effect of the Dietary Treatments on Nutrient Digestibility

Birds fed 2,000 g/t XOS presented improved ileal protein digestibility, likely a consequence of release of protein entrapped in xylan (Van Hoeck et al., 2021), through XOS stimulating xylan-degrading bacteria species. Additionally, providing fiber fractions as the primary source of fuel for the microbiota reduces competition between the host and microbiota for other valuable nutrients, such as amino acids. Other possible explanations are heightened endogenous protease activity (Van Hoeck et al., 2021), the ceca signaling to the gizzard to hold feed for longer (Rodrigues and Choct, 2018) or increased absorption area and epithelial cell turnover in the gastrointestinal tract (Ribeiro et al. 2018; Zhou et al. 2021) due to XOS stimulating proliferation of beneficial bacteria species.

Table 10. Effect of wheat bran (WB), xylanase (XYL) and xylo-oligosaccharides (XOS) on xylanase and cellulase activity in the ceca of laying hens fed the dietary treatments for 56 d (at 47 w of age).

WB (%)	XOS (g/t)	XYL (BXU/kg)	Xylanase (U/g)	Cellulase (U/g)
10	0	0	6.88[d]	16.48[f]
10	50	0	12.56[bc]	37.78[bc]
10	2000	0	8.16[cd]	24.36[def]
10	0	16,000	20.44[ab]	30.01[cde]
10	50	16,000	5.71[d]	41.69[abc]
10	2000	16,000	17.85[abc]	51.68[a]
0	0	0	5.96[d]	18.92[ef]
0	50	0	17.85[abc]	34.39[cd]
0	2000	0	5.96[d]	21.23[ef]
0	0	16,000	22.92[a]	18.92[ef]
0	50	16,000	9.05[cd]	46.74[ab]
0	2000	16,000	24.80[a]	16.02[f]
WB (%)	10		11.93	33.67
	0		14.42	26.04
XOS (g/t)	0		14.05	21.08
	50		11.29	40.15
	2000		14.19	28.32
XYL (BXU/kg)	0		9.56	25.53
	16,000		16.80	34.18
SEM			0.332	0.284
P-value				
WB			0.509	<0.001
XYL			0.820	<0.001
XOS			<0.001	<0.001
WB × XOS			<0.001	<0.001
WB × XYL			0.045	<0.001
XYL × XOS			0.022	0.354
WB × XYL × XOS			<0.001	<0.001

[a-f]Means within the same column, within the same parameter, with no common subscript, differ significantly ($P < 0.05$).

Feeding both 2,000 g/t XOS and WB increased ileal insoluble NSP degradability, suggesting this combination heightened accessibility of nutrients otherwise entrapped by insoluble NSP. This exhibits the benefits of providing both XOS to stimulate xylan-degrading bacteria species and fermentable fiber to fuel them. This may partly explain the beneficial effects observed on shell breaking strength at d56 when feeding combined 2,000 g/t XOS with WB, through increased protein available for eggshell membrane formation (Rose and Hincke, 2009). Feeding 2,000 g/t XOS had a negative impact on shell breaking strength at d56 in birds fed the control diet, indicating that feeding high XOS alone is not beneficial.

The lack of effect of the dietary treatments on ileal energy, starch and soluble NSP digestibility may be because of the short duration the diets were fed. Another explanation is the control diet was balanced and met the bird's nutritional requirements, so additional benefits could not be achieved.

Effect of the Dietary Treatments on Ceca Microbiota

Stimulating probiotic bacteria without providing substrates for them can induce detrimental effects on microbiota balance and thus cecal environmental conditions, potentially resulting in increased abundance of competing pathogenic bacteria species (Mikkelsen et al., 2004; Silversides et al., 2006; Teng and Kim, 2018; Jurburg et al., 2019; Khan et al., 2020). This was illustrated by the negative impact of feeding 2,000 g/t XOS in the absence of XYL on Bifidobacteria concentration in the ceca. In the presence of XYL there is sufficient fuel manufactured for the Bifidobacteria, allowing them to flourish in the ceca (Machado et al., 2020). The increased abundance of cecal *Bifidobacteria* potentially explains the observed heightened cellulase and xylanase activity. In the absence of XYL, feeding 50 g/t XOS was more beneficial than 2,000 g/t XOS at enhancing cecal xylanase and cellulase activity, but the opposite was true when XYL was present. This confirms that XYL should be present when feeding high levels of XOS and highlights the synergistic relationship between XYL and XOS. The reduction in xylotriose concentration in the ceca caused by feeding combined XOS and XYL possibly highlights that this size of XOS fraction was the most rapidly consumed by the bacteria.

Table 11. Effect of wheat bran (WB), xylanase (XYL) and xylo-oligosaccharides (XOS) on short chain fatty acid concentration in the ceca of laying hens fed the dietary treatments for 56 d (at 47 wk of age).

		Acetic	Propionic	Iso-Butyric	Butyric	Iso-Valeric	Valeric	Lactate	Succinic
WB (%)	10	36.81	15.59	0.55[a]	6.68	0.39[a]	0.83	0.31	0.45
	0	34.42	14.22	0.40[b]	5.39	0.25[b]	0.68	0.29	0.36
XOS (g/t)	0	36.96	16.15	0.46	6.50	0.31	0.73	0.33	0.32
	50	35.85	14.51	0.50	5.92	0.37	0.80	0.32	0.51
	2000	34.04	14.04	0.46	5.67	0.29	0.74	0.25	0.38
XYL (BXU/kg)	0	34.56	14.07	0.47	6.17	0.32	0.74	0.25[b]	0.36
	16,000	36.67	15.74	0.47	5.89	0.32	0.77	0.36[a]	0.45
SEM		0.829	0.449	0.014	0.230	0.010	0.022	0.011	0.031
P-value									
XYL		0.527	0.358	0.971	0.766	0.941	0.780	0.021	0.455
WB		0.475	0.449	0.004	0.169	0.001	0.122	0.575	0.501
XOS		0.770	0.606	0.677	0.754	0.241	0.801	0.288	0.438
WB × XOS		0.797	0.655	0.806	0.692	0.674	0.484	0.473	0.831
WB × XYL		0.956	0.446	0.341	0.808	0.405	0.868	0.467	0.511
XYL × XOS		0.433	0.300	0.531	0.144	0.851	0.367	0.437	0.920
WB × XYL × XOS		0.445	0.261	0.657	0.346	0.571	0.122	0.733	0.401

[a-b]Means within the same column, within the same parameter, with no common subscript, differ significantly ($P < 0.05$)

Table 12. Effect of wheat bran (WB), xylanase (XYL) and xylo-oligosaccharides (XOS) on microbiota concentration (log10(genomic DNA copy number)/g digesta) in the ceca of laying hens fed the dietary treatments for 56 d (at 47 wk of age).

XOS (g/t)	XYL (BXU/kg)	Total	Bacillus	Bacteriodes	Bifidobacteria	Ruminococcus	Lactobacillus	Enterobacteriaceae
0	0	11.51	8.19	10.43	9.32[a]	9.43	9.40	6.90
50	0	11.49	8.30	10.40	9.26[a]	9.36	9.55	7.59
2000	0	11.55	8.24	10.45	8.99[b]	9.37	9.38	7.24
0	16,000	11.53	8.25	10.47	9.14[ab]	9.30	9.41	6.60
50	16,000	11.53	8.24	10.43	9.08[ab]	9.40	9.45	6.33
2000	16,000	11.52	8.27	10.40	9.43[a]	9.42	9.55	5.66
WB (%)	10	11.52	8.20	10.45	9.28	9.35	9.43	6.76
	0	11.52	8.29	10.41	9.13	9.40	9.48	6.68
XOS	0	11.52	8.22	10.45	9.23	9.36	9.40	6.75
	50	11.51	8.27	10.42	9.17	9.38	9.50	6.96
	2000	11.53	8.25	10.42	9.21	9.39	9.46	6.45
XYL	0	11.51	8.24	10.43	9.19	9.38	9.44	7.24[a]
	16,000	11.52	8.25	10.43	9.21	9.37	9.47	6.19[b]
SEM		0.007	0.016	0.009	0.015	0.007	0.016	0.057
P-value								
WB		0.966	0.352	0.387	0.087	0.230	0.543	0.786
XYL		0.843	0.935	0.944	0.787	0.755	0.809	0.001
XOS		0.897	0.927	0.825	0.815	0.853	0.708	0.376
WB × XOS		0.592	0.831	0.646	0.999	0.523	0.896	0.179
WB × XYL		0.485	0.904	0.183	0.506	0.111	0.608	0.877
XYL × XOS		0.805	0.878	0.683	0.006	0.141	0.501	0.199
WB × XYL × XOS		0.403	0.831	0.788	0.835	0.552	0.539	0.701

[a-b]Means within the same column, within the same parameter, with no common subscript, differ significantly ($P < 0.05$)

XYL supplementation increased lactate and reduced *Enterobacteriaceae* concentration in the ceca. This lactate possibly induced a decrease in pH, which reduced the ability of acid-sensitive bacteria to prosper, thus stimulating growth of *Bifidobacteria,* encouraging the observed heightened endogenous xylanase and cellulase activity, and reducing *Enterobacteriaceae* counts, through being bacteriostatic and bactericidal (Dittoe et al., 2018). However, in the presence of XYL, feeding 50 g/t XOS had a negative impact on endogenous xylanase activity. A possible explanation is that in the birds fed 50 g/t XOS the amount of fuel generated by the XYL exceeded the number of xylan-utilizing bacteria present to consume it, resulting in an abundance of substrate available to fuel other bacteria species, which outcompeted the xylanase-producing species. Supplementation of 50 g/t appears to be the optimum for the cellulase-producing bacteria.

Effect of the Dietary Treatments on Bird Performance and Egg Quality

The lack of effect of the treatments on egg production may be because egg production was already high in birds fed the control diet, at 98%, so no additional benefits were achievable, or because of the short feeding period (8 wk) used in this study. Increased albumen height and darker yolk and shell color observed with XYL supplementation throughout the study

Table 13. Effect of wheat bran (WB), xylanase (XYL) and xylo-oligosaccharides (XOS) on XOS concentration (mg/g TiO_2) in the ileum of laying hens fed the dietary treatments for 56 d (at 47 wk of age).

WB (%)	XYL (BXU/kg)	Xylobiose (X_2)	Xylotriose (X_3)	Xylotetraose (X_4)	Xylopentaose (X_5)	Xylohexaose (X_6)	XOS Total (X_2-X_6)
10	0	17.91[b]	9.38[b]	4.10[b]	2.34[b]	1.45	35.18[b]
10	16,000	80.32[a]	50.06[a]	18.35[a]	6.19[a]	2.62	157.53[a]
0	0	27.21[b]	10.42[b]	2.59[b]	1.08[b]	0.45	41.76[b]
0	16,000	35.42[b]	18.27[b]	6.72[b]	2.36[b]	1.03	63.79[b]
WB (%)	10	49.11	29.72	11.23	4.26	2.03[a]	96.35
	0	31.32	14.35	4.65	1.72	0.74[b]	52.77
XOS (g/t)	0	33.72	20.64	7.39	2.68	1.23	65.66
	50	24.23	15.30	6.44	2.80	1.47	50.24
	2000	62.69	30.15	9.99	3.49	1.46	107.79
XYL (BXU/kg)	0	22.56	9.90	3.35	1.71	0.95[b]	38.47
	16,000	57.87	34.17	12.54	4.27	1.82[a]	110.66
SEM		0.959	0.478	0.162	0.071	0.047	1.590
P-value							
WB		0.003	<0.001	<0.001	<0.001	<0.001	<0.001
XYL		<0.001	<0.001	<0.001	<0.001	0.009	<0.001
XOS		<0.001	0.001	0.023	0.350	0.793	<0.001
WB × XOS		0.343	0.286	0.110	0.105	0.131	0.228
WB × XYL		<0.001	<0.001	<0.001	0.010	0.370	<0.001
XYL × XOS		0.394	0.260	0.161	0.223	0.512	0.304
WB × XYL × XOS		0.196	0.267	0.543	0.648	0.915	0.222

[a-b]Means within the same column, within the same parameter, with no common subscript, differ significantly ($P < 0.05$)

Table 14. Effect of wheat bran (WB), xylanase (XYL) and xylo-oligosaccharides (XOS) on XOS concentration (mg/g TiO_2) in the ceca of laying hens fed the dietary treatments for 56 d (at 47 wk of age).

WB (%)	XOS (g/t)	XYL (BXU/kg)	Xylobiose (X_2)	Xylotriose (X_3)	Xylotetraoase (X_4)	XOS Total (X_2-X_4)
10		0	32.32	7.82[b]	0.60	40.73
10		16,000	39.98	13.74[a]	0.21	53.93
0		0	24.45	9.37[ab]	0.77	34.59
0		16,000	25.06	7.17[b]	0.44	32.67
	0	0	30.18	5.63[b]	0.41	36.22
	50	0	26.29	9.44[ab]	0.29	36.01
	2000	0	28.70	10.71[ab]	1.37	40.77
	0	16,000	36.21	15.27[a]	0.32	51.80
	50	16,000	25.91	8.30[b]	0.66	34.87
	2000	16,000	35.45	7.80[b]	0.00	43.24
WB (%)	10		36.15[a]	10.78	0.41	47.33[a]
	0		24.76[b]	8.27	0.61	33.63[b]
XOS (g/t)	0		33.19	10.45	0.36	44.01
	50		26.10	8.87	0.47	35.44
	2000		32.07	9.25	0.68	42.00
XYL (BXU/kg)	0		28.38	8.59	0.69	37.66
	16,000		32.52	10.45	0.33	43.30
SEM			0.748	3.477	0.068	1.055
P-value						
WB			0.007	0.199	0.608	0.014
XYL			0.315	0.340	0.354	0.304
XOS			0.319	0.785	0.791	0.410
WB × XOS			0.998	0.457	0.341	0.875
WB × XYL			0.391	0.039	0.942	0.169
XYL × XOS			0.737	0.020	0.171	0.423
WB × XYL × XOS			0.160	0.087	0.391	0.070

[a-b]Means within the same column, within the same parameter, with no common subscript, differ significantly ($P < 0.05$)

presents elimination of the anti-nutritional effects of xylan on nutrient availability, increasing ability of pigment to be absorbed and used in egg formation (Mahmood and Guo, 2020). The improved albumen height observed with XYL agrees with Lei et al. (2018) and Silversides et al. (2006), but contrasts with Cufadar et al. (2010) and Scheideler et al. (2005) who saw no impact of xylanase on albumen height. This discrepancy may be because the diets used in these two studies were corn-based, so had less xylan substrate for xylanase to work on compared to the wheat-based diet used in this study.

A lower concentration of total XOS in the ceca was observed in the absence of WB, which may explain why eggs were lighter in birds fed the control diet, highlighting the importance of providing fermentable fiber in laying hen diets. Feeding combined WB, 2,000 g/t XOS and XYL resulted in notably lighter eggs at d14 compared to feeding WB and 2,000 g/t XOS. This may be because the XYL increased presence of soluble NSP, through solubilization of insoluble NSP, which increased digesta viscosity, reducing ability of nutrients to be absorbed (Nguyen et al., 2021a; Jha and Mishra, 2021). It should be noted that the control diet used in this study contained wheat, which is conducive to producing soluble xylan. Egg weight at d14 and free oligosaccharide digestibility at d56 was low in birds fed XYL without WB or XOS, reiterating that XYL can induce negative impacts if there is not adequate stimulation of probiotic hosts to exploit the XOS manufactured by XYL. This endorses that efficacy of XYL is dictated by the presence and physiochemical properties of the dietary xylan (Bedford, 2018; Choct et al., 2004).

Feeding 2,000 g/t XOS resulted in eggshell color becoming darker in birds fed WB, but lighter in birds fed the control diet. Hooge (2007) reported that some beneficial probiotic bacteria species improve shell color, reiterating that feeding combined WB and 2000 g/t XOS stimulates probiotic bacteria proliferation. This combination may also increase digestibility of elements such as iron, copper and zinc, which function as chelating carriers in porphyrin molecules, the main eggshell pigment in brown eggs (Samiullah et al., 2015). In contrast, reduced yolk color observed at d14, 42, and 56 in this study with WB application indicates that replacing a portion of the diet with WB diluted the pigment in the diet.

CONCLUSION

Results from this study highlight the importance of providing laying hens with sufficient dietary fermentable fiber, to fuel beneficial microbiota species and reduce competition between the host and microbiota for valuable nutrients. It also suggests that supplementing laying hen diets with a combination of XYL and XOS can be used as a tool to manipulate the microbiota to be more proficient at utilizing dietary xylan, reducing its anti-nutritional effects, and increasing nutrient utilization, productive performance, and gastrointestinal health.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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