Comparing the efficacy of stimbiotic and a combination of xylanase and beta-glucanase, in broilers fed wheat-barley based diets with high or low AME

N. K. Morgan,*[,1] G. A. Gomes,[†] and J. C. Kim [†]

[*]*Department of Animal Science, University of New England, Armidale, NSW 2350, Australia; and* [†]*AB Vista, Marlborough, Wiltshire SN48 4AN, UK*

ABSTRACT A stimbiotic is defined as a product that stimulates a fiber-degrading microbiome to increase fiber fermentability. The aim of this study was to examine if it is more advantageous to feed a stimbiotic (xylanase + xylo-oligosaccharides [**STB**]) or a combination of xylanase and beta-glucanase (**Xyl + BG**) to broilers fed wheat-barley based diets with differing AME levels. Cobb 500 broilers (n = 480, 80 birds per treatment) were fed 6 dietary treatments in a 2 × 3 factorial arrangement; 2 AME levels, 'High' or 'Low', which differed by 100 kcal ME/kg, and 3 additive supplementations, with no supplemental additives, STB or Xyl + BG. Diets were fed as 3 phases, starter (d 0−14), grower (d 14−21) and finisher (d 21−35). On bird age d 14, 21 and 35, total pen body weight and feed intake were determined, and feed conversion ratio corrected for mortality (**cFCR**) was calculated. On d 21 and d 35 ileal viscosity and beta-glucan content and caecal SCFA concentration were determined. Additive suplementation had no impact on cFCR in birds fed the low AME diet, but in birds fed the high AME diet the cFCR value was reduced in the presence of the additives (P = 0.001 and P = 0.015, at d 14−21 and d 21−35, respectively). At d 21, cecal SCFA concentration was consistently higher (P = 0.015), and ileal beta-glucan level lower (P = 0.002), in birds fed the diet supplemented with STB compared to those without additives. At d 35, ileal viscosity was lower in birds fed STB compared to those fed the diet without supplementation of additives, irrespective of diet AME level (P = 0.017). These results suggest that both STB and Xyl + BG ameliorate the antinutritive effects of the non-starch polysaccharides (**NSP**) present in wheat-barley based diets, resulting in improved bird performance. However, supplementation with STB induces a comparatively greater positive effect on NSP hydrolysis and SCFA production.

Key words: beta-glucanase, short chain fatty acid, viscosity, xylanase, xylo-oligosaccharide

2021 Poultry Science 100:101383
https://doi.org/10.1016/j.psj.2021.101383

INTRODUCTION

It is well established that the presence of non-starch polysaccharides (**NSP**), such as arabinoxylans and beta-glucans, in feed ingredients fed to poultry induce negative effects on nutrient utilisation and growth performance. Of particular concern is the high molecular weight, water-soluble NSP fraction, due to its ability to increase digesta viscosity. This has detrimental effects on digestion and absorption processes, and causes increased incidences of sticky droppings and poor litter quality (Munyaka et al., 2016). As a consequence, feed supplementation with NSP-degrading enzymes has become common practice in poultry diets, due to their ability to improve nutrient utilization (Jozefiak et al., 2010; Mendes et al., 2013; Kiarie et al., 2014).

Wheat is a dominant grain used worldwide in poultry diets, and xylanase application is ubiquitous in wheat-based diets, to overcome the issues associated with its high arabinoxylan content. However, reduced availability and increased cost of wheat put pressure on the poultry feed industry to seek alternative, cost-effective feeding ingredients. One such alternative is barley, but its use has been limited due to negative effects seen on bird performance, thought to be a consequence of its high beta-glucan level (Jacob and Pescatore, 2014). Consequently, many nutritionists believe it is necessary to supplement beta-glucanases into poultry diets containing barley.

Arabinoxylans are the predominant NSP in wheat and barley (Izydorczyk and Dexter, 2008). Xylanases cleave the internal glycosidic linkages in xylan, producing short-chain xylo-oligosaccharides (**XOS**) (Jommuengbout et al., 2009). These XOS can be utilised more efficiently by gut microbiota, having a direct

Received May 5, 2021.
Accepted July 4, 2021.

[1]Corresponding author: nmorga20@une.edu.au

impact on the overall energy utilisation of cereals. Selective fermentation of XOS by intestinal bacteria positively influences the composition and activity of the gastrointestinal microbiota, improving health and performance in the host, meaning XOS fulfil the definition of a prebiotic. Fermentation of XOS by these bacteria results in the production of short chain fatty acids (**SCFA**), which act as a source of energy (De Maesschalck et al., 2015). Accordingly, there is recent evidence presenting that supplementing broiler diets with XOS has beneficial effects on bird performance and dietary energy utilization (Morgan et al., 2019; Bautil et al., 2020).

Gonzalez et al. (2019) recently proposed a new category of feed additives, stimbiotics, which are defined as products that are able to stimulate a fiber-degrading microbiome to increase fiber fermentability, at doses which are too low to contribute in a meaningful manner to increased SCFA content. Stimbiotics are able to hydrolyze arabinoxylans, while also providing short oligossacharides. They therefore have dual purpose, reducing the antinutritive effects of NSP, especially AX, while also priming the microbiota towards the production of more SCFA. This suggests that stimbiotic supplementation may improve the fermentability of the NSP present in a wheat/barley diet, overcoming the need to supply a beta-glucanase.

The aim of this study was to examine if it is more advantageous to feed a stimbiotic (combined xylanase and XOS) (**STB**) or a combination of xylanase and beta-glucanase (**Xyl + BG**) to broilers fed wheat-barley based diets. The diets were formulated to have either a high or low AME level, with the intention of assessing whether reduction in performance induced by reduced AME can be recovered by supplementation with STB or Xyl + BG, through increasing energy extraction by increasing fermentation. The hypothesis of this study was that both supplements would ameliorate the negative effects of the NSP in wheat and barley, but supplementation of stimbiotic would be superior at promoting fiber fermentation in the gastrointestinal tract, through providing additional substrates for beneficial xylan-degrading bacteria, and therefore energy extraction from the feed.

MATERIALS AND METHODS

Animals and Housing

Cobb 500 mixed sex broilers ($n = 480$) were obtained from a local commercial hatchery on the day of hatch. Upon arrival, birds were weighed and randomly distributed into 48 floor pens (120 cm length × 77 cm width), with 10 birds per pen, bedded on clean wood shavings. The room was thermostatically controlled to produce an initial temperature of 32°C, gradually reduced to 21°C by d 21, and maintained until d35. The lighting regimen used was 23 h of light at approximately 40 lux on d 1, with darkness increasing 1 h a day until 6 hours of darkness was reached, and then 18 h of light per day at 10 lux was maintained for the remainder of the study. Feed and water were provided ad libitum throughout the trial period. The diets were fed as starter from d 0 to 14, grower from d 14 to 21 and finisher from d 21 to 35. The diets were cold pelleted and fed as a crumble (⌀0.1−0.2 mm) from d 0 to 7 and then pellet (⌀3-mm pellet) for the remainder of the trial period. The experimental procedures were approved by the University of New England Animal Ethics Committee, Australia (Approval number: AEC20-048).

Experimental Design and Diets

A 2 × 3 factorial arrangement of treatments was applied; the factors were AME level, 'High' (Starter 3000, Grower 3050 and Finisher 3100 kcal ME/kg) or 'Low' (Starter 2900, Grower 2950 and Finisher 3000 kcal ME/kg); and additive supplement, no additive, stimbiotic (endo-1,4-β-xylanase produced from *Trichoderma reesei* + fermentable XOS) (Signis, AB Vista, Marlborough, UK) (STB) or endo-1,4-β-xylanase + endo-1,3(4)-beta-glucanase produced by *Trichoderma reesei* (Axtra XB, Danisco Animal Nutrition, Marlborough, UK) (Xyl + BG). The experimental diets were formulated to meet or exceed the nutritional requirements for Cobb 500 broilers. The diet formulations are presented in Table 1.

Prior to feed formulation, ingredients were ground through a 0.5-mm screen and the nutrient concentration analyzed by near-infrared spectroscopy to predict proximates, dAA concentrations and AME using AMINONIRPROX, AMINONIRNIR, and AMINONIRNRG (Evonik Nutrition & Care, Hanua, DE). The soluble and insoluble NSP and free oligosaccharide concentration were determined in both the feed ingredients prior to formulation and in the final diets, by measuring the constituent sugar components as alditol acetates by gas-liquid chromatography (Model CP3800, Varian Inc., Palo Alto, CA). This followed 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 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 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,

Table 1. Ingredient composition of experimental diets (g/100g, as fed basis).

	High AME			Low AME		
Ingredient	Starter	Grower	Finisher	Starter	Grower	Finisher
Wheat 11.5%	28.36	33.43	36.53	31.33	35.53	38.54
Barley	30.00	30.00	30.00	30.00	30.00	30.00
SBM	32.40	27.70	24.60	31.20	27.40	24.30
Canola oil	3.80	3.90	4.20	2.00	2.10	2.50
Limestone	0.50	0.30	0.10	0.50	0.30	0.10
MBM	3.00	3.00	3.00	3.00	3.00	3.00
L-Lysine HCl	0.25	0.26	0.25	0.28	0.26	0.25
DL methionine	0.26	0.25	0.26	0.26	0.25	0.26
L-threonine	0.16	0.15	0.13	0.17	0.15	0.13
L-valine	0.00	0.00	0.00	0.00	0.00	0.00
Salt	0.36	0.20	0.15	0.35	0.20	0.14
Sodium bicarbonate	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride	0.08	0.03	0.00	0.08	0.03	0.00
Quantum blue 5G	0.01	0.01	0.01	0.01	0.01	0.01
Vitamin premix[1] 0.5 kg/MT	0.07	0.05	0.05	0.07	0.05	0.05
Mineral premix[2] 0.75 KG/MT	0.10	0.07	0.07	0.10	0.07	0.07
Titanium dioxide	0.50	0.50	0.50	0.50	0.50	0.50
Salinomycin	0.05	0.05	0.05	0.05	0.05	0.05

[1]Vitamin premix per kg diet: vitamin A, 12 MIU; vitamin D, 5 MIU; vitamin E, 75 mg; vitamin K, 3 mg; nicotinic acid, 55 mg; pantothenic acid, 13 mg; folic acid, 2mg; riboflavin, 8 mg; cyanocobalamin, 0.016 mg; biotin, 0.25 mg; pyridoxine, 5mg; thiamine, 3mg; antioxidant, 50 mg.

[2]Trace mineral concentrate supplied per kg diet: Cu (sulfate), 16 mg; Fe (sulfate), 40 mg; I (iodide), 1.25 mg; Se (selenate), 0.3 mg; Mn (sulfate and oxide), 120 mg; Zn (sulfate and oxide), 100 mg; cereal-based carrier, 128 mg; mineral oil, 3.75 mg.

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 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 redissolved 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. 1-methylimidazole and 5 mL of $C_4H_6O_3$ were 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 were added, the sample was centrifuged, and the supernatant was analyzed by gas chromatography (Model CP3800, Varian Inc., Palo Alto, CA).

Dry matter content of the diets was determined by weighing the fresh sample, oven drying at 105°C until constant weight and then reweighing the sample and calculating % dry matter content. Crude protein was determined in the diets as nitrogen (**N**) using the combustion method (LECO Corp. FP-2000N analyser, St. Joeseph, MI), and multiplying this value by a factor of 6.25. Diet gross energy contents were measured using an isoperibolic bomb calorimeter (Model 6400, Parr Instruments, Moline, IL), standardized with benzoic acid. The analyzed nutrient composition of the test diets is presented in Table 2, and analyzed constituent soluble and insoluble sugar composition is shown in Table 3.

Response Variables

Bird mortality and weight of dead birds was recorded throughout the trial period. Total pen weight and feed intake (**FI**) were determined on d 14, 21, and 35 post-hatch, and, used to calculate feed conversion ratio

Table 2. Nutrient composition of experimental diets (as fed basis).

AME level	Dry matter (g/100g)	Protein (g/100g)	Energy (MJ/kg)	Soluble NSP (g/kg)	Insoluble NSP (g/kg)	Free OS (g/kg)
Starter						
High	88.84	24.52	17.24	19.88	56.47	45.56
Low	89.50	25.39	16.93	21.37	67.67	46.87
Grower						
High	88.60	22.90	16.94	20.87	68.20	39.04
Low	88.25	22.27	16.64	25.47	68.24	44.05
Finisher						
High	88.03	21.47	17.02	26.16	69.96	39.56
Low	88.16	21.61	16.68	27.63	71.89	42.25
Dietary ingredient						
Wheat	90.07	11.53	16.82	25.61	65.96	20.68
Barley	89.68	12.07	16.43	31.78	75.73	21.55
SBM	89.14	47.53	17.37	7.30	89.46	90.31

Table 3. Soluble and insoluble monomer composition of dietary treatments (g/kg).

	Rhamnose		Fucose		Ribose		Arabinose		Xylose		Mannose		Galactose		Glucose	
AME Level	sNSP	iNSP	sNSP	iNSP	sNSP	iNSP	sNSP	iNSP	sNSP	iNSP	sNSP	iNSP	sNSP	iNSP	sNSP	iNSP
Starter																
High	0.04	0.28	0.04	0.59	0.46	0.28	2.82	14.69	3.65	17.81	1.10	1.80	2.00	9.29	12.13	18.75
Low	0.04	0.34	0.05	0.75	0.47	0.30	3.84	17.20	4.47	21.13	1.03	1.94	1.83	10.72	12.21	23.67
Grower																
High	0.04	0.34	0.04	0.69	0.45	0.30	3.26	17.91	3.70	21.73	1.20	2.15	2.09	10.61	12.57	22.93
Low	0.04	0.35	0.05	0.71	0.46	0.31	4.15	17.76	5.19	21.56	1.28	1.96	2.26	11.17	15.07	22.89
Finisher																
High	0.05	0.37	0.04	0.77	0.44	0.33	2.96	18.84	3.62	22.69	1.80	2.08	2.14	11.29	18.18	22.32
Low	0.05	0.37	0.04	0.75	0.43	0.33	3.15	18.88	4.45	23.35	1.75	2.16	2.18	11.78	18.84	23.21

corrected for mortality (**cFCR**), accounting for weight of dead birds. On d 35, litter samples were collected from 5 different points per pen and placed in a sealed plastic bag. Dry matter content was determined in the entire litter sample, using the method described above.

On d 21 and 35, two birds per pen were euthanized. The ileal and cecal digesta samples were collected, pooled per pen and homogenised. For the ileum, fresh subsamples were collected for analysis of viscosity, and the remainder was frozen at −20°C, freeze-dried to constant weight and ground through a 0.5 mm screen. For viscosity analysis, the ileal digesta samples were collected into 2 mL Eppendorf tubes, which were then centrifuged at 10,000 × *g* for 10 min at room temperature. Viscosity was then measured in 0.5 mL of the resulting supernatant, using a Brookfield DV3T Rheometer (Brookfield Ametek, Instrumentation & Specialty Controls Division, Middleboro, MA), with CPA-40Z Spindle, at 35°C. Viscosity measurements were expressed in centipoise (**cPs**) unit (1 cPs = 1/100 dyne sec/cm^2 = 1 mPa.s) prior to statistical analysis. Measurements were collected at 0.5, 1, 5, and 10 revolutions per minute. Beta-glucan concentration was measured in the dried ileum samples using the Megazyme Mixed-Linkage Beta-Glucan assay kit (**K-BGLU**; Megazyme, Wicklow, Ireland, UK), analyzed on 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).

SCFA concentrations were measured in the cecal digesta using the method described by Jensen et al. (1995), with some modifications. Briefly, approximately 2 g of fresh homogenized cecal digesta sample, maintained at approximately 5°C, was homogenised with 1 mL of internal standard (0.01 mol/L ethylbutyric acid). The samples were then centrifuged at 3,900 × *g* for 20 min at 5°C, and 1 mL of resulting supernatant was mixed with 0.5 mL concentrated HCl (36%) and 2.5 mL of ether. The sample was then centrifuged at 2,000 × *g* for 15 min at 5°C, and 400 μL of the resulting supernatant was transferred into a GC vial. Following this, 40 μL of N-tert-butyldimethlsilyl-N-methyltrifuoroacetamide (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). The total SCFA concentration was derived from the sum of all the individual SCFAs in the sample, expressed as μmol/g digesta.

Statistical Analysis

All data were analyzed using JMP Pro version 15.2. Pen represented the replicate unit for statistical analysis. After Shapiro-Wilk testing to confirm normality, Standard Least Square Means Platform was used to evaluate the impact of dietary AME level and additive supplementation on the measured parameters, with pen start weight as a co-variate and block effect as a random variable. The model accounted for main effects and its interactions (AME, Additive, and AME × Additive). Means were separated using *t*test at an alpha level of 0.05. Outliers were excluded based on studentised residuals (>3 residuals or <-3 residuals); there was one outlier. Livability was analyzed using Chi-Square test. Correlations between the measured parameters were investigated using Pearson product-moment correlation coefficient. Statistical significance was declared at $P < 0.05$.

RESULTS

Performance and Litter DM

The impact of the dietary treatments on bird performance at each dietary phase (d 0−14, d 13−21, and d 21−35), as well as litter DM content at d 35, is presented in Table 4. There was 7.2% mortality across the entire study period. At d 0 to 14, birds fed the high AME diet presented higher BWG compared to those fed the low AME diet ($P < 0.001$). At d 14 to 21, birds fed the high AME diet supplemented with Xyl + BG had greater BWG compared to those fed the low AME diet with Xyl + BG, or high AME diet without additives ($P = 0.003$). At this age, birds fed the high AME diet without additives presented a higher cFCR value compared to those fed any other treatment, and the cFCR value was higher in birds fed the high AME diet supplemented with STB compared to those fed the high AME diet with Xyl + BG or low AME diet without additives ($P = 0.001$). At d 21 to 35, cFCR value was higher in birds fed the high AME diet without additives compared to those fed the high AME diet supplemented with either STB or Xyl + BG, or the low AME diet

Table 4. Effect of dietary AME level and additive supplementation (stimbiotic [STB] or xylanase + beta-glucanase [Xyl + BG]) on individual feed intake (FI), body weight gain (BWG) and feed conversion ratio corrected for mortality (cFCR) at each dietary phase and litter dry matter content (%) at d35.

		d 0–14			d 14–21			d 21–35			d 35
AME	Additive	FI (g)	BWG (g)	cFCR	FI (g)	BWG (g)	cFCR	FI (g)	BWG (g)	cFCR	Litter DM (%)
High	0	461	421	1.10	746	500[c]	1.46[a]	2290	1370	1.67[a]	76.64
	STB	463	428	1.08	742	527[abc]	1.41[b]	2297	1441	1.55[bc]	76.06
	Xyl + BG	446	420	1.08	757	556[a]	1.36[c]	2249	1456	1.54[c]	74.81
Low	0	427	382	1.12	739	545[ab]	1.36[c]	2450	1519	1.61[ab]	72.46
	STB	436	399	1.09	750	537[ab]	1.40[bc]	2279	1432	1.60[bc]	71.24
	Xyl + BG	449	411	1.09	754	525[bc]	1.40[bc]	2331	1463	1.61[ab]	73.26
SEM		8.88	7.26	0.01	12.55	11.38	0.01	46.50	40.28	0.03	2.73
AME											
High		457	423[a]	1.09	749	527	1.41	2279	1422	1.59	75.84
Low		437	397[b]	1.10	748	536	1.39	2353	1471	1.61	72.32
SEM		5.40	4.42	0.00	8.40	7.80	0.01	25.98	23.60	0.02	1.72
Additive											
0		444	401	1.11	742	522	1.41	2370	1444	1.64	74.55
STB		450	414	1.09	746	532	1.40	2288	1437	1.57	73.65
Xyl + BG		447	415	1.09	756	540	1.38	2290	1460	1.58	74.04
SEM		6.44	5.26	0.01	9.59	8.82	0.01	32.26	28.65	0.02	2.02
P-values											
AME		0.010	<0.001	0.083	0.929	0.345	0.054	0.064	0.146	0.248	0.114
Additive		0.816	0.107	0.051	0.509	0.231	0.179	0.157	0.846	0.009	0.939
AME × Additive	0.091	0.125	0.657	0.799	0.003	0.001	0.187	0.108	0.015	0.805	

[a-b]Mean values within a main effect not sharing a common letter are different ($P \leq 0.05$).

supplemented with STB ($P = 0.015$). The dietary treatments had no impact on litter quality at d 35.

Ileal Digesta Viscosity and Beta-Glucan Concentration

Table 5 presents the impact of the dietary treatments on ileal digesta viscosity and beta-glucan concentration. At d 21, ileal viscosity was higher in birds fed the high AME diet compared to the low AME diet ($P < 0.001$), and was lower in birds fed the diets supplemented with any of the additives tested compared to those without them ($P = 0.001$). At d 35, birds fed the diets supplemented with STB, or the high AME diet supplemented with Xyl + BG, presented lower ileal viscosity compared to those fed any other treatment. Also, birds fed the low AME diet with Xyl + BG presented lower ileal viscosity compared to those fed the high AME diet without additives ($P = 0.017$).

At d 21, an interaction between AME level and additive supplementation revealed that birds fed the high AME diet without additives presented a higher ileal beta-glucan level compared to those fed any other treatment. Additionally at this age, in birds fed the low AME diet, beta-glucan concentration was lower when feeding STB compared to when feeding the diet without supplemental enzymes ($P = 0.002$). At d 35, ileal beta-glucan concentration was higher when feeding the high AME diet compared to the low AME diet ($P = 0.015$), and was lower when feeding the diets supplemented with either additive treatment compared to the diets without additive supplementation ($P = 0.029$).

Table 5. Effect of dietary AME level and additive suplementation (stimbiotic [STB] or xylanase + beta-glucanase [Xyl + BG]) on ileal digesta viscosity (cP) and beta-glucan concentration (% DM) at d 21 and d35.

		Viscosity (cP)		Beta-Glucan (% DM)	
AME	Additive	d 21	d 35	d 21	d 35
High	0	4.50	4.21[a]	24.95[a]	21.84
	STB	2.77	2.63[c]	18.79[bc]	18.97
	Xyl + BG	2.91	2.80[c]	17.90[bc]	21.08
Low	0	3.42	3.68[ab]	19.62[b]	21.77
	STB	2.52	2.82[c]	17.17[c]	17.04
	Xyl + BG	2.60	3.36[b]	18.98[bc]	17.84
SEM		0.19	0.18	0.85	0.88
AME					
High		3.39[a]	3.22	20.55	20.63[a]
Low		2.85[b]	3.29	18.59	18.88[b]
SEM		0.11	0.10	0.54	0.43
Additive					
0		3.96[a]	3.95	22.29	21.81[a]
STB		2.64[b]	2.73	17.98	18.00[b]
Xyl + BG		2.75[b]	3.08	18.44	19.46[b]
SEM		0.13	0.12	0.63	0.58
P-values					
AME		<0.001	<0.001	<0.001	0.015
Additive		0.001	0.633	0.006	0.029
AME × Additive	0.060	0.017	0.002	0.236	

[a-b]Mean values within a main effect not sharing a common letter are different ($P \leq 0.05$).

Cecal SCFA Concentration

As illustrated in Table 6, at d 21 total SCFA concentration in the ceca was higher in birds fed the diets supplemented with STB compared to those fed the diets without supplemental additives ($P = 0.015$), but the dietary treatments had no impact on total SCFA concentration at d 35. Additionally, acetic, propionic, and valeric acid concentration in the ceca at d 21 was greater in birds fed the diet with STB compared to those fed either the diet without additives or with Xyl + BG ($P = 0.007$, $P = 0.007$ and $P = 0.012$, respectively). At d

Table 6. Effect of dietary AME level and additive supplementation (stimbiotic [STB] or xylanase + beta-glucanase [Xyl + BG]) on cecal short chain fatty acid (SCFA) concentration at d 21 and d35 (μmol/g fresh sample).

		Total SCFA		d21			d35
AME	Additive	d 21	d 35	Acetic acid	Propionic acid	Valeric acid	Succinic acid
High	0	62.68	72.37	35.07	2.85	0.49	5.91
	STB	82.26	95.24	46.84	3.92	0.67	13.99
	Xyl + BG	68.20	86.96	34.73	2.52	0.37	10.72
Low	0	43.84	71.04	27.29	2.43	0.40	7.58
	STB	82.44	87.53	51.58	4.06	0.58	12.61
	Xyl + BG	68.81	77.82	37.88	2.20	0.22	9.93
SEM[1]		10.25	10.85	6.42	0.54	0.12	2.48
AME							
High		71.05	84.86	38.88	3.10	0.51	10.21
Low		65.03	78.80	38.92	2.90	0.40	10.04
SEM[1]		7.09	5.98	4.73	0.36	0.09	1.84
Additive							
0		53.26[b]	71.71	31.18[b]	2.64[b]	0.45[ab]	6.75[b]
STB		82.35[a]	91.39	49.21[a]	3.99[a]	0.62[a]	13.30[a]
Xyl + BG		68.50[ab]	82.39	36.31[b]	2.36[b]	0.29[b]	10.32[ab]
SEM[1]		8.01	7.25	5.21	0.42	0.10	2.02
P-values							
AME		0.424	0.509	0.993	0.621	0.183	0.923
Additive		0.015	0.235	0.007	0.007	0.012	0.013
AME × Additive	0.475	0.929	0.450	0.828	0.929	0.733	

[a-b]Means within a main effect or interaction not sharing a common letter are different ($P \leq 0.05$).
[1]Pooled standard error of mean.

35, succinic acid concentration was greater in birds fed STB compared to those fed the diets without enzyme supplementation ($P = 0.013$).

DISCUSSION

The current lack of consideration of the NSP content and composition in feed ingredients during the formulation of diets for poultry is of concern, given vast evidence presenting its direct influence on bird performance, litter quality, and gut health (Morgan et al., 2021). This is reiterated by the ubiquitous application of xylanase in commercial poultry diets, and beta-glucanase in barley-based diets, despite inconsistencies in response to these enzymes, coupled with a lack of knowledge about the level and accessibility of target arabinoxylans and beta-glucans in complete poultry diets. This suggests that a deeper understanding of the efficacy and mechanisms of these enzymes is required to ensure their application is economically and environmentally beneficial to the poultry industry. Furthermore, it has recently been established that the positive effects observed as a consequence of xylanase application are not only attributable to its ability to reduce digesta viscosity and release entrapped nutrients, but also a consequence of the generation of prebiotic XOS (Ribeiro et al., 2018; Bautil et al., 2020). Subsequently, there has been recent interest in directly feeding XOS, as opposed to relying on the bird to produce them in situ. Using XOS as complementary supplement with xylanase has also shown promise; presence of additional XOS increases the amount of fuel available for beneficial microbiota, heigntening ability to ferment dietary fiber (stimbiotic effect) (Morgan et al., 2019; Craig et al., 2020). Thus, the aim of this study was to examine if it was more efficacious to feed stimbiotic or a combination of xylanase and beta-glucanase in wheat and barley-based diets. The ability of these supplements to enhance energy utilization, through increased fiber fermentation, and how this translates into effects on performance and the gastrointestinal environment was also assessed, through feeding diets with differing AME levels.

Barley use in poultry diets remains low, primarily due to concerns about its high NSP content, particularly beta-glucans (Jacob and Pescatore, 2014). However, these diets contained 30% barley and performance values were not dissimilar to those seen on commercial farms, particularly in birds fed the enzyme supplements. It is arabinoxylans (**AX**), as opposed to beta-glucans, that are generally the predominant NSP in barley; for example, the barley used in this study had an analyzed total AX content of 5.9%, compared with 2.7% analyzed beta-glucan content. The low AME diets in this study contained a notably higher soluble NSP concentration compared to the high AME diets, but ileal viscosity at d 21 was statistically higher in birds fed the high AME diet. This may be due to the comparatively higher AX level in the low AME diets, which meant birds fed these diets possessed a microbiota that was somewhat more adept at utilising soluble NSP, as highlighted by Bautil et al. (2019). The consequence of this heightened ileal viscosity was reduced BWG and poorer FCR at d 21 in the absence of additives. Another possible explanation is that the higher fat content in the high AME diet may have had a negative impact on viscosity and microbiota composition; soluble NSP reduces fat digestibility, especially in young birds with immature microbiota (Danicke et al., 1999; Jiménez-Moreno et al., 2009). However, this effect disappeared at d 35, as the difference in soluble NSP between the 2 diets was lessened in the finisher feeds, and the microbiota in older birds was

better adapted to utilizing dietary NSP. A number of factors could have contributed towards the lack of significant impact of the dietary treatments on litter quality and high variability in litter moisture between individual pens in this study, including pen placement within the room and number of birds per pen (due to the presence of spare birds or mortalities).

Of interest is the ability of XOS to stimulate xylan digestion and heighten development of a fiber-fermenting microbiome further, particularly in younger birds, thus enhancing the ability of adult birds to effectively utilize dietary fiber (Bautil et al., 2020). This was illustrated in this study by the lowest ileal beta-glucan concentration and viscosity at d 35 observed in birds fed the diets supplemented with STB, numerically lower than observed in those fed xylanase and beta-glucanase. The lack of significant difference in SCFA concentration between birds fed the diet without additives and the diet with Xyl + BG highlights that the oligosaccharides generated were not at sufficient levels or in the correct form to stimulate or fuel probiotic bacteria. However, the significant increase in total SCFA, acetic, propionic and valeric acid at d 21, and succinic acid at d 35, as a consequence of feeding STB compared to no additives highlights that STB supplementation was effectively and selectively boosting fermentation by beneficial microorganisms. This is in agreement with the work of Morgan et al. (2019) and Craig et al. (2020). The beneficial technological features of XOS include stability at acidic pH, heat resistance, ability to achieve significant biological effects at low daily doses, low calorie content, and no toxicity (Carvalho et al., 2013). However, the disparities in SCFA production between birds fed the 2 different supplements did not translate into differences in performance, suggesting the concentrations of SCFA were not sufficient to provide a notable source of energy.

In conclusion, this study confirms that feeding stimbiotic or a combination of xylanase and beta-glucanase is able to ameliorate the negative effects of the NSP in wheat and barley in the broiler gastrointestinal tract. However, it appears that STB may be superior to the enzyme combination, in that it resulted in comparatively greater SCFA production and beta-glucan hydrolysis, even without the inclusion of a beta-glucanase in the diet, and reduced digesta viscosity, through its ability to generate a fiber-fermenting microbiome. Further research is warranted into how to achieve the greatest economic and environmental benefits from STB application in meat chicken diets.

DISCLOSURES

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

REFERENCES

Bautil, A., J. Verspreet, J. Buyse, P. Goos, M. R. Bedford, and C. M. Courtin. 2019. Age-related arabinoxylan hydrolysis and fermentation in the gastrointestinal tract of broilers fed wheat-based diets. Poult. Sci. 98:4606–4621.

Bautil, A., J. Verspreet, J. Buyse, P. Goos, M. R. Bedford, and C. M. Courtin. 2020. Arabinoxylan-oligosacharides kick-start arabinoxylan digestion in the aging broiler. Poult. Sci. 99:2555–2565.

Carvalho, A. F. A., P. de Oliva Neto, D. Fernandes da Silva, and G. M. Pastore. 2013. Xylo-oligosaccharides from lignocellulosic materials: chemical structure, health benefits and production by chemical and enzymatic hydrolysis. Food Res. Int. 51:75–85.

Craig, A. D., F. Khattak, P. Hatie, M. R. Bedford, and O. A. Olukosi. 2020. Xylanase and xylo- oligosaccharide prebiotic improve the growth performance and concentration of potentially prebiotic oligosaccharides in the ileum of broiler chickens. Br. Poult. Sci. 61:70–78.

Danicke, S., W. Vahjen, O. Simon, and H. Jeroch. 1999. Effects of dietary fat type and xylanase supplementation to rye-based broiler diets on selected bacterial groups adhering to the intestinal epithelium. on transit time of feed, and on nutrient digestibility. Poult. Sci. 78:1292–1299.

De Maesschalck, C. D., V. Eeckhaut, L. Maertens, L. De Lange, L. Marchal, C. Nezer, S. De Baere, S. Croubels, G. Daube, J. Dewulf, F. Hasebrouck, R. Ducatelle, B. Taminau, and F. V. Immerseel. 2015. Effects of xylo-oligosaccharides on broiler chicken performance and microbiota. Appl. Enviro. Microbiol. 81:5880.

Englyst, H. N., M. E. Quigley, and G. J. Hudson. 1994. Determination of dietary fibre as non-starch polysaccharides with gas−liquid chromatographic, high-performance liquid chromatographic or spectrophotometric measurement of constituent sugars. Analyst. 119:1497–1509.

Gonzalez-Ortiz, G., G. A. Gomes, T. T. dos Santos, and M. R. Bedford. 2019. New strategies influencing gut functionality and animal performance. Pages 233−254 in The Value of Fibre—Engaging the Second Brain for Animal Nutrition. Wageningen Academic Press, The Netherlands.

Izydorczyk, M. S., and J. E. Dexter. 2008. Barley β-glucans and arabinoxylans: molecular structure, physicochemical properties, and uses in food products−a review. Food Res. Int. 41:850–868.

Jacob, J., and A. J. Pescatore. 2014. Barley β-glucan in poultry diets. Ann. Trans. Med. 2:20.

Jensen, M. T., R. P. Cox, and B. B. Jensen. 1995. Microbial production of skatole in the hind gut of pigs fed different diets and its relation to skatole deposition in back fat. Anim. Sci. 16:293–304.

Jiménez-Moreno, E., J. M. González-Alvarado, A. González-Serrano, R. Lázaro, and G. G. Mateos. 2009. Effect of dietary fiber and fat on performance and digestive traits of broilers from one to twenty-one days of age. Poult. Sci. 88:2562–2574.

Jommuengbout, P, S. Pinitglang, K. L. Kyu, and K. Ratanakhanokchai. 2009. Substrate-binding site of family 11 xylanase from Bacillus firmus K-1 by molecular docking. Biosci. Biotech. Biochem. 73:833–839.

Jozefiak, D., A. Rutkowski, S. Kaczmarek, B. B. Jensen, R. M. Engberg, and O. Hojbjerg. 2010. Effect of β-glucanase and xylanase supplementation of barley- and rye-based diets on caecal micro-biota of broiler chickens. Br. Poult. Sci. 51:546–557.

Kiarie, E., L. F. Romero, and V. Ravindran. 2014. Growth performance, nutrient utilization, and digesta characteristics in broiler chickens fed corn or wheat diets without or with supplemental xylanase. Poult. Sci. 93:1186–1196.

Mendes, A. R., T. Ribeiro, B. A. Correia, P. Bule, B. Macas, L. Falcao, J. P. B. Freire, L. M. A. Ferreira, C. M. G. A. Fontes, and M. M. Lordelo. 2013. Low doses exogenous xylanase improve the nutritive value of triticale-based diets for broilers. J. Appl. Poult. Res. 22:92–99.

Morgan, N. K., M. Choct, M. Toghyani, and S-B. Wu. 2018. Pages 34-37 in effects of dietary insoluble and soluble non-starch polysaccharides on performance and ileal and excreta moisture contents in broilers. Proc. 29th Annu. Austr. Poult. Sci. Symp. Univ. Sydney, Sydney, Australia.

Morgan N., M. M. Bhuiyan, T. N. A. Nguyen, Middlebrook T. and R. Hopcroft. Dietary soluble non-starch polysaccharide level and composition influences grower and finisher phase performance, excreta moisture content and total tract nutrient digestibility in broilers [e-pub ahead of print]. Br. Poult. Sci. doi:10.1080/00071668.2021.1919995; Accessed August 3, 2021.

Morgan, N. K., C. Keerqin, A. Wallace, S.-B. Wu, and M. Choct. 2019. Effect of arabinoxylo-oligosaccharides and arabinoxylans on net energy and nutrient utilization in broilers. Anim. Nut. 5:56–62.

Munyaka, P. M., N. K. Nandha, E. Kiarie, C. M. Nyachoti, and E. Khafipour. 2016. Impact of combined β-glucanase and xylanase enzymes on growth performance, nutrients utilization and gut microbiota in broiler chickens fed corn or wheat-based diets. Poult. Sci. 95:528–540.

Ribeiro, T., V. Cardoso, L. M. A. Ferreira, M. M. S. Lordelo, A. S. P. Moreira E.Coelho, M. R. M Domingues, M. A. Coimbra, M. R. Bedford, and C. M. G. A. Fontes. 2018. Xylo-oligosaccharides display a prebiotic activity when used to supplement wheat or corn-based diets for broilers. Poult. Sci. 97:4330–4341.

Theander, O., P. Åman, E. Westerlund, R. Andersson, and D. Pettersson. 1995. Total dietary fiber determined as neutral sugar residues, uronic acid residues, and Klason lignin (the Uppsala method): collaborative study. J. AOAC Int. 78:1030–1044.