Soluble non-starch polysaccharide modulates broiler gastrointestinal tract environment

H. T. Nguyen,* M. R. Bedford,† S.-B. Wu,* and N. K. Morgan*,[1]

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

ABSTRACT The aim of this study was to evaluate the importance of considering dietary soluble non-starch polysaccharides (**sNSP**) when formulating broiler diets, due to their impact on the gastrointestinal tract environment. Cobb 500 broilers ($n = 480$, 80 birds per treatment) were fed either wheat- or corn-soybean meal-based diets formulated to contain either a high, medium or low sNSP content, resulting in 6 dietary treatments in a 2 × 3 factorial arrangement of treatments. On d14 and d 35 of age, pH of the gizzard, ileum and caeca, ileum viscosity, caecal short chain fatty acid (**SCFA**) concentration, and ileal and caecal microbiota profile were determined. Ileal viscosity at d 35 was greater in birds fed high sNSP compared to low sNSP ($P = 0.022$). At both d 14 and d 35, birds fed the wheat-based diet presented higher ileal viscosity ($P < 0.001$) and lower ileal pH ($P = 0.027$ and $P < 0.001$, respectively) compared to those fed the corn-based diet. At d 14, birds fed low sNSP exhibited higher caecal pH ($P = 0.010$) and propionic, isobutyric and valeric acid concentrations ($P = 0.035$, $P = 0.007$ and $P = 0.011$, respectively), and lower ileal *Lactobacillus* content ($P = 0.043$), compared to birds fed high sNSP. This effect was also seen for total SCFA ($P = 0.017$) and acetic acid ($P = 0.005$) concentrations in the caeca at d 14, but only in birds fed wheat-, not corn-, based diets. At d 35, total caecal SCFA concentration was greater in birds fed the wheat-based diet with high sNSP level compared to those fed the corn-based diet with high or low sNSP level ($P = 0.028$). In comparison to birds fed corn, birds fed wheat presented greater caecal concentrations of acetic, butyric, lactic, and succinic acids ($P = 0.001$, $P < 0.001$, $P = 0.003$ and $P = 0.007$, respectively) and *Bifidobacteria* at d 35 ($P = 0.003$) and succinic acid at d14 ($P = 0.041$). However, caecal populations of *Ruminococcus* and concentrations of valeric acid at d14 and isobutyric acid at d 35 were greater in birds fed the corn- compared to wheat-based diets ($P = 0.043$, $P = 0.019$ and $P < 0.001$, respectively). These results illustrate that dietary sNSP concentration, as well as its composition, have a direct impact on gastrointestinal viscosity and pH, and fuel beneficial microbial species, resulting in production of SCFA. It appears to be particularly important to consider sNSP level when formulating wheat-based diets for broilers.

Key words: broiler, non-starch polysaccharide, intestinal bacteria, short chain fatty acid, viscosity

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INTRODUCTION

Recently, there has been heightened interest in the beneficial effects of dietary fiber in poultry rations, as opposed to just its role as an antinutrient and nutrient diluent. Despite extensive research in this field presenting the beneficial effects of dietary fiber on bird performance and gastrointestinal health, fiber is still not considered to be an important nutrient during feed formulation. This is largely due to lack of sufficient information about the actual dietary fiber content, and physicochemical properties of this fiber, in ingredients fed to poultry. Another issue is the disparity between the methods and terms used when describing dietary fiber. "Crude fiber" values are the most commonly used during feed formulation, but these values are essentially meaningless, because the crude fiber method measures just a variable portion of the insoluble fraction, and does not account for the soluble fiber fraction. The true definition of dietary fiber is the sum of non-starch polysaccharides (**NSP**) and lignin (Theander et al., 1994), meaning that NSP levels must be measured and accounted for during feed formulation in order to accurately estimate the dietary fiber received by the bird. It is not possible to assess or predict NSP effects in the bird by simply identifying the NSP level in the diet. It is necessary to understand the physicochemical properties and structure of NSP, as different polymers induce different effects within the luminal environment (Chutkan

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

et al. 2012). Of particular interest is the solubility of the NSP in water, as much of the soluble NSP (**sNSP**) can cause increased digesta viscosity, thus reducing accessibility of enzymes to substrates and utilization of dietary nutrients. Consequently, their presence often limits the use of plant-derived ingredients, particularly fiber-rich by- and co-products. However, these products could be utilized if the NSP composition is understood and accounted for, and the application of supplements, such as enzymes, is properly conducted.

Modern poultry diets are very nutrient dense, in order to meet the requirements of rapidly growing birds, but formulating this way naturally forces fiber out of the diet. Recent research has illustrated that moderate levels of sNSP are essential for ensuring digesta transit rate is slow enough to ensure optimal absorption of nutrients through the gastrointestinal tract wall, and for intestinal health and physical development (Mateos et al., 2012). Soluble NSP also act as a fuel for beneficial bacteria, through selective fermentation of the resulting oligosaccharides derived from depolymerisation of the sNSP. This results in production of short chain fatty acids (**SCFA**), which act as an energy source and signaling molecules, alongside reducing the ability of pathogenic bacteria to proliferate (Bao and Choct, 2010). Thus, there is potential for sNSP to be exploited as a gut health stimulator if used appropriately. The application of NSP-degrading enzymes has become common practice in poultry diets, in an attempt to combat the anti-nutritional effects of NSP on nutrient digestibility and performance, yet the target substrates are not accounted for in feed formulations, so it is possible that these enzymes are not being used in the most economically beneficial manner. Only very few studies examining the impact of NSP-degrading enzymes have actually provided information about the NSP composition of test diets (Kiarie et al., 2014). This makes it difficult to draw conclusions about the efficacy of these products and the impact they have on bird performance and health, and impossible to derive explanations for variations in responses to the same test object between different studies.

A deeper understanding of how dietary sNSP influences the gastrointestinal environment is required, in order to assess and predict how this impacts utilisation of dietary nutrients, and thus bird performance and gut health. Consequently, this study focused on the impact of sNSP on gastrointestinal pH and viscosity, and ileal and caecal microbiota and SCFA composition. This was examined by feeding birds either corn or wheat-based diets, to provide differing NSP substrates with different solubilities, formulated to contain similar protein and energy levels but differing in NSP levels, specifically sNSP. The aim of this study was to examine if the sNSP level of a diet directly influences the gastrointestinal environment, thus highlighting the importance of considering sNSP during feed formulation (Tables 1 and 2).

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

Ingredient	Corn-based diet			Wheat-based diet		
	High sNSP	Medium sNSP	Low sNSP	High sNSP	Medium sNSP	Low sNSP
Corn	51.25	52.00	51.40	-	-	-
Wheat	-	-	-	52.14	50.02	50.00
Sorghum	-	2.34	8.00	0.10	5.00	8.00
Barley	8.00	5.57	-	8.00	5.13	-
Wheat bran	0.13	2.24	2.73	1.54	2.03	2.96
Oat bran	3.47	-	-	-	-	-
Soybean meal	19.26	21.03	23.89	19.46	20.15	27.66
Soy protein concentrate	6.00	6.00	6.00	6.00	6.00	3.14
Canola meal solvent	5.00	4.00	1.39	5.00	4.00	0.01
Canola oil	2.39	2.35	2.09	3.47	3.31	3.63
Limestone	1.08	1.12	1.14	1.23	1.2	1.30
Dicalcium phosphate 18P/21Ca	1.69	1.63	1.67	1.36	1.45	1.50
Salt	0.32	0.32	0.33	0.30	0.30	0.37
TiO_2	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin Premix[1]	0.050	0.050	0.050	0.050	0.050	0.050
Mineral Premix[2]	0.075	0.075	0.075	0.075	0.075	0.075
Choline	0.125	0.120	0.116	0.103	0.105	0.100
L-Lysine HCl	0.237	0.227	0.297	0.219	0.225	0.209
DL-Methionine	0.298	0.309	0.32	0.315	0.325	0.354
L-Threonine	0.100	0.101	0.09	0.129	0.131	0.130
Analyzed Values (as-is)						
DM, %	88.67	87.19	87.74	88.57	88.43	88.28
GE, MJ/kg	16.77	16.45	16.50	16.82	16.75	16.68
Crude protein, %	20.93	21.36	21.91	23.18	23.40	23.46
Starch, g/kg	38.06	37.73	37.45	36.45	35.03	35.24
Soluble NSP, g/kg	8.47	6.22	5.78	11.69	9.95	9.32
Insoluble NSP, g/kg	66.84	70.03	70.65	73.03	75.75	74.51
Total NSP, g/kg	75.31	76.25	76.43	84.73	85.69	83.83
Calcium, %	1.04	0.99	0.96	1.00	1.01	1.03
Total phosphorus	0.72	0.72	0.66	0.75	0.74	0.75
Available P, % (calculated)	0.45	0.45	0.45	0.45	0.46	0.46

[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.

Table 2. Ingredient and nutrient composition of experimental Grower diets (g/100 g, as fed basis).

	Corn-based diet			Wheat-based diet		
Ingredient	High sNSP	Medium NSP	Low NSP	High sNSP	Medium sNSP	Low sNSP
Corn	49.72	54.62	58.28	-	-	-
Wheat	-	-	-	59.65	57.08	55.01
Sorghum	-	-	-	-	4.00	7.83
Barley	8.00	4.03	-	8.00	3.34	-
Wheat bran	1.93	2.82	3.60	-	-	-
Oat bran	2.00	1.03	-	-	-	
Soybean meal	18.76	18.25	19.82	14.11	16.68	22.68
Soy Protein Concentrate	6.00	6.00	5.17	6.00	6.00	0.53
Canola meal solvent	6.00	6.00	6.00	4.79	5.51	6.00
Canola oil	3.80	3.41	3.28	3.51	3.61	4.03
Limestone	1.04	1.05	1.06	1.17	1.15	1.15
Dicalcium Phosphate 18P/21Ca	1.47	1.46	1.44	1.25	1.25	1.27
Salt	0.32	0.32	0.33	0.30	0.30	0.30
TiO_2	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin Premix	0.050	0.050	0.050	0.050	0.050	0.050
Mineral Premix	0.075	0.075	0.075	0.075	0.075	0.075
L-lysine HCl	0.083	0.112	0.114	0.214	0.131	0.199
DL-Methionine	0.223	0.228	0.230	0.264	0.244	0.27
L-Threonine	0.028	0.039	0.040	0.114	0.077	0.106
Analyzed Values (as-is)						
DM, %	90.92	87.62	89.72	90.21	89.90	90.58
GE, MJ/kg	17.46	16.88	17.15	16.97	16.84	17.05
Crude protein, %	21.61	20.81	21.04	20.99	20.71	21.14
Starch, g/kg	36.44	36.04	36.32	39.15	39.91	37.03
Soluble NSP, g/kg	8.74	8.01	6.32	12.75	11.45	9.95
Insoluble NSP, g/kg	71.40	76.09	77.23	74.69	68.17	68.67
Total NSP, g/kg	80.14	84.10	83.56	87.45	79.62	78.63
Calcium, %	1.00	1.04	0.95	0.95	1.02	0.94
Total phosphorus, %	0.72	0.75	0.70	0.95	1.02	0.94
Available P, % (calculated)	0.42	0.42	0.42	0.42	0.42	0.42

[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.

MATERIALS AND METHODS

Animals and Housing

Cobb 500-day-old mixed sex broilers ($n = 480$) were obtained from a local commercial hatchery at day of hatch. Upon arrival, birds were weighed and randomly distributed into 48 floor pens (120 cm length × 77 cm width), with ten birds per pen, bedded on clean wood shavings. The room was thermostatically controlled to produce an initial temperature of 32°C, upon arrival, and gradually reduced to 22°C by d 21, where it was maintained until d 35. The lighting regimen used was 23 h of light at approximately 40 lux on d1, with darkness increasing 1 h a day until 6 h 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. Starter diet was fed as crumble from d 0 to 7 and as pellets from d 8 to 14, and grower diet was fed as pellets from d 15 to 35. The experimental procedures were approved by the University of New England Animal Ethics Committee, Australia (Approval number: AEC18-058).

Experimental Design and Diets

A 2 × 3 factorial arrangement of treatments was applied; the factors were: 1) grain type, wheat or corn; and 2) sNSP level, High (8.47 and 11.69 g/kg Starter diet and 8.74 and 12.75 g/kg Grower diet, in corn and wheat diets, respectively), Medium (6.22 and 9.95 g/kg Starter diet and 8.01 and 11.45 g/kg Grower diet, in corn and wheat diets, respectively), or Low (5.78 and 9.31 g/kg Starter diet and 6.32 and 9.95 g/kg Grower diet, in corn and wheat diets, respectively). The experimental diets were formulated to meet or exceed the nutritional requirement for Cobb 500 broilers.

Prior to feed formulation, ingredients were ground through a 0.5-mm screen and the nutrient concentration analyzed by near-infared spectroscopy (NIRS, Evonik AminoProx, Frankfurt, Germany). The soluble and insoluble NSP concentration of the feed ingredients was analyzed prior to formulating the diets, as were the final diets, by measuring the constituent sugar components as alditol acetates by 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). Dry matter content of the diets was determined by oven drying at 105°C until constant weight. Starch was measured in the diets using the Megazyme total starch assay (Megazyme International Ireland Ltd, Wicklow, Ireland), and crude protein was determined 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. The calcium content

of the diets was measured using the microwave digestion technique (Milestone UltraWAVE, Milestone Srl, Sorisole (BG), Italy) and the total phosphorus concentration was determined using an inductively coupled plasma optical emission spectrometer (ICP-OES, Agilent, Victoria 3170, Australia). Diet gross energy content was determined using an adiabatic bomb calorimeter (Model 6400, Parr Instruments, Moline, IL), standardized with benzoic acid.

Gastrointestinal Tract pH

On d 14 and d 35, two birds per pen were randomly selected, weighed individually and euthanized by electrical stunning followed by cervical dislocation. The gizzard, ileum and caeca were excised, and pH immediately measured in duplicate per bird by inserting a spear tip piercing pH electrode (Sensorex Garden Grove, CA), connected to a pH meter (Mettler-Toledo, UK), directly into the digesta within the gastrointestinal segment, ensuring the probe did not touch the intestinal wall. For the ileum, the measurement was taken at the proximal section (3−4 cm from the Meckel's diverticulum), and in the caeca it was measured in both caeca pouches. The pH probe was rinsed with ultra-pure water between samples.

Sample Collection

Digesta samples were collected from the entire ileum and both caecal pouches, pooled per pen into a 50 mL plastic container and thoroughly mixed before taking subsamples. For the ileum digesta, subsamples of approximately 1.5 mL were collected for analysis of microbiota composition, and the remaining used for analysis of viscosity. For the caeca, subsamples of approximately 1.5 mL were collected for analysis of microbiota composition, and the remaining used for analysis of SCFA. For the microbiota samples, the sample was immediately snap-frozen in liquid nitrogen post-collection, and then stored at −20°C until further analysis. The viscosity and SCFA samples were stored on ice during the sample collection period, and then stored at −20°C until further analysis.

Ileal Digesta Viscosity

Ileal digesta viscosity was measured in triplicate for each pen. Ileal digesta samples were put into 2 mL Eppendorf tubes, 3 replicates per pen, and the tubes were then centrifuged at 10000 × g for 10 min at room temperature. Viscosity was 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 25°C. Viscosity measurements were expressed in centipoise (cPs) unit (1 cPs = 1/100 dyne sec/cm^2 = 1 mPa.s) prior to statistical analysis.

Caecal SCFA

SCFA and lactic acid concentrations were measured in duplicate in the caecal digesta, using the method described by Jensen et al. (1995), with some modifications. Briefly, approximately 2 g of fresh homogenized digesta sample, maintained at approximately 5°C, was homogenized with 1 mL of internal standard (0.01 mol/L ethylbutyric acid). The sample was then centrifuged at 3900 × 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 2000 × g for 15 minutes 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 minutes, 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.

Ileal and Caecal Microbial Abundance

Analysis of microbiota composition was determined in duplicate in both the ileal and caecal digesta samples. DNA extraction from the caecal and ileal 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 modification, 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, MA). Only DNA elutions that emitted ratios between 1.6 and 1.8 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 *Enterobacteria* 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 $\log_{10}$ (genomic DNA copy number)/g digesta. The species-specific 16 rRNA primers utilized are described in detail by Kheravii et al. (2017).

Statistical Analysis

Statistical analysis was performed using Minitab (version 18.1, 2017 Minitab Inc., US). Data were tested for

normality using the Anderson-Darling test. All data were then analyzed as a 2×3 factorial arrangement using General Linear Model. Tukey's multiple range tests was used to determine the differences between individual treatment means when interactions were observed. Linear and quadratic relationships between the dietary sNSP concentration and measured parameters were also determined using polynomial regression function if the main effect of sNSP was significant and no interaction was present. Pen served as the experimental unit, and there were eight replicate pens per treatment. Significant differences between mean values were declared at $P \leq 0.05$.

RESULTS

Ileal Digesta Viscosity

Table 3 presents that ileal digesta in birds fed the corn-based diets was approximately 3 times less viscous compared to that determined in birds fed the wheat-based, at both d 14 and d 35 ($P < 0.001$). At d35, ileal viscosity was greater in birds fed the high sNSP diet compared to those fed the low sNSP diet ($P = 0.022$), but this was not observed at d 14. Positive linear relationships between sNSP level and ileal viscosity were observed at d35 ($P = 0.011$). No interaction was observed between grain and sNSP level at either time point.

Gastrointestinal Tract pH

As illustrated in Table 4, birds fed the wheat-based diets presented lower ileal pH at d14 ($P = 0.027$) and d35 ($P < 0.001$) compared to birds fed the corn-based diets. Additionally, caeca pH at d 14 was higher in birds fed the low sNSP diets compared to those fed the high sNSP diets ($P = 0.010$), also illustrated by a significant negative linear relationship ($P = 0.029$). Dietary treatment had no impact on gizzard pH or caeca pH at d 35. No interaction between grain type and sNSP level was observed at either d 14 or d35.

Table 3. Effect of dietary ingredient and soluble non-starch polysaccharide level (sNSP) on ileal viscosity in broilers at d 14 and 35 (cP).

Treatment	d 14[2]	d 35[2]
Grain		
Corn	2.722[b]	2.228[b]
Wheat	6.314[a]	6.898[a]
sNSP level		
High	4.464	4.343[a]
Medium	4.038	3.972[ab]
Low	3.953	3.493[b]
SEM[1]	0.029	0.037
P-value		
Grain	<0.001	<0.001
sNSP level	0.101	0.022
sNSP level linear	-	0.011
sNSP level quadratic	-	0.523
Grain × sNSP level	0.622	0.329

[a-b]Mean values within a main effect not sharing a common letter are different ($P \leq 0.05$).
[1]Pooled standard error of mean.
[2]Back-transformed values of $\log_{10}$ values for the means.

Table 4. Effect of dietary ingredient and soluble non-starch polysaccharide (sNSP) level on broiler gastrointestinal pH at d 14 and 35.

Treatment	d14			d35		
	Gizzard	Ileum	Caeca	Gizzard	Ileum	Caeca
Grain						
Corn	2.474	6.085[a]	6.327	3.092	7.021[a]	6.082
Wheat	2.246	5.902[b]	6.219	3.117	6.427[b]	5.885
sNSP level						
High	2.460	5.979	6.040[b]	2.991	6.604	5.965
Medium	2.245	5.970	6.267[ab]	3.129	6.743	5.983
Low	2.375	6.031	6.512[a]	3.193	6.825	6.003
SEM[1]	0.167	0.104	0.169	0.163	0.147	0.151
P-value						
Grain	0.061	0.027	0.373	0.835	<0.001	0.072
sNSP level	0.338	0.995	0.010	0.446	0.225	0.941
sNSP linear	-	-	0.029	-	-	-
sNSP quadratic	-	-	0.262	-	-	-
Grain ×sNSP level	0.698	0.246	0.635	0.530	0.976	0.964

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

Table 5 shows that at d 14, there was an interaction between grain source and sNSP on total SCFA concentration in the caeca ($P = 0.017$); SCFA concentration was higher in birds fed the low sNSP level compared to those fed the high sNSP level, but only in birds fed the wheat-, not corn-, based diets. Furthermore, caecal acetic acid concentration was higher in birds fed the wheat-based diet with low sNSP level compared to birds fed the wheat-based diet with high sNSP level or corn-based diet with low sNSP level ($P = 0.005$). Caecal propionic, isobutyric and valeric acid concentration at d 14 was greater in birds fed the diets with low sNSP level compared to those fed the high sNSP level ($P = 0.035$, $P = 0.007$ and $P = 0.011$, respectively). A linear relationship between caecal propionic acid concentration and dietary sNSP level was also observed at d 14 ($P = 0.014$). Valeric acid level was higher ($P = 0.019$) and succinic acid level was lower ($P = 0.041$) in birds fed corn compared to those fed wheat.

Table 6 presents an interaction between grain source and sNSP level at d 35, showing that total caecal SCFA concentration was greater in birds fed the wheat-based diet with high sNSP level compared to those fed the corn-based diet with high or low sNSP level ($P = 0.028$). Moreover, at d 35 birds fed the low sNSP level diets presented lower levels of isovaleric acid in the caeca compared to those fed the medium sNSP level diet ($P = 0.025$). Furthermore, birds fed the wheat-based diets had increased quantities of acetic ($P = 0.001$), lactic ($P = 0.003$), and succinic ($P = 0.007$) acid in the caeca compared to those fed corn, but caecal isobutyric acid concentration was higher in birds fed the corn- compared to wheat-based diet ($P < 0.001$).

Ileal and Caecal Microbial Abundance

On d 14, birds fed the diets with low sNSP level presented reduced abundance of *Lactobacillus* spp. in the

Table 5. Effect of dietary ingredient and soluble NSP level on short chain fatty acid profiles in the caeca at day 14 (μmol/g fresh sample).

Treatment		Formic	Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Valeric	Lactic	Succinic	Total
Grain	sNSP level										
Corn	High	0183	113.1^{ab}	6.00	0.621	26.72	5.73	0.588	1.425	17.87	172.3^{ab}
	Medium	0.672	109.3^{ab}	6.48	0.845	27.22	6.78	0.730	1.916	14.41	168.3^{ab}
	Low	0.80	102.8^{b}	6.57	0.87	25.31	5.84	0.878	1.419	16.88	161.4^{ab}
Wheat	High	0.382	100.6^{b}	3.92	0.400	22.15	4.82	0.331	1.313	19.05	153.0^{b}
	Medium	0.086	107.1^{ab}	5.08	0.566	24.71	5.96	0.383	1.678	27.94	173.5^{ab}
	Low	0.61	130.6^{b}	7.22	0.867	26.37	6.52	0.77	1.883	18.41	193.3^{a}
SEM[1]		0.418	6.64	0.77	0.123	0.186	1.371	0.132	0.307	3.67	9.51
Grain											
Corn		0.551	108.4	6.35	0.778	26.42	6.12	0.732^{a}	1.587	16.39^{b}	167.3
Wheat		0.359	112.8	5.41	0.611	24.41	5.77	0.494^{b}	1.625	21.80^{a}	173.3
sNSP level											
High		0.283	106.9	4.96^{b}	0.510^{b}	24.44	5.28	0.459^{b}	1.369	18.46	162.6
Medium		0.379	108.2	5.78^{ab}	0.705^{ab}	25.97	6.36	0.556^{ab}	1.797	21.17	170.9
Low		0.705	116.7	6.89^{a}	0.868^{a}	25.84	6.18	0.824^{a}	1.651	17.65	177.3
P-value											
Grain		0.538	0.381	0.115	0.063	0.138	0.695	0.019	0.789	0.041	0.401
sNSP level		0.511	0.220	0.035	0.007	0.584	0.740	0.011	0.381	0.507	0.237
sNSP level linear		-	-	0.014	0.927	-	-	0.630	-	-	-
sNSP level quadratic		-	-	0.086	0.744	-	-	0.444	-	-	-
Grain × sNSP level		0.589	0.005	0.155	0.405	0.225	0.861	0.601	0.596	0.094	0.017

[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.

Table 6. Effect of dietary ingredient and soluble NSP level on short chain fatty acid profiles in the caeca at d 35 (μmol/g fresh sample).

Treatment		Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Valeric	Lactic	Succinic	Total
Grain	sNSP level									
Corn	High	86.17	9.305	1.881	29.62	4.399	2.244	0.667	3.65	129.6^{c}
	Medium	101.13	9.423	2.163	32.12	5.337	2.578	0.846	5.78	159.4^{abc}
	Low	88.01	8.738	2.033	28.23	3.424	2.162	0.490	3.80	146.4^{bc}
Wheat	High	113.11	9.727	1.243	46.79	5.845	2.128	1.270	8.89	189.0^{a}
	Medium	105.89	9.846	1.398	43.31	5.910	2.121	1.206	6.95	176.6^{ab}
	Low	103.26	9.577	1.617	36.01	4.384	2.196	0.909	7.91	165.3^{abc}
SEM[1]					3.249	0.686	0.189	0.178	1.72	9.82
Grain										
Corn		91.77^{b}	9.155	2.026^{a}	29.99^{b}	4.387	2.328	0.668^{b}	4.41^{b}	145.2
Wheat		107.42^{a}	9.717	1.419^{b}	42.04^{a}	5.380	2.148	1.128^{a}	7.91^{a}	177.0
sNSP level										
High		99.64	9.516	1.562	38.20	5.122^{ab}	2.186	0.969	6.27	159.3
Medium		103.51	9.635	1.825	37.72	5.624^{a}	2.349	1.026	6.36	168.0
Low		95.63	9.157	1.780	32.12	3.904^{b}	2.179	0.700	5.86	155.9
P-value										
Grain		0.001	0.253	<0.001	<0.001	0.053	0.192	0.003	0.007	<0.001
sNSP level		0.332	0.771	0.121	0.075	0.025	0.512	0.177	0.939	0.358
sNSP level linear		-	-	-	-	0.513	-	-	-	-
sNSP level quadratic		-	-	-	-	0.711	-	-	-	-
Grain × sNSP level		0.110	0.923	0.418	0.261	0.764	0.327	0.778	0.378	0.028

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

ileum compared to birds fed the diets with high sNSP level ($P = 0.043$) (Table 7). In the caeca at d 14, the number of copies of genomic DNA per gram digesta of *Ruminococcus* spp. was 1.3% greater in birds fed the wheat-based diets compared to those fed the corn-based diets ($P = 0.043$) (Table 8).

There was no effect of grain type or sNSP level on ileum microbial composition at d 35 (data not shown). However, Table 9 shows that birds fed the corn-based diets presented a 3.7% reduction in caecal *Bifidobacteria* spp. abundance compared to birds fed the wheat-based diets ($P = 0.003$) at d 35.

DISCUSSION

The lack of consideration of the NSP content of feed ingredients during formulation of diets for poultry is of concern, due to its impact, particularly that of sNSP, on bird performance, litter quality and gut health. The results obtained in the current study reiterate this, by presenting the ability of dietary NSP to modulate the intestinal environment, in terms of digesta viscosity, pH, SCFA production and microbial composition.

It is generally accepted that feeding poultry diets that are rich in NSP, especially sNSP with large and complex

Table 7. Effec of dietary ingredient and soluble non-starch polysaccharide level on ileum microbiota profile at d 14 (14 ($\log_{10}$ GDC (genome DNA copies)/g fresh sample).

Treatment	*Lactobacillus*	*Bifidobacteria*	*Bacillus*	*Ruminococcus*	*Enterobacteriaceae*	*Bacteriodes*	Total anaerobic bacteria
Grain							
Corn	7.492	6.770	5.563	6.654	4.990	5.582	8.867
Wheat	7.335	6.843	5.475	6.648	4.934	5.503	8.740
sNSP level							
High	7.541[a]	6.789	5.593	6.650	4.688	5.619	8.830
Medium	7.453[ab]	6.855	5.561	6.663	5.050	5.615	8.873
Low	7.247[b]	6.775	5.403	6.64	5.148	5.394	8.709
SEM[1]	0.133	0.231	0.104	0.055	0.515	0.289	0.196
P-value							
Grain	0.106	0.657	0.251	0.866	0.951	0.701	0.364
sNSP level	0.043	0.913	0.096	0.866	0.857	0.596	0.608
sNSP linear	0.846	-	-	-	-	-	-
sNSP quadratic	0.858	-	-	-	-	-	-
Grain × sNSP level	0.375	0.066	0.805	0.515	0.087	0.432	0.650

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

Table 8. Effect of dietary ingredient and soluble non-starch polysaccharide level on caeca microbiota profile at d 14 ($\log_{10}$ GDC (genome DNA copies)/g fresh sample).

Treatment	*Lactobacillus*	*Bifidobacteria*	*Bacillus*	*Ruminococcus*	*Enterobacteriaceae*	*Bacteriodes*	Total anaerobic bacteria
Grain							
Corn	9.448	8.799	8.053	9.706[a]	8.655	10.614	11.225
Wheat	9.556	8.725	8.024	9.585[b]	8.643	10.405	11.166
sNSP level							
High	9.486	8.701	7.977	9.620	8.598	10.507	11.147
Medium	9.518	8.779	8.076	9.683	8.708	10.485	11.252
Low	9.503	8.805	8.063	9.633	8.642	10.536	11.188
SEM[1]	0.104	0.075	0.199	0.082	0.213	0.289	0.069
P-value							
Grain	0.148	0.194	0.834	0.043	0.935	0.313	0.239
sNSP level	0.938	0.312	0.825	0.643	0.837	0.980	0.225
Grain × sNSP level	0.186	0.775	0.654	0.522	0.060	0.865	0.871

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

Table 9. Effect of dietary ingredient and soluble non-starch polysaccharide level on caeca microflora profile at d 35 ($\log_{10}$ GDC (genome DNA copies)/g fresh sample).

Treatment	*Lactobacillus*	*Bifidobacteria*	*Bacillus*	*Ruminococcus*	*Enterobacteriaceae*	*Bacteriodes*	Total anaerobic bacteria
Grain							
Corn	9.758	9.363[b]	8.785	9.775	8.135	10.211	11.254
Wheat	9.784	9.711[a]	8.794	9.687	8.369	10.162	11.243
sNSP level							
High	9.702	9.623	8.815	9.702	8.358	10.251	11.248
Medium	9.761	9.451	8.706	9.780	8.204	10.173	11.252
Low	9.85	9.537	8.848	9.711	8.195	10.135	11.246
SEM[1]	0.112	0.155	0.157	0.091	0.392	0.102	0.101
P-value							
Grain	0.751	0.003	0.521	0.186	0.415	0.519	0.876
sNSP level	0.342	0.461	0.947	0.570	0.872	0.447	0.998
Grain × sNSP level	0.103	0.919	0.107	0.488	0.300	0.096	0.339

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

structures, results in increased digesta viscosity, and subsequent reduced ability for enzymes to access substrates, thus suppressing nutrient digestion and absorption (Choct, 2006; Schneeman, 2001). This was demonstrated by Bedford et al. (1991), who identified that digesta viscosity in the foregut negatively correlates with growth rate and feed conversion efficiency in broilers. An important point to note here is that viscosity of the intestinal contents correlated most closely with soluble NSP that was in excess of 500,000 Daltons (Bedford et al., 1991). This fraction represented between 11 and 25% of the total soluble NSP in the intestine, emphasizing the importance of separating soluble NSP into high and low molecular weight fractions if further insight is to be gained concerning the potential effects of dietary NSP content and composition on bird performance. The results in the present study further confirmed this, with

the sNSP level of the diet presenting a significant linear impact on ileum viscosity at d 35 (Table 3). As expected, the wheat-based diets induced higher viscosity in the ileum compared to the corn-based diets at both bird ages, due to the higher NSP content, and possibly greater proportion of higher molecular weight NSP, in wheat compared to corn (Kiarie et al., 2014), primarily in the form of arabinoxylans (Annison and Choct, 1991). It is generally believed that younger birds are more susceptible to viscosity compared to older birds, mainly due to their less developed intestine and less mature microbiota, as illustrated by Bautil et al. (2019). However, the opposite was observed in this study, with a significant impact of sNSP level on viscosity seen at d 35 but not at d 14 (Table 3). The discrepancies between this study and the study conducted by Bautil et al. (2019) may be because the birds in this study were raised on wood-shavings, as opposed to a cage environment. Ingestion of shavings by the birds in this study likely meant the effects of the dietary sNSP in the digesta were diluted, and the intestinal microbiota was better adapted to utilize fiber, compared to birds not consuming fibrous bedding materials (Torok et al., 2009; Cressman et al., 2010). Heightened feed consumption, and thus reduced proportion of ingested bedding material to GIT volume, with increasing bird age may explains why viscosity effects were then more evident in older birds. Another notable difference between two studies was the protein and fat sources used, with the current study using plant-based diets with soy protein and canola oil, compared to potato protein, fish meal and animal fat used by Bautil et al. (2019). This suggests that the gastrointestinal microbiota and environment would have been very different between these two studies, with the birds in this study potentially possessing a microbiota that was better adapted to solubilizing insoluble NSP into soluble NSP. Nonetheless, the results in the current study are in agreement with Lee et al. (2017), who reported effects of xylanase on ileal viscosity at d 42 but not at d 14 or d 21, in birds fed vegetarian wheat-based diets.

Heightened digesta retention in the GIT of wheat-fed, compared to corn-fed, birds results in heightened depolymerisation of NSP into oligosaccharides, which were utilized by beneficial bacteria. This is illustrated in this study by the comparatively higher concentration of SCFA in the caeca of birds at d35 when fed the wheat-based diets, particularly at high sNSP level (Table 6). This is in agreement with the work of Choct et al. (1996). The lower ileal pH observed in birds fed wheat compared to those fed corn at both d14 and d35 may reflect the SCFA data. A reduced pH in the lower intestine is also thought to facilitate increased absorption of pH-influencing metabolites, such as calcium, in birds fed wheat. These findings suggest that wheat provides a more favorable environment and oligosaccharides for beneficial bacteria to proliferate, and these positive attributes may outweigh the negative effects of the associated heightened small intestinal viscosity when compared to corn.

The notably lower pH values observed at d14 in the caeca of birds fed the diets containing a high sNSP level, compared to a low level (Table 4), suggests increased fermentation rates and production of SCFA. The consequence of this may be reduced predominance of pathogenic bacteria in the caeca, given how acid-sensitive many pathogenic bacteria are (Corrier et al., 1990; Apajalahti, 2005; Kermanshahi et al. 2018). Consequently, beneficial bacteria were able to flourish, as illustrated in this study by heightened levels of ileal Lactobacillus spp (Table 7) (Jin et al., 1996). In turn, there is less competition between the host and the pathogenic bacteria for nutrients, resulting in improved bird performance (Yang et al. 2008). This finding agrees with Rebolé et al. (2010), who saw heightened ileal and caecal Bifidobacteria and Lactobacilli counts, and n-butyric acid and D-lactic acid levels, as well as improved body weight gain, in broilers as a result of feeding sNSP as inulin to a wheat and barley-based diet. However, these findings are in contrast with that of Li et al. (2019), who reported a reduced abundance of *Lactobacillus* in the ceca of broilers fed inulin in corn-based diets. The discrepancy presented here may reflect that the inulin was fermented prior to leaving the ileum, meaning the effects in the caeca were lower compared to that observed with prebiotic oligosaccharides, such as xylo-oligosaccharides, which reach the caeca relatively unaltered. The difference in bird age was also likely a contributing factor, where birds in the study were comparatively older, at 40 d of age.

In this study, caecal isobutyric and valeric acid level and pH at d 14 was highest in birds fed the low sNSP level (Tables 4 and 5), and the contents of isobutyric and valeric acid were higher in birds fed corn compared to those fed wheat at d 35 and d 14, respectively (Table 6). This agrees with the findings of Kiarie et al. (2014), who observed elevated levels of branched chain fatty acids in birds fed corn compared to those fed wheat. It is well documented that branched chain fatty acids are produced as a consequence of protein or amino acid metabolism, which starts to dominate when supplies of fermentable carbohydrate substrates are limiting (Qaisrani et al., 2015). This suggests that feeding insufficient levels of sNSP disturbs the balance between carbohydrate and protein fermentation, with the latter producing harmful end products such as amines, ammonia, and phenolics which can damage the gut and result in nutrients being directed away from bird growth and toward defence and repair of the gut. In the worst case scenario, the putrefactive pathogenic bacteria move caudally and prosper, competing with the host for these expensive nutrients in the small intestine. This highlights the importance of ensuring adequate levels of fermentable fiber are fed to poultry, particularly when feeding corn-based diets, in order to maintain a positive microbiota environment. This appears to be particularly important in younger birds, as shown in this study by greater responses to dietary sNSP in terms of caecal SCFA concentration at d 14 compared to d 35 (Tables 5 and 6).

In conclusion, the results from this study reconfirm that birds requires a source of fermentable fire to optimize the gastrointestinal environment in terms of pH, viscosity and the resulting desirable microbial profile. This enables facilitation of SCFA production, which can act as a source of energy and inhibitor of pathogenic bacteria, thus increasing bird performance. It is possible to achieve this

by ensuring sufficient, but not excessive, quantities of sNSP to be formulated in the diet. It is especially important to consider this for young birds, of which the microbiota can be modulated and managed. Further work is needed to identify the optimal balance between high and low molecular weight soluble NSP, and where in the intestinal tract these fractions are most beneficial.

DISCLOSURES

The authors declare no conflicts of interest.

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