Effects of fiber type, particle size, and inclusion level on the growth performance, digestive organ growth, intestinal morphology, intestinal viscosity, and gene expression of broilers

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ABSTRACT The aim of this study was to evaluate the effect fiber type, particle size, and inclusion level on the performance parameters, intestinal development and gene expression in broiler chickens. A total of 648 one-day old Cobb male broilers were randomly assigned to a control diet and 8 other dietary treatments divided in 2 fiber types (cellulose vs. soyhulls), 2 particle sizes (100 and 600 μm), and 2 inclusion levels (4 and 8% crude fiber). Birds were reared to 21 days of age in battery cages (n = 6 replicates). Growth performance parameters and intestinal viscosity were measured on da 7, 14, and 21. On d 14 and 21, digestive organ weights were recorded for analyses of organ growth. On d 21, intestinal samples were taken for analyses of histology, and jejunal mucosas were collected for analyses of nutrient transporters. Data were analyzed as a 2 × 2 × 2 factorial design using JMP 2021. Treatments were compared against the control group using one-way analysis of variance, whereas the main effect interactions were evaluated as a factorial excluding the control group to be able to assess the effect of the independent variables without the variability introduced by the control group. The groups fed 8% crude fiber from cellulose (8% **CL**) had the lowest weight gain regardless of the particle size ($P < 0.01$). The control group had the highest feed intake among the treatments ($P < 0.01$). The groups fed 8% crude fiber from soyhulls (8% **SH**) with a coarse particle size had the heaviest relative gizzard weight among the treatments ($P = 0.045$). The groups fed 8% SH had the heaviest small intestine weights regardless of the particle size ($P = 0.009$). No differences were observed in the relative weights of the ceca. The highest viscosity was observed in the group fed 8% SH with a fine particle size ($P < 0.001$). The group fed 4% SH with a coarse particle size had the longest duodenal villus ($P < 0.001$). The shortest jejunal villus height was observed in the group fed 8% CL with a fine particle size ($P < 0.001$). Ileal villus was highest in the groups fed high CL levels regardless of the particle size ($P < 0.001$). The highest digestibility of dry matter was observed in the group 4% SH with fine particle ($P = 0.017$). The group 4% CL with fine particle had the highest digestibility of crude protein ($P = 0.033$). The highest expression of peptide transporter 1 was observed in the group fed 8% CL with a coarse particle size ($P = 0.008$). In conclusion, fiber type, particle size, and inclusion levels are important factors in the regulation of intestinal morphology, viscosity, nutrient transporters, and growth performance.

Key words: fiber, particle size, inclusion level, intestinal morphology, broiler

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INTRODUCTION

Current tendencies to incorporate cheaper feed ingredients in the formulation of poultry diets have led to the adoption of fibrous feed ingredients. Different feedstuffs vary in the type, amount, and proportions of dietary fiber (**DF**) that they contain (Knudsen, 2014; Jaworski et al., 2015; Nguyen et al., 2019), which provides a wide array of potential physiological and nutritional implications when used in broiler diets (Hetland et al., 2003; Owusu-Asiedu et al., 2006; Jiménez-Moreno et al., 2016). Dietary fiber has been associated with changes in growth performance (Hetland and Svihus, 2001; Jiménez-Moreno et al., 2016), intestinal morphology (Sklan et al., 2003; Sittiya et al., 2020), and nutrient digestibility (Cao et al., 2003; Tejeda and Kim, 2020) that are generally ignored when using fibrous by-products as feed ingredients.

Fiber type, amount used, and particle size are the most crucial factors to bear in mind when using dietary fiber as a functional nutrient in the nutrient matrix (O'Dell et al., 1959; Hetland et al., 2004; Tejeda and

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Kim, 2021). Fiber type can be explained in terms of the ability of the fibrous components to form interactions with water molecules (i.e., soluble or insoluble) (Chaplin, 2003). Viscous soluble fibers have been associated with impairment in growth performance due to disruption of normal enzymatic activity and nutrient digestibility (Hetland et al., 2004; Saki et al., 2011). Insoluble fibers used in low amounts (i.e., 3−5%) have been shown to modulate intestinal morphology and nutrient utilization (Chiou et al., 1996; Tejeda and Kim, 2020). Particle size seems to have a paramount role in modulation of intestinal motility and subsequently nutrient utilization (Kheravii et al., 2018a). This beneficial effect has been reported to be important along the different portions of the gastrointestinal tract for both, big and small particle sizes (Amerah et al., 2007).

It has been clearly demonstrated that the dietary fiber modulates intestinal development (Sklan et al., 2003; Sadeghi et al., 2015) and general nutrient metabolism (Hetland et al., 2004; Georgieva et al., 2014; Kheravii et al., 2018a) depending on the type and amount incorporated in the diet. However, little is known about the role that particle size plays when using different fiber types and inclusion level. Therefore, the objective of this study was to evaluate the effects of 2 sources of fiber (cellulose [**CL**] and soybean hulls [**SH**]), 2 inclusion levels (4 and 8% crude fiber [**CF**]), and 2 particles sizes (100 and 600 μm) on the growth performance, digestive organ growth, intestinal viscosity, intestinal morphology, nutrient digestibility, and gene expression of broilers.

MATERIAL AND METHODS

General Procedures

The experiment was approved by the Institutional Animal Care and Use Committee (**IACUC**) of the University of Georgia (Athens, GA). A total of 648, one-day-old male Cobb500 broiler chicks were allocated in a completely randomized factorial designed with 9 dietary treatments and 6 replicates of 12 birds each. There were 3 main factors namely, fiber type (CL and SH), inclusion levels (4 and 8% CF), and particle size (100 and 600 μm). The chicks were allocated in 54 cages equipped with one drinker and one feeder, providing ad-libitum access to water and mash feed from 1 to 21 days of age. Temperature and lighting programs followed the recommendation of Cobb Broiler Management Guide (Cobb-vantress, 2018).

Dietary Treatments

All diets were corn and soybean meal-based formulated to meet the nutrient requirements specified by Cobb500 performance and nutritional guide (Cobb-Vantress, 2018). The control diet was formulated to contain 2% CF. The control diet was used as a basal diet to which purified cellulose (CL: 99% cellulose, Solka floc, Skidmore, Schollcraft, MI) was added as a source of CF by replacing an inert filler (sand) to achieve 4 and 8% CF (4% CL and 8% CL) in the diets. Solka floc 100-fcc and solka floc 900-fcc with an average particle size of 100 and 600 μm, respectively, were added separately to their corresponding dietary treatment as sources of purified CL. The rest of experimental diets were added increasing amounts of SH to achieve 4 and 8% CF (4% SH and 8% SH). Particle sizes averaging 100 and 600 μm of SH were obtained using a machine mill with different screen sizes (Fitzpatrick model M comminuting machine mill, the W. J. Fitzpatrick company, Chicago, IL). Proximate analyses of SH were conducted to measure the gross nutrient contents (Table 1). For amino acids and apparent metabolizable energy corrected for nitrogen (**AME_n**), the nutrient matrix composition used for SH was obtained using cecectomized roosters at the poultry research center at the University of Georgia (Table 2). Diets were provided as mash during the entire rearing period (0−21 d). All diets were isonitrogenous and isocaloric and are shown in Table 3. For ileal nutrient digestibility determination, chromic oxide (Cr_2O_3, Sigma Aldrich, St. Louis, MO) was added at 0.3% as an indigestible marker to all diets.

Table 1. Proximate analyses of the nutrient composition of soybean hulls.

Item*	Value
GE (Kcal/Kg)	3,698
AMEn (Kcal/kg)	658
Dry matter (%)	87.95
Crude protein (%)	16.3
Crude fiber (%)	35.8
Calcium (%)	0.88
Phosphorus (%)	0.55
nPP (%)	0.37

*Abbreviations: AME_n, apparent metabolizable energy corrected for nitrogen; GE, gross energy; nPP, non-phytate phosphorus.

Table 2. Analyzed values for amino acid content, digestibility (%) and digestible amino-acid content of soybean hulls based on cecectomized rooster assay.

Amino acid	Percent amino acid	Digestibility (%)	Digestible amino acid content (%)
Alanine	0.450	39.386	0.177
Arginine	0.480	68.344	0.328
Aspartic acid	0.950	54.040	0.513
Cysteine	0.160	41.461	0.066
Glutamic acid	1.140	51.294	0.585
Histidine	0.260	46.506	0.121
Isoleucine	0.400	43.850	0.175
Leucine	0.660	52.328	0.345
Lysine	0.730	52.738	0.383
Methionine	0.130	57.583	0.075
Phenylalanine	0.390	53.601	0.209
Proline	0.550	55.592	0.306
Serine	0.530	49.335	0.261
Threonine	0.360	50.722	0.183
Tryptophan	0.060	56.100	0.034
Tyrosine	0.360	46.732	0.168
Valine	0.460	33.951	0.156

Table 3. Ingredient composition of diets fed to male Cobb × Cobb broilers from 1 to 20 d of age[1].

Ingredient, %	CTL	4% CL	8% CL	4% SH	8% SH
Corn	49.56	49.56	49.56	53.59	38.14
Soybean meal	35.09	35.09	35.09	32.37	30.48
Solka floc	—	2.03	6.07	—	—
Soybean hulls	—	—	—	5.62	17.77
Soybean oil	5.01	5.01	5.01	3.76	9.24
Defluorinated phosphate	1.05	1.05	1.05	0.168	0.63
Biofos 16/21P	0.55	0.55	0.55	1.29	0.98
Calcium carbonate	0.91	0.91	0.91	1.24	0.72
L-Thr	0.12	0.12	0.12	0.15	0.20
DL-Met	0.32	0.32	0.32	0.33	0.36
Lysine HCl	0.20	0.20	0.20	0.26	0.31
Vitamin premix[2]	0.25	0.25	0.25	0.25	0.25
Mineral premix[3]	0.15	0.15	0.15	0.15	0.15
Sodium chloride	0.23	0.23	0.23	0.33	0.28
Filler (sand)	6.56	4.74	0.5	0.5	0.50
Calculated nutrient composition					
Dry matter (%)	90	90	90	90	90
ME energy (Kcal/kg)	3,000	3,000	3,000	3,000	3,000
Protein (%)	21.0	21.0	21.0	21.0	21.0
Crude Fiber (%)	2.0	4.0	8.0	4.0	8.0
Calcium (%)	0.90	0.90	0.90	0.90	0.90
Dig. Phosphorus (%)	0.45	0.45	0.45	0.45	0.45
Dig. Met (%)	0.63	0.63	0.63	0.63	0.63
Dig. TSAA (%)	0.90	0.90	0.90	0.90	0.90
Dig. Lys (%)	1.22	1.22	1.22	1.22	1.22
Dig. Thr (%)	0.86	0.86	0.86	0.86	0.86

[1]All diets, except control, were added fiber at a particle size of 100 and 600 μm.

[2]Vitamin premix provided the following per kilogram of DSM premix: Vit. A, 2,204,586 IU; Vit. D_3, 200,000 ICU; Vit. E, 2,000 IU; Vit. B12, 2 mg; Biotin, 20 mg; Menadione, 200 mg; Thiamine, 400 mg; Riboflavin, 800 mg; d-Pantothenic Acid, 2,000 mg; Vit. B6, 400 mg; Niacin, 8,000 mg; Folic Acid, 100 mg; Choline, 34,720 mg.

[3]Mineral premix includes per kg of premix: Ca, 0.72 g; Mn, 3.04 g; Zn, 2.43 g; Mg, 0.61 g; Fe, 0.59 g; Cu, 22.68 g; I, 22.68 g; Se, 9.07 g.

Growth Performance and Organ Weights

The birds and feed were weighed weekly per cage to determine mortality-corrected body weight gain (**BWG**), mortality-corrected feed intake (**FI**), and mortality-corrected feed conversion ratio (**FCR**) and results are presented per week. Mortality was recorded twice daily. For organ growth analyses, empty gizzard, small intestine, and ceca were obtained from one average bird per cage (n = 6 per treatment) and weighed to determine the relative organ weight on d 14 and 21.

Intestinal Morphology

On d 21, samples from the mid-duodenum, jejunum and ileum (~ 2 cm long) were collected from one average bird per replicate cage (n = 6 per treatment). Intestinal contents were flushed with phosphate-buffered saline (**PBS**) and intestinal sections were stored in 10% neutral-buffered formalin and left in solution for a minimum period of 48 h for tissue fixation. During slide preparation, increasing amounts of ethanol were used to dehydrate the tissues, then diaphanized in dimethylbenzene, and fixed in paraffin. Finally, tissue sections with a thickness of 4 μm were set on slides and were stained using Hematoxylin and Eosin (**H&E**) procedures. Pictures were taken using a light microscope (10× eyepiece and 1.6× magnification; Leica DC500 camera, Leica Mycrosystems Inc., Buffalo Groove, IL). Measurements for villi height and crypt depth were taken using ImageJ software (Image Processing and Analysis in JAVA − ImageJ 1.52r, National Institutes of Health, Bethesda, MD).

Intestinal Viscosity

On 7, 14, and 21 days of age, one bird per cage was randomly selected and euthanized, and intestinal digesta was collected from the Meckel's diverticulum to the ileocolonic junction. Fresh digesta were centrifuged at 12,600 × *g* for 5 min and the supernatants were collected for viscosity measurements using a cone and plate Brookfield DV-II + Programmable viscometer at 10 rpm using a CPE-40 spindle (Brookfield engineering laboratories, Inc, Middleboro, MA). A water bath control connected to the cone was used to keep the temperature of the samples at 40°C.

Nutrient Digestibility

On d 21, six birds per replicate cage were euthanized, and ileal digesta were collected from two-thirds of the distal ileum (from Meckel's diverticulum to about 1 inch anterior to ileocecal junction). The digesta samples were dried for analyses of dry matter, crude protein, and energy. The chromium oxide concentration was measured according to Dansky and Hill (1952), and gross energy was evaluated using a bomb calorimeter (IKA Calorimeter C1, IKA Works Inc., Wilmington, NC). The crude protein (N × 6.25) was analyzed using a LECO nitrogen analyzer (LECO, St. Joseph, MI). The apparent ileal digestibility (**AID**) of dry matter, crude protein, and apparent metabolizable energy (**AME**) was calculated using the following equation:

$$AID,\ \% = 100\left[1 - \left(\frac{Cr_{feed}}{Cr_{dig}}\right) \times \left(\frac{Nutrient_{dig}}{Nutrient_{feed}}\right)\right]$$

where Cr_{feed} and Cr_{dig} is the chromium dioxide in feed and ileal digesta, respectively; and $nutrient_{dig}$ and $nutrient_{feed}$ are the nutrient in ileal digesta and feed, respectively.

Quantitative Reverse-Transcriptase Polymerase Chain Reaction

On d 21, samples from the jejunal mucosa were collected from one randomly selected bird per cage, snap-frozen in liquid nitrogen, and stored at −80°C previous to analysis. Jejunal mucosa samples were used to analyze the expression of Na±dependent glucose transporter 1 (**SGLT-1**), and peptide transporter 1 (**Pept-1**) genes using quantitative reverse-transcriptase polymerase chain reaction (**qRT-PCR**). Total RNA was extracted from the samples previously stored at −80°C using QIAzol Lysis Reagent (Qiagen, Germatown, MD)

Table 4. Primer pairs used for RT-qPCR analyses.

Gene[1]	Gene bank identification	Primer sequence, forward/reverse	Product size (bp)
SGLT-1	AJ236903.1	GCCGTGGCCAGGGCTTA/ CAATAACCTGATCTGTGCACCAGT	71
Pept-1	KF366603.1	CCCCTGAGGAGGATCACTGTT/ CAAAAGAGCAGCAGCAACGA	66
GAPDH	NC_052532.1	GCTAAGGCTGTGGGGAAAGT/ TCAGCAGCAGCCTTCACTAC	161

[1]Abbreviations: GAPDH, glyceraldehyde 3-phosphate dehydrogenase; Pept-1, peptide transporter-1; SGLT-1, sodium-dependent glucose transporter 1.

according to the manufacturer's instruction. After extraction, RNA quantity and purity were determined using Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Pittsburgh, PA). The cDNA was synthesized from total RNA and subsequently diluted to 10 ng/μL for qRT-PCR analysis. Glyceraldehyde 3-phosphate dehydrogenase (**GAPDH**) was used as the housekeeping gene. The forward and reverse primers for the genes are shown in Table 4. The qRT-PCR was performed on an Applied Biosystems StepOnePlus (Thermo Fisher Scientific, Waltham, MA) with iTaq Universal SYBR Green Supermix (BioRad, Hercules, CA) using the following conditions: 95°C for 15 s, 58°C for 20 s, and 72°C for 15 s during 40 cycles for GAPDH; 95°C for 15 s, 60°C for 20 s, and 72°C for 15 s during 40 cycles for Pept-1; and 95°C for 15 s, 58°C for 20 s, and 72°C for 15 s during 40 cycles for SGLT-1. All reactions were done in duplicate. and relative gene expression data were analyzed using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The mean ΔCt of control group was used to calculate the $\Delta\Delta$Ct value.

Statistical Analyses

Fiber type, particle size, and inclusion level were the fixed effects in the model. A pen was used as an experimental unit for growth performance and nutrient digestibility; a bird was used as an experimental unit for organ growth, intestinal morphology, intestinal viscosity, and gene expression. Data were analyzed as a completely randomized block design with 8 treatments organized as a 2 × 2 × 2 factorial design. One-way analysis of variance was used to determine the effect of fiber inclusion compared to the control group, whereas the main effect interactions were evaluated excluding the control group to be able to assess the effect of the independent variables without the variability introduced by the control group. The main effects model used for statistical analyses is as follows:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \delta_t + \alpha\beta\delta_{ijt} + \varepsilon_{ijt}$$

where Y_{ij} represents the value for each random variable; μ is the overall mean; α_i, β_j, δ_t, and $\alpha\beta\delta_{ijt}$ are the fiber type, inclusion level, particle size, and their interactions, respectively such that $\Sigma\alpha_i$=0; and the random errors ε_{ijt} are identically and independently normally distributed with a mean 0 and a variance σ. All statistical procedures were performed using JMP Pro, version 15 (SAS Institute Inc., Cary, NC). In case of significant differences, means were separated using the Tukey's test HSD option. For all hypothesis tests, statistical significance was considered at $P < 0.05$.

RESULTS

Growth Performance

The results for growth performance are presented per week in Table 5. The upper portion of the table includes the control group, and the second portion of the table includes the main effects and their interactions only, without the control group. On d 21, the control group had the heaviest weigh gain during the rearing period but did not differ from the treatments containing 4% CF regardless of the fiber source ($P > 0.05$). However, the treatments fed 8% CL had the lowest weight gain at the end of the experiment ($P < 0.001$). The control group had the highest feed intake during the entire rearing period ($P < 0.05$). The groups fed 4% CF with a fine particle size had lower FCR on 7 days of age ($P = 0.015$). However, such differences disappeared in the rest of the experiment ($P > 0.05$). The results from the main effects show that fiber type did not affect significantly any of the growth performance parameters ($P > 0.05$). The statistics for the main effects indicate that fine particle size (100 μm) increased the weight gain on d 7 and 14 and improved the FCR on d 7 compared to the coarse particle size ($P = 0.021$); however, such differences disappeared at the end of the experiment ($P > 0.05$). Fiber level affected all the growth performance parameters where the highest fiber level (8% CF) resulted in lower weigh gain, lower feed intake, and poorer FCR compare to the groups fed 4% CF ($P < 0.05$). The interaction, fiber type × particle size, was significant on d 7 for weight gain, where coarse SH improved weight gain compared to the fine SH, and coarse CL decreased weight gain compared to fine CL ($P = 0.0021$). Particle size × level interaction on d 7 for feed intake shows that fine particles at low levels had higher feed intake compared to coarse particles at high levels on d 14 ($P = 0.023$). Three-way interaction among main effects on d 14 and 21 for feed intake shows that fine soyhulls at the low level had the heaviest weight gain, whereas the lowest weight gain was for the group fed coarse and high levels of CL ($P = 0.032$). Three-way interactions also indicate that the group fed coarse and high levels of CL had the highest feed intake among dietary treatments ($P = 0.044$). However, no differences were observed

Table 5. Effects of dietary fiber parameters on the growth performance of male broilers reared to 21 d of age[1].

			BW gain (g/bird)			Feed intake (g/bird)			Feed conversion			Mort., %
Fiber type[2]	Particle size (μm)	Level	D 7	D 14	D 21	D 7	D 14	D 21	D 7	D 14	D 21	D0-21
Control	**-**	**-**	124^{a}	432^{a}	884^{a}	144^{a}	656^{a}	1639^{a}	1.17^{ab}	1.52	1.85	2.78
CL	**100**	**4%**	114^{ab}	396^{ab}	813^{abc}	128^{ab}	527^{bc}	1350^{ab}	1.12^{a}	1.33	1.67	2.78
CL	**100**	**8%**	104^{bc}	324^{cd}	658^{d}	122^{bc}	480^{bc}	1233^{b}	1.18^{ab}	1.48	1.87	4.20
CL	**600**	**4%**	107^{bc}	377^{bc}	818^{abc}	131^{ab}	558^{ab}	1640^{a}	1.23^{b}	1.50	2.00	5.60
CL	**600**	**8%**	85^{d}	279^{d}	641^{d}	105^{c}	429^{c}	1268^{b}	1.24^{b}	1.54	1.98	2.78
SH	**100**	**4%**	112^{abc}	407^{ab}	870^{ab}	127^{ab}	566^{ab}	1497^{ab}	1.14^{a}	1.39	1.72	5.56
SH	**100**	**8%**	96^{cd}	303^{d}	665^{cd}	118^{bc}	473^{bc}	1292^{b}	1.23^{b}	1.56	1.94	4.20
SH	**600**	**4%**	115^{ab}	368^{bc}	767^{abcd}	133^{ab}	524^{bc}	1345^{ab}	1.16^{ab}	1.42	1.75	2.50
SH	**600**	**8%**	97^{cd}	305^{d}	724^{bcd}	119^{bc}	484^{bc}	1427^{ab}	1.23^{b}	1.60	1.98	8.33
Standard error			3.50	12	34	4.30	27.00	99.00	0.03	0.06	0.10	1.35
P-value			<0.01	<0.01	<0.01	<0.01	<0.01	0.030	0.015	0.090	0.162	0.436
Main effects												
Fiber type	CL		103	344	732	121	498	1373	1.19	1.46	1.88	3.84^{b}
	SH		105	346	757	124	512	1390	1.19	1.49	1.85	5.15^{a}
Particle size (μm)	100		107^{a}	358^{a}	752	124	511	1343	1.16^{a}	1.44	1.80	3.83
	600		101^{b}	332^{b}	737	122	499	1420	1.21^{b}	1.51	1.93	4.80
Level	4%		112^{a}	387^{a}	817^{a}	130^{a}	544^{a}	1458^{a}	1.16^{a}	1.41^{a}	1.78^{a}	4.11
	8%		96^{b}	303^{b}	672^{b}	116^{b}	466^{b}	1305^{b}	1.22^{b}	1.54^{b}	1.94^{b}	4.88
Source of variation (*P*-value)												
Fiber type (T)			0.340	0.841	0.247	0.297	0.403	0.788	0.969	0.433	0.637	0.002
Particle size (P)			0.021	0.004	0.489	0.529	0.424	0.242	0.019	0.062	0.073	0.980
Inclusion level (L)			<0.01	<0.01	<0.01	<0.01	<0.01	0.023	0.006	0.002	0.028	0.468
Type × Particle size			0.002	0.436	0.6916	0.077	0.862	0.197	0.057	0.346	0.209	<0.01
T × P × L			0.340	0.052	0.032	0.181	0.035	0.044	0.680	0.492	0.410	<0.01

Boldface indicates the particle size and inclusion levels of dietary fiber.
[1]Values are the least-square means of 6 replicate cages per treatment, each cage with 12 birds.
[2]Abbreviations: CL, cellulose, SH, soyhulls.
[a-d]Means within a column not sharing a common superscript differ significantly ($P < 0.05$).

in the FCR ($P > 0.05$). At the end of the experiment, the mortality was higher for groups fed SH, especially treatments given the highest amounts of soyhulls ($P < 0.05$).

Intestinal Histomorphology

Results for intestinal morphology are shown in Table 6. Duodenal villus was highest for the treatment fed 4% SH with a coarse particle size, whereas the

Table 6. Effects of dietary fiber parameters on the intestinal histomorphology of male broilers reared to 21 d of age[1].

			Duodenum			Jejunum			Ileum		
Fiber type[2]	Particle size (μm)	Level	Villus (μm)	Crypt (μm)	Ratio (μm)	Villus(μm)	Crypt (μm)	Ratio (μm)	Villus (μm)	Crypt (μm)	Ratio (μm)
Control	**-**	**-**	3067^{ab}	269^{a}	11.8^{ab}	1817^{a}	220	8.8^{ab}	1340^{a}	231^{ab}	6.3^{ab}
CL	**100**	**4%**	2848^{bc}	250^{ab}	11.9^{ab}	1650^{abc}	214	8.0^{abcd}	1017^{b}	206^{b}	5.17^{bc}
CL	**100**	**8%**	2643^{c}	262^{ab}	10.5^{b}	1597^{abc}	235	7.1^{bcd}	1029^{b}	202^{b}	5.27^{bc}
CL	**600**	**4%**	2841^{bc}	236^{b}	13.0^{a}	1453^{cd}	217	7.2^{bcd}	1045^{b}	204^{b}	5.3^{bc}
CL	**600**	**8%**	2949^{ab}	243^{ab}	12.6^{a}	1644^{abc}	231	7.3^{abcd}	1058^{b}	211^{ab}	6.69^{a}
SH	**100**	**4%**	2962^{ab}	256^{ab}	12.3^{ab}	1758^{ab}	203	9.0^{a}	1004^{b}	208^{ab}	5.03^{c}
SH	**100**	**8%**	3079^{ab}	241^{ab}	13.3^{a}	1237^{d}	215	6.2^{d}	1023^{b}	210^{ab}	5.04^{c}
SH	**600**	**4%**	3163^{a}	248^{ab}	13.1^{a}	1654^{abc}	224	8.0^{abcd}	1138^{b}	243^{a}	4.96^{c}
SH	**600**	**8%**	2900^{abc}	247^{ab}	12.2^{ab}	1493^{bcd}	232	6.9^{cd}	1041^{b}	244^{a}	4.42^{c}
Standard error			64.00	8.00	0.50	71	10	0.4	45	11	0.5
P-value			<0.01	0.030	<0.01	<0.01	0.202	<0.01	<0.01	<0.01	0.031
Main effects											
Fiber type	CL		2682	238	11.79	1352	229^{b}	6.25	977^{a}	208	4.97^{a}
	SH		2731	231	12.29	1369	248^{a}	5.98	857^{b}	197	4.63^{b}
Particle size (μm)	100		2619^{b}	227^{b}	11.96	1323^{b}	228^{b}	6.26	907	197	4.87
	600		2793^{a}	242^{a}	12.11	1398^{a}	249^{a}	5.97	927	207	4.73
Level	4%		2852^{a}	240^{a}	12.4^{a}	1411^{a}	247^{a}	6.04^{b}	868^{b}	204	4.49^{b}
	8%		2561^{b}	229^{b}	11.67^{b}	1310^{b}	230^{b}	6.19^{a}	965^{a}	201	5.11^{a}
Source of variation (*P*-value)											
Fiber type (T)			0.077	0.188	0.106	0.497	0.003	0.132	<0.001	0.0607	0.0421
Particle size (P)			<0.001	0.005	0.632	0.002	0.001	0.098	0.3644	0.0955	0.3982
Inclusion level (L)			<0.001	0.038	0.018	<0.001	0.010	0.387	<0.001	0.637	<0.001
Type × Particle size			0.030	0.417	0.199	0.078	0.238	0.922	0.1958	0.1931	0.497
Type × Level			0.043	0.712	0.298	<0.001	0.591	0.002	<0.001	0.7467	0.0567
T × P × L			0.206	0.441	0.122	0.146	0.002	<0.001	0.7287	0.5506	0.6563

Boldface indicates the particle size and inclusion levels of dietary fiber.
[1]Values are the least-square means of 6 replicate birds per treatment.
[2]Abbreviations: CL, cellulose, SH, soyhulls.
[a-d]Means within a column not sharing a common superscript differ significantly ($P < 0.05$).

shortest duodenal villus was observed in the group fed 8% CL with a fine particle size ($P < 0.001$). The control group had the deepest duodenal crypt among the treatments ($P = 0.034$). The smallest duodenal villus to crypt ratio was observed in the group fed 8% CL with a fine particle ($P < 0.01$). The control group and the group fed 8% SH with a fine particle had the highest and shortest jejunal villus, respectively ($P < 0.01$). No differences were observed in the jejunal crypts among the dietary treatments ($P = 0.2015$). The group fed 8% SH with a fine particle size had the smallest jejunal villus to crypt ratio among dietary treatments ($P < 0.01$). Ileal villus was highest in the control group compared to the rest of the dietary treatments ($P < 0.001$). Ileal crypt depth was highest for the groups fed coarse SH regardless of the inclusion level ($P < 0.001$). Ileal villus to crypt ratio was highest for 8% CL with coarse particle and lowest for all treatments containing SH regardless of the inclusion level or particle size. The results for the mean effects show that SH-fed treatments had highest crypt depth and shorter ileal villus and ileal villus to crypt ratio compared to groups fed CL ($P < 0.05$). Coarse particle size increased duodenal and jejunal villus height and crypt depth, ($P < 0.01$). High fiber level decreased duodenal villus height, crypt depth, and their ratio, and jejunal villus height and crypt depth; however, it increased jejunal villus to crypt ratio and ileal villus height and ileal villus to crypt ratio ($P < 0.05$). The interaction, type × particle size, was significant for duodenal villus height where the groups fed SH with fine particle had higher duodenal villus compared to the groups fed CL with fine particle ($P = 0.0301$). The interaction, type × inclusion level, shows that 4% SH-fed groups had higher duodenal villus height compared to those fed CL-4%, and shorter jejunal villus and villus to crypt ratio ($P < 0.05$). Three-way interaction, type × particle size × inclusion level, shows that coarse SH at a low level had the highest jejunal crypt depth, and the 8% SH with coarse particle had the highest villus to crypt ratio ($P < 0.05$).

Digestive Organ Growth

The results for organ growth are shown in Table 7. The groups fed the diets containing SH had the heaviest gizzard on d 14 but only the group fed SH at 8% CF with coarse particle maintained such increased weight until d 21 ($P < 0.05$). The groups containing 4 and 8% CF as SH in both particle sizes had the heaviest small intestines weight compared to the rest of the treatments on d 21 ($P = 0.009$). No statistical differences were observed in the ceca weights relative to body weight among the dietary treatments ($P > 0.05$). The results of the main effects show that SH-fed groups had a heavier gizzard on d 14 compared to the CL-fed groups ($P = 0.006$). Fiber type did not significantly affect small intestine or ceca relative weights ($P > 0.05$). Fiber with coarse particle (i.e., 600 μm) increased the relative weights of the gizzard on d 21 ($P = 0.026$) compared to fine particle (i.e., 100 μm). The groups fed 8% CF had heavier gizzard and intestines on d 21 ($P < 0.05$) compared to those fed 4% CF regardless of fiber type. The interaction, fiber type × particle size, was significant; fine CL decreased the

Table 7. Effects of dietary fiber parameters on the relative weights of male broilers reared to 21 d of age[1].

			Gizzard, %		Small intestine, %		Ceca, %	
Fiber type[2]	P. size `(μm)	Level	D 14	D 21	D 14	D 21	D 14	D 21
Control	**-**	**-**	3.6[ab]	2.48[ab]	9.86	6.31[b]	0.93	0.74
CL	**100**	**4%**	3.15[b]	2.48[ab]	10.63	7.95[ab]	1.09	1.02
CL	**100**	**8%**	3.30[b]	2.53[ab]	10.96	7.76[ab]	1.17	0.9
CL	**600**	**4%**	3.73[ab]	2.53[ab]	10.96	8.94[ab]	1.03	1.04
CL	**600**	**8%**	3.93[ab]	2.93[ab]	9.98	9.25[ab]	1.41	1.18
SH	**100**	**4%**	3.79[ab]	2.41[b]	9.71	7.88[ab]	1.14	0.97
SH	**100**	**8%**	4.17[a]	2.63[ab]	11.65	10.19[a]	1.09	0.82
SH	**600**	**4%**	3.45[ab]	2.60[ab]	10.57	7.95[ab]	1.08	0.88
SH	**600**	**8%**	4.09[a]	3.27[a]	11.52	9.82[a]	1.19	0.95
Standard error			0.17	0.19	0.69	0.70	0.13	0.13
P-value			<0.001	0.045	0.419	0.009	0.413	0.408
Main effects								
Fiber type	CL		3.53[b]	2.62	10.63	8.48	1.17	1.04
	SH		3.87[a]	2.73	10.86	8.96	1.12	0.90
Particle size (μm)	100		3.60	2.51[b]	10.74	8.44	1.12	0.93
	600		3.80	2.83[a]	10.76	8.99	1.17	1.01
Level	4%		3.53[b]	2.5[b]	10.47	8.18[b]	1.08	0.96
	8%		3.87[a]	2.84[a]	11.03	9.26[a]	1.21	0.98
Source of variation (*P*-value)								
Fiber type (T)			0.006	0.437	0.634	0.338	0.569	0.144
Particle size (P)			0.102	0.026	0.969	0.276	0.554	0.348
Inclusion level (L)			0.007	0.020	0.250	0.037	0.162	0.883
Type × Particle size			0.002	0.500	0.476	0.171	0.719	0.449
Type × Level			0.163	0.436	0.073	0.047	0.285	0.765

Boldface indicates the particle size and inclusion levels of dietary fiber.
[1]Values are the least-square means of 6 replicate birds per treatment.
[2]Abbreviations: CL, cellulose, SH, soyhulls.
[a-d]Means within a column not sharing a common superscript differ significantly ($P < 0.05$).

weights of the gizzard on d 14 ($P = 0.002$). However, this effect disappeared on d 21. Fiber type × level interaction indicates that the group fed the high level of SH increased the weight of small intestines, whereas the low level of SH had the lowest intestine weight ($P = 0.047$). No other significant interactions were observed among the main effects ($P > 0.05$).

Intestinal Viscosity

Results for intestinal viscosity are shown in Table 8. On d 7, the group fed 8% CF as SH with a coarse particle size had the highest intestinal viscosity, and the lowest was for the group fed 8% CF with coarse CL ($P = 0.045$). On d 14, the groups containing 8% CF as SH had the highest intestinal viscosity regardless of the particle size ($P < 0.001$). However, on d 21, the group having 8% CF as SH with a fine particle size had the highest intestinal viscosity ($P < 0.001$). Results from the main effects show that viscosity was higher in the groups fed SH compared to those fed CL on d 7, 14, and 21 ($P < 0.05$). The main effects show that particle size was not statistically significant in affecting intestinal viscosity ($P > 0.05$). Higher fiber inclusion increased the intestinal viscosity on d 14 and 21 ($P < 0.05$). The interaction. fiber type × level, was significant where the 8% SH-fed group had the highest intestinal viscosity on d 7, 14, and 21 ($P < 0.01$). No other significant interactions were observed among the main effects ($P > 0.05$).

Nutrient Digestibility

The results for nutrient digestibility are shown in Table 9. In the present experiment, all diets were formulated to be isonitrogenous and isocaloric. Statistical differences were observed in the digestibility of dry matter where the group fed 4% SH with fine particle had the highest DM digestibility, whereas the treatment fed 8% SH with coarse particle had the lowest DM digestibility ($P = 0.0169$). The digestibility of crude protein was improved for the group fed 4% CL with a fine particle and was worst for the groups fed 8% SH ($P = 0.0326$). No statistical differences were observed in the digestibility of energy ($P > 0.05$). The results from the main effects indicate that particle size is an important factor in the modulation of nutrient digestibility where the smaller particle (100 μm) increased nutrient digestibility compared to the larger one (600 μm) ($P < 0.05$). Inclusion level significantly affected the digestibility of DM where higher inclusions reduced such parameter ($P = 0.007$).

Gene Expression

The results for gene expression of nutrient transporters are shown in Table 10. No significant differences were observed in the expression of SGLT-1. However, the expression of Pept-1 was higher for the group fed 8% CL with coarse particle compared to the control group ($P = 0.008$). The results from the main effects show that none of the individual main effects have a significant impact in the expression of nutrient transporters ($P > 0.05$). However, the interaction, fiber type × particle size, shows that coarse particle of CL increased the expression of Pept-1 compared to coarse particle of SH ($P = 0.0154$).

Table 8. Effects of dietary fiber parameters on the intestinal viscosity of male broilers reared to 21 d of age[1].

			Viscosity, mPas		
Fiber type[2]	Particle size (μm)	Level	D 7	D 14	D 21
Control	**-**	**-**	6.65[ab]	2.26[b]	2.25[c]
CL	**100**	**4%**	9.07[ab]	2.36[b]	2.02[c]
CL	**100**	**8%**	3.63[bc]	2.19[b]	2.39[c]
CL	**600**	**4%**	7.47[ab]	3.35[b]	3.03[bc]
CL	**600**	**8%**	1.78[c]	2.13[b]	2.41[bc]
SH	**100**	**4%**	7.85a[b]	3.45[b]	2.40[c]
SH	**100**	**8%**	11.38[ab]	6.44[a]	7.75[a]
SH	**600**	**4%**	6.08[ab]	3.06[b]	3.4b[c]
SH	**600**	**8%**	13.23[a]	7.38[a]	5.74[ab]
Standard error			2.80	0.86	0.083
P-value			0.045	<0.001	<0.001
Main effects					
Fiber type	CL		5.58[b]	2.54[b]	2.43[b]
	SH		9.79[a]	4.87[a]	4.55[a]
Particle size (μm)	100		7.93	3.67	3.23
	600		7.71	3.94	3.8
Level	4%		7.63	3.04[b]	2.75[b]
	8%		8.02	4.74[a]	4.45[a]
Source of variation (*P*-value)					
Fiber type (T)			0.020	<0.001	<0.001
Particle size (P)			0.622	0.474	0.993
Inclusion level (L)			0.948	0.006	0.001
Type × Level			0.003	<0.001	<0.001

Boldface indicates the particle size and inclusion levels of dietary fiber.
[1]Values are the least-square means of 6 replicate birds per treatment.
[2]Abbreviations: CL, cellulose, SH, soyhulls.
[a-d]Means within a column not sharing a common superscript differ significantly ($P < 0.05$).

Table 9. Effects of dietary fiber parameters on the nutrient digestibility of male broilers reared to 21 d of age[1].

Fiber type[2]	Particle size (μm)	Level	Dry matter, %	Crude protein, %	Energy, kcal/kg
Control	**-**	**-**	63.83[ab]	82.30[abcd]	3057
CL	**100**	**4%**	69.85[ab]	86.59[a]	3006
CL	**100**	**8%**	67.44[ab]	84.38[abc]	2842
CL	**600**	**4%**	68.20[ab]	83.61[abcd]	2811
CL	**600**	**8%**	61.23[ab]	80.75[bcd]	2641
SH	**100**	**4%**	71.24[a]	85.11[ab]	2938
SH	**100**	**8%**	64.19[ab]	84.42[abc]	2797
SH	**600**	**4%**	62.89[ab]	79.38[d]	2720
SH	**600**	**8%**	59.02[b]	79.58[cd]	2589
Standard error			2.72	1.80	170
P-value			0.0169	0.0326	0.0730
Main effects					
Fiber type	CL		66.68	83.83	2825
	SH		64.33	82.12	2761
Particle size (μm)	100		68.18[a]	85.12[a]	2896[a]
	600		62.84[b]	80.83[b]	2690[b]
Level	4%		68.04[a]	83.67	2869
	8%		62.97[b]	82.28	2717
Source of variation (*P*-value)					
Fiber type (T)			0.2008	0.1577	0.4001
Particle size (P)			0.0052	0.0008	0.0097
Inclusion level (L)			0.0077	0.2484	0.0515
Type × Particle size			0.4373	0.4105	0.9230

Boldface indicates the particle size and inclusion levels of dietary fiber.
[1]Values are the least-square means of 6 replicate cages per treatment, each cage with 12 birds.
[2]Abbreviations: CL, cellulose, SH, soyhulls.
[a-d]Means within a column not sharing a common superscript differ significantly ($P < 0.05$).

DISCUSSION

Growth Performance

To evaluate the impact of fiber type, inclusion level, and particle size, all diets were formulated to be isonitrogenous and isocaloric. However, despite the fact that diets with similar nutrient content were used, there were differences in growth performance among the dietary treatments. Treatments having low fiber inclusion (4% CF) did not differ from the control group. This is in accordance with other researchers that have reported that small inclusions of dietary fiber do not affect negatively the growth performance of broilers (Amerah et al., 2009; Sacranie et al., 2012). However,

Table 10. Effects of dietary fiber parameters on the jejunal gene expression of male broilers reared to 21 d of age[1].

			Gene	
Fiber type[2]	Particle size (μm)	Level	SGLT-1	Pept-1
Control	**-**	**-**	1.00	1.00[b]
CL	**100**	**4%**	0.447	3.17[ab]
CL	**100**	**8%**	0.245	1.56[ab]
CL	**600**	**4%**	0.294	2.63[ab]
CL	**600**	**8%**	1.244	4.11[a]
SH	**100**	**4%**	0.442	3.27[ab]
SH	**100**	**8%**	0.075	2.39[ab]
SH	**600**	**4%**	0.237	1.19[ab]
SH	**600**	**8%**	0.085	1.6a[b]
Standard error			0.344	0.700
P-value			0.080	<0.010
Main effects				
Fiber type	CL		0.558	2.87
	SH		0.210	2.11
Particle size (μm)	100		0.302	2.38
	600		0.465	2.60
Level	4%		0.355	2.56
	8%		0.412	2.41
Source of variation (*P*-value)				
Fiber type (T)			0.122	0.124
Particle size (P)			0.463	0.656
Inclusion level (L)			0.795	0.752
Type × Particle size			0.244	0.015

Boldface indicates the particle size and inclusion levels of dietary fiber.
[1]Values are the least-square means of 6 replicate birds per treatment.
[2]Abbreviations: CL, cellulose, SH, soyhulls.
[a-d]Means within a column not sharing a common superscript differ significantly ($P < 0.05$).

the treatments fed 8% CF using CL with regardless of particle size had the worst weight gain among dietary treatments whereas those fed 8% CF as SH with a course particle size did not differ from those fed 4% CF. This can be attributed to the differences in fiber types between CL and SH. CL contains a tertiary structure linked together by an extensive number of hydrogen bonds, providing stability, low aqueous solubility and mostly resistance to acid hydrolysis (Festucci-Buselli et al., 2007) which might encapsulate nutrients in the upper digestive tract and reduce the break down at the level of the gizzard leading to interference in the breakdown of other nutrients. The control group had the highest feed intake compared to the other fiber-containing treatments during the entire rearing period in the current study. Other studies also reported that broilers given choice feeding between control and diet containing rice hulls had a lower feed intake compared to the control group (González-Alvarado et al., 2008; Sadeghi et al., 2015). However, other researchers have pointed out the ability of broilers to increase feed intake as a means to compensate for the nutrient dilution when using dietary fiber (Amerah et al., 2009; Sacranie et al., 2012). This is associated to the differences in nutrient content in experimental diets (no isocaloric diets). In our experiment, all diets were isocaloric and isonitrogenous; thus, increases in feed intake should not be expected. The reduction in feed intake of the treatments containing 8% CL in either particle size and 8% SH with a particle size of 100 μm was not expected and can be attributed to the impact of dietary fiber on intestinal motility and passage rate. In contrast to these results, Amerah et al. (2009) reported an increased in feed intake when the control diet was diluted with CL in the ratio 6:10. In our experiment, however, filler (sand) was replaced with the adequate level of CL to maintain the same nutrient content. Differences in FCR were observed just in the first week of the rearing period where the groups fed 4% CF with a particle size of 100 μm had the lowest FCR regardless of the fiber source. The opposite was true for the groups fed 8% CF which had the poorest FCR. However, such differences were not seen in the rest of the experiment. Birds fed CL at 8% had the lowest weight gain irrespective of the particle size. In the case of SH, the group fed 8% CF as SH with a coarse particle did not differ from the groups fed 4% CF. The results from the main effects excluding the control group show that fine particle size of fiber improved weigh gain on d 7 and 14 and FCR on d 7. However, such differences disappeared at the end of the experiment. Similar to these results, Donadelli et al. (2019) found that fine particles reduced the FCR when using different fiber types. Other researchers have suggested that insoluble fiber with coarse particle, in some cases, can help in the improvement of growth performance by modulating intestinal functionality (Choct, 2015). In our experiment, it is important to mention that for CL the groups fed the same amounts of fiber (i.e., 4% or 8%) had similar weight gain in both particle sizes. However, for SH it was observed better results when SH is provided in a coarse particle size compared to the fine particle size. These differences might be associated to the fiber matrix found of soyhulls which is composed of pectins, CL, and hemicellulose (Stein and Parsons, 2008) which interact differently in the gastrointestinal tract compared to purified CL. Groups fed 8% CF had lower weight gain, feed intake, and higher (poorer) FCR compared to those fed 4% SH. In fact, the mortality for groups fed 8% SH was higher. The presence of soluble fiber in SH are the main reason behind increases in mortality, just when given in high amounts as observed in this experiment. Other results have previously been reported that high levels of dietary fiber reduce growth performance parameters in diets with the same nutrient level (Sklan et al., 2003) as well as diets where fiber has been replaced without nutrient adjustment (Hetland and Svihus, 2001), which indicates the ability of high fiber levels to encapsulate the nutrients making them unavailable for absorption (Hetland et al., 2004).

The interaction, type × particle size, was significant on day 7, where coarse SH improved weight gain compared to fine soyhulls. Furthermore, coarse CL decreased weight gain compared to fine CL. These results point out the importance of particle size based on the type of fiber used in the diets. Particle size × level interaction on d 7 for feed intake shows that fine particles at low levels had higher feed intake compared to coarse particles at high levels in the present study. Different researchers have indicated the potential of coarse fibers to modulate digesta passage rate and nutrient digestion by increasing the retention time in the upper digestive tract (i.e., gizzard) (Hetland et al., 2004; Gonzalez-Alvarado et al., 2007). This might explain the higher intake for diets containing fine particle size which fail in stimulating retention of feed components at the level of the gizzard. In the present study, three-way interaction among the main effects on d 14 and 21 for feed intake shows that fine SH at the lower level had the heaviest weight gain, whereas the lowest weight gain was for the group fed coarse and high levels of CL. In chicks, it has been clearly demonstrated that nutrient digestibility increases from 58% to up to 90% when coarse particles are ground to finer particles (Mitchell et al., 1972). It is important to highlight that this is true for nutrient-containing feedstuffs. In the present experiment, SH and CL were used as sources of fiber. SH is a substantial source of fiber (75% NDF) but also contains 16% crude protein and 658 kcal/kg ME (Table 1); on the other hand, CL is a purified source of CL (99% CL) which explains the reason behind the differences in performance when using fine particle size of soyhulls at low levels (4% CF). Three-way interactions also show that the group fed coarse and high levels of CL had the highest feed intake among the dietary treatments despite the fact that all diets were isocaloric and isonitrogenous; however, this was not true for the group fed SH, indicating that high levels of pure insoluble fibers can modulate feed intake in broilers as reported for other researchers (Hetland et al., 2003; Donadelli et al., 2019).

Digestive Organ Growth and Digesta Viscosity

All groups containing CL had a similar relative weight of the gizzard irrespective of the inclusion level or particle size. This has been seen by other researchers when using small amounts (<5%) of CL (Cao et al., 2003). However, in the present study, when comparing between fiber sources, the group fed 8% CF as SH with a coarse particle size had the heaviest gizzard relative weight, whereas the group fed fine SH at 4% CL had the lowest one. These results are in accordance with other study reporting that chickens fed fine particles developed smaller gizzard compared to those fed coarser particles (O'Dell et al., 1959). This is because the gizzard functions as a sieve that retains and grinds coarse particles until they have achieved a determined size before moving to the small intestine (Hetland et al., 2004). Therefore, the presence of fine particles fails to stimulate the muscles of the gizzard, resulting in poorer gizzard development. It was also observed in the present experiment that the groups containing 8% CF as SH had the heaviest small intestine weight (including duodenum, jejunum, and ileum), irrespective of the particle size, compared to the rest of the treatments on d 21. This could be attributed to the increase in intestinal viscosity observed in the present experiment for SH-containing diets during the entire rearing period when fed at the level of 8% CF. This is because the carbohydrate portion of SH is made up of 30% pectins (Stein and Parsons, 2008) which increases intestinal viscosity, reducing the passage rate of the digesta, and subsequently provoking the growth of the small intestine as a means to offset the changes in volume caused by the accumulation of feed in such organ (Owusu-Asiedu et al., 2006).

In the present study, the interactions among the main effects were observed; the increase of fine SH in the diet caused significantly higher digesta viscosity compared to coarse SH, and fine and coarse CL. This is because the interaction of fiber with water is determined not only by fiber type (i.e., soluble or insoluble) but for its physical properties as well, where smaller particle size can increase water absorption due to higher surface area available to interact with water molecules (Strange and Onwulata, 2002). Interestingly, no significant differences were observed in the relative weights of the ceca. Similar to these results, Gonzalez-Alvarado et al. (2007) did not observed significant differences in the ceca weights of broilers fed 3% SH compared to the control group; however, birds fed insoluble fiber (oat hulls) did have a lower ceca weight compared to the control group. This contrast could be attributed to the fact that these authors used oat hulls as source of insoluble fiber which contains other non-cellulosic components including lignin, protein, and fat (Welch et al., 1983), resulting in different results. From the main effects it is clear that fiber with coarse particle (i.e., 600 μm) increased the relative weights of the gizzard on d 21 compared to fine particle (i.e., 100 μm). This was more pronounced in the diets containing SH as the source of fiber. This can be attributed to the fact that natural fibers have a higher level of polymerization (Hivechi and Bahrami, 2016) resulting in higher stimulation of the muscles of the gizzard. The fiber level was certainly of influence in the stimulation of the gizzard and also the relative weight of the small intestine. This indicates the need to compensate for the increase of the digesta volume caused by the bulkiness of the fiber particles as observed for other researchers using different fiber types (Hetland et al., 2004; Svihus, 2011; Rezaei et al., 2018).

Intestinal Histomorphology

The treatment 4% SH with coarse particle increased duodenal villus height compared to the group fed 8% CL with fine particle in the current study. This might be associated with the stimulation of the reverse peristalsis provoked by the presence of coarse fiber particles which results in increased villus development (Sacranie et al., 2012). It was also observed that the presence of fine particles in the form of CL reduced the duodenal villus to crypt ratio, indicating a reduction in duodenal functionality. High levels of SH with fine particle size reduced the jejunal villi. However, low levels of SH with fine particle improved jejunal villus to crypt ratio when compared to high levels with fine particle. This inclusion level-dependent differences could be attributed to the excessive abrasive effect of fiber caused when high levels of soyhulls are added to the diet, causing a reduction in villus height as observed by other authors when using other fiber types (Montagne et al., 2003; Sadeghi et al., 2015). This is supported by Tejeda and Kim (2020) who reported that soyhulls fed at 4% crude fiber resulted in improvements in intestinal morphology, but the opposite was true when fed at 6 and 8% CF. Furthermore, the soluble carbohydrates present in SH can also be a reason behind the reduction in jejunal villus height. It has been suggested that soluble carbohydrates can increase the rate of epithelial cell losses, negatively affecting villus growth (Montagne et al., 2003). In the present experiment, it was observed that the highest intestinal viscosity was for groups fed high levels of SH with fine particle and it can, therefore, be concluded that viscosity played an important role in the atrophy of jejunal villi. The ileal villus was highest for the control group compared to the rest of the treatments. However, the ileal villus to crypt ratio was higher for the 8% CL with coarse particle and smallest for all treatments containing SH. The presence of coarse particles of soluble fiber seems to reduce the development of the ileal villus to crypt ratio. This could be associated to the stimulation of pathogenic bacteria of the undigested carbohydrates at the end of the digestive tract. In accordance with the results in the present experiment, Sadeghi et al. (2015) reported that soluble carbohydrates from sugar beep pulp decreased the ileal villus in broiler compared to the control group. The impairment in the development of ileal villus could, therefore, be associated to the increase in bacterial activity that

interferes with the normal intestinal development (Pan and Yu, 2014).

Nutrient Digestibility and Gene Expression

Dietary fibers have been reported to modulate nutrient digestibility in broilers and other poultry species (Cao et al., 2003; Hetland et al., 2003; Amerah et al., 2009; Sacranie et al., 2012). In the present study, the dry matter digestibility was higher for the 4% SH group compared to the 8% SH treatment. From these results, it is clear that the digestibility of dry matter is negatively affected by the presence of soluble fibers (i.e., pectins) present in the SH. Similarly, Silva et al. (2013) reported a quadratic decrease in dry matter digestibility with increases of pectin in the diets of broilers. Interestingly, in the present experiment we observed that small amounts of SH increase the dry matter digestibility which could be associated to a slight increase in the retention time that allows for more breakdown of the dry matter. However, the digestibility of crude protein was higher for the 4% CL group with fine particle compared to the 4% SH group with coarse particle. The fact that coarse particles had lower digestibility of crude protein can be associated to the increase in endogenous amino acid losses caused by higher epithelial cell turnover driven by particle size instead of fiber level (Montagne et al., 2003). In this case, diets with fine CL particles would have lowered endogenous amino acid flow and, therefore, higher protein digestibility. It has been suggested that large particles could slow down the passage rate of digesta at the level of the upper gastrointestinal tract (i.e., gizzard), which would create a prolonged the exposure of nutrients to digestive enzymes, increasing nutrient digestibility (Amerah et al., 2007); however, the effects at the level of the small intestine seem to affect nutrient metabolism differently. Numerical differences in energy digestibility indicate that higher inclusions of fiber reduce energy digestibility despite the fact that diets were formulated to be isocaloric. This might be due to interactions of dietary fiber with other more digestible carbohydrates and/or fat in the diet that renders such nutrients unavailable (Hetland et al., 2004), especially when dietary fiber is given in high amounts. The main effects indicate that coarse particles decreased the digestibility of energy compared to fine particles. Despite the fact that coarse particles can stimulate the upper digestive tract, improving gizzard relative weight, as observed in the present study, the reduction in energy digestibility associated with coarse particles could be attributed to the smaller surface area of coarse particles when compared to smaller particles, which results in a reduction in the accessibility to digestive enzymes (Carré et al., 2005; Amerah et al., 2007).

No significant differences were observed in the expression of jejunal sodium-dependent glucose transporter 1. However, the expression of Pept-1 was higher for the group fed 8% CL with coarse particle compared to the control group. Interestingly, this group had the poorest performance and no improvement in crude protein digestibility among the dietary treatments, which indicates the upregulation of nutrient transporters as a means to compensate the reduction on performance. Kheravii et al. (2018a) reported the reduction in weight gain and the upregulation of intestinal cationic aminoacid transporter 1, and peptide transporter-2 in broilers fed 2% sugar bagasse with a coarse particle size. In accordance with these authors, the interaction, fiber type × particle size, showed that coarse particle of CL increased the expression of Pept-1 compared to coarse particle of SH in the current study. However, the upregulation of Pept-1 did not result in an improved crude protein digestibility. On the contrary, low levels of insoluble fiber in a fine particle size improved crude protein digestibility, indicating that these improvements in digestibility might be associated to the endogenous amino acid losses; the reduction in the endogenous amino acid losses by fine CL inclusion might result in a lower need of amino acid uptake by the transporters. On the other hand, the higher abrasive effect of coarse particles increases the need for amino acid uptake, upregulating the expression of Pept-1. Furthermore, birds from the fine CL group had the lowest feed intake which can be attributed to the slower digesta passage rate due to the bulkiness of the diets containing CL. Similar to these results, Khempaka et al. (2009) reported a reduction in feed intake with increases in died cassava pulp containing 27% insoluble fiber. Therefore, the reduction in feed intake leads to a lower nutrient intake and a higher need to compensate for the lack of nutrients stimulating the upregulation of nutrient transporters such as Pept-1.

CONCLUSIONS

Dietary fiber type, inclusion level, and particle size are important factors determining the functionality of the fibrous feed components. In the present study, it was observed that 4% SH diets with fine particles had similar weigh gain compared to the control group and improved the feed efficiency during the first week of the experiment. An improvement in jejunal villus to crypt ratio was also observed in such diets, indicating a positive modulation of the gastrointestinal tract. High levels of SH increased relative weights of the gizzard and small intestine but reduced performance and increase mortality due to increases in intestinal viscosity. This indicates that SH can be added to diets to achieve 4% CF (i.e., 5 −6% in a corn-soybean meal diet) without causing any deleterious effect and with a high potential to improve intestinal functionality. Expression of Pept-1was not related to digestibility of crude protein due to potential endogenous loses caused by abrasion of dietary fiber. In summary, type of fiber, inclusion level, and particle size should be considered when using fibrous feedstuffs since these are determining factors affecting growth performance, intestinal morphology, nutrient digestibility, nutrient metabolism, and further research is granted to understand the role of fibrous feed ingredient in the

nutrient matrix. This will provide us a way to be able to incorporate cheaper feed ingredients.

DISCLOSURES

There is no conflict of interest.

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