Coarse particle inclusion and lignocellulose-rich fiber addition in feed benefit performance and health of broiler chickens

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ABSTRACT Measures to improve gut health and nutrient digestibility have been sought due to in-feed antibiotics being phased out in poultry. The appropriate physical structure of feed ingredients and addition of dietary fiber may be beneficial in enhancing gut health in poultry. In this study, the effect of a lignocellulose-rich fiber source and corn particle size on broiler performance, gizzard development, nutrient digestibility, cecal microflora, and litter quality was evaluated. A total of 684 day-old male Ross 308 chicks were randomly allocated to 6 treatments with 6 replicate pens, each housing 19 birds. A 2 × 3 factorial arrangement of treatments was applied with the factors of: corn particle size (coarse: 2,982 μm or fine: 941 μm geometric mean diameter), and 3 levels of lignocellulose (0%, 1% or 2%). Significant particle size × fiber interaction was observed for feed conversion ratio (FCR) at d 10 ($P < 0.05$). The birds fed coarsely ground corn (CGC) had lower FCR than those fed finely ground corn (FGC) only at 2% of lignocellulose but not at 1% or no lignocellulose addition. Birds fed FGC were heavier ($P < 0.001$) at d 10. In contrast, at d 24 and 35, birds fed CGC had lower FCR than those fed FGC ($P < 0.05$). Ileal gross energy and protein digestibility increased in birds fed CGC compared with those fed FGC at d 24 ($P < 0.05$). Relative gizzard weight was higher ($P < 0.05$) on d 24 and 35 in birds fed CGC as compared to those fed FGC. Birds consumed 2% dietary lignocellulose had decreased counts of cecal *Clostridium* spp. compared to those with 1% lignocellulose ($P < 0.05$) at d 24. On d 35, both levels of lignocellulose had significantly decreased ($P < 0.05$) litter moisture content compared to the control. In conclusion, birds fed pelleted diets containing CGC exhibited improved FCR, and increased nutrient digestibility, which may have been caused by larger gizzards. Furthermore, dietary lignocellulose addition is beneficial to litter quality.

Key words: Lignocellulose, particle size, broiler performance, gizzard, microflora

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INTRODUCTION

Consumer pressure and legislation to ban the use of in-feed antibiotics in the poultry industry has increased the incidence of enteric disorders in broilers. Alternative feed management approaches to improve the health and digestive efficiency are a continuous endeavour for nutritionists worldwide. Altering the physical structure of feed ingredients and addition of dietary fiber have gained interest in the poultry industry and research community so as to improve gut health and nutrient digestibility. Previous studies have reported negative effects of fiber on nutrient digestibility (Jørgensen et al., 1996) and growth performance (Sklan et al., 2003). However, recent studies have indicated beneficial effects of fiber on performance and digestive functions in broilers (Kheravii et al., 2016; Mateos et al., 2012) due to a better gizzard development (Gonzalez-Alvarado et al., 2008; Sacranie et al., 2012) and gut reflex and enzyme production (Svihus, 2011). Fiber consumption may thus improve nutrient digestibility (Jiménez-Moreno et al., 2013a) and growth performance (Jiménez-Moreno et al., 2016) in chickens. It has also been recognized that the type and amount of fiber in the diet affect gastrointestinal development and growth performance of broilers (Jiménez-Moreno et al., 2013b). In fact, different types of fiber have various physicochemical properties (Raninen et al., 2011) that determine bulk density, water absorption, fermentation capability, digesta pH, digesta viscosity, digesta passage rate, short-chain fatty acid production, and microflora status (Mateos et al., 2012). Mateos et al. (2012) concluded that fiber lignification is a key characteristic that affects bird productivity and physiology. Lignocellulose is composed of carbohydrates (cellulose, hemicellulose) and aromatic polymers (lignin). The physical properties and exact composition of lignocellulose depend on the source of fiber (Jørgensen et al., 2007). A lignocellulose

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product (ARBOCEL®) has been reported to exhibit a positive effect on fecal consistency, litter quality, gut microflora, fermentation activity, and protein digestibility in chickens (Bogusławska-Tryk et al., 2015; Farran et al., 2013; Milosevic et al., 2015).

Ingredient particle size is important during both feed manufacture and in achieving optimal utilization by birds. Particle size effects on performance have been more closely examined in the EU since the ban of in-feed antibiotics. From a nutritional perspective, it is generally believed that smaller particles allow digestive enzymes to better access their substrates, thereby increasing nutrient digestibility and growth performance efficiency (Behnke, 2001). However, it has been documented that broilers may have a requirement for dietary physical structure to enhance gut development and health (Choct, 2009). A fully functional or well-developed gizzard has been hypothesized to improve nutrient digestibility via enhanced gastrointestinal tract function and greater digesta transit time (Amerah et al., 2008). Dietary structural components, such as coarse particles, whole grain and fiber, have attracted considerable attention as they may improve gizzard function. Generally, a large and well-developed gizzard is able to grind feed particles more thoroughly (Amerah et al., 2007a), to elevate pancreatic enzyme secretion through increased release of cholecystokinin (Svihus, 2011), to increase proteolysis by pepsin, trypsin, and other endogenous proteases in the small intestine, and to improve gastrointestinal tract motility (Ferket, 2000; Gonzalez-Alvarado et al., 2008). It has also been reported that enhanced gizzard function in birds fed coarsely ground corn (**CGC**)in pelleted diets improved protein digestibility, energy utilization, live performance, and litter quality (Xu et al., 2015a,b).

Feed physical structural and fiber may exert physiologic influence on 1) feed transit time and gizzard size and development; 2) secretion of HCl and endogenous enzymes; 3) feed intake, growth performance and nutrient digestibility; 4) gut reflux, health, and integrity, and 5) microflora balance and litter quality. This study evaluated the effect of a lignocellulose-rich fiber source and corn particle sizes and their interactions on growth performance, cecal microflora, gizzard development, and litter quality in broiler chickens.

MATERIALS AND METHODS

Corn and Lignocellulose

The whole corn was obtained from a commercial supplier (Grazag Company in Armidale, NSW, Australia) and ground in a roller mill (Bunge Albury Mills, NSW, Australia) to produce CGC and FGC. Lignocellulose was obtained from Agromed GMBH distributor in Queensland, Australia. The product's commercial name is OptiCell, which is a registered trademark of Agromed GMBH, Austria. According to the manufacturer's product datasheet, OptiCell contains 85% total dietary fiber or 59% crude fiber, which includes 30% lignin.

Experimental Design and Bird Management

A total of 684 day-old male Ross 308 chicks were obtained from a local hatchery (Baiada Hatchery in Tamworth, NSW, Australia). Birds were randomly allocated to 6 treatments with 6 replicate pens of 19 birds each. The experiment employed a 2 × 3 factorial arrangement of treatments with the factors of: corn particle size, CGC (2,982 μm geometric mean diameter), and finely ground corn (**FGC**; 941 μm geometric mean diameter); and 3 levels of lignocellulose (0%, 1% or 2%). The geometric mean diameter (**GMD**) of corn particle size was determined according to the American Society of Agricultural Engineers (2003). Chickens were reared in pens measuring 100 cm × 150 cm up to 35 d. Hardwood shavings were used for bedding with an initial depth of approximately 7 cm in each pen. Pens were equipped with hanging tube feeders and nipple drinkers. Feed and water were provided ad libitum. The lighting, relative humidity, and temperature followed Ross 308 strain guidelines (Aviagen, 2014).

Diets

Diets were based on corn, soybean meal, and meat meal and were formulated to meet the nutrient recommendations for Ross 308 (Aviagen, 2014). Table 1 shows the ingredient and nutrient composition of the experimental diets. The feeding program consisted of a starter (d 0 to 10), grower (d 11 to 24), and finisher phase (d 25 to 35). Titanium dioxide ($\mathbf{TiO_2}$) was incorporated into all grower diets as an indigestible marker at a rate of 5 kg/t diet for nutrient digestibility assessment. Lignocellulose fiber was added over the top to the complete feed.

Animal Ethics

This experiment was approved by the Animal Ethic Committee of the University of New England (Approval No: AEC 15-058). All bird procedures including health, care and use of laboratory animals were fulfilled with the Australian Code for the Care and Use of Animals for Scientific Purposes (NHMRC, 2013).

Growth Performance

On d 10, 24, and 35, birds and leftover feed were weighed while mortality was recorded daily. The average weight gain (g/bird), feed intake (g/bird) and feed conversion ratio (g feed/g weight gain) were calculated based on collected data. Feed conversion ratio (**FCR**) was adjusted for mortality by adding weight of dead birds back to live birds within each period. Feed intake was calculated as FCR × weight gain.

Table 1. Composition and nutrient content of corn base diet (g/kg).

Ingredients	Starter	Grower	Finisher
Corn	602	619	672
Soybean meal	3262	2932	248
Xylanase[a]	0.500	0.500	0.500
Meat and bone meal	30.0	36.0	30.0
Canola oil	7.78	20.3	25.6
Limestone	9.70	8.13	7.77
Dical phosphate	6.08	2.70	1.94
Phytase[b]	0.100	0.100	0.100
Salt	3.20	2.89	2.78
Na bicarbonate	2.00	2.00	2.00
Vitamin premix[c]	2.00	2.00	2.00
Choline	1.30	1.20	1.18
L-lysine HCl 784	3.04	2.25	2.08
D,L-methionine	3.92	3.36	3.05
L-threonine	2.04	1.48	1.17
TiO_2	–	5.00	–
Nutrients			
ME (kcal/kg)	3,000	3,100	3,200
Crude protein	222	210	190
Crude fat	29.8	42.5	47.7
Crude Fiber	20.7	20.0	19.2
Arginine	13.7	12.9	11.4
Lysine	12.8	11.5	10.2
Methionine	6.85	6.16	5.63
Methionine + Cysteine	9.50	8.70	8.00
Tryptophan	2.44	2.28	1.99
Isoleucine	8.60	8.06	7.25
Threonine	8.60	7.70	6.80
Valine	9.92	9.39	8.49
NSP soluble	4.25	4.03	3.81
NSP insoluble	56.3	54.3	53.2
Calcium	9.60	8.70	7.80
Available Phosphorus	4.80	4.35	3.90
Sodium	2.20	2.10	2.00
Chloride	3.50	3.19	3.04
Choline	1.70	1.60	1.50

[a]Feedzyme 1000G (Xylanase) 500 g/mt
[b]Phyzyme XP5000G (100 g/mt) Dupont
[c]Vitamin and mineral premix (supplied activity per ton of feed): Cu (sulphate), 8 g; Fe (sulphate), 60 g; I (iodide), 1.0 g; Se (selenate), 0.3 g; Mn (manganese), 80 g; Zn (sulphate and oxide), 60 g; Mo (molybdenum), 1 g; Co (cobalt), 0.3 g; vitamin A (retinol), 12 MIU; vitamin D3 (cholecalciferol), 3.5 MIU; vitamin E (tocopherol acetate), 40 g, vitamin K3 (menadione), 2 g; vitamin B1 (thiamine), 2 g; vitamin B2 (riboflavin), 6 g; vitamin B3 (niacin), 50 g; vitamin B5 (pantothenic acid), 11 g; vitamin B6 (pyridoxine hydrochloride), 5 g; vitamin B9 (folate), 1.5 g; biotin (vitamin H), 100 mg; vitamin B12 (cyanocobalamin), 20 mg; antioxidant, 25 g.

Sample Collection

On d 24, 2 birds per replicate were randomly selected from each pen, weighed, and humanely killed by cervical dislocation. Ileal contents were collected by gently squeezing the digesta into 50 mL plastic containers and stored at –20°C for digestibility analysis. The cecal contents from each replicate were pooled into a 2 mL Eppendorf cap lock tube, snap-frozen in liquid N_2, and stored at –20°C for bacterial quantification. On d 24 and 35, two birds were dissected to obtain gizzard weights and pH.

Gizzard Measurements

Empty gizzards were weighed and recorded along with bird weights. Gizzard contents of 2 birds were collected and homogenized to measure pH by using a calibrated pH meter (EcoScan pH 6). The relative gizzard weight was calculated as mass per unit of live body weight (g/100 g of live body weight).

Titanium Dioxide Analysis

The spectrophotometric method described by Short et al. (1996) was followed to measure the TiO_2 in the diet and ileal digesta samples. Between 0.1 g and 0.2 g of the freeze-dried digesta and diet samples were accurately weighed in porcelain crucibles and placed in Muffle furnace at 580°C for 13 h. Upon cooling, 5 mL of 7.4 M H_2SO_4 was added to the samples then boiled on hotplate at 200°C for 30 min and another 30 min at 250°C to dissolve completely. The solutions were cooled at room temperature with 5 mL Milli-Q H_2O added before filtering (Whatman 541, hardened, ashless, 90 mm, Whatman International Ltd Maidstone, UK) into 50 mL volumetric flasks and then 10 mL H_2O_2 (30% v/v) was added to each flask and the mixture adjusted to 50 mL with Milli-Q H_2O and mixed thoroughly. The absorbance aliquots of solutions obtained and of similarly prepared standards were recorded against 410 nm using Hitachi 150-20 UV spectrophotometer (Hitachi Science Systems Ltd., Ibaraki, Japan). The TiO_2 content was calculated from the standard curve.

Ileal Digestibility of Nutrients

The nitrogen content of diets and freeze-dried ileal digesta samples was analyzed using a LECO® FP-2000 automatic nitrogen analyzer (Leco Corporation, St. Joseph, MI). The crude protein of diets and ileal digesta samples was determined by multiplying the nitrogen contents by 6.25 and the digestibility coefficient of nutrients was calculated using the following equation:

$$\begin{aligned}&\text{Digestibility coefficient}\\&= 1-[(\text{Digesta nutrient (g/kgDM)}/\\&\quad \text{Digesta}TiO_2\text{(g/kgDM)}]/\\&\quad [(\text{Diet nutrient (g/kg DM)}/\\&\quad \text{Diet } TiO_2\text{(g/kg DM)}]\end{aligned}$$

Extraction of Cecal Bacterial DNA

The cecal DNA was extracted using QIAxtractor DNA Reagents, and QIAxtractor DNA plasticware kits, (Qiagen, Inc., Doncaster, VIC, Australia). Approximately 300 mg of glass beads (0.1mm) and 60 mg of frozen cecal contents were placed in a 2 mL Eppendorf tube. Then 300 μL Qiagen Lysis Buffer (270 μL DXL and 30 μL digestive enzyme) was pipetted to samples with prior disrupted the cells by bead beater mill (Retsch GmbH & Co, Haan, Germany) for 5 min

Table 2. Sequence of primers used for the qPCR analysis of selected microbial populations in cecal digesta samples.

Target group or organism	Primer sequence (5′–3′)	Annealing temperature (C°)	Reference
Bacillus spp.	F-GCA ACG AGC GCA ACC CTT GA R-TCA TCC CCA CCT TCC TCC GGT	63	Zhang et al. (2015)
Bacteroides spp.	F-GAG AGG AAG GTC CCC CAC R-CGC TAC TTG GCT GGT TCA G	63	Layton et al. (2006)
Bifidobacterium spp.	F-GCG TCC GCT GTG GGC R-CTT CTC CGG CAT GGT GTT G	63	Requena et al. (2002)
Clostridium spp.	F-ATG CAA GTC GAG CGA KG R-TAT GCG GTA TTA ATC TYC CTT T	60	Rinttilä et al. (2004)
Enterobacteriaceae	F-CAT TGA CGT TAC CCG CAG AAG AAG C R-CTC TAC GAG ACT CAA GCT TGC	63	Bartosch et al. (2004)
Lactobacillus spp.	F-CAC CGC TAC ACA TGG AG R-AGC AGT AGG GAA TCT TCC A	63	Wise and Siragusa (2007)
Ruminococcus spp.	F-GGC GGC YTR CTG GGC TTT R-CCA GGT GGA TWA CTT ATT GTG TTA A	63	Ramirez-Farias et al. (2009)
Salmonella spp.	F-CGT TTC CTG CGG TAC TGT TAA TT R-AGA CGG CTG GTA CTG ATC GAT AA	56	Bartosch et al. (2004)
Total bacteria	F-CGG YCC AGA CTC CTA CGG G R-TTA CCG CGG CTG CTG GCA C	63	Lee et al. (1996)

at frequency 30/S. The samples were incubated in heating block at 55°C for 2 h and then centrifuged in 20,000 × *g* for 5 min. An aliquot of 200 μL supernatant was transferred into loading block and extraction was performed using the Xtractor Rcorbett Robotics (Model-CAS1820, Australia). In brief, the reactions (DXB, DXW, DXF, or DXE) were loaded into a dedicated cassette and the filtration and elution blocks were placed inside the machine. An aliquot of 400 μL of binding buffer (DXB) was mixed with 200 μL supernatant in the loading block and incubated for 6 min. Then 500 μL of lysed samples was loaded into the capture plate and vacuumed at 30 kPa for 3 min. Another 200 μL of DXB was added to the capture plate and vacuumed at 35 kPa for 3 min. An aliquot of 600 μL DXW was loaded into the capture plate and vacuumed at 30 kPa for 2 min prior to a final washing by 600 μL DXF, followed by vacuuming at 35 kPa for 1 min and again at 25 kPa for 5 min to dry the samples. The extracted cecal DNA was eluted into an elution block by 60 μL DXE after being vacuumed at 30 kPa for 2 min. The extracted DNA was stored at –20°C until required.

Quantification of Cecal Bacteria

The quantitative real-time polymerase chain reaction (**PCR**) of domain bacteria, *Bifidobacterium* spp., *Lactobacillus* spp., *Bacillus* spp., *Ruminococcus* spp., *Bacteroides* spp., *Salmonella* spp., *Clostridium* spp., and Enterobacteriaceae was achieved by using the methods of Wise and Siragusa (2006). The extracted cecal DNA was diluted 20 times in sterile water. The Rotorgene 6000 real-time PCR machine (Corbett, Sydney, Australia) was employed for qPCR assay of the desired bacteria from the extracted cecal DNA. PCR was performed in duplicate for each sample in 10 μL of reaction where PCR was repeated when the difference between the threshold cycle (**CT**) values of the duplicates were >0.5. For PCR reactions, a SYBR Green-containing Mix (SensiMix SYBR No-Rox, Bioline, Sydney, Australia) was applied. The reaction in a volume of 10 μL contained 5 μL of 2× SensiMix, 300 mM of each primer, and 2 μL of DNA template. Table 2 shows the specific 16S rRNA primers were used for quantification of different groups of bacteria.

PCR was performed in a Rotorgene 6500 real-time PCR machine and a threshold cycle average from the duplicate samples was used for data analysis. Serial dilutions of linearized plasmid DNA (pCR®4-TOPO Vector, Life Technologies, Carlsbad, CA) inserted with respective bacterial amplicons were used to construct a standard curve. The concentrations of the plasmid DNA were measured using NanoDrop ND-8000 (Thermo Fisher Scientific, Waltham, MA) prior to the serial dilutions. The number of target DNA copies was calculated from the mass of DNA taking into account the size of the amplicon insert in the plasmid. Bacteria numbers were expressed as $\log_{10}$ (genomic DNA copy number)/g digesta.

Litter Quality

On d 35, a composite sample of approximately 1 kg of litter was obtained by pooling subsamples collected from 6 locations (around feeder, drinkers and end of the pen) in each pen. Each sample was weighed accurately before drying in oven at 105°C for 24 h. The moisture content (**MC**) was calculated (Barker et al., 2013).

Statistical Analyses

All data were analyzed using the General Linear Models (**GLM**) procedure of SPSS statistics version 22 (IBM Corporation) for the main effect of particle size and lignocellulose supplementation and interactions. Differences between mean values were determined using the Tukey test at the level of $P < 0.05$.

Table 3. Response of broiler performance to lignocellulose and corn particle size from d 0-10.

Treatments	FCR	Weight gain (g/bird)	Feed intake (g/bird)	Livability %
CGC + 0% lignocellulose	1.065[a,b,c]	272	290	100
CGC + 1% lignocellulose	1.086[a]	264	286	99
CGC + 2% lignocellulose	1.049[c]	274	288	97
FGC + 0% lignocellulose	1.053[b,c]	282	297	98
FGC + 1% lignocellulose	1.070[a,b,c]	278	298	98
FGC + 2% lignocellulose	1.081[a,b]	278	300	99
SEM[1]	0.007	3	2	1
Main effect				
Particle size				
CGC	1.067	270[b]	288[b]	99
FGC	1.068	279[a]	298[a]	99
SEM[2]	0.005	2	1	1
Lignocellulose				
0%	1.059	277	294	99
1%	1.078	271	292	99
2%	1.065	276	294	98
SEM[3]	0.006	2	2	1
P value				
Particle size	0.815	<0.001	<0.001	1.00
Lignocellulose	0.051	0.082	0.683	0.476
Particle size × lignocellulose	0.004	0.158	0.439	0.119

[a-c]Means sharing the same superscripts are not significantly different from each other at $P < 0.05$
[1]SEM = Standard error of the mean for particle size and lignocellulose effect (n = 6).
[2]SEM = Standard error of the mean for particle size effect (n = 18).
[3]SEM = Standard error of the mean for lignocellulose effect (n = 12).

Table 4. Responses of broiler performance to lignocellulose and particle size from d 0 to 24 and d 0 to 35.

Main effect	Feed intake (g/bird)		Weight gain (g/bird)		FCR		Livability	
	d 24	d 35	d 24	d 35	d 24	d 35	d 24	d 35
Particle size								
CGC	1875	3794	1511	2752	1.243[b]	1.379[b]	99	98
FGC	1872	3770	1484	2698	1.262[a]	1.398[a]	98	98
SEM[1]	10	21	10	21	0.005	0.006	1	1
Lignocellulose								
0%	1873	3770	1505	2741	1.247	1.376	99	99
1%	1867	3778	1493	2716	1.251	1.392	99	98
2%	1882	3797	1494	2719	1.260	1.397	97	96
SEM[2]	12	26	14	28	0.006	0.007	1	1
P value								
Particle size	0.832	0.423	0.092	0.095	0.007	0.023	0.574	0.795
Lignocellulose	0.689	0.749	0.803	0.784	0.304	0.091	0.201	0.086
Particle size × lignocellulose	0.522	0.437	0.544	0.555	0.490	0.907	0.575	0.624

[a,b]Within a column, values with different superscripts are significantly different from each other at $P < 0.05$.
[1]SEM = Standard error of the mean for particle size effect (n = 18).
[2]SEM = Standard error of the mean for lignocellulose effect (n = 12).

RESULTS

Broiler Performance

The broiler performance results are presented in Tables 3 and 4. At d 10, there was significant particle size × fiber interaction observed for FCR ($P < 0.01$). The birds fed CGC had lower FCR than those fed FGC only at 2% of lignocellulose but not at 1% or no lignocellulose addition. Furthermore, 2% lignocellulose addition in feed showed improved FCR compared to those with 1% of lignocellulose only when birds were fed CGC. Birds fed FGC were heavier than those fed CGC ($P < 0.001$) and birds fed CGC consumed less feed than those fed FGC ($P < 0.001$) at d 10. However, at d 24 and 35, the birds fed the CGC had lower FCR than those fed the FGC ($P < 0.01$ and <0.05, respectively). Furthermore, weight gain of birds fed CGC tended to be greater, although not significant, compared to those fed FGC on both d 24 and 35 ($P = 0.092$ and 0.095, respectively). There was no significant particle size × fiber interaction observed for any performance parameters at d 24 and 35 ($P > 0.05$). No differences in livability were detected ($P > 0.05$).

Relative Gizzard Weight and pH

Table 5 shows that broilers fed CGC had heavier relative gizzard weights on d 24 ($P < 0.05$) and 35

Table 5. The effect of lignocellulose and particle size on gizzard weight and pH at d 24 and 35.

Treatments	Relative gizzard weight		Gizzard digesta pH	
	D24	D35	D24	D35
CGC + 0% lignocellulose	1.853	1.195	3.372	3.707
CGC + 1% lignocellulose	1.845	1.219	3.240	3.655
CGC + 2% lignocellulose	1.866	1.173	3.250	3.725
FGC + 0% lignocellulose	1.733	1.043	3.360	3.770
FGC + 1% lignocellulose	1.780	1.061	3.490	3.760
FGC + 2% lignocellulose	1.791	1.068	3.412	3.976
SEM[1]	0.044	0.044	0.080	0.087
Main effect				
Particle size				
CGC	1.855[a]	1.196[a]	3.287	3.696
FGC	1.768[b]	1.057[b]	3.421	3.835
SEM[2]	0.024	0.025	0.046	0.052
Lignocellulose				
0%	1.793	1.119	3.366	3.738
1%	1.813	1.140	3.365	3.708
2%	1.829	1.121	3.331	3.851
SEM[3]	0.033	0.037	0.061	0.066
P-value				
Particle size	0.025	0.001	0.058	0.072
Lignocellulose	0.723	0.873	0.893	0.286
Particle size × lignocellulose	0.811	0.815	0.278	0.577

[a,b]Means sharing the same superscripts are not significantly different from each other at $P < 0.05$.
[1]SEM = Standard error of the mean for particle size and lignocellulose effect (n = 6).
[2]SEM = Standard error of the mean for particle size effect (n = 18).
[3]SEM = Standard error of the mean for lignocellulose effect (n = 12).

($P < 0.001$) than those fed FGC. The gizzard digesta pH tended to be lower, although not significant, by a magnitude of 0.134 and 0.139 units in broilers received the CGC compared those received the FGC at d 24 and 35, respectively ($P = 0.058$ and 0.072). The relative gizzard weight and gizzard digesta pH were not affected by the inclusion of lignocellulose in the diets ($P > 0.05$) and no interaction between particle size and lignocellulose was observed at d 24 and 35 ($P > 0.05$).

Crude Protein Digestibility and Gross Energy

Table 6 shows a significantly increased ileal gross energy and protein digestibility ($P < 0.05$) in birds fed the CGC compared to those fed the FGC. Lignocellulose addition did not affect gross energy or protein digestibility ($P > 0.05$). No interaction was detected between lignocellulose and particle size on ileal gross energy and protein digestibility ($P > 0.05$).

Cecal Microflora

Birds fed the CGC diet had higher ($P < 0.01$) counts of cecal *Ruminococcus* spp. compared to those fed the FGC diet at d 24 (Table 7). However, no significant difference in cecal *Bifidobacterium* spp., *Bacteroides* spp., *Bacillus* spp., *Clostridium* spp., *Lactobacillus* spp., *Salmonellae* spp., Enterobacteriaceae, or total bacteria counts was observed between birds fed the CGC and FGC at d 24 ($P > 0.05$). Birds fed 2% lignocellulose had lower ($P < 0.05$) cecal *Clostridium* spp. counts than those fed 1% lignocellulose being numerically lower than control group. Furthermore, birds fed 2% lignocellulose had lower ($P < 0.05$) cecal *Ruminococcus* spp. counts than the control group. However, lignocellulose had no effect on counts of cecal *Bifidobacterium* spp., *Bacteroides, Bacillus* spp., *Lactobacillus* spp., *Salmonellae* spp., Enterobacteriaceae, or total bacteria counts ($P > 0.05$). No significant particle size × fiber interaction was observed for all groups of bacteria ($P > 0.05$).

Litter Moisture Content

At d 35, the birds fed lignocellulose at both 1% and 2% levels had lower moisture (%) in the litter ($P < 0.001$) compared to those fed diets without lignocellulose (Table 8). However, particle size of corn did not affect litter moisture ($P > 0.05$). No interaction was detected between particle size and lignocellulose on litter moisture content ($P > 0.05$).

DISCUSSION

The results of the current study clearly demonstrated the effects of corn particle size on broiler performance in both early and late age. Jacobs et al. (2010) reported feeding chicks with a geometric mean diameter (GMD) of 1,387 μm reduced weight gain compared to those chicks with a GMD of 858 μm at d 7. Douglas et al. (1990) also found that young chicks fed CGC with a GMD of 1,470 μm had reduced body weight and feed utilization compared to those fed FGC with a GMD of about 900 μm. Nir et al. (1994) also reported that

Table 6. The effect of lignocellulose and particle size on ileal protein and gross energy digestibility at d 24.

Treatments	Crude protein digestibility	Gross energy digestibility
CGC + 0% lignocellulose	0.823	0.773
CGC + 1% lignocellulose	0.842	0.781
CGC + 2% lignocellulose	0.823	0.770
FGC + 0% lignocellulose	0.809	0.755
FGC + 1% lignocellulose	0.817	0.759
FGC + 2% lignocellulose	0.821	0.765
SEM[1]	0.008	0.007
Main effect		
Particle size		
CGC	0.829[a]	0.775[a]
FGC	0.816[b]	0.760[b]
SEM[2]	0.005	0.004
Lignocellulose		
0%	0.816	0.764
1%	0.830	0.770
2%	0.822	0.767
SEM[3]	0.006	0.005
P-value		
Particle size	0.032	0.043
Lignocellulose	0.186	0.748
Particle size × lignocellulose	0.300	0.550

[a,b]Means sharing the same superscripts are not significantly different from each other at $P < 0.05$
[1]SEM = Standard error of the mean for particle size and lignocellulose effect (n = 6).
[2]SEM = Standard error of the mean for particle size effect (n = 18).
[3]SEM = Standard error of the mean for lignocellulose effect (n = 12).

Table 7. Bacterial quantification ($\log_{10}$ CFU) in cecal content of birds fed lignocellulose and corn particle size at d 24.

Main effect	*Bifidobacterium*	*Lactobacillus*	*Bacillus*	*Ruminococcus*	*Bacteroides*	*Clostridium*	*Salmonella*	*Enterobacteria*	Total bacteria
Particle size									
CGC	8.02	8.66	9.27	9.93[a]	8.87	6.58	5.35	7.72	11.12
FGC	7.97	8.52	9.14	9.78[b]	9.03	6.59	5.28	7.74	11.13
SEM[1]	0.05	0.07	0.09	0.04	0.11	0.11	0.06	0.13	0.02
Lignocellulose									
0%	8.01	8.71	9.29	9.95[a]	8.87	6.61[a,b]	5.29	7.76	11.15
1%	8.07	8.56	9.32	9.87[a,b]	9.04	6.81[a]	5.37	7.94	11.14
2%	7.91	8.50	9.00	9.75[b]	8.96	6.34[b]	5.29	7.49	11.08
SEM[2]	0.06	0.08	0.11	0.05	0.14	0.12	0.07	0.16	0.02
P-value									
Particle size	0.423	0.152	0.321	0.009	0.339	0.970	0.391	0.920	0.843
Lignocellulose	0.150	0.207	0.081	0.020	0.697	0.029	0.700	0.146	0.086
Particle size × lignocellulose	0.296	0.939	0.461	0.362	0.373	0.139	0.503	0.090	0.603

[a,b]Within a column, values with different superscripts are significantly different from each other at $P < 0.05$.
[1]SEM = Standard error of the mean for particle size effect (n = 18).
[2]SEM = Standard error of the mean for lignocellulose effect (n = 12).

the coarse corn particles significantly depressed growth performance compared to medium and fine particles up to d 7. Impaired live performance, such as lower weight gain and poorer FCR in young broilers fed dietary coarse particle corn could be related to their limited ability to efficiently grind and utilize coarse particles owing to an underdeveloped gizzard (Douglas et al.,1990; Lott et al., 1992). There was, however, significant particle size × fiber interaction observed for FCR on 10 d. The birds fed CGC with 2% lignocellulose had better FCR than those fed FGC with 2% lignocellulose or birds fed CGC with 1% lignocellulose. This may be due to the combination of high water consumption and water holding capacity of lignocellulose fiber, thereby increasing the retention time of the coarse particles in the gut as indicated by Partners (2012) that lignocellulose increased water consumption by 6.9% in broilers. This source of fiber might form a matrix with fibrous characteristics and swell within the aqueous medium to trap more water and create a network around coarse particles. This thereby increases the digestibility of nutrients in the gastrointestinal tract. In contrast, older birds fed CGC showed better FCR than those fed FGC, and weight gain of older birds fed coarse corn tended ($P = 0.09$) to be higher. This effect may be due to the fact that the gizzard of grown birds is more developed to grind coarse particles, which, in turn, further enhances its development, resulting in better conversation of feed. However, previous findings on the effect of the particle size on

Table 8. The effect of lignocellulose and particle size on litter moisture content at d 35.

Treatments	Litter moisture content %
CGC + 0% lignocellulose	38.5
CGC + 1% lignocellulose	34.6
CGC + 2% lignocellulose	33.4
FGC + 0% lignocellulose	39.1
FGC + 1% lignocellulose	32.6
FGC + 2% lignocellulose	35.9
SEM[1]	1.1
Main effect	
Particle size	
CGC	35.5
FGC	35.9
SEM[2]	0.9
Lignocellulose	
0%	38.8[a]
1%	33.6[b]
2%	34.7[b]
SEM[3]	0.7
P-value	
Particle size	0.731
Lignocellulose	0.001
Particle size × lignocellulose	0.247

[a,b]Means sharing the same superscripts are not significantly different from each other at $P < 0.05$

[1]SEM = Standard error of the mean for particle size and lignocellulose effect (n = 6).

[2]SEM = Standard error of the mean for particle size effect (n = 18).

[3]SEM = Standard error of the mean for lignocellulose effect (n = 12).

growth performance are conflicting and inconsistent in older birds. Amerah et al. (2007b) stated that the different wheat particle sizes (839 or 1,164 μm) with pelleted diets had no effect on growth performance. Furthermore, Naderinejad et al. (2016) stated that the different corn particle sizes (490, 651, or 796 μm) with pelleted diets had no effect on growth performance. Interestingly, Lott et al. (1992) observed that birds performed better when corn particle size decreased from 1,196 to 716 μm. Also, Chewning et al. (2012) stated that the reduction of corn particle size resulted in an improvement in body weight up to 21 d, but not at 44 d. However, in their study, the particles were very fine (267 and 570 μm). In contrast, Xu et al. (2015b) stated that the replacement of 50% of fine corn particles (294 μm) with coarse corn particles (1,362 μm) improved feed conversion and increased weight gain compared to fine particle size alone. In the current study, the better performance of birds fed CGC was likely to be the result of better gizzard development.

Diet structure, such as particle size or fiber type, is important for gizzard development (Choct, 2009; Naderinejad et al., 2016). As gizzard development is enhanced, its pH decreases between 0.2 and 1.2 units (Gabriel et al., 2003; Naderinejad et al., 2016; Svihus, 2011). In the present study, birds fed CGC had heavier gizzards relative to body weight and tended to have lower pH compared to birds fed FGC. The outcomes of the present study are supported by the previous reports (Naderinejad et al., 2016; Xu et al., 2015b; and Parsons et al., 2006). Enhanced gizzard activity results in a more thorough digesta grinding and a longer digesta retention time to produce a critical size of the digesta prior to passing through the pyloric sphincter (Clemens et al., 1975; Hetland et al., 2003). This leads to an increase in the volume of gizzard contents and the frequency of contractions, leading to muscular adaptation to cope with the extra grinding demand from feed.

In the current study, the coefficients of ileal gross energy and protein digestibility were improved in the birds fed CGC compared with those fed FGC in pelleted diets. Once again, it confirms that benefits of a well-functioning gizzard. Indeed, a heavy, well-developed gizzard enhances gut motility (Ferket, 2000) through increased release of cholecystokinin (Svihus et al., 2004), which occurs in the duodenal and pyloric region of fowls (Denbow, 2000) and acts through the vagus nerve to stimulate the pancreatic enzyme secretion and gastroduodenal reflux (Duke, 1992; Li and Owyang, 1993). Coarse particles delay the transit time of digesta in the gizzard, which increases of the exposure time of nutrients to digestive enzymes (Nir et al.,1994), thereby improving energy utilization and nutrient digestibility (Carré, 2000). Furthermore, it has been reported that a lower pH of gizzard contents may encourage pepsin activity (Gabriel et al., 2003), which increases the denaturation and hydrolysis of dietary proteins and hence improves protein digestion. A number of studies have shown improved nutrient utilization when birds are fed structural materials in their diets (Rougière et al., 2009; Svihus and Hetland, 2001). The higher energy and protein digestibility values observed in this study corroborate previous findings (Xu et al., 2015a,b; Rougière et al., 2009; Naderinejad et al., 2016; and Parsons et al., 2006). The findings of the present study prove that the beneficial performance effects of feeding CGC to broilers are due to the improved digestibility of nutrients through enhanced gizzard function and suitable digesta pH for the digestive enzymes.

The structural components of feed that impart beneficial effects on the gizzard consist largely of fiber, which is the sum of NSP and lignin. In addition to the direct effects that fiber has on the gizzard, certain components of fiber bring about gut health benefits via the production of prebiotics in situ. Slavin (2013) stated that higher intakes of dietary fiber play a vital role in intestinal health via improved intestinal barrier function and host immunity, reduced pathogen load (e.g., Clostridia), and enhanced short chain fatty acid production. In this study, 2% supplemental lignocellulose led to significantly lower *Clostridium* spp. counts in the ceca, which suggests the possible role of lignocellulose on the control of *Clostridium* spp. in the gut. It is therefore speculated that diets high in certain types of structural components may help alleviate the risk of some diseases, such as necrotic enteritis.

The control of litter moisture content is a priority in the modern poultry industry to reduce productivity loss and avoid environmental and bird welfare problems. Not all sources of fiber improve litter and fecal

quality (Hartini et al., 2003). Jiménez-Moreno et al. (2013a) reported that the inclusion of sugar bulb beet in the diet increased the moisture content in the excreta compared with the inclusion of oat hulls. At d 35 in the present study, the litter in the pens housing the birds received lignocellulose had a lower moisture content compared to those fed the control diets. Similar observations have been reported elsewhere (Farran et al., 2013). A couple of possible explanations may be speculated. Firstly, fiber can hold a large amount of water in its matrix and this capaciy depends on the type of fiber used. A longer digesta retention time coupled with a better water holding capacity in the gut would encourage water re-use in the ceca and reduce water excretion. Secondly, the gizzard of the birds fed a higher-fiber diet would act as a pace-maker organ for nutrient digestion and absorption, regulating water absorption to an optimum. This moderates the unnecessary urge of birds to drink excessively and hence reduce the amount of water excreted in the litter.

In conclusion, coarse grinding of corn was beneficial in improving digestion and bird performance. The use of a lignocellulose product altered the gut microbial environment in a way it improved gut health and litter quality. A well-developed gizzard as a result of feeding broilers diets with a coarsely ground corn was responsible for low gizzard pH. This was probably the underlying reasons for improved bird performance observed in this study. However, it is likely that the cost of feed will increase, and it would be beneficial if cereal by-product can be used instead of such purified fiber sources. Studies on whether such cereal by-products may have similar effects as lignocellulose will be interesting and further investigations are warranted.

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