Dietary sugarcane bagasse and coarse particle size of corn are beneficial to performance and gizzard development in broilers fed normal and high sodium diets

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ABSTRACT An experiment was conducted to evaluate the effects of sugarcane bagasse (SB) and particle size on broiler performance, gizzard development, ileal microflora, litter quality, and bird welfare under a wet litter challenge model. A total of 672 one-day-old Ross 308 male broilers was allocated to 48 pens using a 2 × 2 × 2 factorial arrangement of treatments with corn particle size—coarse 3,576 μm (CC) or fine 1,113 μm (FC) geometric mean diameter, SB - 0 or 2% and sodium (Na) - 0.16 or 0.40% with increased Na level to induce wet litter. A 3-way particle size × Na × SB interaction ($P < 0.05$) was observed for weight gain at d 10. Birds fed FC showed a higher weight gain compared to birds fed CC when 0.40% Na without SB diet or 0.16% Na with 2% SB diet was offered. A significant particle size × SB interaction was observed at d 24 on feed conversion ratio (FCR; $P < 0.001$) and weight gain ($P < 0.05$). FCR was reduced by 2% SB supplementation in birds fed CC but increased in birds fed FC. Further, weight gain of birds fed 2% SB was higher in birds fed CC but not in those fed FC. On d 35, birds fed 2% SB had a higher weight gain ($P < 0.001$) compared to those without SB, and a SB × particle size interaction on relative gizzard weight ($P < 0.05$) and pH ($P < 0.05$) was present. SB reduced gizzard pH and increased the relative gizzard weight in birds fed the FC diet but not the CC diet ($P < 0.05$). Counts of ileal *Bacillus* spp. were increased in birds fed SB ($P < 0.05$) on d 24. No effects of SB and particle size on litter quality and bird welfare were observed, but higher Na increased litter moisture and footpad dermatitis (**FPD**) scores ($P < 0.001$). These findings suggest that SB independently or in combination with CC improves performance in older birds regardless of Na level in diets, possibly through improved gizzard development and gut microflora of birds.

Key words: sugarcane bagasse, particle size, gizzard, microflora, performance

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INTRODUCTION

The legislation to ban the use of in-feed antibiotics in the poultry industry in the European Union and voluntary removal of antibiotics in animal feed have led to the emergence of wet litter problems due to enteric disorders. Thus, broiler performance and profitability are affected (Hofacre et al., 2003; Collett, 2012). There has been a concerted effort to find alternative strategies to modulate gut microflora, augment the immune response, and reduce pathogens through management and nutritional intervention, such as inclusion of fiber and large particle sizes in the diet, in an attempt to improve health and digestive efficiency of broilers. There is no clear consensus on the effects of ingredient particle size and dietary fiber on broiler performance. Large particle sized ingredients and high levels of fiber were reported to have negative effects on broiler performance (Lott et al., 1992; Sklan et al., 2003). However, it is well documented that structural components of feed, such as coarse fiber or ingredient particle size, modulate the gastrointestinal development and function and can improve gut health and litter quality (Mateos et al., 2012; Xu et al., 2015a,b; Jiménez-Moreno et al., 2016; Kheravii et al., 2016). The inclusion of fiber or coarse particles in diet has been shown to increase digesta retention time in the upper part of the gastrointestinal tract (**GIT**) (i.e., from crop to gizzard), stimulate gizzard function (Nir et al., 1994; Hetland et al., 2005), and increase the secretion of HCl in the proventriculus (Duke, 1986) in broilers. It has been shown that low pH in the upper GIT improves solubility and absorption of mineral salts (Guinotte et al., 1995) and pepsin activity (Sklan et al., 1978). Therefore, feed modulations by addition of fiber and coarse particles may be beneficial to growth performance of the broiler chickens.

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It has been reported that sodium (**Na**) stimulates intestinal adenosine triphosphatases and is involved in small intestinal nutrient transport (Gal-Garber et al., 2003) and thus improves broiler performance (Vieira et al., 2003). However, a high level of Na in the broiler diet is associated with the occurrence of wet litter and bird welfare issues (Collett, 2012). Currently, the control of litter moisture is a priority in the broiler industry to reduce productivity losses and minimize bird welfare issues due to footpad dermatitis (**FPD**), hock burn (**HB**), and ammonia production. Wet litter was found to increase FDP, HB, and breast irritations and reduce broiler performance (de Jong et al., 2014). Different strategies to minimize litter moisture to improve welfare and performance of the birds have been examined. These include the use of appropriate bedding materials, such as wood shavings, sugarcane bagasse (**SB**), rice husk, and pelleted wheat straw (Monira et al., 2003; Teixeira et al., 2015; Kheravii et al., 2017); the use of coarse particle ingredients in broiler diet (Xu et al., 2015a); and the inclusion of an appropriate source of fiber in the diet (Jiménez-Moreno et al., 2013a). The degree of lignification of fiber is a key characteristic that affects bird productivity and physiology (Mateos et al., 2012). It has been reported that feeding of fiber rich in lignin exhibited a positive effect on fecal consistency, litter quality, gut microflora, and its fermentation activity in chickens (Bogusławska-Tryk et al., 2015; Milosevic et al., 2015). The structural components of feed, including particle size or fiber, have been shown to affect retention time, pH in the gizzard, and bacterial population in the gut (Jacobs et al., 2010). Singh et al. (2014) concluded that the inclusion of coarse corn (**CC**) in broiler diets increased *Lactobacillus* spp. and decreased *Clostridium* spp. and *Campylobacter* spp. It appeared that dietary fiber has the potential to maintain balanced gut flora to enhance health and wellbeing of the animals through improved gut health (Gibson et al., 2004; Bogusławska-Tryk et al., 2015). This study aimed to evaluate the effect of SB and corn particle size on growth performance, litter quality, gizzard development, gut microflora, and welfare of birds fed normal and high salt feed.

MATERIALS AND METHODS

Experimental Design and Bird Management

A total of 672 one-day-old male Ross 308 chicks was obtained from Baiada Hatchery in Tamworth, NSW, Australia. Chicks were randomly assigned to 8 treatments in a 2 × 2 × 2 factorial arrangement of treatments consisting of: SB - 0 or 2% inclusion in the diets; corn particle size—coarse at 3,576 μm or fine at 1,113 μm geometric mean diameter (**GMD**); and Na - 0.16 or 0.4% inclusion in diets. The GMD of corn particle size was determined according to the American Society of Agricultural Engineers (2003). The broiler chicks were reared in pens measuring 75 cm × 120 cm up to d 35. Wood shavings were used as bedding material to a depth of approximately 7 cm in each pen. Pens were equipped equally with a plastic tube feeder and nipple drinkers. Feed and water were provided *ad libitum*. The lighting, relative humidity, and temperature were maintained in accordance with Ross 308 guidelines (Aviagen, 2014).

Sugarcane Bagasse

The SB was provided by FCR Consulting Group, Brisbane. The composition of SB was determined ("as is" basis) for total non-starch polysaccharides (**NSP**) and lignin following the method described by Englyst et al. (1994) and Kirk and Obst (1988), respectively. The SB contained 6.1 g/kg free sugar, 191 g/kg lignin, 534 g/kg insoluble NSP, and 1.9 g/kg soluble NSP.

Diets and Bird Performance

The ingredient and nutrient composition of the experimental diets are shown in Table 1. All of the 8 diets based on corn, soybean meal, and meat meal were formulated to meet the minimum nutrient profiles of the Ross 308 specifications (Aviagen, 2014). Herein, the composition of diets were diluted when 2% SB was added over the top of the complete feed. All the diets were thoroughly mixed and cold-pelleted (65°C). Birds were fed in the phases of starter (d 0 to 10), grower (d 11 to 24), and finisher (d 25 to 35). Birds and leftover feed were weighed and the average weight gain, feed intake, and FCR were calculated based on the measurements at the end of the starter, grower, and finisher phases. Mortality was recorded on a daily basis.

Animal Ethics

This experiment was approved by the Animal Ethic Committee of the University of New England (Approval No: AEC 15–053). All bird management 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).

Gizzard Measurements

Empty gizzards without proventriculi were weighed and recorded along with bird weights. Gizzard contents of 2 birds were collected 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).

Water Intake and Fresh Excreta Moisture

From d 17 to 24, water consumption was measured by pen replicate and the water: body weight ratio calculated. Water intake was determined as the difference

Table 1. Composition and nutrient content of corn base diet (%).[1]

Ingredients	Starter (d 0 to 10)		Grower (d 11 to 24)		Finisher (d 25 to 35)	
	Normal Na	High Na	Normal Na	High Na	Normal Na	High Na
Corn	60.6	59.3	62.3	61.0	67.5	66.3
Soybean meal	32.6	32.8	29.3	29.5	24.7	24.9
Meat and bone meal	3.00	3.00	3.60	3.60	3.00	3.00
Canola oil	0.644	1.08	1.91	2.30	2.46	2.84
Limestone	0.970	0.968	0.814	0.812	0.777	0.775
Dical phosphate	0.607	0.611	0.269	0.271	0.193	0.196
Phytase[2]	0.01	0.010	0.010	0.010	0.010	0.010
Salt	0.154	0.568	0.161	0.703	0.176	0.752
Na bicarbonate	0.219	0.505	0.200	0.300	0.200	0.250
Vitamin-mineral[3]	0.200	0.200	0.200	0.200	0.200	0.200
Choline	0.111	0.111	0.103	0.103	0.101	0.101
L-lysine HCl 784	0.305	0.303	0.226	0.222	0.209	0.205
D, L-methionine	0.392	0.394	0.336	0.337	0.305	0.306
L-threonine	0.204	0.204	0.148	0.147	0.117	0.117
TiO_2	–	–	0.500	0.500	–	–
Nutrients						
ME (kcal/kg)	3000	3000	3100	3100	3200	3200
ME (MJ/kg)	12.55	12.55	12.97	12.97	13.39	13.39
Crude protein	22.2	22.2	21.0	21.0	19.0	19.0
Crude fat	2.85	3.26	4.14	4.51	4.67	5.03
Crude fiber	2.07	2.06	2.01	1.99	1.92	1.91
SID arginine	1.37	1.37	1.27	1.29	1.14	1.14
SID lysine	1.28	1.28	1.15	1.15	1.02	1.02
SID methionine	0.684	0.685	0.616	0.616	0.563	0.563
SID methionine + Cysteine	0.950	0.950	0.870	0.870	0.800	0.800
SID tryptophan	0.244	0.245	0.226	0.226	0.198	0.199
SID isoleucine	0.860	0.860	0.807	0.808	0.725	0.726
SID threonine	0.860	0.860	0.770	0.770	0.680	0.680
SID valine	0.992	0.992	0.939	0.939	0.848	0.849
Starch	35.8	35.1	36.8	36.0	39.8	39.1
NSP soluble	0.426	0.424	0.404	0.402	0.381	0.38
NSP insoluble	5.64	5.60	5.45	5.40	5.33	5.29
Calcium	0.960	0.960	0.870	0.870	0.780	0.780
Available phosphorus	0.480	0.480	0.435	0.435	0.390	0.390
Sodium	0.160	0.400	0.160	0.400	0.160	0.400
Chloride	0.250	0.500	0.242	0.569	0.243	0.591
Choline	0.170	0.170	0.160	0.160	0.150	0.150

[1]The composition of diets were diluted by 1.96% when 2% SB was added over the top of the complete feed for SB treatment(s).
[2]Phyzyme XP5000G (100 g/mt) Dupont.
[3]Vitamin-mineral concentrate supplied per kilogram of diet: retinol, 12,000 IU; cholecalciferol, 5000 IU; tocopheryl acetate, 75 mg, menadione, 3 mg; thiamine, 3 mg; riboflavin, 8 mg; niacin, 55 mg; pantothenate, 13 mg; pyridoxine, 5 mg; folate, 2 mg; cyanocobalamine, 16 μg; biotin, 200 μg; cereal-based carrier, 149 mg; mineral oil, 2.5 mg; Cu (sulphate), 16 mg; Fe (sulphate), 40 mg; I (iodide), 1.25 mg; Se (selenate), 0.3 mg; Mn (sulphate and oxide), 120 mg; Zn (sulphate and oxide), 100 mg; cereal-based carrier, 128 mg; mineral oil, 3.75 mg; SID = Standard ileal digestible.

between water supplied and water remaining in the bottle. On d 24, 2 birds from each pen were transferred to another room and placed in metabolic cages. After 2 d of adaptation, on d 26, the fresh excreta were collected from cleaned trays placed beneath the cages. The excreta from each cage were collected and weighed before and after being oven-dried at 100°C for 24 h. The moisture content was then determined according to the method described by Barker et al. (2013).

Extraction of Ileal Bacterial DNA

Around 1 g of homogenized ileal digesta was collected into a 2-mL Eppendorf cap lock tube, snap-frozen in liquid N2, and stored at −20°C until required for DNA extraction. Ileal digesta DNA was extracted using a QIAamp DNA Stool Mini Kit, Cat No. 51,504 (Qiagen, Hilden, Germany) with some modification. Approximately 300 mg of glass beads (0.1 mm) and 190 mg of frozen ileal contents were placed in a 2-mL Eppendorf tube. Then 400 μl ASL buffer was added followed by disruption of the cells by bead beater mill for 45 s at a frequency of 30/S. An aliquot of 1,000 μL of ASL buffer was added to the samples and vortexed for 1 min. The samples were incubated at 95°C for 5 min and vortexed and centrifuged at 20,000 × g for 1 min. An InhibitEX Tablet was dissolved in the supernatant of each sample and vortexed immediately until the tablet was completely suspended. The suspension was incubated for 1 min at room temperature to allow inhibitors to adsorb to the InhibitEX matrix. The samples were centrifuged at 20,000 × g for 3 min to pellet inhibitors bound to InhibitEX matrix. The supernatant was transferred into a new 1.5 mL microcentrifuge tube and centrifuged at 20,000 × g for 3 min. An aliquot of 15 μL of proteinase K was added to 200 μl supernatant, and then 200 μL of buffer AL were added followed by incubation at 70°C for 10 min. The lysate was mixed with 200 μl of absolute ethanol and then centrifuged in a QIAamp spin column at 20,000 × g for 1 min with the flow-through discarded. Wash buffer AW1 (500 μL) and AW2 (500 μL) were applied at independent steps to

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

Target group or organism	Primer sequence (5′–3′)	Amplicon length (bp)	Annealing temperature (°C)	Reference
Bacillus spp.	F- GCA ACG AGC GCA ACC CTT GA R- TCA TCC CCA CCT TCC TCC GGT	92	63	Zhang et al. (2015)
Bacteroides spp.	F- GAG AGG AAG GTC CCC CAC R- CGC TAC TTG GCT GGT TCA G	108	63	Layton et al. (2006)
Bifidobacterium spp.	F- GCG TCC GCT GTG GGC R- CTT CTC CGG CAT GGT GTT G	106	63	Requena et al. (2002)
Clostridium spp.	F- ATG CAA GTC GAG CGA KG R- TAT GCG GTA TTA ATC TYC CTT T	120	60	Rinttilä et al. (2004)
Enterobacteriaceae	F- CAT TGA CGT TAC CCG CAG AAG AAG C R- CTC TAC GAG ACT CAA GCT TGC	190	63	Bartosch et al. (2004)
Lactobacillus spp.	F- CAC CGC TAC ACA TGG AG R- AGC AGT AGG GAA TCT TCC A	186	63	Wise and Siragusa (2007)
Ruminococcus spp.	F- GGC GGC YTR CTG GGC TTT R- CCA GGT GGA TWA CTT ATT GTG TTA A	157	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	67	56	Bartosch et al. (2004)
Total bacteria	F- CGG YCC AGA CTC CTA CGG G R- TTA CCG CGG CTG CTG GCA C	204	63	Lee et al. (1996)

purify DNA, through centrifugation at 20,000 × g for 1 and 3 min, respectively, to remove the wash buffer and to dry the silica membrane completely. Finally, 50 μL of Elution Buffer were used to elute DNA into a 1.5-mL Eppendorf tube. The extracted DNA was stored at −20°C until required.

Quantitative PCR (qPCR) of Ileal Microflora

The dominant bacteria groups in the small intestine, *Bifidobacterium* spp., *Lactobacillus* spp., *Bacillus* spp., *Ruminococcus* spp., *Bacteroides* spp., *Salmonella* spp., *Clostridium* spp., and Enterobacteriaceae, were quantified using the qPCR method. The 16S rRNA primers were used and are shown in Table 2. The extracted ileal DNA was diluted 20 times in autoclaved water, and the amplification of the desired bacteria from the extracted DNA was performed using a Rotorgene 6500 real-time PCR machine (Corbett Research, Sydney, Australia). For PCR reactions, a reagent mix containing SYBR Green (SensiMix SYBR No-Rox, Bioline, Sydney, Australia) was used. 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. The PCR was performed in duplicate, and if the difference of threshold cycle (**Ct**) values between the duplicates was >0.5, the assay of the sample was repeated.

PCR was performed in a Rotorgene 6500 real-time PCR machine (Corbett, Sydney, Australia). The Ct average from the duplicate samples was used for data analysis. Serial dilutions of linearized plasmid DNA (pCR®4-TOPO Vector, Life Technologies, Carlsbad, CA, USA) 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, USA) 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.

Footpad Dermatitis and Hock Burn Scoring

On d 35, 3 birds from each pen were scored for FPD and HB. The procedures described by Allain et al. (2009) and Kjaer et al. (2006) were used to score FPD and HB, respectively. For FPD, a 10-point (ranging from 0 to 9) scale was used based on extent and appearance of lesions: 0 indicates no lesions and 9 the most macroscopic deep lesions. For HB, score 1 refers to no lesions, score 2 minor lesions, and score 3 major lesions.

Litter Quality (structure) and Moisture Content

Litter quality (structure) was scored per pen by visual inspection at d 35. Litter was scored using a 4-point scale (ranging from 0 to 3): 0 = dry; 1 = slightly moist/caked; 2 = more moist/caked; and 3 = wet. On d 35, a composite sample of approximately 1 kg of litter was obtained by pooling subsamples collected from 5 locations (around feeder, drinkers, and end of the pen) in each pen. Each sample was weighed accurately before drying in an 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, Armonk, New York) for the main effects of particle size, SB supplementation, and Na levels, along with their interactions. Differences between mean values were determined using the LSD test at the level of $P < 0.05$.

Table 3. Effect of corn particle size, SB, and dietary Na on broiler performance at d 10.

Treatments Particle size	Na	SB	FCR	Weight gain (g)	Feed intake (g)	Livability %
CC	0.16%	0%	1.049	277[a,b,c]	290	99
FC	0.16%	0%	1.053	279[a,b,c]	294	99
CC	0.16%	2%	1.072	267[d]	287	99
FC	0.16%	2%	1.074	281[a,b]	302	100
CC	0.40%	0%	1.040	270[c,d]	281	99
FC	0.40%	0%	1.030	283[a]	292	99
CC	0.40%	2%	1.036	273[b,c,d]	282	100
FC	0.40%	2%	1.054	277[a,b,c]	292	100
	Main effect SB					
0%			1.043[b]	277	289	99
2%			1.059[a]	275	291	100
	Na					
0.40%			1.040[b]	276	287[b]	99
0.16%			1.062[a]	276	293[a]	99
	Particle size					
CC			1.049	272[b]	285[b]	99
FC			1.053	280[a]	295[a]	99
P-value						
SB			0.001	0.234	0.519	0.187
Na			0.000	0.874	0.010	0.657
Particle size			0.485	0.001	<0.001	0.657
SB × Na			0.217	0.712	0.784	0.657
SB × particle size			0.170	0.785	0.279	0.657
Na × particle size			0.883	0.901	0.894	0.657
SB × Na × particle size			0.110	0.034	0.184	0.657

[a–d]Within a column, values with different superscripts are significantly different from each other at $P < 0.05$.

RESULTS

Broiler Performance

Broiler performance results are presented in Tables 3 and 4. On d 10, FCR decreased ($P < 0.001$) in birds fed the diet containing 0.40% Na compared to those fed the diet containing 0.16% Na, while it increased ($P < 0.001$) in birds fed the diet supplemented with 2% SB compared those fed the diet without SB supplementation. Meanwhile, birds fed the diet containing 0.40% Na had lower feed intake than those fed the diet containing 0.16% Na. A 3-way particle size × Na × SB interaction ($P < 0.05$) on weight gain was observed. Interestingly, birds fed fine corn (**FC**) showed higher weight gain compared to birds fed CC only when 2% SB was supplemented in diets containing 0.16% Na, or when no SB was supplemented in the diet containing 0.40% Na. On d 24, birds fed the diet supplemented with 2% SB had higher feed intake ($P < 0.05$) than those without SB in their diet. A particle size × SB interaction was observed for both FCR ($P < 0.001$) and weight gain ($P < 0.05$). In this respect, regardless of the levels of Na, the birds fed CC had a lower FCR only when 2% SB was supplemented, whereas the supplementation of 2% SB in the FC diet increased FCR. Meanwhile, addition of 2% SB increased weight gain only in the birds fed CC but not in those fed FC. On d 35, the birds receiving 2% SB had higher weight gain ($P < 0.001$) and feed intake ($P < 0.001$) compared to those without SB. The SB × particle size interaction on FCR continued to d 35, when CC resulted in lower FCR only when 2% SB was supplemented ($P < 0.05$). No effect of dietary Na on bird performance was observed on either d 24 or 35.

Effect of Corn Particle Size, SB, and Na on Gizzard

The effects of corn particle size, SB, and Na level on the relative gizzard weight and its content pH are presented in Table 5. A particle size × SB interaction was observed for both relative gizzard weight and pH ($P < 0.05$). Regardless of the levels of Na, addition of 2% SB reduced gizzard pH and increased the relative gizzard weight in the FC fed birds but not in the CC fed birds. Na levels had no effect on the relative gizzard weight or pH on d 35.

Water Intake and Moisture Content of Excreta

Water consumption and moisture content of the excreta were affected by SB and Na level but not by particle size (Table 6). The average daily water consumption (ml/kg body weight) from d 17 to 24 and moisture content of the excreta (%) at d 26 were higher in birds fed SB compared to those fed SB-free diets ($P < 0.05$). Meanwhile, the birds fed the 0.40% Na diet had higher water consumption and moisture content of the excreta than those fed the 0.16% Na diet. There was no interaction among particle size, SB, and Na level on

Table 4. Effect of corn particle size, SB, and dietary Na on broiler performance at d 24 and 35.

Treatments		D 0 to 24				D 0 to 35			
Particle size	SB	FCR	Weight gain (g)	Feed intake (g)	Livability %	FCR	Weight gain (g)	Feed intake (g)	Livability %
CC	0%	1.255[b]	1431[b]	1797	97	1.368[a]	2726	3728	97
FC	0%	1.255[b]	1453[b]	1820	97	1.366[a]	2691	3672	96
CC	2%	1.224[c]	1506[a]	1842	98	1.333[b]	2869	3823	96
FC	2%	1.274[a]	1456[b]	1855	100	1.368[a]	2816	3852	98
Main effect SB									
0%		1.254	1442[b]	1808[b]	97	1.367[a]	2708[b]	3700[b]	97
2%		1.249	1481[a]	1849[a]	99	1.350[b]	2843[a]	3837[a]	97
Na									
0.40%		1.251	1457	1821	98	1.355	2764	3745	97
0.16%		1.252	1466	1836	98	1.362	2787	3792	97
Particle size									
CC		1.240[b]	1469	1819	98	1.350[b]	2798	3776	97
FC		1.263[a]	1455	1838	99	1.367[a]	2754	3762	97
P-value									
SB		0.391	0.014	0.027	0.094	0.037	<0.001	0.001	0.695
Na		0.802	0.532	0.413	0.807	0.383	0.482	0.227	1.000
Particle size		<0.001	0.370	0.314	0.466	0.034	0.168	0.722	0.695
SB × Na		0.916	0.989	0.943	0.466	0.240	0.575	0.993	0.243
SB × particle size		<0.001	0.023	0.773	0.466	0.018	0.778	0.273	0.434
Na × particle size		0.794	0.457	0.375	0.807	0.119	0.316	0.765	0.243
SB × Na × particle size		0.790	0.614	0.674	0.807	0.988	0.380	0.309	0.434

[a–c]Within a column, values with different superscripts are significantly different from each other at $P < 0.05$.

Table 5. Effect of corn particle size, SB, and dietary Na on gizzard pH and relative gizzard weight at d 35.

Treatments Particle size	SB	Gizzard pH	Relative gizzard weight
CC	0%	3.526[b]	1.242[a]
FC	0%	4.034[a]	1.106[b]
CC	2%	3.670[b]	1.297[a]
FC	2%	3.694[b]	1.278[a]
Main effect SB			
0%		3.778	1.170[b]
2%		3.683	1.287[a]
Na			
0.40%		3.749	1.234
0.16%		3.712	1.228
Particle size			
CC		3.596[b]	1.264[a]
FC		3.865[a]	1.192[b]
P-value			
SB		0.230	<0.001
Na		0.638	0.835
Particle size		0.001	0.007
SB × Na		0.537	0.723
SB × particle size		0.002	0.036
Na × particle size		0.918	0.998
SB × Na × particle size		0.623	0.675

[a,b]Within a column, values with different superscripts are significantly different from each other at $P < 0.05$.

Table 6. Effect of corn particle size, SB, and dietary Na on water consumption and excreta moisture.

Treatments Particle size	Na	SB	Excreta moisture %	Water intake (ml/kg body weight/d)
CC	0.16%	0%	85	81
FC	0.16%	0%	86	82
CC	0.16%	2%	85	86
FC	0.16%	2%	85	84
CC	0.40%	0%	89	94
FC	0.40%	0%	90	100
CC	0.40%	2%	88	108
FC	0.40%	2%	88	104
Main effect SB				
0%			88[a]	89[b]
2%			87[b]	96[a]
Na				
0.40%			89[a]	102[a]
0.16%			86[b]	83[b]
Particle size				
CC			87	92
FC			87	93
P value				
SB			0.037	0.003
Na			<0.001	<0.001
Particle size			0.363	0.903
SB × Na			0.188	0.156
SB × particle size			0.492	0.130
Na × particle size			0.998	0.840
SB × Na × particle size			0.703	0.482

[a,b]Within a column, values with different superscripts are significantly different from each other at $P < 0.05$.

water consumption and moisture content of the excreta ($P > 0.05$). boffset="-3pt"

Effect of Corn Particle Size, SB, and Dietary Na level on Ileal Microflora

Significant changes of ileal *Bacillus*, *Bifidobacterium*, and *Clostridium* were observed in response to the treatments at d 24 (Tables 7 and 8). Ileal *Bacillus* increased in birds fed diets containing SB compared with those fed diets without SB ($P < 0.05$). A particle size × Na level interaction was observed for the counts of *Bifidobacterium* ($P < 0.05$) and *Clostridium*

Table 7. Effect of corn particle size, SB, and dietary Na on the quantification of ileal microflora (log10 CFU) at d 24.

Treatments Particle size	Na	SB	*Lactobacillus*	*Bacillus*	*Ruminococcus*	*Bacteroides*	Enterobacteriaceae	*Salmonella*	Total bacteria
CC	0.16%	0%	8.45	6.09	4.74	5.09	5.52	6.27	9.18
FC	0.16%	0%	8.28	6.00	4.52	4.94	5.86	6.42	8.87
CC	0.16%	2%	8.33	6.27	4.67	4.97	6.04	6.38	8.80
FC	0.16%	2%	7.97	6.63	5.49	5.68	6.50	6.86	8.60
CC	0.40%	0%	8.18	6.14	4.80	5.26	6.08	6.51	8.99
FC	0.40%	0%	8.39	6.26	4.74	5.09	5.93	6.33	9.06
CC	0.40%	2%	8.38	6.24	5.04	5.42	6.16	6.31	8.89
FC	0.40%	2%	8.54	6.42	4.67	4.92	5.69	6.49	9.00
Main effect SB									
0%			8.33	6.12[b]	4.70	5.09	5.84	6.38	9.02
2%			8.30	6.39[a]	4.96	5.25	6.10	6.51	8.82
Na									
0.40%			8.37	6.26	4.81	5.17	5.96	6.41	8.95
0.16%			8.26	6.25	4.85	5.17	5.98	6.48	8.86
Particle size									
CC			8.33	6.18	4.81	5.18	5.95	6.37	8.96
FC			8.29	6.33	4.85	5.16	5.99	6.52	8.88
P-value									
SB			0.858	0.048	0.218	0.388	0.171	0.232	0.231
Na			0.376	0.924	0.820	0.975	0.921	0.501	0.468
Particle size			0.746	0.287	0.845	0.878	0.815	0.143	0.622
SB × Na			0.137	0.306	0.385	0.369	0.073	0.177	0.485
SB × particle size			0.645	0.328	0.392	0.460	0.783	0.111	0.832
Na × particle size			0.083	0.945	0.227	0.083	0.057	0.151	0.306
SB × Na × particle size			0.798	0.456	0.111	0.093	0.550	0.981	0.913

[a,b]Within a column, values with different superscripts are significantly different from each other at $P < 0.05$.

Table 8. Effect of corn particle size and dietary Na on the quantification of ileal microflora (log10 CFU) at d 24.

Treatments Particle size	Na	*Bifidobacterium* spp.	*Clostridium* spp.
CC	0.16%	6.30[b]	5.49[b]
FC	0.16%	6.85[a]	5.92[a]
CC	0.40%	6.63[a,b]	5.57[b]
FC	0.40%	6.38[b]	5.43[b]
Main effect SB			
0%		6.48	5.55
2%		6.61	5.65
Na			
0.40%		6.50	5.50[b]
0.16%		6.58	5.71[a]
Particle size			
CC		6.47	5.53
FC		6.62	5.68
P-value			
SB		0.437	0.358
Na		0.651	0.050
Particle size		0.361	0.153
SB × Na		0.201	0.158
SB × particle size		0.391	0.918
Na × particle size		0.019	0.008
SB × Na × particle size		0.318	0.442

[a,b]Within a column, values with different superscripts are significantly different from each other at $P < 0.05$.

($P < 0.01$). 0.40% Na reduced *Bifidobacterium* and *Clostridium* populations in birds fed the FC diet but not in those fed the CC diet, while CC inclusion in the diet reduced both groups of bacteria in birds fed diets with 0.16% Na but not 0.40% Na.

Litter Score and Moisture Content

As expected, 0.40% Na in the diet increased litter moisture content ($P < 0.001$) and produced caked litter ($P < 0.001$), as shown in Table 9. Inclusion of SB and particle size had no effect on the litter score or moisture content ($P > 0.05$). A SB × Na interaction tended to be significant ($P = 0.070$) for litter moisture content. 2% SB supplementation in diets tended to reduce litter moisture content when the birds were fed the diet with 0.40% but not 0.16% Na.

Incidence of Footpad Dermatitis and Hock Burn

0.40% Na in the diet led to a higher incidence of FPD in birds ($P < 0.001$) than the diet containing 0.16% Na, and there was a tendency ($P = 0.056$) for higher incidence of HB in the birds fed the 0.40% Na diet compared to the 0.16% Na diet (Table 10). Corn particle size and SB had no effect on FPD or HB ($P > 0.05$). No interactions were observed among particle size, SB, and Na level on FPD or HB ($P > 0.05$).

DISCUSSION

This study investigated the effects of Na level, inclusion of SB, and larger corn particle size in diets on bird performance, gizzard development, ileal microflora, and litter quality. The study showed that corn particle size and SB treatments significantly affected broiler

Table 9. Effect of corn particle size, SB, and dietary Na on litter characteristics at d 35.

Treatments Particle size	Na	SB	Litter moisture (%)	Litter score
CC	0.16%	0%	29.13	1.167
FC	0.16%	0%	31.39	1.167
CC	0.16%	2%	35.05	1.333
FC	0.16%	2%	33.06	1.417
CC	0.40%	0%	80.51	2.250
FC	0.40%	0%	84.76	2.167
CC	0.40%	2%	73.29	2.167
FC	0.40%	2%	76.08	2.167
Main effect SB				
0%			56.45	1.688
2%			54.37	1.771
Na				
0.40%			78.66[a]	2.188[a]
0.16%			32.16[b]	1.271[b]
Particle size				
CC			54.50	1.730
FC			56.32	1.729
P-value				
SB			0.514	0.372
Na			<0.001	<0.001
Particle size			0.567	1.000
SB ×× Na			0.070	0.183
SB × particle size			0.653	0.654
Na × particle size			0.594	0.654
SB × Na × particle size			0.826	1.000

[a,b]Within a column, values with different superscripts are significantly different from each other at $P < 0.05$.

Table 10. Effect of corn particle size, SB, and dietary Na on the incidence of FPD and HB at d 35.

Treatments Particle size	Na	SB	FPD[1]	HB[2]
CC	0.16%	0%	0.222	1.056
FC	0.16%	0%	0.444	1.056
CC	0.16%	2%	0.556	1.056
FC	0.16%	2%	0.556	1.056
CC	0.40%	0%	2.389	1.167
FC	0.40%	0%	2.833	1.167
CC	0.40%	2%	2.444	1.056
FC	0.40%	2%	2.611	1.222
Main effect SB				
0%			1.472	1.111
2%			1.542	1.097
Na				
0.40%			2.569[b]	1.153
0.16%			0.444[a]	1.056
Particle size				
CC			1.403	1.083
FC			1.611	1.125
P-value				
SB			0.800	0.780
Na			<0.001	0.056
Particle size			0.450	0.403
SB × Na			0.579	0.780
SB × particle size			0.649	0.403
Na × particle size			0.724	0.403
SB × Na × particle size			0.960	0.403

[a,b]Within a column, values with different superscripts are significantly different from each other at $P < 0.05$.
[1]FPD: Footpad dermatitis.
[2]HB: Hock burn.

performance in a different way during early and late stages of bird growth. It was demonstrated that 2% SB supplementation and CC particle inclusion in the feed improved bird performance, possibly through improved gizzard development and altered gut bacterial load. Elevating the dietary Na level from 0.16 to 0.40% increased water intake, increased the moisture level of the excreta and litter, and worsened litter quality without apparent performance compromise.

During the starter period (0 to10 d), 0.40% Na in the diet produced lower FCR of the birds compared to 0.16% dietary Na. This is in agreement with the findings of Maiorka et al. (2004) who concluded that 0.40% total Na in the broiler diet at an early age improved FCR. Vieira et al. (2003) also reported that an increase in Na content of the diet improved the growth and FCR during the starter phase of broiler growth. It has been demonstrated that Na has an important role in feed intake just after hatching, as it stimulates the secretion and activity of certain digestive enzymes (Mushtaq et al., 2013). Na also stimulates the secretion of intestinal adenosine triphosphatases and is involved in nutrient transport in the small intestine (Gal-Garber et al., 2003). As the dietary Cl (0.50 vs. 0.25% in the starter; 0.57 vs. 0.24% in the grower; and 0.59 vs. 0.24% in the finisher) was also higher in high Na diets, higher Cl could also be a factor in improving the performance in the birds fed high Na diets. Koreleski et al. (2011) concluded that a high level of Cl in broiler feed improved performance in the starter phase (d 1 to 15) but did not have an effect during the rest of the growth period (d 16 to 42). Thus, it may be suggested that elevated levels of Na and Cl above the recommendation level for Ross 308 broilers can be used in the starter phase to improve performance. However, the effect may not last till later stages as has been shown in the current study and reported in other studies.

A particle size × Na × SB interaction for weight gain at d 10 was observed, when birds receiving 0.40% Na in the FC diet without SB recorded higher weight gain compared to those fed the 0.16% Na in the CC diet with SB. This may be due to: 1) reduced particle size of corn in the FC diet increasing surface area, allowing greater access to digestive enzymes (Goodband et al., 2002); 2) the limitation imposed by an underdeveloped gizzard was less of an issue in younger birds when the corn was finely ground; and 3) a high Na level stimulated intestinal adenosine triphosphatases, enhancing small intestinal nutrient transport (Gal-Garber et al., 2003). In addition, higher FCR was observed in the birds receiving 2% SB. This may be due to the addition of 2% causing: 1) minor challenge to young birds due to undeveloped digestive systems thus less efficiency in feed conversion; and 2) the dilution of nutrients in the diet. On d 24 and 35, on the other hand, both CC and SB improved broiler performance, and the combination of both had a greater benefit than when they were applied alone. However, literature findings (Nir et al., 1994; Chewning et al., 2012; Xu et al., 2015b) for the effect of corn

particle size on broiler performance are inconsistent. Amerah et al. (2007) and Naderinejad et al. (2016) stated that particle size treatments within pelleted diets had no effect on growth performance, whereas Lott et al. (1992) observed that the birds performed better when corn particle size was decreased from 1,196 to 716 μm GMD up to d 21. In the current study, CC inclusion in the diet benefited the birds in the later stages of the grow-out. This inconsistency may be attributed to the type of diets used, age of birds, and genetic differences of birds due to the breeding program during different times.

In recent years, the beneficial effects of structural materials on gut health and nutrient utilization in poultry have become an important topic. The so-called structural materials refer to the intact plant cell walls that are coarse in their physical form and insoluble fiber (NSP and lignin) in their chemical nature. The beneficial effects appear to depend on type, physical structure, and amount of fiber in the diet (Hetland and Svihus, 2001; Jiménez-Moreno et al., 2013b). For instance, Jimenez-Moreno et al. (2009) and Jiménez-Moreno et al. (2013b) showed that the inclusion of moderate levels of insoluble fiber in the broiler diets improves performance, especially FCR. The recent studies have demonstrated the beneficial effect of a moderate level of inclusion of dietary fiber in the diet on performance and digestive functions in broilers (Mateos et al., 2012; Jiménez-Moreno et al., 2016). This effect comes from the ability of structural materials to stimulate: a) the gizzard to improve its development and function (Gonzalez-Alvarado et al., 2008; Sacranie et al., 2012) and b) gut reflex and enzyme production (Svihus, 2011; Jiménez-Moreno et al., 2013a) to enhance nutrient digestibility. The benefit achieved by the addition of SB as a source of fiber in the diet in the current study may be due to these structural material abilities to improve the function of bird digestive systems.

The combination of CC and SB improved broiler performance, as demonstrated by lower FCR in birds fed CC together with SB. It is hypothesized that coarse particle size and structural fiber may have: 1) extended digesta retention time, leading to prolonged exposure of nutrients to endogenous enzymes; 2) promoted gut reflux, re-exposing undigested nutrients to digestive enzymes for better digestion; 3) created a better microenvironment for enzyme activity around coarse corn particles of SB that has a strong water-holding capacity; and 4) enhanced gizzard activity (Svihus et al., 2002; Gabriel et al., 2003; Engberg et al., 2004), leading to secretion of more digestive juices and better ability to grind feed particles. In the current study, birds fed CC had heavier gizzards and lower digesta pH than those receiving FC diets. These results are in agreement with the findings of Naderinejad et al. (2016), in which finely ground corn increased the pH and reduced the weight of the gizzard compared to corn ground to medium and coarse sizes. In addition, Dahlke et al. (2003) and Parsons et al. (2006) reported that gizzard weight increased linearly as corn particle size increased. Therefore, considering particle size and fiber during formulation of broiler grower and finisher diets may benefit growth and feed conversion.

Interestingly, there was a particle size × Na interaction for counts of *Bifidobacterium* and *Clostridium* spp. The birds fed FC had increased counts of both bacterial groups compared to those fed CC when 0.16% Na was used. Jacobs et al. (2010) stated that incorporating coarse particle size, as compared to fine particle size, into broiler diets may have an indirect effect on birds in reducing enteric disease through altering the gut microflora. More specifically, a larger particle size may increase counts of beneficial flora or decrease the counts of pathogenic flora, such as *Clostridium* and *Escherichia*, or both. However, our data could not confirm this observation, as both *Bifidobacterium* and *Clostridium* spp. showed lower counts in birds fed CC in diets with 0.16% Na. *Bifidobacteria* have been considered as a probiotic and members of this group of bacteria have been linked to a healthy gut (Vlasova et al., 2016), while *Clostridia* have been reported as unfavorable to birds (Prescott et al., 2016). The current study observed a decrease in both beneficial and unfavorable bacterial groups when CC was fed. Therefore, whether coarse particle size alters the microflora in a beneficial way requires further investigation, possibly at a global scale.

In general, dietary fiber may deliver a number of health benefits by altering the composition of the gut microflora. Fiber appears to have a beneficial impact on the host through selectively stimulating the growth and/or activity of one or a limited number of bacteria in the gut (Glenn and Roberfroid, 1995). In the current work, SB as a source of fiber increased the number of *Bacillus* spp., which suggests that the SB may be a good candidate as a source of prebiotic that can be used to improve gut health and thus the performance of chickens. The observed effects of ingredient particle size or fiber on intestinal microflora may be elucidated by one or both of the following mechanisms: First, reduced pH through enhanced gizzard development and increased secretion of HCl in the proventriculus may lead to an antimicrobial effect on some pathogenic bacteria entering the distal part of the digestive tract (Engberg et al., 2002); second, competitive exclusion promoted by colonization and proliferation of beneficial microflora may reduce proliferation of harmful bacteria (Bjerrum et al., 2005; Santos et al., 2008). A higher *Bacillus* number caused by SB addition in the diet in this study may be an example of the competitive exclusion mechanism.

In conclusion, the inclusion of SB independently or in combination with CC improves bird performance demonstrated in the current study. The results suggest enhanced gizzard development as a mode of action. The combination of CC and SB was more beneficial than either of them on its own to growth performance and feed efficiency of broilers. Further, SB significantly increased the number of ileal *Bacillus* spp., suggesting SB is a promising prebiotic for broiler chickens.

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