

Nutrient digestibility response to sugarcane bagasse addition and corn particle size in normal and high Na diets for broilers

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ABSTRACT Improving diet digestibility is important to the broiler industry. Therefore, this study focused on optimizing the physical structure of feed ingredients and addition of dietary fiber as strategies to improve nutrient digestibility in low and high sodium diets. A total of 672 day-old Ross 308 male broilers was allocated to 48 pens using a 2 × 2 × 2 factorial arrangement of treatments with 2 particle sizes of corn (coarse 3,576 μm or fine 1,113 μm geometric mean diameter), 2 levels of sugarcane bagasse (SB) (0 or 2%), and 2 levels of Na (0.16 or 0.4%). Protein digestibility coefficient was measured using pooled distal ileal digesta of 3 birds per pen on d 24. Meanwhile, starch and gross energy digestibility coefficients were measured using pooled duodenal, distal jejunal, and distal ileal digesta of 3 birds

per pen on d 24. Coarsely ground corn (CC) resulted in improved ileal protein digestibility ($P < 0.05$). Addition of 2% SB increased starch digestibility in the duodenum ($P < 0.05$), distal jejunum ($P < 0.001$), and distal ileum ($P < 0.001$), and increased protein digestibility in distal ileum ($P < 0.01$). A significant particle size × SB × Na interaction was observed for ileal energy digestibility ($P < 0.05$). The SB increased ileal energy digestibility only in birds fed the diet with finely ground corn (FC) and 0.16% Na. These findings demonstrate that SB and CC are able to improve nutrient digestibility. It can be recommended for the poultry industry to use SB and coarsely ground corn in feed to improve the utilization of nutrients.

Key words: sugarcane bagasse, corn particle size, starch digestibility, protein digestibility

2018 Poultry Science 97:1170–1176
<http://dx.doi.org/10.3382/ps/pex403>

INTRODUCTION

The physical structure of feed and the use of dietary fiber in poultry diets have attracted a great deal of interest in recent years as a means to improve nutrient digestibility and enhance gut health of birds. Previous studies have reported negative effects of ingredient coarse particles and high fiber levels on nutrient digestibility in chickens (Jørgensen et al., 1996; Behnke, 2001). Generally, the reduction of feed particle size has been widely believed to enhance the access of digestive enzymes to substrates due to the increased surface area of feed particles, while dietary fiber has been considered as an anti-nutritional factor in poultry diet. However, a number of recent studies have shown beneficial effects of dietary fiber and coarse particle size on nutrient digestibility (Parsons et al., 2006; Rougière et al., 2009; Jiménez-Moreno et al., 2013; Xu et al., 2015a; Naderinejad et al., 2016). These measures lead to enhance gizzard development and function (Gonzalez-

Alvarado et al., 2008; Sacranie et al., 2012) and improve gut reflux and endogenous enzyme production (Svihus, 2011). The inclusion of high levels of fiber or coarsely ground ingredients in broiler feed increases foregut digesta retention time, which in turn increases the exposure time of nutrients to digestive enzymes in the upper part of the gastrointestinal tract, stimulates gizzard function (Rogel et al., 1987a; Nir et al., 1994; Hetland et al., 2005; Kheravii et al., 2017), and increases the secretion of HCl in the proventriculus (Duke, 1986), thereby improving 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 denaturation and hydrolysis of dietary proteins and improves protein digestion. Structural components of feed may increase the release of cholecystokinin (Svihus et al., 2004), which acts through the vagus nerve to stimulate pancreatic enzyme secretion and gastro-duodenal reflux (Duke, 1992; Li and Owyang, 1993). Increased secretion of pancreatic juice containing digestive enzymes would be expected to maximize the rate of digestion and absorption of nutrients in the small intestine, resulting in improved bird performance. It has been reported that sodium (Na) stimulates intestinal adenosine triphosphatases

(ATPase) and is involved in small intestinal nutrient transport (Gal-Garber et al., 2003), which may increase nutrient digestibility and hence growth performance. However, high dietary Na levels might increase gut leakage and depress nutrient digestibility and absorption, which could negatively affect growth performance. It has been well documented that water intake increased with corresponding Na concentration in broiler diets (Vieira et al., 2003). It is hypothesized that dietary structural components, such as the addition of fiber and/or inclusion of coarse particle size, increase digesta retention time, allowing more water reabsorption, and enhance the development of the gizzard, which is believed to act as a pacemaker organ for nutrient digestion and absorption, regulating water absorption to an optimum. The aim of this study was to evaluate the effect of dietary structural components, i.e., the addition of sugarcane bagasse (SB), and inclusion of different corn particle sizes, on the digestibility coefficient of nutrients in broilers fed 0.16 or 0.40% Na diet.

MATERIALS AND METHODS

Experimental Design and Bird Management

A total of 672 day-old male Ross 308 chicks was obtained on d of hatch from Baiada Hatchery in Tamworth, NSW, Australia. The chicks were assigned to a $2 \times 2 \times 2$ factorial arrangement of treatments. Factors were: SB, 0 and 2%; particle size, coarsely ground corn, (CC, at 3,576 μm geometric mean diameter) and finely ground corn (FC, at 1,113 μm geometric mean diameter); and Na, 0.16 and 0.40%. Salt and sodium bicarbonate were used to adjust Na level of the diets. The geometric mean diameter (GMD) of corn particle size was determined according to the American Society of Agricultural Engineers (2003). The birds were randomly allocated to each treatment, resulting in 6 replicate pens (75 cm \times 120 cm) with 14 birds per pen. Hardwood shavings were used for bedding with an initial depth of approximately 7 cm in each pen. Pens were equipped with a single plastic tube feeder and 2 cup drinkers. Feed and water were provided ad libitum. The lighting, relative humidity, and temperature were controlled according to Ross 308 guidelines (Aviagen, 2014).

Diets

Diets were corn, soybean meal, and meat meal based, formulated to meet the nutrient recommendations for Ross 308 (Aviagen, 2014). Table 1 shows the ingredient and nutrient composition of the experimental diets. Herein, the composition of the diets was diluted when 2% SB was added over the top of the complete feed. All the diets were thoroughly mixed and cold-pelleted (65°C). The feeding program consisted of starter (d 0 to 10) and grower (d 11 to 24) diets. Titanium dioxide

(TiO₂) was incorporated into all grower diets as an indigestible marker at a rate of 5 kg/t diet for nutrient digestibility assessment.

Sugarcane Bagasse

The SB was provided by FCR Consulting Group, Brisbane. The composition of SB was measured (as-is basis) for total non-starch polysaccharides (NSP) and lignin following the methods 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.

Animal Ethics

This experiment was approved by the Animal Ethics Committee of the University of New England (Approval No: AEC 15-053). All procedures involving the birds, including health, care, and use of laboratory animals, were fulfilled within the Code of Practice for the Use of Animals for Scientific Purposes issued by the Australian Bureau of Animal Health (NHMRC, 2013).

Sample Collection

On d 24, 3 birds per replicate were randomly selected from each pen, weighed, and euthanized by cervical dislocation. The digesta contents from the duodenum, distal jejunum, and distal ileum were collected by gently squeezing the digesta into 50 mL plastic containers and stored at -20°C for nutrient digestibility analysis.

Titanium Dioxide Analysis

The spectrophotometric method described by Short et al. (1996) was followed to measure the TiO₂ in the diet, duodenal, jejunal, and ileal digesta samples. Freeze-dried digesta and diet samples were accurately weighed to a range between 0.1 g and 0.2 g in porcelain crucibles and placed in a muffle furnace at 580°C for 13 hours. Upon cooling, 5 mL of 7.4 M H₂SO₄ were added to the samples, and they were then boiled on a hotplate at 200°C for 30 min followed by 30 min at 250°C to completely dissolve the samples. The solutions were left at room temperature for an h to cool and then 5 mL Milli-Q H₂O were added. The solutions were then filtered through grade 541 filter paper (Whatman 541, hardened, ashless, 90 mm, Cat No. 1541 090, Whatman International Ltd., Maidstone, UK) into 50 mL volumetric flasks. Ten mL of H₂O₂ (30% v/v) were added to each flask and the mixture diluted up to 50 mL with Milli-Q H₂O and mixed. Absorbance of the samples and standards was measured on a Hitachi 150-20 UV spectrophotometer (Hitachi Science Systems Ltd., Ibaraki, Japan) set to 410 nm. The TiO₂ content was calculated from the regression analysis of the standard curve.

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

Ingredients	Starter (d 0 to 10)		Grower (d 11 to 24)	
	Normal Na	High Na	Normal Na	High Na
Corn	60.60	59.30	62.30	61.00
Soybean meal	32.60	32.80	29.30	29.50
Meat and bone meal	3.00	3.00	3.60	3.60
Canola oil	0.644	1.08	1.91	2.30
Limestone	0.970	0.968	0.814	0.812
Dical phosphate	0.607	0.611	0.269	0.271
Phytase ²	0.010	0.010	0.010	0.010
Salt	0.154	0.568	0.161	0.703
Na bicarbonate	0.219	0.505	0.200	0.300
Vitamin-Mineral ³	0.200	0.200	0.200	0.200
Choline	0.111	0.111	0.103	0.103
L-lysine HCl 784	0.305	0.303	0.226	0.222
D, L-methionine	0.392	0.394	0.336	0.337
L-threonine	0.204	0.204	0.148	0.147
TiO ₂	-	-	0.500	0.500
Calculated nutrients				
ME (kcal/kg)	3000	3000	3100	3100
ME (MJ/kg)	12.55	12.55	12.97	12.97
Crude protein	22.20	22.20	21.00	21.00
Crude fat	2.85	3.26	4.14	4.51
Crude Fiber	2.07	2.06	2.01	1.99
SID arginine	1.37	1.37	1.27	1.29
SID lysine	1.28	1.28	1.15	1.15
SID methionine	0.684	0.685	0.616	0.616
SID methionine + Cysteine	0.950	0.950	0.870	0.870
SID tryptophan	0.244	0.245	0.226	0.226
SID isoleucine	0.860	0.860	0.807	0.808
SID threonine	0.860	0.860	0.770	0.770
SID valine	0.992	0.992	0.939	0.939
Starch	35.80	35.10	36.80	36.00
NSP soluble	0.426	0.424	0.404	0.402
NSP insoluble	5.64	5.60	5.45	5.40
Calcium	0.960	0.960	0.870	0.870
Available Phosphorus	0.480	0.480	0.435	0.435
Sodium	0.160	0.400	0.160	0.400
Chloride	0.250	0.500	0.242	0.569
Choline chloride 70%	0.170	0.170	0.160	0.160

¹Compositions of diets were diluted when 2% SB was added over the top of the complete feed for SB treatment(s).

²Phyzyme XP5000G (100 g/mt) Dupont. The matrix values for calcium and phosphorous availability were 1,100 and 1,200%, respectively.

³Vitamin-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; cyanocobalamin, 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.

Digestibility of Nutrients

Crude protein (**CP**), TiO₂, gross energy (**GE**), and starch were determined in each diet and freeze-dried duodenal, jejunal, and ileal digesta samples. The Dumas combustion technique using a LECO® FP-2000 automatic nitrogen analyzer (Leco Corporation, St. Joseph, MI) was employed to measure the nitrogen content of diet and ileal digesta samples. The CP of diets and digesta samples was calculated by multiplying the nitrogen value by 6.25. The gross energy of the feed and digesta samples was determined on an IKA®-WERKE bomb calorimeter (C7000, GMBH & CO., Staufen, Germany). The GE values of the samples were obtained as Kcal/kg. The starch content of feed and digesta samples was measured using the Megazyme Total Starch

Assay Kit (Megazyme Int. Ireland Ltd., Wicklow, Ireland). The digestibility coefficient of nutrients was calculated on a freeze-dried basis using the following equation:

$$\text{Digestibility coefficient} = 1 - \left[\frac{(\text{Digesta nutrient (g/kg or Kcal/kg)}) / (\text{Digesta TiO}_2 \text{ (g/kg)})}{(\text{Diet nutrient (g/kg or Kcal/kg)}) / (\text{Diet TiO}_2 \text{ (g/kg)})} \right]$$

Statistical Analysis

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 SB level, particle size, and Na level, along with their

Table 2. Ileal protein digestibility in broilers at d 24.¹

Main effect	Ileal protein digestibility
SB	
0%	0.815 ^b
2%	0.840 ^a
Na	
0.40%	0.827
0.16%	0.828
Particle size	
CC	0.837 ^a
FC	0.818 ^b
SEM ²	0.006
<i>P</i> -value	
SB	0.004
Na	0.932
Particle size	0.030
SB × Na	0.284
SB × particle size	0.870
Na × particle size	0.178
SB × Na × particle size	0.260

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

¹Sample size = 6 replicate pens with 3 birds each/treatment.

²SEM = Standard error of the mean.

interactions. Differences between mean values per pen were determined using Tukey's multiple range test at the level of $P < 0.05$. Paired comparisons between treatment means were performed using Tukey's multiple-range test in which interactions were present.

RESULTS

Ileal Crude Protein Digestibility

Ileal protein digestibility was significantly improved in the birds fed CC compared with those fed FC ($P < 0.05$), and in the birds that received 2% SB compared to those fed the diet without SB ($P < 0.05$) (Table 2). No interactions were observed among particle size, SB level, and Na level on protein digestibility at d 24.

Starch Digestibility in Small Intestine

The birds fed 2% SB had higher starch digestibility in the duodenum ($P < 0.05$), distal jejunum, ($P < 0.001$), and distal ileum ($P < 0.001$) compared to those fed diets without SB addition, from d 0 to 24 (Table 3). Meanwhile, no differences in duodenal, jejunal, or ileal starch digestibility were detected between birds fed diets containing CC and FC ($P > 0.05$), or different levels of Na ($P > 0.05$). No interactions were detected among particle size, SB level, or Na level for starch digestibility in the small intestine ($P > 0.05$).

Gross Energy Digestibility

The digestibility of GE of the digesta from different parts of the intestine at d 24 is presented in Table 4. All treatments showed a negative digestibility value for GE in the duodenum, and no differences were detected among treatments ($P > 0.05$). No significant interac-

Table 3. Starch digestibility in broilers at d 24.¹

Main effect	Duodenum	Distal jejunum	Distal ileum
SB			
0%	0.4880 ^b	0.867 ^b	0.970 ^b
2%	0.5668 ^a	0.904 ^a	0.983 ^a
Na			
0.40%	0.518	0.886	0.978
0.16%	0.536	0.884	0.975
Particle size			
CC	0.527	0.887	0.979
FC	0.528	0.883	0.974
SEM ²	0.024	0.008	0.003
<i>P</i> -value			
SB	0.024	0.001	0.001
Na	0.595	0.832	0.457
Particle size	0.976	0.645	0.132
SB × Na	0.770	0.536	0.521
SB × particle size	0.459	0.416	0.171
Na × particle size	0.204	0.834	0.313
SB × Na × particle size	0.551	0.828	0.169

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

¹Sample size = 6 replicate pens with 3 birds each/treatment.

²SEM = Standard error of the mean.

tions were detected among particle sizes, SB level, and Na level on duodenal GE digestibility. Particle size, SB level, and dietary Na level had no impact on duodenal or jejunal GE digestibility at d 24. However, a significant 3-way interaction was observed on ileal GE digestibility ($P < 0.05$), in that birds fed the FC diet with 0.16% Na and without SB supplementation had the lowest ileal GE digestibility compared to all other treatments. Also, significant SB × Na, particle size × Na, and SB × particle size interactions were observed on ileal GE digestibility ($P < 0.05$) at d 24.

DISCUSSION

This study showed that high Na level was effective on the distal ileum only in terms of GE digestibility; dietary SB effect was effective on ileal protein digestibility and starch digestibility (duodenum, jejunum, and ileum); and particle size was effective on the ileal protein digestibility only. This improved nutrient and energy digestibility may be responsible for the better bird performance, as shown in a parallel study reported recently (Kheravii et al., 2017), where the focus of the study was on performance, while this study focused on digestibility with the same birds. An interesting observation was that both CC and SB improved ileal protein digestibility. The findings of this study corroborate the previous findings of Rougrière et al. (2009) who reported that CC improved digestibility of protein in D-genetic broiler lines, due to increased gizzard and pancreas sizes. Xu et al. (2015a,b) also reported that feeding CC increased apparent ileal digestibility of nitrogen. Coarse particles delay the transit time of digesta in the gizzard, which increases the exposure time of nutrients to digestive enzymes (Nir et al., 1994), thereby improving nutrient digestibility (Carré, 2000). Gabriel et al. (2003) reported that a lower pH of gizzard contents may increase

Table 4. GE digestibility in broilers at d 24.¹

Treatments			Duodenum	Distal jejunum	Distal ileum
Particle size	Na	SB			
CC	0.16%	0%	−1.108	0.656	0.796 ^a
FC	0.16%	0%	−0.977	0.651	0.744 ^b
CC	0.16%	2%	−0.869	0.687	0.785 ^a
FC	0.16%	2%	−0.996	0.679	0.788 ^a
CC	0.40%	0%	−0.744	0.686	0.791 ^a
FC	0.40%	0%	−0.247	0.692	0.796 ^a
CC	0.40%	2%	−1.237	0.687	0.788 ^a
FC	0.40%	2%	−0.685	0.669	0.788 ^a
SEM ²			0.347	0.013	0.007
Main effect					
SB					
0%			−0.769	0.671	0.780
2%			−0.947	0.681	0.787
Na					
0.40%			−0.728	0.684	0.791 ^a
0.16%			−0.988	0.668	0.777 ^b
Particle size					
CC			−0.989	0.679	0.789
FC			−0.726	0.673	0.779
SEM ²			0.181	0.007	0.004
<i>P</i> -value					
SB			0.501	0.359	0.201
Na			0.328	0.140	0.015
Particle size			0.321	0.538	0.081
SB × Na			0.278	0.050	0.028
SB × particle size			0.847	0.513	0.049
Na × particle size			0.325	0.971	0.033
SB × Na × particle size			0.766	0.589	0.014

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

¹Sample size = 6 replicate pens with 3 birds each/treatment.

²SEM = Standard error of the mean.

the denaturation and hydrolysis of dietary proteins and thus improve protein digestion. In fact, insoluble fiber may increase pancreatic enzymes secretion, particularly trypsin and chymotrypsin (Bogulsawska-Tryk, 2005), which may help to improve protein digestibility. Similar effects of CC and SB may have occurred in the current study. The improved protein digestibility would be expected to benefit growth performance and reduce nitrogen excretion and NH_3 production in the litter and thus improve bird welfare. It has been reported that increased nitrogen excretion and NH_3 formation in the litter leads to increased incidence and severity of footpad dermatitis in broilers (Nagaraj et al., 2007). Therefore, application of coarse particle size and increased fiber in broiler diet formulation may reduce such issues in the broiler industry.

There is evidence to suggest that starch may not be fully digested in poultry (Naderinejad et al., 2016), thus considering factors that could alter total tract or ileal starch digestibility is required in feed formulation. In the current study, SB supplementation increased starch digestibility in the duodenum, jejunum, and ileum. It has been reported that increased dietary fiber may result in increased pancreas size and heightened enzymatic secretions (Kratzer et al., 1967). It is possible that SB might form a fibrous matrix and expand within the aqueous medium, trapping water and creating an optimum hydrated environment for enzyme ac-

tivity, thus increasing starch digestibility. Other mechanisms also have been explored (Rogel et al., 1987a,b; Hetland and Svihus, 2001; Hetland et al., 2003) with regard to improvements in starch digestibility observed when birds are fed insoluble dietary fiber. Hetland et al. (2003, 2005) reported that dietary oat hulls elevated the level of bile acids in the gizzard, suggesting that fibrous dietary components increase digesta reflux, possibly through increased gizzard activity. It was speculated that increased bile acids in the gizzard may underline the improvement of starch digestibility. Hetland et al. (2005) also suggested that insoluble fiber may stimulate pancreatic secretion of amylase, thus improving starch digestibility.

In the current study, negative values for energy digestibility in the duodenum were detected in all treatment groups. The reason for this has not been elucidated, and reports showing negative duodenal energy digestibility have been scarce in the literature. It is likely that negative duodenal GE digestibility is due to endogenous secretions that dilute nutrients and the digestibility marker (Cadogan and Choct, 2015). In the current study, it is hypothesized that the energy portion of digesta from feed in the duodenum is smaller than the energy contributed by endogenous secretions.

It was shown that lower ileal GE digestibility was observed only in the birds receiving the diet containing 0.16% Na and FC without SB supplementation

compared to all other groups, which showed the same level of energy digestibility. This finding implies that coarse particle size, increased fiber, and increased sodium levels in the diet can improve energy digestibility. It is well known that dietary Na plays a role in nutrient transport, such as glucose, across the brush border membrane of the intestinal epithelial cell. It appears that slightly higher sodium than recommended may promote energy utilization. It has been reported that Na stimulates intestinal adenosine triphosphatases and is involved in small intestinal nutrient transport (Gal-Garber et al., 2000, 2003). Furthermore, the structural components of feed might stimulate the pancreatic enzyme secretion and gastro-duodenal reflux through the release of cholecystokinin (Svihus et al., 2004). Hence, increasing pancreatic juice secretion would be expected to increase digestion and absorption of nutrients in the small intestine. It is hypothesized that the observed improvement in nutrient digestibility in the small intestine by structural components in feed (coarse particle size and/or SB) could be related to: 1) a more developed gizzard (Kheravii et al., 2017), which may increase the retention time and prolong the exposure of the digesta to endogenous enzymes; 2) increased pancreatic secretions through stimulation of cholecystokinin or pancreatic secretion regulators, such as melatonin and glucagon; and, 3) high water consumption, which might help the physical structure of fiber (SB) to create a network around digesta and increase the digestibility of nutrients in the small intestine. In conclusion, this study demonstrate that SB and CC are efficient to improve nutrient digestibility. Therefore, it can be recommended for the broiler industry to use SB and coarsely ground corn in feed so as to improve the utilization of nutrients.

ACKNOWLEDGMENTS

The Higher Committee for Education Development in Iraq (HCED) for providing Sarbast Kheravii with the scholarship for his PhD study. Dr Santiago Ramirez of FCR Consulting Group for providing SB for this study. Mr. Matt Hilliar for proofreading the manuscript.

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