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Chemical Composition, *in Vitro* Fermentation Characteristics, and *in Vivo* Digestibility Responses by Dogs to Select Corn Fibers

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The objective was to examine the chemical composition, *in vitro* fermentation characteristics, and *in vivo* digestibility responses of fiber-rich corn coproducts resulting from corn wet milling. Native corn fibers, native corn fibers with fines, hydrolyzed corn fibers, and hydrolyzed extracted corn fibers were analyzed chemically and their capacity to produce short-chain fatty acids determined. Ash content was low (<1.2%), crude protein content varied little, but fat and fiber concentrations varied widely. Most fiber was in the insoluble form, with glucose being predominant followed by xylose. Total short-chain fatty acid production ranged from 211.6 to 699.52 μmol/g of dry matter, whereas branched-chain fatty acid production was low. Four corn fibers (native and processed) were included in a canine diet matrix at the 7% inclusion level. Nutrient digestibility, food intake, and fecal characteristics were not affected by corn fiber inclusion in canine diets, suggesting that they should be considered as potential dietary fiber sources in dog foods.

KEYWORDS: Corn fiber; dogs; canine; digestibility; in vitro; in vivo; composition; fecal characteristics; dietary fiber

INTRODUCTION

In the gastrointestinal tract, dietary fibers can alter the physical characteristics of the gastric and small intestinal contents. This can affect physical and physiological responses in the animal such as satiety, laxation, attenuation of blood glucose concentration, and normalization of blood lipid concentrations (*1*–*4*).

Pet food manufacturers use dietary fiber sources from grains, fruits and vegetables, celluloses, gums, and other sources. Examples are beet pulp, corn gluten feed, corn bran, soy and peanut hulls, wood cellulose, and wheat middlings (*5*). Beet pulp is commonly used as a fiber source in high-quality dog diets. Alternative fiber sources, however, are increasingly being researched as possible replacements for beet pulp (*6*). Corn bran, a coproduct from dry milling of corn, is an ingredient currently used in select dog foods; however, there is no research published on its utilization by dogs.

Corn fiber is a coproduct originating from wet milling of corn. Ethanol production in the U.S. is growing rapidly due to increasing gasoline prices and the national Renewable Fuels Program (*7*). With the increase in ethanol production, the volume of coproducts also has increased dramatically, creating a necessity for the ethanol industry to find new uses for these coproducts and an opportunity for pet food manufacturers to acquire a potentially abundant, high-quality, and consistent dietary fiber source. Prior to use of corn fibers as dietary fiber sources in pet food, it is imperative to understand the impact of different processing methods on characteristics of fiber-rich corn coproducts and the effects of feeding these fiber-rich corn coproducts on nutritional responses of dogs. However, there is a lack of information about digestibility, fermentability, and physiological responses to corn fiber inclusion in dog food.

The purpose of this study was to examine chemical composition, *in vitro* fermentation characteristics, and *in vivo* digestibility responses of select fiber-rich corn coproducts obtained from the corn wet milling industry.

MATERIALS AND METHODS

Substrates. Fifteen select corn fibers (CF) resulting from ethanol production [Archer Daniels Midland (ADM), Decatur, IL] were evaluated. Of these, seven samples from different operating facilities and batches were native corn fiber (NCF) that consisted of the wet milled corn pericarp or outer covering. Corn was steeped, dewatered, and milled to separate the germ from the remainder of the corn. The germ was separated by hydrocloning. The degermed, milled corn was passed over a screen to separate out any small starch granules and then milled again to release more starch from the fiber. The fiber then was washed repeatedly with water over screens to separate out any remaining

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unbound starch and fine fiber. The washed, coarse fiber was pressed to remove excess water. The fine fiber was separated by size from the starch granules also and was then dewatered with a centrifuge.

Four samples consisted of hydrolyzed corn fiber (HCF), which is NCF hydrolyzed at 150 °C for 30 min with direct steam injection, then pressed, and washed with 75 °C water to remove the solubilized hydrolysate.

Four samples consisted of hydrolyzed extracted corn fiber (HECF), which is HCF extracted with a 7:1 ratio of ethanol to fiber at 60 °C in a countercurrent Crown extractor, model 5 (Crown Iron Works, Roseville, MN). The extracted material then was dried at 150 °C in a twin-screw desolventizer-toaster with a jacket for indirect heating (ADM, Decatur, IL) and then ground.

Substrates used in the *in vivo* digestion experiment also were evaluated. Native CF, NCF with fines (NCFF), HCF, and HECF were the samples tested. Native corn fiber with fines consisted of 90% NCF and 10% of fine CF particles. The fine CF is composed of the cell walls from the endosperm.

Chemical Analyses. Fiber samples that arrived in the wet state were placed in a forced air oven at 55 °C until dry. All fiber samples were ground in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ) through a 2 mm screen and analyzed for dry matter (DM) and organic matter (OM) according to AOAC methods (*8*). Acid-hydrolyzed fat (AHF) concentrations were determined using acid hydrolysis (*9*) followed by ether extraction (*10*). Crude protein (CP) concentrations were calculated using LECO (nitrogen analyzer model FP-2000; Leco Corp., St. Joseph, MI) nitrogen (N) values (N × 6.25) (*8*). Total (TDF), soluble (SDF), and insoluble dietary fiber (IDF) concentrations of diets, substrates, and fecal samples from the *in vivo* study were measured according to Prosky et al. (*11*).

For sugar analyses, samples were hydrolyzed using the procedure of Hoebler et al. (*12*). Hydrolyzed monosaccharides and free sugars were quantified using a Dionex DX500 HPLC system (Dionex Corp., Sunnyvale, CA). Standards for quantification included glucose, fructose, sucrose, inositol, fucose, arabinose, rhamnose, galactose, xylose, and mannose. Free monosaccharides were injected at a volume of 25 μL. All assays were conducted using a CarboPac PA-1 column and guard column following methods cited by Smiricky et al. (*13*).

***In Vitro* Digestion Experiment.** Purpose-bred, healthy, female dogs ($n = 3$; Butler Farms USA, Clyde, NY) with hound bloodlines, an average initial body weight of 23.1 kg (18.2–26.6 kg), and an average age of 4.4 years (1–6 years) served as sources of feces from which inoculum was prepared. Dogs consumed a commercially available dry extruded dog food with chicken, corn meal, ground whole grain sorghum, and chicken byproduct meal constituting the main ingredients of the diet. Dogs were housed individually in kennels in a temperature-controlled room (21 °C) at the animal care facility in the Edward R. Madigan Laboratory, University of Illinois. Animal care procedures were approved by the University of Illinois Animal Care and Use Committee prior to initiation of the experiment.

Approximately 500 mg of substrate was placed into tubes in triplicate and exposed to pepsin/hydrochloric acid and pancreatin to simulate hydrolytic digestion (*14*). A set of tubes without substrate was used as blanks, whereas another set of tubes did not continue into the fermentation phase of the experiment in order to measure enzymatic digestion. The substrate remaining after simulated gastric and small intestinal digestion was used for *in vitro* fermentation.

On designated collection days, fresh feces from dogs were collected in plastic bags, which were sealed after expressing excess air, and maintained at 37 °C until inoculum was prepared. Anaerobic inoculum was prepared from fresh fecal samples within 15 min of defecation.

Each substrate was fermented *in vitro* for 0 and 12 h in triplicate with the fecal microbiota obtained from each of the three donors. Triplicate tubes containing no substrate were fermented with each inoculum source and at each time point to enable appropriate corrections for short-chain fatty acid (SCFA) production not arising from the substrates. The composition of the semidefined medium used for the fermentation is presented in **Table 1**. All components except for the vitamin solutions were mixed before autoclave sterilization of the medium. Filter-sterilized vitamin solutions were added just before dispensing the medium, which was maintained under anaerobic conditions at all times after preparation.

Table 1. Composition of Medium Used for *in Vitro* Fermentation of Select Corn Fibers

component	concn in medium
solution A (mL/L)[a]	330.0
solution B (mL/L)[b]	330.0
trace mineral solution (mL/L)[c]	10.0
water-soluble vitamin mix (mL/L)[d]	20.0
folate/biotin solution (mL/L)[e]	5.0
riboflavin solution (mL/L)[f]	5.0
hemin solution (mL/L)[g]	2.5
short-chain fatty acid mix (mL/L)[h]	0.4
resazurin (mL/L)[i]	1.0
distilled water (mL/L)	296.0
yeast (g/L)	0.5
trypticase (g/L)	0.5
Na_2CO_3 (g/L)	4.0
cysteine hydrochloride · H_2O (g/L)	0.5

[a] Composition (g/L): NaCl, 5.4; KH_2PO_4, 2.7; $CaCl_2 \cdot H_2O$, 0.18; $MgCl_2 \cdot 6H_2O$, 0.12; $MnCl_2 \cdot 4H_2O$, 0.06; $CoCl_2 \cdot 6H_2O$, 0.06; $(NH_4)_2SO_4$, 5.4. [b] Composition: K_2HPO_4, 2.7 g/L. [c] Composition (mg/L): EDTA (disodium salt), 500; $FeSO_4 \cdot 7H_2O$, 200; $ZnSO_4 \cdot 7H_2O$, 10; $MnCl_2 \cdot 4H_2O$, 3; H_3PO_4, 30; $CoCl_2 \cdot 6H_2O$, 20; $CuCl_2 \cdot 2H_2O$, 1; $NiCl_2 \cdot 6H_2O$, 2; $Na_2MoO_4 \cdot 2H_2O$, 3. [d] Composition (mg/L): thiamin hydrochloride, 100; D-pantothenic acid, 100; niacin, 100; pyridoxine, 100; *p*-aminobenzoic acid, 5; vitamin B_{12}, 0.25. [e] Composition (mg/L): folic acid, 10; D-biotin, 2; NH_4HCO_3, 100. [f] Composition: riboflavin, 10 mg/L, in 5 mmol/L HEPES. [g] Hemin, 500 mg/L, in 10 mmol/L NaOH. [h] 250 mL/L each of *n*-valerate, isovalerate, isobutyrate, and DL-α-methylbutyrate. [i] Resazurin, 1 g/L, in distilled H_2O.

Aliquots (10 mL) of medium were aseptically transferred into Balch tubes and capped with butyl rubber stoppers. All tubes were stored at 4 °C for approximately 12 h to enable hydration of the substrates before initiating fermentations. Tubes were placed in a 37 °C water bath approximately 30 min before inoculation. Each fecal sample was diluted 1:10 (w/v) in anaerobic dilution solution (*15*) by blending for 15 s in a Waring blendor under a stream of CO_2. Blended, diluted feces were filtered through four layers of cheesecloth and sealed in 125 mL serum bottles under CO_2.

Diluted feces (4 mL) were inoculated into tubes containing either 10 mL of semidefined medium only (blank tubes) or 10 mL of semidefined medium and the substrate remaining after simulated gastric and small intestinal digestion. Tubes were incubated at 37 °C with periodic mixing for the respective fermentation times. At the appropriate time, tubes were removed from the 37 °C incubator and processed immediately for analyses. The pH of tube contents was measured with a standard pH meter (Denver Instrument Co., Arvada, CO) at 0 and 12 h.

A 2.0 mL subsample was taken from each tube for SCFA analyses. Samples to be analyzed for SCFA were mixed with 0.5 mL of 250 g/L metaphosphoric acid, precipitated at room temperature for 30 min, and then centrifuged at 20100*g* for 20 min. The supernatant was decanted and frozen at −20 °C in microfuge tubes. After freezing, the supernatant was thawed and centrifuged in microfuge tubes at 13000*g* for 10 min. Concentrations of SCFA were determined using gas–liquid chromatography (*16*). Briefly, concentrations of acetate, propionate, butyrate, isobutyrate, isovalerate, and valerate were determined in the supernatant of the tubes using a Hewlett-Packard 5890A Series II gas–liquid chromatograph and a glass column (180 cm × 64 mm i.d.) packed with 10% SP-1200/1% H_3PO_4 on 80/100 mesh Chromosorb WAW (Supelco Inc., Bellefonte, PA). Short-chain fatty acid concentrations were corrected for the quantities of SCFA produced in the blank tubes.

The remaining 28 mL was combined with 120 mL of 95% ethanol and precipitated for 1 h to recover unfermented residues. Residues were filtered through Whatman 541 filter paper and washed sequentially with 78% ethanol, 95% ethanol, and acetone. Residues were dried at 105 °C until a constant weight was obtained. Then, they were ashed (500 °C) and weighed back to determine OM disappearance.

A second set of samples (those used in the *in vivo* digestion experiment) was studied following the same procedure used for the

larger sample set except that, in this case, substrates were fermented *in vitro* for 0, 8, and 16 h with inoculum prepared by pooling fresh fecal samples from all three dogs. For this set of samples, OM disappearance (OMD) alone was measured.

Calculations. In vitro OMD was calculated as [1 − (OM residue − OM blank/DM sample)] − OMD at 0 h.

Statistics. Data were analyzed as a completely randomized design using the Mixed Models procedure of SAS/STAT software, version 9.1 for Windows (*17*). The statistical model for the hydrolytic−enzymatic stage in the second set of samples was

$$y_{ij} = \mu + \tau_i + e_{ij}$$

where y_{ij} is the *J*th observation of the *I*th substrate with a total of 15 observations, μ is the grand mean for *in vitro* OMD, τ_i is the fixed effect of the Ith substrate with 4 degrees of freedom, and e_{ij} is the experimental error with NID (0, σ^2_e) and 10 degrees of freedom.

Fermentative digestion in the second set of samples was analyzed as a completely randomized design with a 5 × 2 factorial arrangement with 5 substrates and 2 pull times. The statistical model was

$$y_{ijk} = \mu + \tau_i + \pi_j + \tau\pi_{ij} + e_{ij}$$

where y_{ijk} is the *K*th observation in the *J*th pull time of the *I*th substrate with a total of 30 observations, μ is the grand mean for *in vitro* OMD, τ_i is the fixed effect of the *I*th substrate with 4 df, π_j is the fixed effect of the *J*th pull time with 1 degree of freedom, $\tau\pi_{ij}$ is the fixed effect of the interaction between the *I*th substrate and the *J*th pull time with 4 degrees of freedom, and e_{ijk} is the experimental error with NID (0, σ^2_e) and 20 degrees of freedom.

Normal distribution of residuals and homogeneity of variances were tested, and assumptions for analysis of variances were fulfilled. Treatment least-squares means for main effects and interactions are reported and were compared using a Bonferroni adjustment to ensure the overall protection level. Standard error of the mean (SEM) values are associated with least-squares means as calculated in the Mixed Models procedure. Differences among means with a *P*-value of less than 0.05 were considered significant, and *P*-values greater than 0.05 but less than or equal to 0.10 were considered trends.

***In Vivo* Digestion Experiment.** *Animals.* Fifteen purpose-bred beagles (Kennelwood, Inc., Champaign, IL) with an average age of 6.4 years (3.0–8.0 years) and an average starting body weight of 13.2 kg (10.3–16.1 kg) were used in this experiment. The University of Illinois Institutional Animal Care and Use Committee approved all animal care procedures prior to initiation of the experiment. Dogs were housed individually in indoor−outdoor pens (approximately 1.2 × 1.5 m indoors and 1.2 × 3.0 m outdoors) in an environmentally controlled facility with a 12 h light:12 h dark cycle. The outdoor portion of the runs consisted of commercial kennel paneling.

Diets and Treatments. Five experimental diets were formulated to meet or exceed the National Research Council (NRC, 2006) nutrient profiles for adult dogs at maintenance (*18*). The basal (control) diet consisted of a commercial-type diet matrix with poultry byproduct meal and brewer's rice as the main ingredients and beet pulp (BP) as the fiber source. To produce the treatment diets, BP was substituted with one of four select fiber sources resulting from ethanol production from corn. These fibers were NCF, NCFF, HCF, and HECF. Chromic oxide was included as a digestion marker at 0.2% of the diet. Complete ingredient and chemical composition data for the diets are presented in **Table 2**. Diets were prepared at Kansas State University Department of Grain Science and Industry (Manhattan, KS) under the supervision of Pet Food & Ingredient Technology, Inc. (Topeka, KS). The diets were in extruded, dry kibble form and were formulated to be isonitrogenous and isocaloric. Dogs were fed 300 g of food once daily, and food refusals from the previous feeding were collected and weighed. Dogs had *ad libitum* access to fresh water.

Experimental Design. The experimental design was a partially balanced incomplete block design consisting of two blocks of 15 dogs each. In the first block, dogs were randomly allotted to one of five experimental diets. For the second block, dogs were randomly allotted to one of five experimental diets, ensuring no dog received the same diet as in the first block. Each 12-day block consisted of two phases: 8 days for diet adaptation and 4 days for fecal collection. Dogs were weighed at the beginning and at the end of each block prior to feeding.

Table 2. Ingredient (% as Fed Basis) and Chemical (% Dry Matter Basis) Composition of Diets Fed to Dogs in Total Tract Digestibility Study

	diet[a]				
item	beet pulp	NCF	NCFF	HCF	HECF
poultry byproduct meal	37.0	37.0	37.0	37.0	37.0
poultry fat	14.0	14.0	14.0	14.0	14.0
ground yellow corn	10.0	10.0	10.0	10.0	10.0
brewer's rice	30.0	30.0	30.0	30.0	30.0
vitamin and mineral premix[b]	0.5	0.5	0.5	0.5	0.5
choline chloride	0.1	0.1	0.1	0.1	0.1
salt	0.7	0.7	0.7	0.7	0.7
potassium chloride	0.6	0.6	0.6	0.6	0.6
chromic oxide	0.2	0.2	0.2	0.2	0.2
test fiber	7.0	7.0	7.0	7.0	7.0
Analyzed Chemical Composition					
dry matter	95.3	95.4	95.8	95.9	95.6
organic matter	90.3	93.1	91.6	90.8	92.0
crude protein	33.3	24.6	30.4	32.7	28.4
acid-hydrolyzed fat	20.1	19.4	20.3	21.7	19.3
total dietary fiber	8.2	9.1	8.3	9.1	10.4
gross energy, kcal/g	5.3	5.3	5.5	5.3	5.4

[a] Abbreviations: NCF, native corn fiber; NCFF, native corn fiber with fines; HCF, hydrolyzed corn fiber; HECF, hydrolyzed extracted corn fiber. [b] Provided per kilogram of diet: 101 mg of Fe (ferrous sulfate); 10 mg of Mn (manganese sulfate); 7.5 mg of Cu (copper sulfate); 2 mg of I (calcium iodate); 225 μg of Se (sodium selenite); 150 mg of Zn (50% zinc sulfate and 50% zinc oxide); 7500 IU of vitamin A; 750 IU of vitamin D_3; 94 IU of vitamin E; 2.3 mg of vitamin K (menadione); 3.8 mg of thiamin; 15 mg of niacin; 30 mg of riboflavin; 12 mg of pantothenic acid; 39 μg of vitamin B_{12}; 1.9 mg of pyridoxine; 300 μg of D-biotin; 300 μg of folic acid.

Sampling Procedures. A sample of approximately 500 g of diet was taken from four different bags of each of the diets and composited, and a 500 g subsample was removed, ground, and stored at 4 °C until analysis. During the 4-day collection phase, all possible voided feces were collected from the floor of the pen and weighed. Feces were scored on a scale from 1 to 5, with 1 being dry, hard pellets; 2, dry, well-formed stool; 3, soft, moist, formed stool; 4, unformed stool; and 5, watery, liquid that can be poured. Feces were stored at −20 °C until composited and ground for analysis.

Chemical Analyses. Frozen feces and some of the CF samples were placed in a forced air oven at 55 °C until dry. Corn fibers, diet, and dried fecal samples were ground in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ) through a 2 mm screen. Samples were analyzed for DM, OM, CP, AHF, and TDF following procedures described previously. Gross energy (GE) concentrations of CF and diets were measured using oxygen bomb calorimeter (model 1261; Parr Instruments, Moline, IL). Food and fecal samples were prepared for chromium analysis according to the method of Williams et al. (*19*), and chromium concentrations were measured using an atomic absorption spectrophotometer (model 3100; Perkin-Elmer, Waltham, MA).

Calculations. Apparent total tract DM digestibilities were calculated as 100 – [100 × marker concentration in the feed (%)/marker concentration in the feces (%)]. Apparent total tract nutrient digestibilities were calculated as 100 – 100[marker concentration in the feed (%) × nutrient concentration in feces (%)/marker concentration in feces (%) × nutrient concentration in the feed (%)].

Statistics. Data were analyzed as a partially balanced incomplete block design using the Mixed Models procedure of SAS/STAT software, version 9.1 for Windows (*17*). The statistical model was

$$y_{ijk} = \mu + B_i + \tau_j + D_k + e_{ijk}$$

where y_{ijk} is the observation of the *K*th dog receiving the *J*th treatment in the *I*th block with a total of 30 observations, μ is the grand mean of the response variable, B_i is the random effect of the *I*th block with NID (0, σ^2_B) and 1 degree of freedom, τ_j is the fixed effect of the *J*th dietary treatment with 4 degrees of freedom, D_k is the random effects of the *K*th dog with NID (0, σ^2_D) and 14 degrees of freedom, and e_{ijk} is the experimental error with NID (0, σ^2_e) and 10 degrees of freedom.

Table 3. Proximate Analyses of Select Corn Fibers

	% dry matter basis for component[a]						
sample[b]	DM	OM	CP	AHF	TDF	IDF	SDF
NCF, batch 1	33.8	98.8	14.5	6.6	52.6	52.6	0.0
NCF, batch 2	38.5	99.4	12.0	7.5	65.2	65.1	0.1
NCF, batch 3	43.5	99.3	11.4	6.5	68.5	67.0	1.5
NCF, batch 4	38.7	99.3	13.7	4.9	69.8	66.8	3.0
NCF, batch 5	97.8	99.1	10.4	8.3	70.3	70.2	0.0
NCF, batch 6	38.3	99.5	12.7	6.2	71.1	70.9	0.2
NCF, batch 7	97.0	99.2	11.1	7.8	73.5	73.2	0.3
HCF, batch 1	22.6	99.1	14.6	5.5	46.4	41.8	4.6
HCF, batch 2	27.1	99.4	15.9	6.3	46.8	42.2	4.6
HCF, batch 3	96.7	99.8	15.8	8.4	64.4	63.8	0.5
HCF, batch 4	96.3	99.8	11.1	8.6	71.5	69.5	2.0
HECF, batch 1	30.6	99.5	13.5	1.6	61.7	56.1	5.7
HECF, batch 2	31.5	99.2	12.7	1.7	63.2	58.3	5.0
HECF, batch 3	94.4	99.6	11.9	3.8	78.8	76.0	2.8
HECF, batch 4	95.0	99.7	12.0	2.6	79.3	77.5	1.8

[a] Abbreviations: DM, dry matter; OM, organic matter; CP, crude protein; AHF, acid-hydrolyzed fat; TDF, total dietary fiber; IDF, insoluble dietary fiber; SDF, soluble dietary fiber. [b] Abbreviations: NCF, native corn fiber; HCF, hydrolyzed corn fiber; HECF, hydrolyzed extracted corn fiber.

For statistical analysis of digestibility data, the model also included CP intake as covariate because of a difference in CP concentration among diets.

Normal distribution of residuals and homogeneity of variances were tested, and assumptions for analysis of variances were fulfilled. It was assumed that there was no interaction between block and treatment, block and dog, and treatment and dog. Treatment least-squares means are reported and were compared using a Bonferroni adjustment to ensure the overall protection level. Standard error of the mean (SEM) values are associated with least-squares means as calculated in the Mixed Models procedure. Differences among means with a *P*-value of less than 0.05 were considered significant, and *P*-values greater than 0.05 but less than or equal to 0.10 were considered trends.

RESULTS AND DISCUSSION

Compositional Analyses. Proximate component analyses of select CF are presented in **Table 3**. Percentage DM ranged from 22.6% to 97.8%. The high variation is due to the fact that some samples arrived directly after wet milling, while other samples were dried before they were shipped. Organic matter concentrations were very uniform, ranging from 98.8% to 99.8%, consistent with values reported previously (*20–24*). Ingredients with low ash concentrations are particularly useful for the manufacture of pet foods. Beet pulp, which is used in many high-quality dog foods, usually has high ash concentrations that can limit its use as a fiber source for dogs. Crude protein concentration of CF ranged from 10.4% to 15.9%, consistent with literature values (*20–24*). Hydrolyzed corn fiber had the highest average CP concentrations (14.4%). Native corn fiber (12.3%) and hydrolyzed extracted corn fiber (12.5%) were similar in CP concentration. The fact that HECF had lower CP concentrations than HCF is maybe because protein is being washed out by ethanol during the fat extraction process. Acid-hydrolyzed fat values ranged from 1.6% to 8.6%, with HECF having lower average concentrations (2.4%) than NCF (6.8%) or HCF (7.2%), as expected. Total dietary fiber concentration ranged from 46.4% to 79.3%. Hydrolyzed corn fiber had lower average concentrations (57.3%) than NCF (67.3%) or HECF (70.8%). Most of the dietary fiber content was in the insoluble form; therefore, it can be inferred that it will not be as highly fermentable by microbiota in the large bowel.

Free sugar concentrations (Supporting Information) varied widely among substrates. Higher average values were noted for HCF. This is because of the hydrolysis process to which they were exposed. Free sugar concentrations also varied widely within CF, showing that batch is an important source of variation that might be associated with inconsistent efficiency of the washing process to remove free sugars after hydrolysis. On the contrary, hydrolyzed monosaccharides corrected for free sugar (HMC) concentrations (**Table 4**) did not vary greatly within and among CF, with NCF having higher concentrations than either HCF or HECF. This can be explained by the hydrolysis process that would reduce the amount of complex carbohydrates present in the sample. Arabinose and xylose concentrations are consistent with hemicellulose concentrations reported previously (*20, 23*). Arabinoxylan is the main hemicellulose in CF. Concentrations of arabinose and glucose were reduced after hydrolysis, as expected, whereas concentrations of xylose and galactose were not. Galactose concentrations were uniform within and among CF. Glucose contents were lower for CF after hydrolysis, as expected. However, on the basis of the average glucose concentration in NCF (282.9 mg/g of DM), it may be inferred that there is starch remaining in NCF because glucose in CF comes mainly from two components, starch and cellulose, and the content of the latter in CF has been reported to be in the range of 10−16% (*20, 23, 25*).

In general, large differences within each CF type in chemical composition were observed. This large variation in CF composition may be due to differences in three aspects of the corn used for ethanol production: variety, harvest time, and geographic location (*26*).

***In Vitro* Digestion Experiment.** Data reporting SCFA production resulting from fermentation of select CF are presented in **Table 5**. For all samples, acetate production was higher than was propionate production, which was higher than for butyrate. This pattern of SCFA production is similar to other fiber sources (*27*). Total SCFA production ranged from 211.6 to 699.52 μmol/g of DM. Average SCFA production for NCF was 467.1, for HCF, 527.5, and for HECF, 352.4 μmol/g of DM. These values are similar to those for xanthan gum (500 μmol/g of OM) (*28*). Total SCFA production was higher in some samples with lower SDF concentrations, which can seem contradictory. However, resistant starch, which behaves as IDF, is fermented to SCFA. Acetate and propionate production followed the same trend as total SCFA production. On the contrary, butyrate production was greater for NCF (46.4 μmol/g of DM) compared to HCF (23.1 μmol/g of DM) and HECF (13.5 μmol/g of DM). However, butyrate production was low for all substrates relative to moderately fermentable fibers such as BP (220 μmol/g of OM) or rice bran (260 μmol/g of OM) (*24*). Branched-chain fatty acid production was low for most samples with the exception of one NCF sample (batch 4). This indicates that there was little fermentation of branched-chain amino acids, valine, leucine, and isoleucine, and that those amino acids were present only in small amounts in the residue. A different sample of NCF (batch 1) resulted in no production of isobutyrate or isovalerate but resulted in the highest valerate production (25 μmol/g of DM). This value is more in line with that of a higher-protein substrate (*29*).

Total Tract Digestibility Study. *Diet Preparation.* The procedure followed in diet preparation consisted of preparing a base mix that was common for all five diets except for the fiber source. Then, each fiber source was added to the base mix, generating the five experimental diets in the following order: BP, HCF, NCFF, HECF, and NCF. Diets differed in DM by 0.6 and OM concentrations by 2.8 percentage units (**Table 2**). Acid-hydrolyzed fat concentration varied from 19.3% to 21.7%, while TDF concentration varied from 8.2% to 10.4%. The diet

Table 4. Hydrolyzed Monosaccharide Content of Select Corn Fibers Corrected for Free Sugar Concentrations

	hydrolyzed monosaccharides, mg/g of dry matter						
sample[a]	fucose	arabinose	galactose	glucose	xylose	mannose	total
NCF, batch 1	0.0	121.7	26.8	421.4	173.7	4.8	748.5
NCF, batch 2	0.0	146.6	36.6	314.7	223.4	7.4	728.7
NCF, batch 3	0.0	150.3	36.2	319.1	240.1	8.0	753.7
NCF, batch 4	0.0	128.6	34.5	225.9	211.4	0.0	600.4
NCF, batch 5	0.7	124.4	35.0	222.5	225.3	7.9	615.7
NCF, batch 6	0.0	150.2	37.8	277.8	245.4	7.4	718.6
NCF, batch 7	0.7	131.0	36.3	198.7	239.2	8.4	614.4
HCF, batch 1	−0.2	64.8	26.4	188.9	163.3	6.5	449.6
HCF, batch 2	−0.2	60.6	26.7	184.7	180.8	5.7	458.2
HCF, batch 3	0.4	48.2	29.5	164.1	208.0	10.8	460.9
HCF, batch 4	0.5	60.6	36.7	169.2	246.0	11.1	524.1
HECF, batch 1	0.0	60.0	27.2	248.0	188.5	8.6	532.4
HECF, batch 2	0.0	63.5	27.4	242.6	181.3	5.1	519.8
HECF, batch 3	0.5	64.1	39.2	174.0	267.9	11.0	556.8
HECF, batch 4	0.0	107.1	43.8	221.4	310.1	10.3	692.6

[a] Abbreviations: NCF, native corn fiber; HCF, hydrolyzed corn fiber; HECF, hydrolyzed extracted corn fiber.

Table 5. Short-Chain and Branched-Chain Fatty Acid Production Resulting from Fermentation of Select Corn Fibers

	fatty acid production, μmol/g of dry matter						
sample[a]	acetate	propionate	butyrate	isobutyrate	isovalerate	valerate	total
NCF, batch 1	375.3	221.1	78.1	0.0	0.0	25.0	699.5
NCF, batch 2	229.6	126.6	52.6	0.0	0.0	7.6	416.4
NCF, batch 3	190.6	114.3	67.5	0.0	0.0	0.0	372.4
NCF, batch 4	338.8	168.2	24.3	2.4	12.9	14.3	560.9
NCF, batch 6	247.2	144.2	47.7	0.0	0.0	5.0	444.1
NCF, batch 7	194.5	100.3	8.4	1.0	1.3	3.6	309.1
HCF, batch 1	374.1	181.4	29.5	0.0	8.6	8.8	602.4
HCF, batch 2	374.2	210.7	29.5	1.7	7.6	10.2	633.9
HCF, batch 3	194.8	122.0	10.3	1.0	9.7	8.4	346.2
HECF, batch 1	317.9	133.5	13.5	0.7	3.2	2.0	470.8
HECF, batch 2	322.0	157.6	13.6	2.5	8.5	4.5	508.7
HECF, batch 3	137.9	62.5	4.6	1.2	8.1	4.1	218.4
HECF, batch 4	114.3	75.0	22.3	0.0	0.0	0.0	211.6

[a] Abbreviations: NCF, native corn fiber; HCF, hydrolyzed corn fiber; HECF, hydrolyzed extracted corn fiber.

containing HECF had the lowest fat and the highest TDF concentrations as did the substrate itself. Gross energy concentration values differed by only 0.2 kcal/g, indicating that diets were isocaloric. However, percentage CP ranged from 24.6% to 33.3%, even though diets were formulated to be isonitrogenous. This difference can be attributed to a mixing problem during the preparation of the experimental diets. Mixing of the base ingredients was done in a different location from where the dog food was prepared, thus requiring transport that apparently resulted in ingredient sifting. Poultry byproduct meal may have settled to the bottom, creating a gradient in CP concentrations in the hopper and, consequently, in the diets. The first diet prepared had the highest CP concentration and the last one prepared the lowest. Nonetheless, CP concentrations are all well above the requirement for dogs at maintenance. Also, CP digestibility was not different among diets ($P = 0.21$) and should not have affected DM digestibility. Consequently, comparisons of digestibility results among treatments should be valid. But, to ensure valid comparisons among treatments, CP intake was used as a covariate in the statistical model, eliminating any possible effect on digestibility coefficients.

Substrate Composition. Chemical composition of CF sources fed to dogs is presented in **Table 6**. Percentage DM ranged from 87.4% to 96.4%. Organic matter was very uniform among CF, ranging from 99% to 99.5%, similar to values for CF samples analyzed previously. Organic matter content of BP (91.4%) was lower than that of CF, as expected. Crude protein followed the same trend as previous samples, ranging from 10.8% to 14.1% among CF compared to 6.1% for BP. Acid-hydrolyzed fat ranged from 2.4% to 6.8%, with HECF having the lowest value, as would be expected due to the fat extraction process. Total dietary fiber ranged from 63% to 88.2%. Native corn fiber with fines had the lowest TDF concentration and HECF the highest. It is important to note that "fines", or fine fiber, generally includes greater amounts of starch and lower amounts of TDF. Gross energy concentrations ranged from 3988 to 4885 kcal/kg, with BP having the lowest value, consistent with its lower OM content. Hydrolyzed corn fiber had the highest GE concentration (4885 kcal/kg), consistent with its higher AHF concentration.

Table 6. Chemical Analyses of Fiber Sources Fed to Dogs in Total Tract Digestibility Study

	fiber source[a]				
	beet pulp	NCF	NCFF	HCF	HECF
dry matter, %	94.5	92.1	87.4	94.2	96.4
% Dry Matter Basis for Nutrient					
organic matter	91.4	99.0	99.3	99.5	99.5
crude protein	6.3	12.0	14.1	12.0	10.8
acid-hydrolyzed fat	2.9	5.6	4.9	6.8	2.4
total dietary fiber	68.8	71.1	63.0	79.9	88.2
gross energy, kcal/g	4.0	4.8	4.8	4.9	4.7

[a] Abbreviations: NCF, native corn fiber; NCFF, native corn fiber with fines; HCF, hydrolyzed corn fiber; HECF, hydrolyzed extracted corn fiber.

In Vitro Digestion of Corn Fibers. In vitro OMD (IVOMD) of the CF sources fed to dogs is presented in **Table 7**. Interaction

Table 7. *In Vitro* Organic Matter Disappearance (%) of Fiber Sources Fed to Dogs in Total Tract Digestibility Study

	fiber source[a]					
item	beet pulp	NCF	NCFF	HCF	HECF	SEM[b]
hydrolytic–enzymatic digestion	20.5 c	25.4 b	31.1 a	15.0 d	7.2 e	0.35
fermentative digestion, 8 h pull time	6.3 a	3.9 a	5.1 a	−0.6 b	0.0 b	0.70
fermentative digestion, 16 h pull time	17.7 a	9.3 b	9.9 b	0.1 c	3.9 c	0.70

[a] Abbreviations: NCF, native corn fiber; NCFF, native corn fiber with fines; HCF, hydrolyzed corn fiber; HECF, hydrolyzed extracted corn fiber. [b] SEM, pooled standard error of the mean.

Table 8. Intake (as Fed Basis) and Total Tract Digestibility by Dogs of Diets Containing Select Corn Fibers

	diet[a]					
item	beet pulp	NCF	NCFF	HCF	HECF	SEM[b]
intake, g/day	216.1	246.5	287.3	270.4	238.8	24.5
Total Tract Digestibility, %[c]						
dry matter	78.4 b	82.3 a	80.9 ab	79.0 b	79.2 b	0.5
organic matter	84.5 ab	86.6 a	86.3 ab	84.4 b	84.3 b	0.5
crude protein	81.2	82.3	83.2	83.5	83.1	0.9
acid-hydrolyzed fat	93.8	94.3	94.4	94.7	94.7	0.2
total dietary fiber	27.7 ab	30.9 a	19.1 b	17.8 b	20.1 ab	2.4

[a] Abbreviations: NCF, native corn fiber; NCFF, native corn fiber with fines; HCF, hydrolyzed corn fiber; HECF, hydrolyzed extracted corn fiber. [b] SEM, pooled standard error of the mean. [c] a, b: means in the same row with unlike letters differ ($P < 0.05$).

Table 9. Fecal Characteristics of Dogs Fed Diets Containing Select Corn Fibers

	diet[a]					
item	beet pulp	NCF	NCFF	HCF	HECF	SEM[b]
fecal output (as is), g/day	140.7	107.7	134.6	137.9	116.9	16.55
fecal output (DM), g/day	44.8	41.9	53.0	54.5	47.4	5.00
fecal output (as is) per g of DM consumed[c]	0.62 a	0.44 b	0.46 b	0.51 ab	0.49 b	0.03
fecal DM, %[c]	33.7 b	38.8 a	40.6 a	39.8 a	40.9 a	1.31
fecal score[d]	3.0	3.1	3.1	3.1	3.0	0.06

[a] Abbreviations: NCF, native corn fiber; NCFF, native corn fiber with fines; HCF, hydrolyzed corn fiber; HECF, hydrolyzed extracted corn fiber. [b] SEM, pooled standard error of the mean. [c] a, b: means in the same row with unlike letters differ ($P < 0.05$). [d] Scores based on the following scale: 1 = dry, hard pellets; 2 = dry, well-formed stool; 3 = soft, moist, formed stool; 4 = unformed stool; 5 = watery, liquid that can be poured.

between fiber source and pull time was significant ($P < 0.01$). Even though *in vitro* OMD was higher at 16 h for all substrates, the increase from 8 to 16 h did not occur in the same manner; hence, the interaction term was significant. *In vitro* OMD after hydrolytic−enzymatic digestion was higher for NCFF, followed by NCF, BP, HCF, and HECF. These differences are to be expected as NCFF would have a higher amount of starch that is susceptible to degradation by hydrolytic−enzymatic means. *In vitro* OMD after 8 h of fermentative digestion was higher for BP, NCFF, and NCF as compared to HCF and HECF whose values were not different than 0. After 16 h of fermentative digestion, *in vitro* OMD was higher for BP (17.7%), followed by NCFF (9.9%) and NCF (9.3%), with lower values for HECF (3.9%) and HCF (0.1%). Results after 16 h of fermentative digestion for NCF and NCFF were similar to those of corn bran reported by Bourquin et al. (*30*). Low *in vitro* OMD values for HCF and HECF may occur because both have been exposed to high temperatures and washed to remove most of the carbohydrates that are easy to hydrolyze. This process leaves in the residue polysaccharides that are more difficult to hydrolyze.

In Vivo Digestion of Diets Containing Corn Fibers. Intake and total tract digestibility by dogs of diets containing select CF are presented in **Table 8**. Average daily food intakes were similar among treatments throughout the study with most of the dogs ingesting all the food they were provided. This is an indication that the concentrations of CF included in diets did not negatively affect palatability. Apparent DM digestibility coefficients were high, with the NCF treatment having a higher ($P < 0.05$) value compared to the remaining treatments except for NCFF. Beet pulp, HCF, and HECF treatments had a lower DM digestibility compared to NCF but not to NCFF. Apparent OM digestibility coefficients followed the same basic trend as DM. Apparent CP and AHF digestibilities were high and not different among treatments, suggesting the absence of antinutritive compounds in all CF sources supplemented at the 7% dietary inclusion level. These results agree with those of Lewis et al. (*31*), who showed no significant difference in CP or fat digestibilities by dogs when CF was compared to starch, pectin, and finely ground or coarsely ground cellulose as fiber sources. On the other hand, slightly lower nutrient digestibilities of diets with different fiber sources compared with a control diet without added fiber were reported by Fahey et al. (*32*). However, similar nutrient digestibility values among BP, tomato pomace, peanut hulls, wheat bran, and alkaline hydrogen peroxide-treated wheat straw were noted in that study. Apparent TDF digestibility was highest for the NCF treatment and lowest for the NCFF and HCF treatments. Values for BP and HECF were intermediate. The HECF treatment tended to have a different TDF digestibility coefficient compared with NCF ($P = 0.09$).

The relationship between *in vitro* OMD of CF and *in vivo* TDF digestibility showed that the *in vitro* digestibility assay accurately predicted the apparent digestibility of BP, NCF, and HCF (data not shown). However, HECF digestibility was underestimated, whereas digestibility of NCFF was overestimated. In the latter case, perhaps the higher amount of starch remaining in the NCFF is bound to the fiber fraction, rendering

it unavailable to the dog. Harsher conditions associated with the *in vitro* procedure may have hydrolyzed this starch, thus overestimating the *in vivo* digestibility of this substrate. However, all diets were extruded, and this should increase digestibility of resistant starch, in which case *in vivo* digestibilities should be higher. In the case of HECF, perhaps the extrusion process associated with diet preparation rendered select carbohydrates more available to the dog, thus resulting in higher digestibility values than predicted by *in vitro* methodology.

Fecal characteristics of dogs fed diets containing select CF are presented in **Table 9**. Fecal output expressed on both a DM and as-is basis was similar among treatments. However, fecal output (as-is basis) per gram of DM consumed was higher ($P < 0.05$) for BP and HCF treatments, with HCF tending ($P < 0.07$) to be different from the BP treatment. Conversely, HECF, NCFF, and NCF treatments had similar but lower values. These differences are to be expected as the fecal DM concentration for dogs on the BP treatment was lower than for all other treatments. Despite the fact that the BP diet generated feces with a higher amount of water, fecal scores were ideal and not different among treatment groups, indicating that inclusion of CF is well tolerated by dogs, producing feces with characteristics considered to be of good quality for dog owners. Similar results were presented by Lewis et al. (*31*).

Implications. Corn fibers can provide an economical and abundant source of DF. Consistent quality fiber sources are a major concern in promoting the benefits of CF inclusion in dog foods. Compositional and *in vitro* fermentation data indicated that CF are high carbohydrate and poorly fermentable by dog microbiota. However, their concentrations of arabinose and xylose may indicate potential usefulness as prebiotics if these carbohydrates exist in CF substrates as arabinoxylan oligosaccharides. Results suggest that incorporation of CF at the 7% inclusion level, when substituted for beet pulp in the diets of healthy adult dogs, does not dramatically impact nutrient digestibility, food intake, or fecal production and characteristics. Thus, CF may be considered as potential fiber sources in high protein−high fat dog foods.

Supporting Information Available: One table containing free sugar content of select corn fibers. This material is available free of charge via the Internet at http://pubs.acs.org.

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