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Select corn coproducts from the ethanol industry and their potential as ingredients in pet foods

M. R. C. de Godoy, L. L. Bauer, C. M. Parsons, and G. C. Fahey Jr.[1]

University of Illinois at Urbana-Champaign, Urbana 61801

ABSTRACT: The objectives of this study were to determine the chemical composition and nutritive value of corn protein product 1 (CPP 1), corn protein product 2 (CPP 2), and corn fiber (CF), novel coproducts of the ethanol industry, and compare these feed ingredients with standard plant protein ingredients [soybean meal (SBM), distillers dried grains with solubles (DDGS), corn gluten meal (CGlM), and corn germ meal (CGeM)], and to compare CF sources (CF control 1 and control 2) with standard fiber sources (peanut hulls, Solka-Floc, and beet pulp) commonly used in pet foods. Corn fiber, CPP 1, and CPP 2 were produced at a pilot-scale modified dry-grind plant, with CPP 2 having a greater degree of purification than CPP 1. Crude protein values for CPP 2 and CPP 1 were 57.3 and 49.7%, respectively. Total dietary fiber concentration was 29% for CPP 2 and 23.5% for CPP 1. Acid-hydrolyzed fat and GE concentrations were similar for these ingredients. In a protein efficiency ratio assay, no differences ($P > 0.05$) in feed intake, BW gain, or CP intake were noted for CPP 2, CPP 1, or CGlM. However, feeding CPP 2 resulted in a greater ($P < 0.05$) G:F ratio and protein efficiency ratio than CPP 1 and CGlM. In a cecectomized rooster assay, CGlM had numerically the greatest standardized total AA, total essential AA, and total nonessential AA digestibilities, but they were not different ($P > 0.05$) from CPP 1 or SBM values. Corn germ meal resulted in the least values, but they were not different from those for DDGS and CPP 1. The greatest values for true nitrogen-corrected ME were obtained with CGlM, followed by CPP 2, DDGS, CPP 1, SBM, and CGeM. Distillers dried grains with solubles and CPP 1 had similar true nitrogen-corrected ME values, and they were not different from values for CPP 2 and SBM. In vitro CP disappearance was greatest ($P < 0.05$) for CGlM (94.1%), intermediate for DDGS (76.8%) and CPP 1 (77.5%), and least for CPP 2 (74.1%) and CGeM (67.7%). Corn fibers contained predominantly insoluble dietary fiber (1% or less of soluble dietary fiber), with a moderate CP concentration. In vitro OM disappearance for the fiber sources, when using inoculum from dog feces, revealed that with the exception of beet pulp, which had a moderate disappearance value after 16 h of fermentation (17.7%), all fiber substrates had a nonsignificant extent of fermentation. In conclusion, novel corn coproducts had properties comparable with the standard protein and fiber sources used in animal nutrition.

Key words: corn coproduct, dog, fiber, in vitro, nutritive value, protein

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INTRODUCTION

The ethanol industry is the fastest growing renewable energy industry in the world (Tolman and Tumbleson, 2006). In 2006, ethanol production increased by 25% from the previous year and by 300% compared with the year 2000. Roughly, 100.8 billion kg of corn was used for ethanol production in 2006, representing nearly 17% of the US corn crop (Renewable Fuels Association, 2007).

Corn is the main raw material used in ethanol production. Ethanol is mainly produced by the dry-milling process, which generates distillers dried grains with solubles (**DDGS**) as a coproduct. Wet milling is responsible for 18% of ethanol production (Renewable Fuels Association, 2007), and this process produces corn gluten meal (**CGlM**), corn germ meal (**CGeM**), corn gluten feed, and corn fiber (**CF**) as coproducts.

In the pet food industry, CGlM has been the most used corn protein source. Usually, it is used in combination with soy products to overcome CGlM deficiencies in essential AA (**EAA**) such as lysine and tryptophan (Case et al., 2000). Most corn coproducts from both processes have been used mainly in ruminant nutrition because of their greater concentration of structural carbohydrates, poor AA profile, and high variability. New technology developed recently by the ethanol industry, however, may open new applications for coproducts

[1]Corresponding author: gcfahey@illinois.edu
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from modern ethanol plants. Improvements in protein quality and reduced fiber and phosphorus contents can make these coproducts appropriate dietary ingredients for swine, poultry, and companion animals.

Little research has been done on evaluating the efficacy of corn coproducts from the ethanol industry in companion animal nutrition. Among the corn coproducts, DDGS and CGlM have relatively high DM and CP digestibilities by dogs (Allen et al., 1981; Yamka et al., 2004). In cats, CGlM had urine-acidifying properties, which is of importance in the urinary tract health of felines (Case et al., 2000; Funaba et al., 2005). The lack of data on these coproducts prevents their maximal use in pet foods. Expansion of the database on chemical composition and nutrient digestibility would promote the use of corn coproducts in the pet food industry. Hence, the objective of this study was to determine the chemical composition, protein quality, and in vitro OM and protein disappearances of corn protein product 1 (**CPP 1**), corn protein product 2 (**CPP 2**), and NutraFiber [a novel CF (**NCF**)] in comparison with standard feed ingredients.

MATERIALS AND METHODS

All surgical and animal care procedures were conducted under a research protocol approved by the Institutional Animal Care and Use Committee, University of Illinois, Urbana.

Substrates

Three novel corn coproducts from the ethanol industry were tested in this study and compared with conventional feed sources. Corn protein product 1 and CPP 2 (corn protein concentrates), 2 novel protein sources, and NutraFiber, an NCF source, were obtained (Quality Technology International Inc., Elgin, IL). Conventional protein sources [CGlM, CGeM, DDGS, and soybean meal (**SBM**)] were obtained commercially. Fiber ingredients used as controls were CF [CF control 1 (**CF-C1**; Archer Daniels Midland Company, Decatur, IL) and CF control 2 (**CF-C2**; Cargill, Minneapolis, MN)], as well as peanut hulls (**PH**), Solka-Floc (**SF**; International Fiber Corporation, St. Louis, MO), and beet pulp (**BP**; Archer Daniels Midland Company, Decatur, IL).

The novel corn coproducts were produced from a modified wet milling plant (hybrid process; Hydromilling, owned by Corn Value Products). This process fractionates the corn kernel components (germ, fiber, and protein + starch) before the fermentation phase used in the ethanol industry but without the use of sulfur dioxide. The main nutritional characteristic of these novel corn coproducts is their high CP and AA concentrations.

Chemical Analyses

Sample ingredients were ground in a Wiley mill (model 4, Arthur H. Thomas, Swedesboro, NJ) through a 1-mm screen. Each substrate was analyzed for DM, ash (AOAC, 2000), CP (from Leco nitrogen values, FP2000, Leco Corp., St. Joseph, MI; AOAC, 2000), total dietary fiber (**TDF**; Prosky et al., 1992), ADF (Goering and Van Soest, 1970), NDF (Goering and Van Soest, 1970; Jeraci et al., 1988), GE (Parr Instrument Manuals, Parr Instrument Co., Moline, IL), and AA and mineral concentrations (from University of Missouri Experiment Station Chemical Laboratories, using methods outlined by AOAC, 2000). Total fat content was measured by acid hydrolysis (American Association of Cereal Chemists, 1983), followed by ether extraction (Budde, 1952). Total nonstructural carbohydrates were measured according to the method of Smith (1969) for plant protein ingredients only. All ingredients were analyzed in duplicate, with a 5% error allowed between duplicates; otherwise, analyses were repeated. For substrates whose fiber concentrations were low and protein concentrations were high (CPP 1, CPP 2, and CGlM), a 10% error between duplicates was accepted for the ADF analysis.

Protein Efficiency Ratio Assay

A total of 120 female crossbred chicks (New Hampshire × Columbian), averaging 92.8 g of BW and 8 d of age, were used. Chicks were housed in groups of 5 in starter batteries with raised wire floors in a controlled environment. The chicks had ad libitum access to food and water throughout the experimental period, which lasted for 9 d, with the animals being weighed at the beginning and end. A basal diet was formulated to meet the nutritional requirements of the chicks, except for CP. Six additional test diets were formulated to provide 10% CP from CPP 1, CPP 2, CGlM, CGeM, DDGS, and SBM at the expense of the cornstarch:dextrose mixture (basal diet). Composition of the diet is presented in Table 1.The DMI was recorded for calculation of G:F and protein efficiency ratio (**PER**). The latter was calculated as grams of gain in BW per gram of protein intake.

The experimental design was a completely randomized design with 6 treatments, 5 chicks per cage, and 4 cages per treatment. Data were analyzed by ANOVA (SAS Inst. Inc., Cary, NC). Treatment differences were determined by using the LSD procedure calculated from the pooled SEM from ANOVA. A probability of $P < 0.05$ was accepted as the level of significance.

Cecectomized Rooster Assay

A precision-fed cecectomized rooster assay, as described by Sibbald (1979), was conducted to quantify

standardized AA digestibilities of 6 plant protein ingredients (CPP 1, CPP 2, CGlM, CGeM, DDGS, and SBM). Twenty-four Single Comb White Leghorn roosters, 50 wk of age, were used. When the birds were 25 wk of age, cecectomy was performed under anesthesia according to the procedure of Parsons (1985). All roosters were given at least 8 wk to recover from surgery before being used in the experiment. All birds were housed individually in cages with raised wire floors. They were kept in an environmentally controlled room and subjected to a 16-h light and 8-h dark photoperiod. Before the beginning of the experiment, the birds had ad libitum access to feed and water.

Roosters were deprived of feed for 24 h and then crop-intubated with approximately 30 g of each plant protein ingredient. Each was fed to 4 roosters. After crop intubation, excreta (urine and feces) were collected for 48 h on plastic trays placed under each cage. The excreta samples were freeze-dried, weighed, and ground to pass through a 60-mesh screen. Each sample was analyzed to determine AA concentrations. In addition, endogenous excretion of AA was measured by using 4 additional roosters held without feed throughout the experimental period. Standardized digestibility of AA was calculated by using the method described by Sibbald (1979). The difference between apparent AA digestibility and standardized AA digestibility is that the latter takes into consideration the amount of AA excreted during fasting. It provides a correction factor for apparent AA digestibility values, avoiding their underestimation, particularly for low-protein ingredients.

Feed and excreta from cecectomized roosters also were analyzed for nitrogen (Leco nitrogen values; AOAC, 2000) and GE by using an adiabatic bomb calorimeter standardized with benzoic acid. True ME corrected for nitrogen (**TME_n**) was calculated by the method of Sibbald (1976). Endogenous corrections for energy and nitrogen were made by using excreta from roosters that had been fasted for 48 h. The non-ME was corrected to nitrogen equilibrium, assuming that nitrogen retained would produce additional urinary energy in the excreta amounting to 8.22 kcal/g of nitrogen. These latter values also were corrected for endogenous energy of nonnitrogenous origin, as measured by using the fasted birds to obtain TME_n. The TME_n values were calculated by the equation

$$TME_n = FEf - (EEf + 8.22\ Nf) + (EEu + 8.22\ Nu)/FC,$$

where FEf is the GE of the total feed consumed; EEf and EEu refer to the energy in the excreta collected from the fed and fasted birds, respectively; Nf and Nu refer to the grams of nitrogen retained by the fed and fasted birds, respectively; and FC is the grams of dry feed consumed.

Data were analyzed as a completely randomized design by ANOVA (SAS Inst. Inc.). Treatment differences were determined by using the LSD procedure calculated from the pooled SEM from ANOVA. A probability of $P < 0.05$ was used as the level of significance.

Table 1. Ingredient composition of the basal diet (as-is basis) fed to young chicks in the protein efficiency ratio assay

Basal diet ingredient	Amount, %
Cornstarch:dextrose (2:1 ratio)	To 100
Test ingredient[1]	To provide 10% CP
Soybean oil	5.00
Limestone	1.22
Dicalcium phosphate	2.45
Salt	0.50
Purified vitamin mix[2]	0.20
Mineral mix[3]	0.15
Magnesium sulfate[4]	0.35
Potassium carbonate	0.90
Purified choline chloride[5]	0.20
DL-Tocopheryl acetate	0.002
Ethoxyquin	0.0125

[1]Protein sources (% incorporated into diet): 21.5% corn protein 1, 18.2% corn protein 2, 39.5% distillers dried grains with solubles, 15% corn gluten meal, 39.5% corn germ meal, 20.3% soybean meal.

[2]Provided per kilogram of diet: thiamine·HCl, 20 mg; niacin, 50 mg; riboflavin, 10 mg; D-calcium-pantothenate, 30 mg; vitamin B_{12}, 0.04 mg; pyridoxine·HCl, 6 mg; D-biotin, 0.6 mg; folic acid, 4 mg; menadione dimethylpyrimidinol bisulfite, 2 mg; cholecalciferol, 15 µg; retinyl acetate, 1,789 µg; ascorbic acid, 250 mg.

[3]Provided per kilogram of diet: manganese (as MnO), 75 mg; iron (as $FeSO_4 \cdot H_2O$), 75 mg; zinc (as ZnO), 75 mg; copper (as $CuSO_4 \cdot 5H_2O$), 8 mg; iodine (as CaI_2), 0.75 mg; selenium (as Na_2SeO_3), 0.3 mg.

[4]$MgSO_4 \cdot 7H_2O$.

[5]Choline chloride and vitamin mix in a carrier with few to no nitrogen-containing compounds were incorporated to provide an otherwise nitrogen-free diet except for nitrogen from the test ingredients.

Multiple Enzymatic Filtration System In Vitro Method

The objective of this assay was to predict nutrient digestibility of food or ingredients at the ileal level, total digestive tract level, or both by using the method of Boisen and Eggum (1991). In triplicate, 300 mg of each substrate was weighed into 50-mL (28 × 100 mm) polypropylene tubes. Blank tubes containing no substrate also were started at this point. Initially, 12.5 mL of phosphate buffer (0.1*M*) was added to each tube. The function of this buffer was to simulate the pH created by salivary amylase in the mouth.

Hydrochloric acid (0.2 *N*) and a hydrochloric acid:pepsin solution (1 g of pepsin + 10 mL of hydrochloric acid in 100 mL of double-distilled water) were subsequently added to the substrate and blank tubes. In animals, the action of these enzymes is responsible for the hydrolytic digestion that occurs in the stomach; for optimal enzymatic activity, a pH of approximately 2 must be maintained. Pepsin and hydrochloric acid are able to hydrolyze at least half the proteins to small peptides. A chloramphenicol solution (Sigma, St. Louis, MO) also was used at this stage to act as a bacteriostatic agent. This is of importance to avoid confounding

factors between enzymatic action and microbial degradation of the substrates. Tubes were sealed with rubber stoppers and incubated at 39°C for 6 h, with mixing on a regular basis.

After the 6-h incubation period, the hydrochloric acid was neutralized with the addition of 0.5 *N* sodium hydroxide, followed by addition of 5 mL of phosphate buffer (pH 6.8, 0.2 *M*) containing porcine pancreatin (5 g/L, Sigma). The purpose of this step was to mimic the enzymatic digestion at the proximal small intestine. At this point, tubes were resealed and incubated for 18 h at 39°C with regular mixing. Tubes were next centrifuged for 15 min at 6,750 × *g* at room temperature and decanted, and the supernatant was discarded. For fiber sources, remaining substrate was lyophilized and used in the in vitro fermentation stage, whereas at this point, the protein source ingredients were precipitated with 95% ethanol for 1 h to recover unfermented residues. Samples were filtered through Whatman 541 filter paper and washed sequentially with 78% ethanol, 95% ethanol, and acetone. Samples retained on the filter paper were dried at 105°C to obtain a constant weight. They then were weighed and analyzed for CP (from Leco nitrogen values; AOAC, 2000).

In Vitro Fermentation

The NCF was tested along with CF-C1 and CF-C2, BP, SF, and PH. These ingredients were chosen because of their potential or actual use in dog foods. Three purpose-bred, healthy dogs (average age = 5 yr; average BW = 25.1 kg) were used as fecal donors. Inoculum was prepared by using pooled feces from dog donors. The dogs consumed a commercial diet (Iams Weight Control, The Iams Co., Lewisburg, OH) and were not exposed to antibiotics for 6 mo before this experiment.

The composition of the medium used to culture the microbiota was published previously (Sunvold et al., 1995a). All medium components except for the vitamin solutions and short-chain fatty acids (**SCFA**) were mixed before autoclave sterilization of the medium. Filter-sterilized vitamin solutions were added just before dispensing the medium along with SCFA, which were maintained under anaerobic conditions at all times after preparation. Aliquots (26 mL) of the medium, added to maintain microbial viability, were aseptically transferred to 50-mL tubes containing fiber substrates, which were analyzed previously by using the in vitro multiple enzymatic filtration system. Anaerobic conditions were maintained by purging with carbon dioxide before sealing the tubes with rubber stoppers equipped with one-way gas release valves. All tubes were stored at 4°C overnight before inoculation to enable hydration of substrates before initiating the fermentation process.

The freshly voided fecal samples from each dog were immediately placed into plastic bags that were sealed after expressing excess air. Fecal samples were then pooled and diluted 1:10 (wt/vol) in a previously warmed (39°C) anaerobic dilution solution (Bryant and Burkey, 1953) by blending it for 15 s in a Waring blender under a stream of carbon dioxide. Blended, diluted feces were filtered through 4 layers of cheesecloth and sealed in 125-mL serum bottles under carbon dioxide.

Sample and blank tubes were aseptically inoculated with 4 mL of diluted, pooled feces. Tubes were incubated at 39°C with periodic mixing. Substrates were fermented in vitro for 8 and 16 h. At appropriate sampling times, tubes were removed from the 39°C incubation and processed immediately for analyses. For each sampling time, a 2-mL subsample was taken from each 50-mL tube and added to 0.5 mL of 25% metaphosphoric acid for SCFA analysis. The remaining 28 mL was combined with 4 vol of 95% ethanol and precipated for 1 h to recover unfermented residues. Samples were filtered through Whatman 541 filter paper and washed sequentially with 78% ethanol, 95% ethanol, and acetone. These precipitation and filtration steps were very similar to the residue recovery steps described for the TDF procedure (Prosky et al., 1992). Samples were dried at 105°C to obtain a constant weight. Samples were weighed and ashed (500°C), and the residue was weighed to determine OM disappearance. In vitro OM disappearance was calculated as $1 - [(\text{OM residue} - \text{OM blank})/\text{initial OM})] \times 100$, where OM residue is the OM recovered after 8 and 16 h of fermentation of each substrate, OM blank is the OM recovered in the corresponding blank after the same fermentation times, and initial OM is the OM of the original substrate placed in the tube.

In vitro data were analyzed as a completely randomized design by using PROC MIXED of SAS. The statistical model included the fixed effects of substrate, sampling time, the interaction between substrate and time for protein substrates when using the multiple enzymatic filtration system in vitro, and the interaction between substrate and time for the fiber substrates in the in vitro fermentation step. The statistical model for the hydrolytic digestion step for the fiber substrates was analyzed as a completely randomized design including the fixed effect of substrate. Treatment least squares means are reported and were compared by using a Tukey adjustment to ensure the overall amount of protection. Standard error of the mean values were associated with least squares means as calculated in PROC MIXED. Differences among means with a *P*-value of less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Chemical Composition of Plant Protein and Fiber Ingredients

Dry matter values for plant protein ingredients varied little (Table 2). Ash values ranged from 1.8 to 7.4%, for CGlM and SBM, respectively. Differences in CP concentrations were observed among samples, ranging from 27.6% (DDGS) to 73.9% (CGlM). Corn protein

Table 2. Chemical composition of select protein sources

Item[1]	CPP 1	CPP 2	CGlM	CGeM	DDGS	SBM
	Protein source[2]					
DM, %	93.4	95.7	90.5	90.1	91.8	88.7
	% (DM basis)					
Ash	3.8	1.9	1.8	3.9	4.3	7.4
CP	49.7	57.3	73.9	28.4	27.6	55.6
TDF	23.5	29.0	0.3	45.0	30.5	19.6
TNC	9.3	8.9	17.8	32.2	19.7	19.5
NDF	16.6	32.5	2.0	38.3	26.8	12.7
ADF	6.0	7.0	0.7	11.5	8.1	5.5
AHF	10.9	10.6	7.8	6.0	15.2	3.8
GE, kcal/kg	5,362	5,489	5,743	4,559	5,175	4,703

[1]TDF = total dietary fiber; TNC = total nonstructural carbohydrates; AHF = acid-hydrolyzed fat.

[2]CPP 1 = corn protein product 1; CPP 2 = corn protein product 2; CGlM = corn gluten meal; CGeM = corn germ meal; DDGS = distillers dried grains with solubles; SBM = soybean meal.

product 2 had a greater CP concentration when compared with CPP 1 (57.3 vs. 49.3%, respectively). The difference observed in CP concentration between CPP 1 and CPP 2 was related to the fact that CPP 2 is a more purified product after processing than is CPP 1, which receives some solubles back from the heavy steep water, which dilutes its CP concentration. Corn protein product 1 and CPP 2 had the least total nonstructural carbohydrate values (9.3 and 8.9%), whereas CGeM had the greatest (32.2%). Total dietary fiber concentration ranged from 0.3% (for CGlM) to 45% (for CGeM). The low TDF value for CGlM is perhaps related to its high concentration of CP. Neutral detergent fiber and ADF values were corrected for residual CP, but not for ash content. In both analyses, CGlM had the least and CGeM had the greatest NDF and ADF values. Neutral detergent fiber and ADF values were greater than the TDF value for CGlM. Correction for ash in ADF and NDF would decrease these values, bringing them closer in line with the TDF value. Acid-hydrolyzed fat (**AHF**) ranged from 3.8% (SBM) to 15.2% (DDGS). With the exception of DDGS, the substrates were not expected to have a great amount of lipid because all of them had been through oil extraction. Corn germ meal and SBM had reduced GE values because of their greater ash and reduced AHF contents compared with the other substrates.

Total AA concentrations (Table 3) were greatest for CGlM (76.3%) and least for CGeM (24.6%). Generally, total AA values were very similar to CP values, indicating that most of the nitrogen-containing compounds in these ingredients were of protein origin. Total EAA and total non-EAA concentrations followed the same pattern as total AA concentrations. Soybean meal had the greatest concentrations of arginine, lysine, and tryptophan. Corn gluten meal had the greatest concentrations of isoleucine, leucine, methionine, phenylalanine, threonine, and valine. Overall, DDGS and CGeM had the least concentrations of EAA. A reduced concentration of lysine is a characteristic of cereal grains, explaining the reduced concentration of this AA in corn coproducts when compared with soybean (oilseed) coproducts such as SBM.

Calcium concentration was negligible among ingredients (Table 4). Phosphorus concentration was greatest for CGeM (1%) and least for CPP 2 (0.4%). Phosphorus concentration of CGlM (0.6%) was in accordance with data reported by Rausch and Belyea (2006). Depending on the phosphorus bioavailability of the corn coproducts, phosphorus concentration may not be of concern in food and feed formulations. However, phosphorus concentration was greater than calcium concentration, resulting in an undesirable ratio between these minerals, which must be corrected during diet formulation. Additionally, mineral concentrations cited herein do not account for their respective bioavailability. Plant mineral sources oftentimes are unavailable to the animal. Overall, mineral concentration was fairly similar to data published by NRC (1994, 2006) and Dale and Batal (2007). Wide variation in mineral composition, however, is not uncommon because it can be influenced by cultivar, growing season, location of production, and characteristics of the soil where the grain was cultivated.

Dry matter values for fiber ingredients (Table 5) ranged from 89.2% (CF-C2) to 98.9% (CF-C1). Ash values varied widely. Crude protein concentration varied from 0% (SF) to 11.0% (CF-C1). The control CF (CF-C1 and CF-C2) had similar CP concentrations (11.0 and 10.4%, respectively). These values were greater than the CP value for the NCF (7.5%), which suggests either that CF-C1 and CF-C2 may have had a greater proportion of CF from endosperm compared with the NCF (which possibly had more fiber from the pericarp), or that CF-C1 and CF-C2 were less purified during processing. Total dietary fiber was greatest for SF (100%) and least for BP (68.8%). Corn fibers had intermediate TDF values. Neutral detergent fiber and ADF for the 3 CF followed the same pattern as TDF. Chemical analyses support this observation.

Table 3. Amino acid composition of select protein sources (DM basis)

	Protein source,[1] %					
AA	CPP 1	CPP 2	CGlM	CGeM	DDGS	SBM
Essential						
Arginine	1.7	2.0	2.4	2.1	1.2	4.1
Histidine	1.2	1.5	1.5	0.8	0.8	1.4
Isoleucine	2.1	2.3	3.0	1.0	1.1	2.6
Leucine	7.0	8.6	12.2	2.1	3.3	4.3
Lysine	0.9	1.1	1.2	1.1	0.9	3.5
Methionine	1.0	1.2	1.8	0.5	0.6	0.8
Phenylalanine	2.7	3.3	4.6	1.2	1.4	2.8
Threonine	1.8	2.0	2.4	1.0	1.1	2.1
Tryptophan	0.3	0.4	0.5	0.3	0.2	0.8
Valine	2.5	2.9	3.3	1.6	1.4	2.7
Nonessential						
Alanine	3.6	4.5	6.4	1.7	2.0	2.4
Aspartic acid	2.8	3.4	4.4	2.0	1.8	6.2
Cysteine	0.8	1.1	1.2	0.4	0.6	0.8
Glutamic acid	7.1	10.7	15.4	3.8	4.3	9.9
Glycine	1.4	1.7	2.0	1.6	1.1	2.3
Ornithine	0.1	0.1	0.1	0.0	0.1	0.1
Proline	4.0	5.2	6.7	1.2	2.5	3.0
Serine	2.2	2.4	3.3	1.2	1.3	2.4
Taurine	0.0	0.0	0.0	0.1	0.0	0.1
Tyrosine	2.4	2.8	3.9	0.9	1.2	2.0
Total AA	45.6	57.2	76.3	24.6	26.9	54.3
Essential AA	21.2	25.3	32.9	11.7	12.0	25.1
Nonessential AA	24.4	31.9	43.4	12.9	14.9	29.2

[1]CPP 1 = corn protein product 1; CPP 2 = corn protein product 2; CGlM = corn gluten meal; CGeM = corn germ meal; DDGS = distillers dried grains with solubles; SBM = soybean meal.

Acid-hydrolyzed fat concentrations ranged from 0.8% (SF) to 6% (CF-C1). Corn fibers, however, had similar fat concentrations, varying at most by one percentage unit. Gross energy values were very similar among CF sources. This outcome was expected because there was no difference in AHF concentration or in ash content among substrates. Beet pulp had the least GE concentration, 3,512 kcal/kg. This value was mainly due to the greater ash content (8.6%) of this substrate.

Amino acid concentrations for fiber ingredients (Table 6) followed the same pattern as CP values for these ingredients. Total AA was greater for CF-C1 (9.46%) and less for NCF (5.05%). Corn fibers will not have a major influence on the protein and AA concentrations of diets, but knowing their AA profile can be a useful piece of information for diet formulation, accounting for a more accurate AA balance in the diet overall.

Of the novel corn coproducts, all have potential use as ingredients in pet food. Based on chemical composition, the quality of CPP 2 was greater than that of CPP 1 in terms of CP and AA concentrations. Corn protein product 1 and CPP 2, however, had greater TDF concentrations. For them to be used widely or to be used in a high-quality diet, the amount of fiber in these ingredients must to be reduced. In reference to NCF, based on its chemical composition, it appears to be a good source of insoluble fiber, with relatively little contamination from other nutrients. However, further

Table 4. Mineral composition of select protein sources (DM basis)

	Protein source[1]					
Item	CPP 1	CPP 2	CGlM	CGeM	DDGS	SBM
Calcium, %	0.1	0.0	0.0	0.0	0.0	0.4
Copper, mg/kg	10.7	12.5	12.2	7.8	5.5	16.9
Magnesium, mg/kg	0.3	0.1	0.1	0.4	0.3	0.3
Manganese, mg/kg	11.8	7.3	4.4	23.3	14.2	38.3
Phosphorus, %	0.6	0.4	0.6	1.0	0.8	0.9
Potassium, %	0.8	0.3	0.2	0.6	1.1	2.6
Sodium, %	0.2	0.2	0.0	0.0	0.1	0.0
Sulfur, %	0.6	0.7	1.1	0.4	1.0	0.5
Zinc, mg/kg	140.3	158.9	28.7	147.7	59.9	27.0

[1]CPP 1 = corn protein product 1; CPP 2 = corn protein product 2; CGlM = corn gluten meal; CGeM = corn germ meal; DDGS = distillers dried grains with solubles; SBM = soybean meal.

Table 5. Chemical composition of select fiber sources

Item[1]	Fiber source[2] NCF	CF-C1	CF-C2	PH	SF	BP
DM, %	90.3	98.9	89.2	90.8	94.9	94.5
	% (DM basis)					
Ash	0.8	0.9	0.9	3.3	0.1	8.6
CP	7.5	11.0	10.4	8.5	0.0	6.3
TDF	82.2	71.4	75.4	87.2	100.0	68.8
NDF	85.0	68.5	78.6	85.0	100.0	47.7
ADF	21.5	15.9	19.9	71.5	94.2	27.4
AHF	5.0	6.0	5.7	3.8	0.8	3.5
GE, kcal/ kg	4,520	4,513	4,666	4,872	3,902	3,512

[1]TDF = total dietary fiber; AHF = acid-hydrolyzed fat.
[2]NCF = novel corn fiber; CF-C1 = corn fiber control 1; CF-C2 = corn fiber control 2; PH = peanut hulls; SF = Solka-Floc (International Fiber Corporation, St. Louis, MO); BP = beet pulp.

research is required to better understand its nutritional behavior once fed to the animal. It is likely to have positive effects similar to those of other insoluble fibers, such as SF, on the reduction of food caloric density as well as on laxation and satiety (Case et al., 2000).

PER and Cecectomized Rooster Assays

In the PER experiment (Table 7), the comparison between CPP 1 and CPP 2 treatments indicated no differences ($P > 0.05$) in feed intake, protein intake, or BW gain. Corn protein product 2 resulted in greater ($P < 0.05$) G:F and PER than CPP 1 and CGlM. When CPP 2 and CPP 1 were compared with CGlM, similar results were obtained for feed intake, protein intake, and BW gain. Overall, SBM resulted in the best performance outcomes, followed by CGeM, which had results similar ($P > 0.05$) to SBM for feed and protein intakes.

The PER experiment indicated that CPP 1, CPP 2, and CGlM were poor-quality proteins, because the PER values were less than 2.0. Distillers dried grains with solubles, CGeM, and SBM had PER values greater than 2.0, indicating they were of greater protein quality than CPP 1, CPP 2, and CGlM. The greatest PER value was for SBM. The PER value for CGeM was not different ($P > 0.05$) from that of DDGS. The decreased PER values for CPP 1, CPP 2, and CGlM were perhaps partially due to the low intake of these ingredients. A similar observation was made by Sasse and Baker (1973). Because palatability is not an issue for poultry owing to the smaller number of gustative papillae, the decreased feed intake values for CPP 1, CPP 2, and CGlM were probably due to their AA profile, where lysine concentrations in relation to total AA or CP concentrations were less than those in DDGS and CGeM.

The PER value for SBM confirms its protein quality, leading to superior performance in chicks. Moreover, lysine concentration of SBM was approximately 3.5%, whereas corn coproducts had an average of approximately 1% lysine. This AA is known to be the first limiting AA in corn and corn coproducts such as CGlM and DDGS in poultry diets (Hallauer, 2000). The reason for the superior PER results for SBM, CGeM, and DDGS compared with other corn coproducts was mainly due to their superior AA profile, mainly the greater digestible lysine content in relation to their CP concentrations. Indeed, the calculated digestible lysine:CP ratios for CPP 1, CPP 2, CGlM, CGeM, DDGS, and SBM were 1.00, 1.54, 1.37, 2.85, 2.54, and 5.63, respectively; these values were consistent with the PER values.

Table 6. Amino acid composition of select corn fiber sources (DM basis)

AA	Corn fiber source,[1] % NCF	CF-C1	CF-C2
Essential			
Arginine	0.30	0.41	0.25
Histidine	0.16	0.33	0.29
Isoleucine	0.18	0.34	0.23
Leucine	0.49	1.15	0.79
Lysine	0.28	0.32	0.23
Methionine	0.10	0.21	0.14
Phenylalanine	0.23	0.47	0.32
Threonine	0.27	0.40	0.31
Tryptophan	0.05	0.06	0.05
Valine	0.27	0.49	0.37
Nonessential			
Alanine	0.33	0.65	0.47
Aspartic acid	0.36	0.54	0.37
Cysteine	0.14	0.23	0.19
Glutamic acid	0.73	1.61	1.17
Glycine	0.28	0.42	0.34
Ornithine	0.00	0.01	0.04
Proline	0.43	1.01	0.85
Serine	0.23	0.39	0.29
Taurine	0.05	0.07	0.06
Tyrosine	0.17	0.35	0.23
Total AA	5.05	9.46	6.99
Essential AA	2.33	4.18	2.98
Nonessential AA	2.72	5.28	4.01

[1]NCF = novel corn fiber; CF-C1 = corn fiber control 1; CF-C2 = corn fiber control 2.

Table 7. Body weight gain, feed intake, G:F, protein intake, and protein efficiency ratio (PER[1]) for select protein sources

Item	Protein source[2]						LSD	SEM[3]
	CPP 1	CPP 2	CGlM	CGeM	DDGS	SBM		
Feed intake, g	107.3[c]	102.3[c]	102.9[c]	201.8[a]	164.9[b]	216.2[a]	18.4	6.2
BW gain, g	8.2[d]	12.2[d]	8.2[d]	57.2[b]	43.3[c]	74.0[a]	7.9	2.6
G:F, g/g	0.076[d]	0.118[c]	0.079[d]	0.283[b]	0.263[b]	0.342[a]	0.35	0.012
Protein intake, g	10.7[c]	10.2[c]	10.3[c]	20.1[a]	16.5[b]	21.6[a]	1.8	0.6
PER	0.76[d]	1.18[c]	0.76[d]	2.83[b]	2.63[b]	3.42[a]	0.35	0.12

[a–d]Means in the same row not sharing common superscript letters differ ($P < 0.05$).

[1]PER = protein efficiency ratio, calculated as grams of gain in BW per gram of protein intake. Data are means of 4 replicates, with each replicate containing 5 chicks.

[2]CPP 1 = corn protein product 1; CPP 2 = corn protein product 2; CGlM = corn gluten meal; CGeM = corn germ meal; DDGS = distillers dried grains with solubles; SBM = soybean meal.

[3]Pooled SEM, n = 4.

Protein efficiency ratio values similar to those in the current study for CGlM and SBM (0.76 and 3.47, respectively) were reported by Augspurger and Baker (2004). Emmert et al. (2000) also reported a PER value of 3.5 for SBM. Greater values, however, were reported by Peter and Baker (2001), who showed a PER value of 1.47 for CGlM and of 3.69 to 3.99 for SBM. In another study, CGlM had a PER value of 1.21 (Peter et al., 2000). Protein efficiency ratios ranged from 1.12 to 1.51 for CGlM, and from 3.75 to 3.92 for SBM in diets containing 10% CP from each ingredient, with varying concentrations of Ca and P (Boling-Frankenbach et al., 2001).

In reference to standardized AA digestibility (Table 8), CGlM had the greatest numerical mean values for total AA, EAA, and non-EAA, but they were not different ($P > 0.05$) from those obtained for CPP 2 and SBM. Corn germ meal resulted in the least digestibility, which was not significantly different from those for DDGS and CPP 1. Corn protein product 1 and CPP 2 were not different from each other in total non-EAA digestibility.

The greatest mean values for TME_n were obtained with CGlM, followed by CPP 2, DDGS, CPP 1, SBM, and CGeM. Distillers dried grains with solubles and CPP 1 had similar TME_n values. These values were

Table 8. Standardized AA digestibility coefficients (%) and true ME (TME_n; kcal/kg) of select protein sources as determined in cecectomized roosters in the precision-fed rooster assay[1]

AA	Protein source[2]						SEM
	CPP 1	CPP 2	CGlM	CGeM	DDGS	SBM	
Essential							
Arginine	86.0[b]	93.1[a]	94.8[a]	90.9[ab]	87.0[b]	90.4[ab]	1.78
Histidine	81.6[b]	88.3[a]	92.7[a]	81.7[b]	80.9[b]	88.7[a]	1.69
Isoleucine	85.5[bc]	91.9[ab]	96.8[a]	79.9[c]	83.1[c]	90.4[ab]	2.20
Leucine	92.1[bc]	95.1[ab]	98.6[a]	82.8[d]	88.5[c]	90.4[bc]	1.87
Lysine	55.5[d]	79.9[bc]	84.1[ab]	73.8[c]	77.9[bc]	89.5[a]	2.88
Methionine	85.4[cd]	92.9[ab]	97.6[a]	83.1[d]	86.3[cd]	88.3[bc]	1.63
Phenylalanine	89.3[bc]	93.8[ab]	97.9[a]	83.6[d]	84.8[cd]	91.4[b]	1.89
Threonine	79.3[bcd]	86.9[abc]	95.7[a]	72.4[d]	77.9[cd]	88.1[ab]	3.39
Tryptophan	75.2[bc]	81.6[b]	89.4[a]	73.7[c]	66.6[d]	93.5[a]	2.32
Valine	84.5[cd]	91.3[ab]	95.3[a]	82.5[d]	82.3[d]	89.2[bc]	1.94
Nonessential							
Alanine	88.1[b]	93.8[a]	97.5[a]	82.7[c]	87.0[bc]	87.4[bc]	1.74
Aspartic acid	77.6[cd]	85.1[bc]	93.8[a]	75.9[d]	75.7[d]	88.7[ab]	2.69
Cysteine	72.4[bc]	86.5[ab]	96.3[a]	59.6[c]	69.5[bc]	87.7[ab]	6.21
Glutamic acid	87.3[cd]	93.8[ab]	98.2[a]	85.5[d]	85.1[d]	92.3[bc]	1.71
Proline	85.7[b]	92.6[ab]	98.2[a]	75.5[c]	86.8[b]	89.6[ab]	3.20
Serine	84.3[bc]	90.9[ab]	96.7[a]	78.2[c]	80.7[c]	91.0[ab]	2.78
Tyrosine	91.0[bc]	95.1[ab]	98.0[a]	80.3[d]	84.5[cd]	92.1[ab]	2.32
Total AA	82.4[b]	90.2[a]	95.4[a]	79.0[b]	81.5[b]	89.9[a]	2.40
Essential AA	81.5[b]	89.5[a]	94.3[a]	80.4[b]	81.5[b]	90.0[a]	2.05
Nonessential AA	83.8[bcd]	91.1[ab]	97.0[a]	76.8[d]	81.3[cd]	89.8[abc]	2.92
TME_n	2,624[bc]	2,879[b]	3,863[a]	1,924[d]	2,678[bc]	2,388[c]	104.03

[a–d]Means in the same row not showing common superscript letters differ ($P < 0.05$).

[1]Data are means of 4 roosters.

[2]CPP 1 = corn protein product 1; CPP 2 = corn protein product 2; CGlM = corn gluten meal; CGeM = corn germ meal; DDGS = distillers dried grains with solubles; SBM = soybean meal.

Table 9. Crude protein disappearance (%) after 6 and 24 h of in vitro hydrolysis of select protein sources

	Protein source[1]						
Time	CPP 1	CPP 2	CGlM	CGeM	DDGS	SBM	SEM
6 h	29.3[c]	24.9[e]	49.3[b]	25.3[d]	49.0[b]	53.3[a]	0.37
24 h	77.5[c]	74.1[d]	94.1[a]	67.7[e]	76.8[c]	87.2[b]	0.37

[a–e]Means in the same row not sharing common superscript letters differ ($P < 0.05$).

[1]CPP 1 = corn protein product 1; CPP 2 = corn protein product 2; CGlM = corn gluten meal; CGeM = corn germ meal; DDGS = distillers dried grains with solubles; SBM = soybean meal.

not different ($P > 0.05$) from those for CPP 2 and SBM.

In previous studies, TME_n values for 5 different DDGS samples ranged from 2,484 to 3,047 kcal/kg. These values were negatively correlated with color, with the darker colored DDGS having decreased TME_n values (Fastinger et al., 2006). Lumpkins et al. (2004) reported a TME_n value of 2,800 kcal/kg for a DDGS with CP and lysine concentrations (27 and 0.94%, respectively) similar to the one used in the present study. A TME_n value of 3,164 kcal/kg was reported by Parsons (1985). Standardized EAA and non-EAA digestibilities were similar to values reported by Fastinger et al. (2006). Only tryptophan (66.6%) and cysteine (69.5%) were less than the standardized values cited by the previous authors: 88.0 and 80.2%, on average, for tryptophan and cysteine, respectively. In the present study, the TME_n of SBM (2,388 kcal/kg) was less than the TME_n value (2,688 kcal/kg) reported by Bruce et al. (2006). Reasons for the decreased value are not clear, because the chemical composition of the SBM used by these authors was close to that in the current study (92.7% DM, 50.3% CP, 20.8% TDF, 3.8% AHF, and 4,700 kcal/kg). Standardized AA digestibilities for SBM measured in the present study are in agreement with values published by Bruce et al. (2006) and Garcia et al. (2007).

The cecectomized rooster assay provides a good estimation of AA digestibility of protein ingredients. This assay, when compared with dog and cat digestibility trials, is less expensive and faster. In addition, it eliminates the confounding factor of protein degradation by bacterial populations in the colon and ceca and therefore can provide information on AA digestibility of a substrate or a food matrix. The data generated in this assay probably can be extrapolated to other nonruminant animals, such as dogs and cats.

From the results obtained by using the PER and cecectomized rooster assays, CPP1 and CPP 2 were of a quality similar or superior to CGlM, the major market competitor of these novel corn protein ingredients. In addition, the poor PER values for CPP 1, CPP 2, and CGlM seemed to be associated more with their poor protein quality (lysine deficiency) and their inability to support growth. Corn protein product 2 and CGlM had standardized total AA, EAA, and non-EAA digestibilities similar to those of SBM in the cecectomized rooster assay.

In Vitro CP Disappearance

In vitro CP disappearance (Table 9) after 6 h of incubation in an HCl:pepsin solution was greater ($P < 0.05$) for SBM (53.3%), followed by CGlM (49.3%), DDGS (49.0%), CPP 1 (29.3%), CGeM (25.3%), and CPP 2 (24.9%). Corn gluten meal was not different ($P > 0.05$) from DDGS, whereas all other substrates were different ($P < 0.05$) from each other. After an additional 18 h of incubation (24 h total) with porcine pancreatin, CGlM had the greatest CP disappearance (94.1%), followed by SBM (87.2%). Distillers dried grains with solubles and CPP 1 had intermediate values (76.8 and 77.5%, respectively) and were not different ($P > 0.05$) from each other. Corn protein product 2 and CGeM had the least CP disappearance values: 74.1 and 67.7%.

The first sample time (6 h) simulated the hydrolytic digestion of proteins that would occur in the stomach by the action of pepsin and HCl in an animal. At the second sample time, CP disappearance represented the action of porcine pancreatin, which mimics the action of pancreatic enzymes. In the animal, these enzymes are released in the proximal regions of the small intestine to further break down proteins to small peptides (di- or tripeptides) or free AA that can be absorbed by the brush-border membrane of the enterocytes.

In digestibility studies, apparent ileal CP digestibility of CGlM by adult dogs was reported to be 73 to 83% (Yamka et al., 2004). In the present study, CP disappearance of CGlM was greater (94.1%). This difference may possibly be related to the use of different methodologies (in vivo vs. in vitro), the influence of other ingredients in the food matrix of the diet, dietary processing conditions, or even nutritional differences related directly to the CGlM used in these studies, because corn coproducts from the ethanol industry are quite variable. The apparent ileal CP digestibility by adult dogs of conventional and low-oligosaccharide SBM ranged from 72.1 to 81.4%, respectively (Zuo et al., 1996). Crude protein disappearance of SBM was slightly greater in our study (87.2%).

From the in vitro data, CPP 1 and CPP 2 resulted in CP disappearance values less than acceptable in pet nutrition. Usually, CP digestibility greater than 80% is desirable (Case et al., 2000). However, no data are available regarding the behavior of these ingredients once incorporated into diets and exposed to extrusion. In vitro data provide basic information about the sub-

Table 10. Organic matter disappearance (%) after in vitro hydrolytic and fermentative digestion of select fiber sources

Item	Fiber source[1]						SEM
	NCF	CF-C1	CF-C2	PH	SF	BP	
Hydrolytic digestion, 24 h	6.2[c]	22.0[a]	12.5[b]	3.3[c]	−6.5[d]	20.5[a]	0.55
Fermentative digestion							
8 h	0.6[b]	0.5[b]	0.0[b]	−0.7[b]	−2.1[b]	6.3[a]	0.73
16 h	3.0[bcd]	5.7[bc]	5.3[c]	1.1[d]	−1.6[d]	17.7[a]	0.73

[a–d]Means in the same row not sharing common superscript letters differ ($P < 0.05$).

[1]NCF = novel corn fiber; CF-C1 = corn fiber control 1; CF-C2 = corn fiber control 2; PH = peanut hulls; SF = Solka-Floc (International Fiber Corporation, St. Louis, MO); BP = beet pulp.

strate alone, not about the substrate once mixed in a diet.

In Vitro OM Disappearance

In vitro OM disappearance of fiber sources (Table 10) was least for SF in the hydrolytic (−6.5%) and fermentative stages (−2.1 and −1.6%, respectively, at 8 and 16 h) and greatest for CF-C1 and BP in the hydrolytic stage. Beet pulp was the only fiber source with significant fermentation (17.7%) after 16 h. Corn fiber-C1 and CF-C2 had intermediate fermentation values (5.7 and 5.3%, respectively), but CF-C1 and CF-C2 did not differ ($P > 0.05$) when compared with the NCF (3.0%). Similar to SF, PH were not fermented over time, having an OM disappearance of 1.1% at 16 h of fermentative digestion. Substrate × time interactions were significant ($P < 0.05$) for OM disappearance.

The greater ($P < 0.05$) OM disappearance during simulated hydrolytic digestion of CF-C1 and BP is perhaps related to the presence of polysaccharides other than structural ones (e.g., starch). The greater BP fermentation value also is associated with a greater proportion of soluble fibers compared with insoluble fibers. Organic matter disappearance improved as the insoluble:soluble fiber ratio decreased (Swanson et al., 2001). The reduced fermentability of the remaining substrates reflects their increased concentration of insoluble fiber, an effect mainly observed in SF because of the crystalline structure of cellulose, which prevents bacterial action. Data available on OM disappearance of SF are fairly consistent. No substantial fermentation or SCFA production has been observed for this substrate (Sunvold et al., 1995a,b,c; Campbell and Fahey, 1997; Swanson et al., 2001). The consistency in OM disappearance values for SF can be justified by the relatively constant chemical composition of this substrate, as well as by its increased concentration of cellulose. Physiologically, the negative values for OM disappearance for SF are not different from zero. Negative numbers are due to random error, as well as to the addition of enzymes that bind to the substrate in an attempt to degrade it. Because enzymes (pepsin or pancreatic) and some bacteria are not able to break down cellulose, there is no disappearance. Consequently, the weight of the residue will be slightly greater than the initial weight of the sample, resulting in false negative values.

Previous research on OM disappearance of BP is in accordance with results of the present study. An OM disappearance of 17.7% after 12 h of fermentation when using dog feces as inoculum was reported by Sunvold et al. (1995a). The same authors also reported OM disappearance values of 18.7, 13.9, and 33% after 6, 12, and 24 h of fermentation, respectively, for BP. Data suggest that an increased length of fermentation results in greater OM disappearance of this substrate (Sunvold et al., 1995c). Even though our data are in agreement with previous studies, it is possible to obtain different OM disappearance values for BP because this ingredient has a variable composition that may result in different rates and extents of fermentation.

To our knowledge, no information has been published on OM disappearance of CF sources. Sunvold et al. (1995a) reported a much greater OM disappearance for PH (average of 14% after a 12-h fermentation period) compared with ours (1.1% after a 16-h fermentation period). The reason for this difference is unclear, but is perhaps associated with variation in the chemical composition between the 2 sources of PH.

Results of fiber fermentation evaluated in vitro reasonably predict in vivo fiber utilization responses by dogs (Sunvold et al., 1995b). Moreover, the in vitro technique is a more accurate tool to quantify both substrate degradation and SCFA production because, in vivo, these fatty acids are rapidly absorbed or utilized by the colonocytes.

The in vitro data for the fiber sources indicated that they behaved as insoluble fibers. The NCF, compared with the control CF sources, had the least OM disappearance during simulated hydrolytic and fermentative digestion, suggesting that this ingredient might be a good substitute for SF, a common ingredient used in pet food for laxation and BW control.

In conclusion, the data gathered herein provide basic information about ingredients of potential importance to the pet food industry. The lack of nutritional information on novel ingredients oftentimes is the limiting step in evaluating the potential efficacy for including novel ingredients in a diet matrix. In pet nutrition, more so than in production animal nutrition, compa-

nies are able to find a niche where novel products can be used once benefits associated with the use of these products have been defined. Hence, for companies to increase their product portfolio, and consequently their revenue, they need to make and support claims about their products based on reliable research information.

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