Analysis for low-molecular-weight carbohydrates is needed to account for all energy-contributing nutrients in some feed ingredients, but physical characteristics do not predict in vitro digestibility of dry matter

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ABSTRACT: An experiment was conducted to quantify nutrient and fiber fractions of feed ingredients and to determine in vitro apparent ileal digestibility (IVAID) and in vitro apparent total tract digestibility (IVATTD) of DM and OM in each ingredient. Ten ingredients that vary in fiber concentration and composition were used: corn, wheat, soybean meal (SBM), canola meal, distillers dried grains with solubles (DDGS), corn germ meal, copra expellers, sugar beet pulp (SBP), synthetic cellulose (SF), and pectin. Correlations between chemical and physical characteristics of ingredients and IVAID and IVATTD of DM and OM were determined. The physical characteristics measured included bulk density, water-binding capacity (WBC), swelling, and viscosity. The analyzed GE was compared with values for GE calculated from all energy-contributing components. Results indicated that the analyzed chemical composition of most ingredients added to 100% or greater, except for DDGS, SBP, and SF, where nutrients added to only 94.29%, 88.90%, and 96.09%, respectively. The difference between the sum of the calculated GE of the analyzed components and the analyzed GE of the ingredients ranged from -2.25 MJ/kg in DDGS to 1.74 MJ/kg in pectin. No correlation was observed between swelling, WBC, or viscosity

and IVAID or IVATTD of DM or OM. The concentration of insoluble dietary fiber (IDF) and total dietary fiber (TDF) was negatively correlated (P < 0.05) with IVAID and IVATTD of DM and OM. There was a tendency for NDF (r = -0.60) and ADF (r = -0.61) to be negatively correlated (P < 0.10) with IVAID of DM. However, no correlation was observed between the concentration of CP, GE, acid-hydrolyzed ether extract, lignin, or soluble dietary fiber and IVAID and IVATTD of DM and OM. The stronger correlations between IDF, TDF, and insoluble non-starch polysaccharides and IVAID and IVATTD of DM and OM than between ADF and NDF and IVAID and IVATTD of DM and OM indicate that the concentration of TDF in feed ingredients is a better predictor of the digestibility of DM and OM than values for NDF and ADF. In conclusion, the calculated GE of some feed ingredients was in agreement with the analyzed GE, which gives confidence that energy-contributing components were accounted for, but for DDGS and SBP, it was not possible to account for all analyzed GE. Concentrations of IDF and TDF, but not the physical characteristics of feed ingredients, may be used to estimate IVAID and IVATTD of DM and OM in feed ingredients.

Key words: energy, in vitro digestibility, physicochemical characteristics, total dietary fiber

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INTRODUCTION

Diets fed to pigs have changed from being based primarily on cereal grains and soybean meal

(SBM) to containing more by-products and alternative ingredients (Zijlstra and Beltranena, 2013). By-products from the grain processing industry such as corn distillers dried grains with solubles (DDGS) and corn germ meal have relatively high concentrations of dietary fiber and may be fed to pigs without affecting growth performance (Weber et al., 2010; Xu et al., 2010; Cromwell et al., 2011) although that is not always the case (Whitney et al., 2006; Linneen et al., 2008). However, the implications of including more fiber in diets fed to pigs are not completely understood. Physical characteristics of dietary fiber such as bulk density, swelling, water-binding capacity (WBC), and viscosity may negatively influence the digestion and availability of nutrients in feed ingredients (Urriola et al., 2013), but limited information about the correlation between physical characteristics of feed ingredients and digestibility of nutrients is available.

Analyzing all chemical components in feed ingredients is challenging and values presented in feed composition tables usually do not add to 100% (Sauvant et al., 2004; Villamide et al., 2010; NRC, 2012), which indicates that not all nutrients or energy-contributing components are accounted for. It is, however, likely that if all energy-containing components in feed ingredients are accounted for, it may be possible to predict the energy in the ingredients with greater accuracy. Therefore, the objectives of this study were to test the hypothesis that calculated GE from all energy-containing components in feed ingredients will equal analyzed GE in the ingredient if all chemical fractions are accounted for. The second hypothesis was that correlations exist between the physicochemical characteristics of feed ingredients and in vitro apparent ileal digestibility (IVAID) and in vitro apparent total tract digestibility (IVATTD) of DM and OM.

MATERIALS AND METHODS

Feed Ingredients

Ten feed ingredients that vary in fiber concentration and composition were obtained. Corn (Premier Cooperative, Philo, IL) and wheat (Siemers, Teutopolis, IL) were the 2 cereal grains used, and conventional dehulled SBM (Solae LLC, Gibson City, IL) and conventional canola meal (Dow AgroSciences, Indianapolis, IN) were obtained to represent oilseed meals that are used as protein sources in swine diets. Corn DDGS (One Earth Energy LLC, Gibson City, IL), corn germ meal (Archer Daniels Midland, Decatur, IL), copra expellers (CoolStance, Stance Equine, Kenmore, Australia), and sugar beet pulp (**SBP**; Midwest Agri-Commodities Company, San Rafael, CA) are coproducts from commodity industries and represent high-fiber ingredients with varying degrees of soluble fiber that are used in the feed industry. Synthetic cellulose (**SF**; Solka-Floc 100 FCC, International Fiber Corporation, North Tonawanda, NY) and pectin (Pacific Pectin Inc., Oakhurst, CA) are purified synthetic sources of insoluble and soluble fiber, respectively, that were also included in the experiment, although these ingredients are usually not included in commercial diets fed to pigs.

Chemical Analyses

All chemical analyses were performed in duplicates. Feed ingredients were analyzed for DM by oven drying at 135 °C for 2 h (Method 930.15; AOAC Int., 2007) and for ash (Method 942.05; AOAC Int., 2007). The concentration of N in all samples was determined using the combustion procedure (Method 990.03; AOAC Int., 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ). Aspartic acid was used as a calibration standard and CP was calculated as N \times 6.25. Amino acids (AA) were analyzed in all samples on a Hitachi Amino Acid Analyzer (Model L8800, Hitachi High Technologies America Inc., Pleasanton, CA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Before analysis, samples were hydrolyzed with 6N HCl for 24 h at 110 °C [Method 982.30 E(a); AOAC Int., 2007]. Methionine and Cys were analyzed as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis [Method 982.30 E(b); AOAC Int., 2007]. Tryptophan was determined after NaOH hydrolysis for 22 h at 110 °C [Method 982.30 E(c); AOAC Int., 2007]. Samples were analyzed for GE on an isoperibol bomb calorimeter (Model 6300, Parr Instruments, Moline, IL) using benzoic acid as the internal standard. Ingredients were also analyzed for total starch (Thivend et al., 1972) and resistant starch (Muir and O'Dea, 1992, 1993). Glucose, fructose, maltose, sucrose, stachyose, and raffinose were analyzed by HPLC using a pulsed amperometric detractor (Dionex Tech Notes 21 and 92, Sunnyvale, CA). Ingredients were also analyzed for ADF and NDF using Ankom Technology methods 12 and 13, respectively, using the Ankom²⁰⁰⁰ Fiber Analyzer (Ankom Technology, Macedon, NY). After ADF analysis, lignin was determined using Ankom Technology method 9 (Ankom Daisy^{II} Incubator, Ankom Technology, Macedon, NY). Total dietary fiber (TDF) was determined by analyzing for insoluble and soluble dietary fiber (IDF and SDF, respectively; Method 991.43; AOAC Int., 2007) using the Ankom^{TDF} Dietary Fiber Analyzer (Ankom Technology, Macedon, NY). Calcium and total P were measured using the inductively coupled plasma (ICP) spectroscopy method (Method 985.01 A, B, and C; AOAC, 2007) after wet ash sample preparation [Method 975.03 B(b); AOAC Int., 2007]. Copper, K, Mg, Mn, and Zn were measured by flame atomic absorption spectroscopy after wet ash sample preparation [Method 975.03 B(b); AOAC Int., 2007]. Sulfur was measured by a gravimetric method (Method 956.01; AOAC Int., 2007) and I was measured by a volumetric method (Method 935.14; AOAC Int., 2007). Selenium was also determined [Method 996.16(G); AOAC Int., 2007] and Cl was measured by manual titration (Method 943.01; AOAC Int., 2007). The chromium concentration in ingredients was determined using an ICP Atomic Emission Spectrometric method (Method 990.08; AOAC Int., 2007). Samples were prepared using nitric acid-perchloric acid [Method 968.08D(b); AOAC Int., 2007]. Acidhydrolyzed ether extract (AEE) was analyzed by acid hydrolysis using 3N HCl (Ankom^{HCl}, Ankom Technology, Macedon, NY) followed by crude fat extraction using petroleum ether (Ankom^{XT15}, Ankom Technology, Macedon, NY). Canola meal was analyzed for glucosinolates (Method Ak 1-92; AOCS, 1998) and all ingredients were also analyzed for phytic acid (Ellis et al., 1977). Sinapine in canola meal and SBM were extracted using dimethylformamide and extracts were analyzed for sinapine thiocyanate by reverse-phase ultra-performance liquid chromatography with UV detection (SOP-208, EPL Bio Analytics Services, Niantic, IL). Soluble condensed tannins were extracted from canola meal and SBM using sodium meta-bisulfite in 70:30 (vol/ vol) acetone: deionized water, leaving insoluble condensed tannins in the residue, and both soluble and insoluble condensed tannins were hydrolyzed using 95:5 (vol/vol) butanol:concentrated HCl with added iron salt before analysis by UV-visible spectrophotometer (SOP-206, EPL Bio Analytics Services, Niantic, IL). Corn, wheat, DDGS, corn germ meal, and SBP were analyzed for fructo-oligosaccharides and inulin by refractive index high-performance liquid chromatography (HPLC) using a Phenomenex Rezex RHM column (Campbell et al., 1997). Briefly, 1.0 g of sample for each analysis was extracted at 85 °C for 15 min and then was cooled and analyzed on the same day. The mobile phase for pure water had a flow rate of 0.6 mL/min. Distillers dried grains with solubles and corn germ meal were also analyzed for glycerol using HPLC (GA-SOP-419, Gorge Analytical, Hood River, OR).

Physical Characteristics

All analyses for physical characteristics were performed in triplicates with the exception of viscosity, which was analyzed in quadruplicates. The measured physical characteristics of the ingredients included bulk density, swelling, WBC, and viscosity. Bulk density was determined by pouring samples into a 250 mL beaker and leveling off the top before weighing the sample as described by Cromwell et al. (2000). Swelling was measured using a procedure modified after Serena and Bach Knudsen (2007). Briefly, 0.3 g of sample was weighed into a 15 mL conical centrifuge tube and dissolved in 10 mL of 0.9% NaCl with 0.02% NaN₃ and placed in a shaking water bath at 39 °C for 20 h. Samples were allowed to settle for 1 h before the swelling capacity was measured by reading the volume the fiber occupied. Water-binding capacity was measured using a procedure modified after Robertson et al. (2000). Briefly, 2 g of sample was hydrated in 50 mL of distilled water for 18 h in preweighed centrifuge tubes. Samples were then centrifuged $(2,000 \times g;$ 20 min) and the supernatant was decanted by carefully inverting the tube to allow water to drain and weights of the pellets were recorded.

Viscosity was measured using a procedure modified after Serena and Bach Knudsen (2007) and was expressed in centipoise (**cP**). Briefly, 2 g of sample was dissolved in 10 mL of 0.9% NaCl and 0.02% NaN₃ solution and extracted in a water bath at 40 °C for 1 h. The sample was then centrifuged at 3,500 × g for 25 min at 23 °C and 0.5 mL of the supernatant was removed by suction. Viscosity of the supernatant was measured using a Brookfield LV-DV-2T viscometer (Brookfield Eng. Lab. Inc., Middleboro, MA) with a Wells-Brookfield Cone/Plate extension and a CPA-40Z cone spindle. Values were reported as the average shear rate of 225, 240, 255, 270, 285, and 300/s. Viscosity of solutions was measured at room temperature (23 °C).

In Vitro Ileal and Total Tract Digestibility

The IVATTD was determined using a 3-step procedure modified from Boisen and Fernández (1997). The procedure simulates gastric and small intestinal digestion and large intestinal fermentation. Three separate subsamples of each ingredient were used providing 3 replicates per ingredient. Samples were incubated in 125 mL Erlenmeyer flasks placed in a water bath at 39 °C with constant shaking for 2 h. Pepsin from porcine gastric mucosa (Sigma-Aldrich, St. Louis, MO) was added to the flasks and the pH was maintained at 2 by adding HCl. After 2 h, the pH was adjusted to 6.8 using NaOH, and pancreatin from porcine pancreas (Sigma-Aldrich, St. Louis, MO) was added to each flask. This step represented the digestion processes in the stomach and the small intestine, respectively. Viscozyme enzyme (Sigma-Aldrich, St. Louis, MO) was added in the third step to degrade soluble fiber and samples were incubated at 39 °C for 18 h (Jaworski et al., 2015). After the third incubation, contents of the flasks were emptied and filtered into preweighed glass crucibles and DM was determined in the residue to calculate IVATTD of DM. Ash analysis was performed on the remaining residue to calculate IVATTD of OM. For IVAID, the same procedure was used, but the process was discontinued after the second step and DM was determined in the residue to calculate IVAID of DM and the remaining residue was analyzed for ash to calculate IVAID of OM.

Calculations and Statistical Analysis

Concentrations of TDF (IDF + SDF), cellulose (ADF – lignin), insoluble hemicelluloses (NDF – ADF), non-starch polysaccharides (NSP; TDF – lignin), insoluble NSP (NSP – SDF), and non-cellulosic NSP (NSP – cellulose) were calculated for all ingredients. Concentration of levans (fructo-oligosaccharides – inulin) was also calculated for corn, wheat, DDGS, corn germ meal, and SBP. The calculated GE was the sum of all energy-contributing components calculated according to Eq. [1], which was modified from Atwater and Bryant (1900):

Calculated GE, = $(AEE \times 39.36 \text{ MJ / kg})$ MJ / kg + (total AA × 23.45 MJ / kg) +[(total starch + fructo -oligosaccharides + NSP)×17.58 MJ / kg] +[(glucose + fructose + sucrose + stachyose + raffinose + tannins + sinapine) ×15.49 MJ / kg] +(lignin × 29.13 MJ / kg) where lignin is the concentration of ADL. The GE contribution from lignin was calculated by multiplying the concentration of lignin in the feed ingredient by the average GE of 4 commercially available lignin preparations (Jung et al., 1999).

Data for physical characteristics and the in vitro analyses were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with each feed ingredient as the fixed effect and replication as the random effect. Correlation coefficients among the physicochemical characteristics of the 10 feed ingredients and the IVAID and IVATTD of DM and OM were determined using the CORR procedure of SAS treating each ingredient as one observation. Each replicate corresponding to a feed ingredient for analysis was considered the experimental unit. Statistical significance and tendency were considered at P < 0.05 and $0.05 \le P < 0.10$, respectively.

RESULTS

Dry matter concentrations ranged from 85.42% in corn to 96.54% in copra expellers and the concentration of CP ranged from 6.56% in corn to 46.90% in SBM (Table 1). Concentrations of NDF and ADF ranged from 6.30% and 5.00% in SBM to 48.14% and 23.79% in copra expellers. Concentrations of glycerol in DDGS and corn germ meal were negligible.

The concentration of IDF ranged from 10.71% in corn to 44.57% in SBP and the concentration of SDF ranged from 0.06% in corn to 3.97% in SBP. Sugar beet pulp had numerically greater concentrations of IDF and SDF than all other coproducts and corn germ meal had numerically greater concentrations of IDF and SDF than DDGS.

Copra expellers and DDGS had numerically greater concentrations of AEE (11.17% and 9.58%, respectively) than the other ingredients, which corresponded with greater GE in these ingredients. The concentration of ash ranged from 1.05% in corn to 7.14% in canola meal.

The analyzed nutrient composition of most ingredients added to 100% or greater, except for DDGS, SBP, and SF, where nutrients added to only 94.29%, 88.90%, and 96.09%, respectively. However, the difference between the calculated GE of the analyzed components and the GE of the ingredients ranged from -2.25 MJ/kg in DDGS to 1.74 MJ/kg in pectin. The percentage of analyzed GE that was accounted for in the calculated GE ranged from 87.58% in SBP to 112.31% in pectin.

					Ingred	ient ^a				
Item	Corn	Wheat	SBM	СМ	DDGS	CGM	CE	SBP	SF	Pectin
Analyzed GE, MJ/kg	15.58	15.90	17.20	17.77	19.00	17.50	19.73	15.66	16.57	14.17
DM, %	85.42	86.81	88.80	88.90	88.77	89.28	96.54	92.48	98.35	91.50
СР, %	6.56	10.80	46.90	40.52	25.52	23.91	21.65	7.27	0.71	1.68
AEE, %	3.06	1.86	1.55	4.06	9.58	2.97	11.17	2.00	0.38	0.14
NDF, %	8.51	11.36	6.30	23.63	32.29	39.60	48.14	45.47	30.49	0.78
ADF, %	2.40	3.06	5.00	17.33	12.97	14.70	23.79	21.54	16.43	0.15
Lignin, %	0.47	0.69	0.16	7.39	2.29	4.29	5.14	2.46	ND	ND
Ash, %	1.05	1.61	6.78	7.14	5.91	2.61	5.63	6.96	0.04	1.62
OM, %	84.37	85.20	82.02	81.76	82.86	86.67	90.91	85.52	98.31	89.88
Tannins ^b , %										
SCT	_	_	0.02	0.05	_	_	_	_	_	_
ICT	_	_	0.04	0.32	_	_	_	_	_	_
Sinapine, %	_	_	ND	1.16	_	_	_	_	_	_
Glucosinolates, µmol/g	_	_	_	7.92	_	_	_	_	_	_
Glycerol, %	_	_	_	_	< 0.04	ND	_	_	_	_
Carbohydrates, %										
Total starch	64.71	60.01	5.80	1.87	5.11	19.20	4.02	3.88	ND	_
Resistant starch	9.72	12.83	4.41	1.79	1.30	2.91	3.54	3.55	ND	_
Glucose	0.19	0.16	ND	ND	0.26	0.06	0.12	0.20	ND	41.79
Fructose	0.15	0.09	ND	ND	0.11	0.41	0.58	0.16	ND	ND
Maltose	ND	ND	0.16	ND	0.37	ND	ND	ND	ND	ND
Sucrose	1.62	0.76	8.18	6.86	ND	0.07	9.36	10.55	ND	ND
Stachyose	ND	ND	6.01	2.34	ND	ND	ND	ND	ND	ND
Raffinose	0.28	0.51	1.42	0.66	ND	0.16	ND	0.29	ND	ND
$FOS^{c}, \%$	2.09	2.53	_	_	1.54	4.33	_	1.53	_	_
Inulin	1.08	1.31	_	_	0.80	2.25	_	0.80	_	_
Levan	1.01	1.22	_	_	0.74	2.08	_	0.73	_	_
TDF, %	10.76	11.40	17.84	26.42	34.66	39.78	43.84	48.54	93.31	51.69
IDF, %	10.71	10.93	16.70	25.44	34.38	38.47	42.05	44.57	93.16	0.09
SDF, %	0.06	0.47	1.14	0.98	0.29	1.31	1.79	3.97	0.15	51.60
Cellulosed	1.93	2.37	4.84	9.94	10.68	10.41	18.65	19.08	16.43	0.15
Insoluble hemicelluloses ^e	6.11	8.30	1.30	6.30	19.32	24.90	24.35	23.93	14.06	0.63
NSP ^f	10.29	10.71	17.68	19.03	32.37	35.49	38.70	46.08	93.31	51.69
Insoluble NSP ^g	10.24	10.24	16.54	18.05	32.09	34.18	36.91	42.11	93.16	0.09
Non-cellulosic NSP ^h	8.36	8.34	12.84	9.09	21.69	25.08	20.05	27.00	76.88	51.54
Calculated values										
Sum ⁱ , %	105.05	102.92	105.84	100.97	94.29	104.22	99.83	88.90	96.09	105.42
Calculated GE ^{<i>j</i>} , MJ/kg	16.82	16.27	17.86	17.21	16.75	17.78	19.17	13.71	16.56	15.91
Difference ^{<i>k</i>} , MJ/kg	1.24	0.37	0.65	-0.56	-2.25	0.28	-0.56	-1.94	-0.01	1.74
Difference ¹ , %	107.97	102.36	103.80	96.85	88.17	101.63	97.16	87.58	99.97	112.31

Table 1. Analyzed nutrient composition of corn, wheat, soybean meal, canola meal, distillers dried grains with solubles, corn germ meal, copra expellers, sugar beet pulp, synthetic cellulose, and pectin, as-fed basis

^{*a*}CM = canola meal; CGM = corn germ meal; CE = copra expellers; ND = not detected.

^bSCT = soluble condensed tannins; ICT = insoluble condensed tannins.

^cFOS = fructo-oligosaccharides; levans = FOS – inulin.

 d Cellulose = ADF – lignin.

^{*e*}Insoluble hemicelluloses = NDF – ADF.

/NSP = non-starch polysaccharides, TDF – lignin.

 g Insoluble NSP = NSP - SDF.

^{*h*}Non-cellulosic NSP = NSP – cellulose.

Summation of moisture, ash, total AA, AEE, TDF, total starch, glucose, fructose, sucrose, stachyose, raffinose, and FOS.

 j Calculated as (AEE × 39.36 MJ/kg) + (total AA × 23.45 MJ/kg) + [(total starch + FOS + NSP) × 17.58 MJ/kg] + [(glucose + fructose + sucrose + stachyose + raffinose) × 15.49 MJ/kg] + (lignin × 29.13 MJ/kg).

^kThe difference between the calculated gross energy of the components and the analyzed gross energy of the ingredient.

'The percentage of analyzed gross energy that is accounted for in the calculated gross energy.

The concentration of Lys ranged from 0.27%in corn to 2.99% in SBM (Table 2). Concentrations of individual minerals varied greatly among ingredients, but as expected, the concentration of P and K were the greatest for all ingredients with the exception of SF and pectin (Table 3). Bulk density was less (P < 0.05) in DDGS than in corn, wheat, SBM, canola meal, corn germ meal, SF, and pectin, but not different from copra expellers and SBP (Table 4). Swelling capacity ranged from 2.48 L/kg DM in corn to 9.01 L/kg DM in pectin and WBC ranged from 1.00 g/g in wheat to 4.09 g/g in SBP. Viscosity was greater (P < 0.05) in SBP (1.45 cP) than in corn (1.12 cP), SBM (1.10 cP), canola meal (1.00 cP), DDGS (1.07 cP), corn germ meal (1.17 cP), and SF (0.93 cP), but not different from that in wheat (1.30 cP) and copra expellers (1.27 cP).

The IVAID of DM was greater (P < 0.05) in copra expellers than in corn, corn germ meal, SBP, and SF, but not different from DDGS (Table 5). The IVAID of DM was greater (P < 0.05) in corn than in SBP and SF, but not different from corn germ

meal. The IVAID of OM was greater (P < 0.05) in corn germ meal than in SBP and SF, but not different from corn. The IVATTD of DM and OM was different (P < 0.05) among all ingredients.

The concentration of NDF was positively correlated (P < 0.05) with concentrations of ADF, IDF, cellulose (r = 0.93), and insoluble hemicelluloses (r = 0.95), but was negatively correlated (P < 0.01) with bulk density (Table 6). The concentration of ADF was positively correlated (P < 0.05) with IDF, cellulose (r = 0.96), and insoluble hemicelluloses (r = 0.79) but was negatively correlated (P < 0.05) with bulk density. The concentration of IDF was positively correlated (P < 0.01) with TDF, cellulose (r = 0.80), NSP (r = 0.78), and insoluble NSP (r = 0.99), whereas SDF was positively correlated (P < 0.05) with swelling and viscosity. There was a tendency (P < 0.10) for GE to be positively correlated with NDF and ADF, and a tendency for GE to be negatively correlated (P < 0.10) with SDF and viscosity. There was also a tendency (P < 0.10) for TDF to be positively correlated with WBC.

Table 2. Analyzed amino acid composition of corn, wheat, soybean meal, canola meal, distillers dried grains with solubles, corn germ meal, copra expellers, sugar beet pulp, synthetic cellulose, and pectin, as-fed basis

	Ingredient ^a												
Item	Corn	Wheat	SBM	СМ	DDGS	CGM	CE	SBP	SF	Pectin			
Indispensable AA, %													
Arg	0.31	0.48	3.44	2.31	1.16	1.59	2.49	0.28	ND	0.06			
His	0.20	0.24	1.22	1.01	0.70	0.68	0.41	0.23	ND	0.04			
Ile	0.25	0.35	2.12	1.46	0.93	0.84	0.66	0.29	ND	0.06			
Leu	0.83	0.68	3.62	2.67	2.92	1.86	1.29	0.49	ND	0.10			
Lys	0.27	0.36	2.99	2.11	0.90	1.02	0.68	0.47	ND	0.12			
Met	0.15	0.19	0.63	0.73	0.44	0.43	0.28	0.16	ND	0.02			
Phe	0.34	0.44	2.36	1.52	1.22	1.04	0.85	0.29	ND	0.06			
Thr	0.25	0.30	1.82	1.56	1.01	0.91	0.65	0.35	ND	0.06			
Trp	0.06	0.15	0.66	0.53	0.20	0.22	0.18	0.07	< 0.02	< 0.02			
Val	0.33	0.45	2.25	1.86	1.31	1.35	1.03	0.43	ND	0.07			
Total	2.99	3.64	21.11	15.76	10.79	9.94	8.52	3.06	0.01	0.60			
Dispensable AA, %													
Ala	0.51	0.40	2.01	1.66	1.71	1.46	0.91	0.37	ND	0.07			
Asp	0.47	0.56	5.21	2.55	1.57	1.71	1.69	0.56	ND	0.12			
Cys	0.16	0.21	0.61	0.90	0.44	0.32	0.32	0.09	ND	0.02			
Glu	1.26	2.71	8.32	6.66	3.33	3.23	3.66	0.74	0.01	0.19			
Gly	0.29	0.45	1.96	1.92	0.99	1.31	0.89	0.33	ND	0.07			
Pro	0.60	0.90	2.29	2.34	1.84	1.14	0.67	0.32	ND	0.08			
Ser	0.33	0.44	2.11	1.36	1.21	1.04	0.85	0.34	ND	0.06			
Tyr	0.13	0.17	1.67	1.07	0.87	0.65	0.44	0.25	ND	0.04			
Total	3.75	5.84	24.18	18.46	11.96	10.86	9.43	3.00	0.01	0.65			
Total AA, %	6.74	9.48	45.29	34.22	22.75	20.80	17.95	6.06	0.02	1.25			
Calculated values													
Lys:CP ratio ^b , %	4.12	3.30	6.38	5.21	3.53	4.27	3.14	6.46	_	7.14			

^{*a*}CM = canola meal; CGM = corn germ meal; CE = copra expellers; ND = not detected.

^bThe Lys:CP ratio was expressed as the concentration of Lys as a percentage of the concentration of CP in each sample (González-Vega et al., 2011).

	Ingredient ^a													
Item	Corn	Wheat	SBM	СМ	DDGS	CGM	CE	SBP	SF	Pectin				
Ca, %	0.01	0.03	0.57	0.61	0.04	0.02	0.05	0.87	0.02	0.09				
P, %	0.26	0.36	0.59	1.04	0.81	0.68	0.50	0.70	ND^b	0.03				
Phytate, %	0.85	1.15	1.62	2.65	0.26	1.66	0.96	< 0.14	< 0.14	< 0.14				
Phytate P ^c , %	0.24	0.32	0.46	0.75	0.07	0.47	0.27	_	_	_				
Non-phytate P ^d , %	0.02	0.04	0.13	0.29	0.74	0.21	0.23	_	_	_				
Na, mg/kg	4.82	10.30	65.50	1,600	2,800	100	300	1,200	200	4,900				
Mg, %	0.09	0.12	0.26	0.56	0.28	0.19	0.26	0.24	< 0.01	0.02				
K, %	0.34	0.40	2.11	1.21	1.12	0.36	2.21	0.51	< 0.01	0.09				
Cl, %	< 0.10	< 0.10	< 0.10	0.39	0.12	< 0.10	0.63	0.10	< 0.10	0.10				
S, %	0.08	0.12	0.38	0.82	0.29	0.29	0.26	0.27	0.01	0.07				

99.20

0.01

6.36

9.82

89.90

< 0.10

< 0.13

< 2.00

0.61

60.60

0.02

6.73

10.60

45.00

< 0.10

< 0.13

< 2.00

1.12

208.00

31.4

33.70

47.50

0.70

0.18

0.59

< 2.00

0.01

281.00

0.06

6 7 9

54.30

9.74

2.30

0.21

0.12

< 2.00

44.60

< 0.01

0.09

1.82

1.10

< 0.10

< 0.13

< 2.00

0.10

18.80

1.20

2.10

2.90

0.80

< 0.10

< 2.00

< 0.10

229.00

0.12

5.53

51.00

55.40

0.13

1.08

< 0.10

< 2.00

^aCM = canola meal; CGM = corn germ meal; CE = copra expellers.

31.8

0.01

5.85

30.60

27.20

< 0.10

< 0.13

< 2.00

0.74

113.00

0.01

13.00

36.30

38.30

< 0.10

< 0.13

< 2.00

3.50

 b ND = not detected.

'Calculated as 28.2% of phytate (Tran and Sauvant, 2004).

18.1

0.02

1.34

3.60

18.30

0.20

< 0.13

<2.00

0.40

^dCalculated as the difference between phytate P and total P.

Table 4. Bulk density, swelling, WBC, and viscosity of corn, wheat, soybean meal, canola meal, distillers dried grains with solubles, corn germ meal, copra expellers, sugar beet pulp, synthetic cellulose, and pectin

		Ingredient ^a											
Item	Corn	Wheat	SBM	СМ	DDGS	CGM	CE	SBP	SF	Pectin	SEM	P-value	
Bulk density, g/L	728.51°	676.41 ^{ef}	782.68 ^a	715.06 ^d	656.10 ^g	705.06 ^d	658.43 ^g	665.76 ^{fg}	681.41°	768.36 ^b	3.79	< 0.01	
Swelling, L/kg DM	2.48 ⁱ	3.00^{h}	4.98°	4.54^{f}	3.76 ^g	5.79 ^d	7.50°	8.08 ^b	4.05 ^g	9.01 ^a	0.18	< 0.01	
WBC, g/g	1.21 ⁱ	1.00 ^j	2.74^{f}	1.82 ^g	1.72^{h}	3.14 ^d	3.61 ^b	4.09 ^a	2.86 ^e	3.39°	0.03	< 0.01	
Viscosity, cP	1.12 ^{cde}	1.30 ^{bc}	1.10 ^{cde}	1.00^{de}	1.07 ^{cde}	1.17 ^{cde}	1.27^{bcd}	1.45 ^b	0.93 ^e	7.00^{a}	0.11	< 0.01	

^{a-j}Means within a row lacking a common superscript letter differ (P < 0.05).

"CM = canola meal; CGM = corn germ meal; CE = copra expellers.

Water-binding capacity was positively correlated (P < 0.01) with swelling capacity, and there was a tendency (P < 0.10) for swelling capacity to be positively correlated with viscosity (P < 0.10).

There was a tendency for bulk density to be positively correlated with IVAID of DM P < 0.10and IVAID of OM P < 0.10; Table 7. However, no correlation was observed between swelling, WBC, or viscosity and IVAID or IVATTD of DM and OM. The concentration of IDF was negatively correlated (P < 0.01) with IVAID of DM and OM and IVATTD of DM and OM. The concentration of TDF was negatively correlated (P < 0.05) with IVAID of DM and IVATTD of DM and OM. The IVAID of DM and OM was negatively correlated (P < 0.05) with the concentrations of cellulose and insoluble NSP. The concentrations of NSP, insoluble NSP, and non-cellulosic NSP were negatively correlated (P < 0.05) with IVATTD of DM and OM. There was a tendency for IVAID of DM to be negatively correlated (P < 0.10) with NDF (r = -0.60), ADF (r = -0.59), insoluble hemicelluloses (r = -0.55), and NSP (r = -0.63). However, no correlation was observed between the concentration of CP, GE, AEE, lignin, or SDF and IVAID or IVATTD of DM and OM. The IVAID of DM

Fe, mg/kg

Cu, mg/kg

Mn, mg/kg

Zn, mg/kg

Cr, mg/kg

Co, mg/kg

Se, mg/kg

Mb, mg/kg

I, mg/kg

	Ingredient ^a												
Item, %	Corn	Wheat	SBM	СМ	DDGS	CGM	CE	SBP	SF	Pectin	SEM	P-value	
IVAID													
DM	47.57^{f}	69.50°	78.63 ^b	59.75 ^d	54.75°	47.27 ^f	56.61 ^e	26.23 ^g	5.03^{h}	85.37ª	1.04	< 0.01	
OM	46.15 ^g	66.57°	76.97 ^b	58.37 ^d	50.93^{f}	46.22 ^g	54.10 ^e	27.57 ^h	4.92 ⁱ	87.38 ^a	0.75	< 0.01	
IVATTD													
DM	87.14 ^d	88.60 ^c	94.45 ^b	80.34 ^f	58.90 ⁱ	62.28 ^h	79.34 ^g	81.28 ^e	7.09 ^j	99.26 ^a	0.33	< 0.01	
OM	86.96 ^d	88.59°	94.17 ^b	79.60 ^f	56.10 ⁱ	60.95 ^h	78.35 ^g	83.39°	7.32 ^j	99.40 ^a	0.28	< 0.01	

Table 5. IVAID and IVATTD of DM and OM in corn, wheat, soybean meal, canola meal, distillers dried grains with solubles, corn germ meal, copra expellers, sugar beet pulp, synthetic cellulose, and pectin

^{a-j}Means within a row lacking a common superscript letter differ (P < 0.05).

^aCM = canola meal; CGM = corn germ meal; CE = copra expellers.

 Table 6. Correlation coefficients between the chemical composition and physical characteristics of feed ingredients

						Correlation	coefficient ^a				
Item	DM	GE	NDF	ADF	IDF	SDF	TDF	Bulk	Swelling	WBC	Viscosity
DM	1.00	0.22	0.53	0.62*	0.77***	0.09	0.88***	-0.33	0.48	0.68**	0.06
GE	_	1.00	0.60*	0.62*	0.33	-0.58*	-0.01	-0.47	-0.14	0.00	-0.59*
NDF	_	_	1.00	0.94***	0.65**	-0.44	0.42	-0.77***	0.27	0.49	-0.47
ADF	_	_	_	1.00	0.69**	-0.43	0.47	-0.67**	0.29	0.51	-0.47
IDF	_	_	_	_	1.00	-0.41	0.81***	-0.54	-0.04	0.34	-0.45
SDF	_	_	_	_	_	1.00	0.20	0.47	0.64**	0.33	1.00***
TDF	_	_	_	_	_	_	1.00	-0.28	0.36	0.57*	0.16
Bulk	_	_	_	_	_	_	_	1.00	0.11	-0.01	0.48
Swelling	_	_	_	_	_	_	_	_	1.00	0.89***	0.62*
WBC	_	_	_	_	_	_	_	_	_	1.00	0.30
Viscosity	_	_	_	_	_	_	_	_	_	_	1.00

 a Bulk = bulk density.

*P < 0.10, **P < 0.05, ***P < 0.01.

	Correlation coefficient ^a													
Item	TDF	Cell	iNSP	Bulk	Swelling	WBC	Viscosity	IVAID of DM	IVAID of OM	IVATTD of DM	IVATTD of OM			
IDF	0.81***	0.80***	0.99***	-0.54	-0.04	0.34	-0.45	-0.87***	-0.88***	-0.92***	-0.91***			
TDF	1.00	0.59*	0.99***	-0.28	0.36	0.57*	0.16	0.65**	-0.62*	-0.76**	-0.75**			
Cell	_	1.00	0.75**	-0.69**	0.29	0.57*	-0.45	-0.70**	-0.71**	-0.54	-0.53			
iNSP	_	_	1.00	-0.50	-0.05	0.34	-0.41	-0.88***	-0.88***	-0.93***	-0.91***			
Bulk	_	_	_	1.00	0.11	-0.01	0.48	0.56*	0.60*	0.46	0.46			
Swelling	_	_	_	_	1.00	0.89***	0.62*	0.15	0.20	0.27	0.28			
WBC	_	_	_	_	_	1.00	0.30	-0.21	-0.17	-0.07	-0.05			
Viscosity	_	_	_	_	_	_	1.00	0.48	0.54	0.38	0.38			
IVAID of DM	_	_	_	_	_	_	_	1.00	1.00***	0.81***	0.79***			
IVAID of OM	-	_	-	-	_	_	_	-	1.00	0.82***	0.80***			
IVATTD of DM	_	_	_	_	_	_	_	_	—	1.00	1.00***			

Table 7. Correlation coefficients between fiber content, physical characteristics, and in vitro ileal and total tract digestibility of DM and OM of feed ingredients

^{*a*}Cell = cellulose; iNSP = insoluble non-starch polysaccharides; bulk = bulk density. *P < 0.10, **P < 0.05, ***P < 0.01. was perfectly correlated (P < 0.01) with IVAID of OM and IVATTD of DM was perfectly correlated (P < 0.01) with IVATTD of OM. The IVAID of DM was positively correlated (P < 0.01) with IVATTD of DM and IVATTD of OM and IVAID of OM was positively correlated (P < 0.01) with IVATTD of DM and IVATTD of OM.

DISCUSSION

Two sources of cereal grains, 2 sources of oilseed meals, 4 sources of coproducts, and 2 sources of synthetic fiber were used to obtain a wide range of IDF and SDF concentrations among ingredients. The ingredients varied in chemical composition and measurable physical characteristics. With the exception of canola meal and copra expellers, the analyzed sum of the components for each feed ingredient differed from 100.00% by more than 1.00%, and analyzed components in corn, wheat, SBM, corn germ meal, and pectin totaled between 2% and 6% more than 100%. There may be a number of reasons for this observation. Dry matter was not measured at each analysis, which may affect the results. Inaccuracies in analyses may also happen due to human factors or nonhomogenized samples. Another possible reason is that CP is calculated by multiplying the concentration of N by 6.25, with the assumption that all protein in the feed is composed of 16% N. It may be argued that the sum of total indispensable and dispensable AA should be used when adding chemical components to 100% instead of CP because only AA can be used in protein synthesis. However, this disregards the nonprotein N and other AA also present in the feed and it is, therefore, most likely more accurate to use the calculated value for CP than the total concentration of AA.

Fructo-oligosaccharides serve as reserve carbohydrate compounds that are synthesized and stored in the vacuole and are often localized in the stems, leaves, roots, and kernels in grasses such as wheat and barley (Heldt and Piechulla, 2011). Fructooligosaccharides may be mobilized to preserve the carbon flow to the kernel during times of insufficient photosynthetic products (Verspreet et al., 2013). To our knowledge, the concentration of fructo-oligosaccharides in DDGS and corn germ meal has not been previously reported. It is possible that DDGS or corn germ meal contain bacterial inulin or levans produced by cocultures of yeast and bacteria used in the fermentation process of ethanol production, which may explain the presence of fructo-oligosaccharides in both ingredients. Saccharomyces cerevisiae is the most used source of yeast in ethanol fermentation, but it is not uncommon for an ethanol plant to encounter microbial contamination (Beckner et al., 2011). Lactic acid bacteria are common contaminants due to their tolerance for ethanol, low pH, and high temperature (Narendranath and Power, 2005). Fructooligosaccharide synthesis has been observed in several lactic acid bacteria including *Lactobacillus reuteri* and *Leuconostoc citreum*, both of which are contaminants of ethanol fermentations (van Hijum et al., 2006; Beckner et al., 2011).

The concentration of fructo-oligosaccharides in corn, wheat, and SBP used in this experiment was greater than what was reported by Campbell et al. (1997), wheat has also been reported to contain 1% to 4% fructo-oligosaccharides on a DM basis (Bornet, 2001), so it appears there are some differences among varieties of wheat. The SBP used in this experiment contained added molasses, which contributed to a high concentration of sucrose, which levansucrase- or inulosucrase-secreting bacteria may convert to fructo-oligosaccharides (van Hijum et al., 2006; BeMiller, 2007). This may be the reason fructo-oligosaccharides were detected in the SBP used in this experiment. Differences in the concentration of fructo-oligosaccharides among different samples of the same ingredient may also be due to sample origin, sampling technique, and extraction method used (Campbell et al., 1997).

Wheat DDGS was reported to contain 4.6% glycerol (Cozannet et al., 2010), but only negligible levels of glycerol were observed in the corn DDGS and corn germ meal used in this experiment. Nevertheless, by complementing the traditional feed analyses with analyses for nutrients that are not typically analyzed, we were able to characterize the entire nutritional profile of the ingredients used in this study with the exception of DDGS, SBP, and SF. Incomplete nutritional profiles have traditionally been a problem with values in most feed composition tables. As an example, the analyzed concentration of nutrients in DDGS and SBP is 90.85% and 74.07% in NRC (2012) and 90.90% and 76.40% in Sauvant et al. (2004), whereas in this experiment, the analyzed components in these 2 ingredients were 94.29% and 88.90%, respectively. However, the fact that 5 of the 10 ingredients analyzed between 102% and 106% also indicates that additional work to improve feed ingredient analyses is needed.

Prediction equations have traditionally been used to predict the energy content of feed ingredients, but in several cases, the analyzed components in the ingredients did not add to 100% (Pedersen et al., 2007; Anderson et al., 2012; Kerr et al., 2013). This may result in erroneous prediction equations because it is possible that some of the components that were not analyzed also contributed energy to the ingredients. To predict the energy value of a feed ingredient, it is, therefore, important that all energy-contributing components are accounted for and the current data indicate that this is possible if traditional analyses are complemented by additional analyses that primarily aim at analyzing soluble carbohydrates.

In the current experiment, values for the analyzed GE of all ingredients were compared with values calculated as the sum of the theoretical GE of each energy-containing nutrient because a difference between the 2 values indicates that an energy-contributing component is unaccounted for. Thus, the comparison of the 2 values gives an indication of the accuracy of the component analyses for each ingredient. As an example, although the measured components of canola meal added to 100.97%, the calculated GE was 0.56 MJ/kg less than the analyzed GE indicating that some energy-contributing components in canola meal were not accounted for. The reason for this observation may be that sinapine, the most common phenolic compound in canola meal (Barthet and Daun, 2011), and tannins also contribute to the analyzed GE because their organic structure consisting of polyphenolic molecules contribute energy during combustion. Combined, sinapine and tannins contributed more than 1.50% to the DM in canola meal, so it is likely that this contributed to the fact that the calculated GE for canola meal was slightly less than the analyzed GE. Nevertheless, with the exception of corn, DDGS, SBP, and pectin, the percentage of calculated GE was within 4% of analyzed GE indicating that for 6 of the 10 ingredients, the analyzed components appear to be accurate. For DDGS and SBP, the analyzed concentrations of all components were less than 100%, which is likely the reason that the calculated GE was less than analyzed GE. So for these 2 ingredients, there are chemical components present in addition to the components analyzed in this experiment. For corn and pectin, it is possible that the reason for the differences between calculated and analyzed GE is that there may have been inaccuracies or overlaps in quantifying concentrations of energy-contributing nutrients (i.e., carbohydrate analysis). As an example, residual fructo-oligosaccharides may remain in the SDF fraction and, although unlikely, it is also possible that starch is not completely hydrolyzed in the TDF analysis resulting in residual resistant starch in the IDF fraction. Another possible reason is that the Atwater factors used to calculate GE from nutrients may not be applicable to every ingredient (Novotny et al., 2012).

A GE value was also assigned to lignin because lignin is combustible and contributes to the GE of an ingredient when analyzed using bomb calorimetry. The GE value for lignin reported by Jung et al. (1999) was used, but this value was derived from the average GE of 4 commercially available lignin preparations that may not be completely representative of the lignin that is present in the 10 feed ingredients used in this experiment. The complete structure of lignin has not been elucidated because it varies greatly in size, component subunits, location in the plant, and among different species of plants (Albersheim et al., 2011). The concentration of lignin in the samples may also be underestimated because the ADL procedure used in this experiment underestimates the concentration of lignin in forages compared with the Klason lignin procedure (Jung et al., 1999). However, it is not known if this is also the case for nonforage feed ingredients. Therefore, it is imperative that a method to characterize and quantify lignin in specific feed ingredients be developed. This may allow for a more accurate analysis of GE, which may result in improved agreement between the analyzed and calculated GE of feed ingredients.

The concentrations of total and resistant starch in wheat are slightly greater than published values, whereas resistant starch in corn was in agreement with reported values (Bednar et al., 2000; Murray et al., 2001). The concentration of resistant starch in corn and wheat may explain the low IVAID of DM and OM and greater IVATTD of DM and OM for these 2 ingredients. The 55% increase from IVAID to IVATTD of DM in SBP indicates that the fiber in SBP is poorly digested in the small intestine, but highly fermentable in the hindgut. This is most likely a result of the high concentration of SDF in SBP because SDF is much more fermentable than IDF (Urriola et al., 2010; Zhang et al., 2013). In contrast, the low IVAID and IVATTD of DM in DDGS indicate that the fiber fraction in DDGS has a low utilization by pigs, which is in agreement with in vivo data (Urriola et al., 2010) and most likely is a result of the high concentration of IDF in the fiber in corn and DDGS (Pedersen et al., 2014; Jaworski et al., 2015). The low IVATTD of SF and the high IVATTD of pectin confirm that cellulose is an indigestible fraction of fiber, whereas pectin is close to 100% fermentable, which further confirms the high fermentability of SDF.

The strong positive correlation between WBC and swelling indicates that one of these hydration properties can be measured to predict the other. Swelling is defined as the volume fiber occupies after hydration under specified conditions, which depends on WBC or the quantity of water that can be bound to a substrate (Bach Knudsen et al., 2013; Capuano, 2017). Processes that alter physical characteristics (i.e., grinding) may also affect the hydration properties of fiber, and therefore, the same batch of sample should be used in subsequent analyses without further processing (Guillon and Champ, 2000).

The stronger correlation between IDF and TDF and IVAID and IVATTD of DM and OM than the correlations between NDF and ADF and IVAID and IVATTD indicates that measuring IDF and TDF in fiber results in an improved prediction of the digestibility of GE compared with values for NDF and ADF. This observation is in agreement with Anderson et al. (2012) and Kerr et al. (2013) who also concluded that TDF predicts energy digestibility better than analyzed values for ADF and NDF, which may be because TDF, unlike ADF and NDF, also includes the SDF fraction. However, values for TDF are less reproducible than values for crude fiber or ADF and NDF (Mertens, 2003). Alternatively, the concentration of insoluble NSP may also be calculated and used to evaluate digestibility of GE because insoluble NSP is also strongly correlated with both IVAID and IVATTD of DM and OM.

The observation that physical characteristics of the feed ingredients were not correlated with IVAID or IVATTD of DM or OM indicates that these parameters do not influence digestibility of DM or OM in feed ingredients. These results are in agreement with data from Serena and Bach Knudsen (2007), who reported that IVATTD of OM and lignin were correlated with soluble and insoluble non-cellulosic NSP, but not with WBC or swelling. It is likely that because of the relatively high concentration of water in the small intestine of pigs, physical characteristics of feed ingredients do not result in measurable changes to nutrient and energy digestibility.

Viscosity is defined as a fluid's resistance to flow due to the physical entanglement among polysaccharides within the solution and is dependent on the primary structure, molecular weight, and concentration of fiber (Dikeman and Fahey, 2006; Bach Knudsen et al., 2013). It is possible that the reason for the lack of correlation between viscosity and IVAID or IVATTD of DM or OM is that although the thermochemical conditions of the in vitro procedure simulate that of the gastrointestinal tract, the physical setup does not allow for an accurate representation of the flow behavior of digesta in the intestinal lumen that defines the rate of digestion and absorption of nutrients (Takahashi, 2011). A lack of correlation may also be a result of very low viscosity measurements from the ingredients used in this experiment. Only an aliquot of the supernatant after centrifugation is used in viscosity measurements (Johansen et al., 1997; Serena and Bach Knudsen, 2007); however, this disregards the effect of large particles on viscosity (Takahashi and Sakata, 2002).

In conclusion, results of this work indicate that it is possible to analyze nutrient composition of some, but not all, feed ingredients to account for all nutrients and GE. However, future refinements of analyses are needed to avoid overlapping fractions in analyses such as analyzed starch and analyzed dietary fiber. Likewise, it is not always that GE in ingredients calculated from analyzed energy-containing components equal analyzed GE, even if the total analyzed components are close to 100%. It is possible that some of these inaccuracies are a result of a lack of knowledge about the GE value of lignin, tannins, sinapine, and possibly other components in the ingredient. Physical characteristics of feed ingredients do not appear to influence estimates for IVAID or IVATTD of DM or OM, but the concentration of fiber fractions (i.e., IDF, TDF, cellulose, and insoluble NSP) may be used to estimate IVAID and IVATTD of DM. If possible, IDF and TDF should be measured instead of ADF and NDF because TDF and IDF are better correlated with digestibility of DM and OM than ADF and NDF.

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