2-2013

The use of a covariate reduces experimental error in nutrient digestion studies in growing pigs

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Abstract
Covariance analysis limits error, the degree of nuisance variation, and overparameterizing factors to accurately measure treatment effects. Data dealing with growth, carcass composition, and genetics often use covariates in data analysis. In contrast, nutritional studies typically do not. The objectives of this study were to 1) determine the effect of feeding diets containing dehulled, degermed corn, corn–soybean meal, or distillers dried grains with solubles on nutrient digestibility coefficients, 2) evaluate potential interactive effects between initial and final treatment diets on the final treatment diet effects, and 3) determine if initial criterion (digestibility or physiological values) would effectively correct for variation among pigs that could thereby affect final treatment diet digestibility coefficients. Seventy-two crossbred barrows [(Yorkshire × Landrace × Duroc) × Chester White] were randomly assigned to 1 of the 3 diets within initial dietary treatment for Phase-2 (P2; 14 d). Fecal and blood samples were collected after feeding the Phase-1 (P1) diets for 14 d (trial d-14) and on d 28 after feeding the P2 diets for 14 d. Fecal samples were dried and analyzed for C, ether extract, GE, N, NDF, P, and S. Plasma samples were analyzed for plasma urea N and triacylglycerides. Pigs were fed diets that differed widely in CP, NDF, and P, resulting in an overall decrease in C, GE, NDF, N, P, and S digestibility and plasma urea N and triacylglycerides as dietary fiber increased in P1 and P2 ($P < 0.10$). There were no differences in P2 criteria due to blocking for the P1 diet. There tended ($P = 0.10$ to 0.20) to be P1 × P2 interactions for NDF and S, indicating that the response of pigs to the P2 diet may depend on the P1 diet. In contrast, when the P1 variable was used as a covariate for P2 data, it was statistically significant for GE, NDF, N, S, and plasma urea N ($P < 0.10$) whereas C and ether extract showed tendencies but not for P digestibility or plasma triacylglycerides. In conclusion, if initial diets are known, subsequent treatments should be balanced for the initial diet because of potential of initial diet × final diet interactions. If the initial diets are not known, then the initial digestibility coefficient would be effective in reducing the variation associated with subsequently obtained data and should be considered as a covariate in future grower–finisher swine digestibility research.

Keywords
covariance, digestibility, fiber, pig

Disciplines
Agriculture | Animal Sciences | Nutrition

Comments
This article is published as Jacobs, B. M., J. F. Patience, M. D. Lindemann, K. J. Stalder, and B. J. Kerr. "The use of a covariate reduces experimental error in nutrient digestion studies in growing pigs." *Journal of animal science* 91, no. 2 (2013): 804-810. doi: 10.2527/jas.2011-4868.

The use of a covariate reduces experimental error in nutrient digestion studies in growing pigs[1]

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ABSTRACT: Covariance analysis limits error, the degree of nuisance variation, and overparameterizing factors to accurately measure treatment effects. Data dealing with growth, carcass composition, and genetics often use covariates in data analysis. In contrast, nutritional studies typically do not. The objectives of this study were to 1) determine the effect of feeding diets containing dehulled, degermed corn, corn–soybean meal, or distillers dried grains with solubles on nutrient digestibility coefficients, 2) evaluate potential interactive effects between initial and final treatment diets on the final treatment diet effects, and 3) determine if initial criterion (digestibility or physiological values) would effectively correct for variation among pigs that could thereby affect final treatment diet digestibility coefficients. Seventy-two crossbred barrows [(Yorkshire × Landrace × Duroc) × Chester White] were randomly assigned to 1 of the 3 diets within initial dietary treatment for Phase-2 (P2; 14 d). Fecal and blood samples were collected after feeding the Phase-1 (P1) diets for 14 d (trial d-14) and on d 28 after feeding the P2 diets for 14 d. Fecal samples were dried and analyzed for C, ether extract, GE, N, NDF, P, and S. Plasma samples were analyzed for plasma urea N and triacylglycerides. Pigs were fed diets that differed widely in CP, NDF, and P, resulting in an overall decrease in C, GE, NDF, N, P, and S digestibility and plasma urea N and triacylglycerides as dietary fiber increased in P1 and P2 ($P < 0.10$). There were no differences in P2 criteria due to blocking for the P1 diet. There tended ($P = 0.10$ to 0.20) to be P1 × P2 interactions for NDF and S, indicating that the response of pigs to the P2 diet may depend on the P1 diet. In contrast, when the P1 variable was used as a covariate for P2 data, it was statistically significant for GE, NDF, N, S, and plasma urea N ($P < 0.10$) whereas C and ether extract showed tendencies but not for P digestibility or plasma triacylglycerides. In conclusion, if initial diets are known, subsequent treatments should be balanced for the initial diet because of potential of initial diet × final diet interactions. If the initial diets are not known, then the initial digestibility coefficient would be effective in reducing the variation associated with subsequently obtained data and should be considered as a covariate in future grower–finisher swine digestibility research.

Key words: covariance, digestibility, fiber, pig

J. Anim. Sci. 2013.91:804–810
doi:10.2527/jas2011-4868

INTRODUCTION

Variation is inevitable in animal research with experiments evaluating body composition (Brown et al., 1985), feed intake (Smith et al., 2011), growth (Rikard-Bell et al., 2009), AA (Waguespack et al., 2011), and meat quality (Lefaucheur et al., 2011) often using covariates in data analysis. Others (Kirkpatrick et al., 1990; Schaeffer and Dekkers, 1994) have reported

[1]The authors express thanks to J. Cook at the National Laboratory for Agriculture and the Environment (Ames, Iowa) for laboratory assistance. Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA, Iowa State University, or the University of Kentucky and does not imply approval to the exclusion of other products that may be suitable. The USDA is an equal opportunity provider and employer.

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Received November 1, 2011.
Accepted December 24, 2012.

covariance to be an alternative repeated record for certain model traits in the life of an animal such as age, BW, or genetic line. The purpose of a covariate is to limit error, the degree of nuisance variation, and overparameterizing factors to accurately measure treatment effects (Meyer and Hill, 1997) in which the variable does not directly impact the study (Ramsey and Schafer, 2002). In general, nutrition studies do not use covariates but instead use blocking relative to initial BW or previous treatment. As with data dealing with growth, genetics, or meat quality, digestibility coefficients obtained in nutritional studies may need to be adjusted for differences among animals to reduce experimental error as long as an appropriate covariate has been selected (Lee et al., 1997). In addition, use of covariance to control experimental error may minimize the number of experimental units required to obtain statistically valid results as questioned by Institutional Animal Care and Use Committees. The objectives of this study were to 1) determine the effect of feeding diets containing dehulled, degermed corn (**DDC**), corn–soybean meal (**CSBM**), or distillers dried grains with solubles (**DDGS**) on digestibility coefficients, 2) evaluate potential interactive effects between pretreatment and posttreatment diets on posttreatment diet effects, and 3) determine if initial criterion (digestibility or physiological values) would be effective in reducing the error due to variation among pigs, thereby improving the precision of digestibility coefficients for the dietary treatments.

MATERIALS AND METHODS

The experiment was conducted under protocols approved by the University of Kentucky Institutional Animal Care and Use Committee.

Feeding Management

Diets (Table 1) were mixed at the University of Kentucky (Lexington, KY) and formulated to contain varying levels of CP, NDF, and P through the use of DDC, CSBM, and DDGS. Diets were formulated to meet requirements relative to NRC (1998) recommendations. The same diet composition was used in each of 2 phases, with Phase-1 (**P1**) diets using chromic oxide and Phase-2 (**P2**) diets using titanium dioxide, each added at 0.5% of the diet to determine digestibility coefficients. Two different inert markers were used to prevent the potential contamination of the marker in the digestive tract in P1 with that of P2, which would have tainted the digestibility coefficients associated with P2 digestibility coefficients, thereby preventing our planned comparisons. Pigs were provided ad libitum access to feed and water throughout the experiment.

Pig Management and Collections

Seventy-two crossbred barrows [(Yorkshire × Landrace × Duroc) × Chester White] were individually penned and randomly assigned to 1 of 3 dietary treatments (Table 1). All pigs were initially separated

Table 1. Composition of Phase-1 and Phase-2 diets, as-fed basis[1]

Item	DDC	CSBM	DDGS
Ingredient, %			
Corn	–	78.40	56.70
Soybean meal, 48% CP	18.00	18.00	15.00
Dehulled, degermed corn	78.19	–	–
Dried distillers grains with solubles	–	–	25.00
Soybean oil	0.50	0.50	0.50
L-Lys HCl	0.11	–	–
Dicalcium phosphate	0.85	0.70	0.15
Limestone	0.70	0.75	1.00
Sodium chloride	0.50	0.50	0.50
Vitamin premix[2]	0.05	0.05	0.05
Trace mineral premix[3]	0.05	0.05	0.05
Marker[4]	0.50	0.50	0.50
Clay[5]	0.50	0.50	0.50
Antibiotic[6]	0.05	0.05	0.05
Calculated composition			
Ca, %	0.50	0.50	0.50
Crude fat, %	1.20	4.10	5.30
CP, %	14.27	15.06	18.76
Lys, %	0.75	0.75	0.75
ME, kcal/kg	3,293	3,332	3,193
NDF, %	4.50	9.10	15.40
P, %	0.34	0.47	0.48
S, %	0.10	0.18	0.21
Analyzed composition[7]			
Crude fat, %	1.28	3.72	4.77
CP, %	13.25	16.00	19.75
GE, kcal/kg	3,770	3,973	4,131
NDF, %	2.51	7.25	12.50
P, %	0.28	0.46	0.49
S, %	0.17	0.20	0.32

[1]DDC = dehulled, degermed corn; CSBM = corn–soybean meal; DDGS = distillers dried grains with solubles.

[2]Supplied per kilogram of diet: vitamin A, 6,600 IU; vitamin D_3, 880 IU; vitamin E, 44 IU; vitamin K (menadione sodium bisulfate complex), 6.4 mg; thiamin, 4.0 mg; riboflavin, 8.8 mg; pyridoxine, 4.4 mg; vitamin B_{12}, 33 μg; folic acid, 1.3 mg; niacin, 44 mg; pantothenic acid, 22 mg; and D-biotin, 0.22 mg.

[3]Supplied per kilogram of diet: Zn, 131 mg as ZnO; Fe, 131 mg as $FeSO_4 \cdot H_2O$; Mn 45 mg, as MnO; Cu, 13 mg as $CuSO_4 \cdot 5H_2O$; I, 1.5 mg as CaI_2O_6; Co, 0.23 mg as $CoCO_3$; and Se, 0.28 mg as Na_2O_3Se.

[4]The addition of 0.5% Cr_2O_3 (≥98% purity; Elementis Chromium LP, Corpus Christi, TX) represents an addition of 3.35 mg Cr/g diet; analyzed content equaled 2.76 mg Cr/kg diet (Phase-1). The addition of 0.5% TiO2 (99% purity; Tronox Pigments GmBH, Krefield, Germany) represents an addition of 2.97 mg titanium/g diet; analyzed content equaled 2.89 mg titanium/kg diet (Phase-2).

[5]AB-20 (Prince Agriproducts, Quincy, IL).

[6]Tylan-40 supplied 44 mg/kg of diet (Elanco, Greenfield, IN).

[7]Diets analyzed at the USDA-ARS (Ames, IA), except for P, which was analyzed by SDK Labs (Hutchison, KS).

into 3 groups of 24 pens and fed P1 diets for 14 d and then randomly reassigned within P1 dietary treatment into the 3 P2 dietary treatments and fed an additional 14 d, resulting in 9 groups of 8 pigs (Fig. 1). On d 14 (P1) and 28 (P2) fresh fecal samples were collected into 454 g plastic containers and placed into a –20°C freezer until analyzed. On each collection day, samples were collected from 0700 to 1200 h to ensure adequate sample size for subsequent analysis. Pigs were also weighed on d 14 and 28 of the experiment. In addition, on d 10 (P1) and d 28 (P2), blood samples were collected between 1000 and 1200 h via venipuncture into 10 mL centrifuge tubes containing sodium heparin (14.3 United States Pharmacopeia units/mL) and centrifuged at 900 × *g* for 18 min at 4°C. The resultant plasma was harvested and stored at –20°C until analyzed.

Chemical Analysis

Before analysis, fecal samples were thawed, homogenized, and dried in a forced-air oven at 70°C for 48 h before grinding. Feed and fecal samples were ground through a 1-mm screen before composition was determined. Gross energy was determined with isoperibol bomb calorimeter (Model 1281; Parr Instrument Co. Moline, IL) with benzoic acid as a standard. Duplicate analyses were performed on all fecal samples for C, N, and S by thermocombustion (VarioMax, Elementar Analysensysteme, Gmbh, Hanau, Germany). Ether extract (**EE**) was determined using petroleum ether (ASE 350; Dionex Corporation, Sunnyvale, CA; Luthria, 2004). Neutral detergent fiber was determined using a fiber analyzer (Ankom 200, Macedon, NY; Van Soest and Robertson, 1979). Phosphorus and chromic oxide (P1) were analyzed at a commercial laboratory (SDK Labs, Hutchinson, KS), chromic oxide by inductively coupled plasma spectroscopy (Ultima 2; Horiba Jobin-Yvon Inc., Edison, NJ) according to standard method (3120B; American Public Health Association, 1992), and P by colorimetric determination (Technicon Auto Analyzer II, Technicon, Tarrytown, NY) using AOAC Int. (1995) method 976.06. Titanium dioxide (P2) was analyzed by digesting the samples in sulfuric acid and hydrogen peroxide and subsequent absorbance was measured using a UV spectrophotometer (method 988.05; AOAC, 1978).

Plasma urea N (**PUN**) concentrations were determined using a method described by Kerr et al. (2004) in which the samples were analyzed colorimetrically using a kit (kit B755'-'10; Point Scientific Inc., Lincoln Park, MI) followed by UV absorbance (Varian Cary 50 Spectrophotometer; Varian Analytical Instruments, Walnut Creek, CA). Plasma triacylglycerides were quantified using an enzymatic kit (GPO; Pointe Scientific Inc., Lincoln Park, MI) wherein lipase converts the triacylglycerides to glycerol and FFA. Glycerol kinase and glycerophosphate oxidase was then used to derive H_2O_2, after which the absorbance was measured at 540 nm.

Calculations and Statistical Analysis

Feed and fecal Cr and Ti concentrations were used to estimate apparent C, EE, GE, N, NDF, P, and S total tract digestibility by indirect marker methodology. Digestible C, EE, GE, N, NDF, P, and S were calculated as [1 – ($marker_{feed}$ × digestibility $criterion_{feces}$)/($marker_{feces}$ × digestibility $criterion_{feed}$)] × 100. Feed and fecal samples were analyzed in duplicates for C, EE, GE, N, P, and S, and NDF was measured in triplicate. Postexperiment evaluation of the treatment digestibility data identified statistical outliers (greater than 3 SD above or below the mean) with a total of 6 data points were removed from the data analysis. Two for pigs fed DDGS and 1 for a pig fed DDC during P1 and 2 for pigs fed CSBM and 1 for a pig fed DDC during P2 were removed from the data set.

Data were analyzed using the Proc MIXED procedure (SAS Inst. Inc., Cary, NC) to evaluate digestibility coefficients or plasma criterion among dietary treatments. The initial model for P1 and P2 included only the dietary treatment fed during that phase. A second model used P1, P2, and their interaction as model variables, along with initial BW as a covariate. A third model used initial criterion and initial BW as covariates. Initial criterion and initial BW were used as fixed effects when the covariance analysis was performed, with treatment means reported as least square means. The individual pig was the experimental unit for all variables. Data with $P \leq$

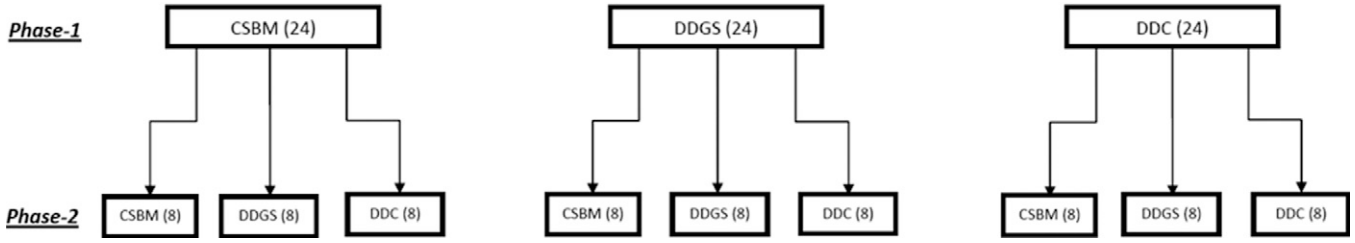

Figure 1. Allotment of a total of 72 crossbred barrows into Phase-1 (d 0 to 14) and Phase-2 (d 14 to 28) diets. DDC = dehulled, degermed corn; CSBM = corn–soybean meal; DDGS = distillers dried grains with solubles. Numbers in parentheses represent the initial number of pigs per treatment.

0.10 were considered to be significant with $P = 0.10$ to 0.20 being considered tendencies. The slopes (*b*-values) of covariates are reported in the appropriate tables. The slope can be either positive or negative and represents the change in the particular digestibility coefficient or plasma concentration value relative to the change in the covariate (e.g., the slope when initial BW is included in the analysis model as a covariate for GE digestibility indicates how the GE digestibility coefficient was influenced by initial BW).

RESULTS AND DISCUSSION

Diet Effects Within Phase

Nutritional studies are crucial for providing knowledge to support the use of ingredients, feed additives, and medications in swine. Furthermore, understanding of nutrient and energy digestibility is important in characterizing the impact of dietary treatments on subsequent nutrient use. All data from 1 pig was removed because of a rectal prolapse (allotted to CSBM during P1 and DDC during P2); therefore, data from 71 pigs were used. In the current study, P1 dietary treatments differed widely in CP, NDF, and P, resulting in an overall decrease in C, GE, and N digestibility as dietary fiber increased (Table 2). Apparent total tract digestibility of EE, NDF, and S were greater in pigs fed DDC compared with pigs fed CSBM and DDGS but did not differ between pigs fed CSBM and DDGS. Phosphorus digestibility was greatest for pigs fed DDGS and least for pigs fed the CSBM diet. In general, the nutrient and GE digestibility decreased ($P < 0.10$) as dietary fiber increased. The current data are supported by Kuan et al. (1983) and Stanogias and Pearce (1985) who reported a similar response to increasing dietary fiber, in which apparent total tract digestibility of CP, DM, and EE decreased because of increasing fiber. Also, increased dietary fiber type has been reported to decrease the digestibility of NDF, DM, and CP (Ehle et al., 1982), AA (Pedersen et al., 2007), and total dietary fiber (Urriola et al., 2010). Plasma urea N and triacylglycerides increased ($P < 0.01$) as dietary fiber increased, but delineating the response is confounded due to the increase in dietary CP and crude fat.

Impact of P2 diets on P2 fecal digestibility and blood measurements, with no consideration of carryover effects from the previous diet, are shown in Table 3. As expected, pigs fed P2 diets showed similar digestibility differences among dietary treatments in comparison to data reported for P1, in which increasing dietary fiber resulted in an overall decrease in nutrient and GE digestibility ($P < 0.10$). Urriola and Stein (2010) also reported that inclusion of 30% DDGS reduced GE digestibility in grower pigs because of the fiber contained in DDGS. In the current experiment, nutrient digestibility was affected ($P < 0.10$) by diet. The total P content in the experimental diets increased with increasing amount of fiber (DDC vs. CSBM and DDGS), even though P digestibility decreased ($P < 0.10$); delineating the response due to total dietary P or NDF is not possible. Pettey et al. (2006) reported a linear increase in fecal P digestibility as dietary P increased, but they used a purified diet with monosodium P and their

Table 2. Apparent total tract digestibility and plasma analysis on d 14 in grow–finish pigs fed diets differing in nutrient composition (Phase-1)[1]

	Phase-1 diet				
Criterion	DDC	CSBM	DDGS	SEM	*P*-value
Total tract digestibility,[2] %					
C	95.5[a]	85.0[b]	81.5[c]	0.4	0.01
Ether extract	57.2[a]	52.2[b]	52.5[b]	1.0	0.01
GE	95.0[a]	86.2[b]	80.0[c]	0.4	0.01
NDF	66.0[a]	58.3[b]	58.0[b]	2.6	0.05
N	89.0[a]	82.9[b]	80.4[c]	0.5	0.01
P	55.7[a]	45.0[c]	47.9[b]	1.0	0.01
S	82.3[a]	81.0[b]	80.0[b]	0.4	0.01
Plasma,[2] mg/dL					
Urea N	15.3[b]	16.3[b]	20.1[a]	0.7	0.01
Triacylglycerides	27.8[b]	47.5[a]	55.3[a]	4.2	0.01

[a–c]Means in the same row not sharing a common superscript differ ($P < 0.10$).

[1]DDC = dehulled, degermed corn; CSBM = corn–soybean meal; DDGS = distillers dried grains with solubles. Average BW was 59.2 and 71.0 kg for d 0 and d 14, respectively. Average daily feed intake was 2.94, 2.79, and 2.66 kg (SE = 0.07 kg) for pigs fed DDC, CSBM, and DDGS diets, respectively.

[2]Mean digestibility coefficients calculated based on fecal grab samples collected on d 14. Blood was collected from pigs on d 10.

Table 3. Apparent total tract digestibility and plasma analysis on d 28 in grow–finisher pigs fed diets differing in nutrient composition (Phase-2)[1]

	Phase-2 diet				
Criterion	DDC	CSBM	DDGS	SEM	*P*-value
Total tract digestibility,[2] %					
C	95.6[a]	88.1[b]	84.1[c]	0.3	0.01
Ether extract	56.1[a]	41.1[c]	51.9[b]	0.9	0.01
GE	95.1[a]	87.0[b]	83.6[c]	0.3	0.01
NDF	70.6[a]	59.0[b]	57.1[b]	1.9	0.01
N	89.3[a]	84.0[b]	83.2[b]	0.4	0.01
P	47.4[a]	42.9[b]	43.0[b]	1.3	0.04
S	81.1[b]	75.8[c]	82.9[a]	0.4	0.01
Plasma,[2] mg/dL					
Urea N	15.1[b]	15.7[b]	19.1[a]	0.6	0.01
Triacylglycerides	40.1[b]	52.6[a]	50.9[ab]	4.3	0.11

[a–c]Means in the same row not sharing a common superscript differ ($P < 0.10$).

[1]DDC = dehulled, degermed corn; CSBM = corn–soybean meal; DDGS = distillers dried grains with solubles. Average BW on d 14 and 28 were 71.0 and 88.6 kg, respectively. Average daily feed intake was 2.91, 2.84, and 2.63 kg (SE = 0.21 kg) for pigs fed DDC, CSBM, and DDGS diets, respectively.

[2]Phase-2 data repeats mean of 8 pigs previously fed each Phase-1 diet. Mean digestibility coefficients calculated based on fecal grab samples collected on d 28. Blood collected from pigs on d 28.

increase in dietary P was much greater than the current experiment. Similar to P1 data, PUN and triacylglyceride concentrations were affected ($P < 0.10$) by P2 diet. The observed increase in PUN concentration as CP increased is in agreement with previous literature (Zervas and Zijlstra, 2002) and agrees with others (Brown and Cline, 1974; Fuller et al., 1979; Coma et al., 1995) who have reported that N metabolism, and consequently PUN, can be affected by dietary AA concentrations.

Potential Impact of Phase-1 Diet on Phase-2

Although digestibility and physiological data were expected to differ among diets as described before, the real focus in the current experiment was whether the composition of the P1 diet may have affected the data obtained during P2 of the experiment. Because information from the literature indicates that age or BW may affect digestibility (Noblet and van Milgen, 2004) and may be used as covariate to adjust for variation in BW within treatment (Boddicker et al., 2011; Young et al., 2011), we elected to include initial BW as a covariate in this and subsequent analysis. Table 4 represents the results of evaluation of the data when previous diet is known, along with potential interactive effects between P1 and P2 diets and the use of initial BW as a covariate.

Day 14 BW as a covariate impacted GE, N, and S digestibility ($P = 0.10$, 0.01, and 0.04, respectively) and tended to affect P ($P = 0.17$), PUN ($P = 0.12$), and triacylglycerides ($P = 0.20$) values. We elected, however, to retain BW as a covariate in this (Table 4) and subsequent (Table 5) analysis, even though it may not have been statistically significant. Although there were no significant interactions between P1 and P2 diet on P2 treatment means, NDF and S tended to affected by P1 × P2 interactions ($P = 0.17$ and 0.14, respectively; Table 4), indicating that P1 diet may affect P2 data. Likewise, there was no impact of P1 diet on any of the P2 criteria measured. The lack of an interaction between P1 and P2 or an overall lack of P1 on P2 data was partially expected as increasing the length of feeding or adaptation to a diet (Gargallo and Zimmerman, 1981) before samples are collected likely limits the impact of a P1 diet on subsequent treatment means. Even with using BW as a covariate and some tendencies for P1 × P2 interactions used in the analysis, most fecal digestibility coefficients and plasma measurements differed because of P2 dietary treatment in a similar manner as described previously. However, the data shown in Table 4 indicate that it is important to account for the initial diet fed and potential interactions between the initial and final diet in animal nutrition research to reduce nuisance variation. It also indicates that longer adaptation time to subsequent dietary treatments may be needed to reduce or prevent previous diet effects on subsequent treatment means.

Use of Covariate Analysis

Similar to that described for diet effects within phase (Table 3) and potential impact of P1 diet on

Table 4. Apparent total tract digestibility and plasma analysis on d 28 during Phase 2 in grow–finish pigs fed diets differing in nutrient composition as affected by the initial diet (Phase-1), its potential interaction with the Phase-2 diet, and initial pig BW[1]

	Phase-2 diet				*P*-value[2]			
Criterion	DDC	CSBM	DDGS	SEM	P1	P2	P1 × P2	P1 BW[3]
Total tract digestibility,[4] %								
C	95.6[a]	88.1[b]	84.3[c]	0.3	0.84	0.01	0.35	0.41
Ether extract	56.2[a]	41.1[c]	51.9[b]	0.9	0.98	0.01	0.31	0.28
GE	95.0[a]	87.0[b]	83.6[c]	0.3	0.59	0.01	0.34	0.10
NDF	70.8[a]	58.8[b]	57.1[b]	1.9	0.30	0.01	0.17	0.54
N	89.2[a]	84.0[b]	83.3[b]	0.4	0.83	0.01	0.26	0.01
P	47.4[a]	43.0[b]	42.9[b]	1.4	0.81	0.04	0.53	0.17
S	81.1[b]	75.8[c]	82.9[a]	0.4	0.63	0.01	0.14	0.04
Plasma,[4] mg/dL								
Urea N	15.1[b]	15.7[b]	19.1[a]	0.6	0.75	0.01	0.64	0.12
Triacylglycerides	40.3[b]	52.6[a]	51.2[ab]	4.4	0.27	0.11	0.70	0.20

[a–c]Means in the same row not sharing a common superscript differ ($P < 0.10$).

[1]DDC = dehulled, degermed corn; CSBM = corn–soybean meal; DDGS = distillers dried grains with solubles. Average BW on d 14 and 28 were 71.0 and 88.6 kg, respectively. Average daily feed intake was 2.91, 2.84, and 2.63 kg (SE = 0.21 kg) for pigs fed DDC, CSBM, and DDGS diets, respectively.

[2]P1 = impact of Phase-1 (P1) diet on Phase-2 (P2) digestibility; P2 = P2 diet effect; P1 × P2 = interaction between P1 and P2 diets on P2 diet means; P1 BW = P1 BW (d 14) as a covariate for P2.

[3]The *b*-values (slope) of P1 BW as a covariate were 0.02, –0.11, 0.05, –0.13, 0.10, –0.20, 0.08, 0.11, and 0.60 for C, ether extract, GE, NDF, N, P, S, plasma urea, and triglycerides, respectively.

[4] P2 data repeat mean of 8 pigs previously fed each P1 diet. Mean digestibility coefficients calculated based on fecal grab samples collected on d 28. Blood collected from pigs on d 28.

Table 5. Apparent total tract digestibility on d 28 in grow–finisher pigs fed diets differing in nutrient composition (Phase-2) using Phase-1 individual criterion and initial BW as covariates[1]

	Diet				*P*-value[2]		
Criterion	DDC	CSBM	DDGS	SEM	P2 diet	P1 criterion[3]	P1 BW[4]
Total tract digestibility,[5] %							
C	95.5[a]	88.1[b]	84.3[c]	0.3	0.01	0.12	0.31
Ether extract	56.2[a]	41.1[c]	51.7[b]	0.9	0.01	0.11	0.49
GE	95.0[a]	87.0[b]	84.0[c]	0.3	0.01	0.04	0.07
NDF	70.0[a]	59.7[b]	57.3[b]	1.8	0.01	0.01	0.62
N	89.2[a]	84.0[b]	83.2[b]	0.4	0.01	0.02	0.01
P	47.4[a]	43.0[b]	42.9[b]	1.3	0.03	0.37	0.16
S	81.0[b]	75.9[c]	82.9[a]	0.3	0.01	0.01	0.01
Plasma,[5] mg/dL							
Urea N	15.1[b]	15.7[b]	19.1[a]	0.6	0.01	0.61	0.32
Triacylglycerides	39.4[b]	53.0[a]	51.2[ab]	4.4	0.08	0.64	0.20

[a–c]Means in the same row not sharing a common superscript differ ($P < 0.10$).

[1]DDC = dehulled, degermed corn; CSBM = corn–soybean meal; DDGS = distillers dried grains with solubles. Digestibility of Phase-2 (P2) diets. Average BW on d 14 and 28 were 71.0 kg and 88.6 kg, respectively. Average daily feed intake was 2.91, 2.84, and 2.63 kg (SE = 0.21 kg) for pigs fed DDC, CSBM, and DDGS diets, respectively.

[2]P2 diet = P2 diet effect; Phase-1 (P1) criterion = use of P1 criterion as a covariate for P2; P1 BW = P1 BW as a covariate for P2.

[3]The *b*-values (slope) of P1 criterion covariates were 0.04, 0.17, 0.05, 0.28, 0.11, 0.11, 0.32, 0.20, and 0.05 for C, ether extract, GE, NDF, N, P, S, plasma urea N, and triglycerides, respectively.

[4]The *b*-values (slope) of P1 BW as a covariate were 0.03, –0.07, 0.05, –0.10, 0.10, –0.20, 0.09, 0.07, and 0.60 for C, ether extract, GE, NDF, N, P, S, plasma urea N, and triglycerides, respectively.

[5]P2 data repeat mean of 8 pigs previously fed each P1 diet. Mean digestibility coefficients calculated based on fecal grab samples collected on d 28. Blood collected from pigs on d 28.

P2 (Table 4), digestibility and plasma variables were expected to differ among diets. However, the critical question was whether or not appropriate covariate or covariates could be used to enhance the accuracy of measuring the effects of diet on digestibility and plasma variables. Previously, Brown et al. (1985) used BW and Dean et al. (2005) used initial PUN concentration as a covariate to adjust for variation in particular criteria of interest. Only recently has this been used in nutritional studies in which P1 digestibility values were used to adjust subsequent digestibility values (Urriola and Stein, 2010). Along with using initial BW as a covariate for P2 data, we evaluated the corresponding P1 criteria as a potential covariate to impact P2 dietary outcomes. As shown in Table 5, using P1 criterion as a covariate for P2 was statistically significant for apparent total tract digestibility of GE, NDF, N, S, and PUN ($P \leq 0.05$) and tended to be significant for C and EE ($P = 0.12$ and 0.11, respectively) but was not statistically significant for P digestibility or plasma triacylglycerides. The statistical significance of these covariates, and in several cases the slight numerical reductions in the SE of the treatment mean, indicates that when a previous digestibility criterion is known, the use of a P1 criterion as a covariate improves our ability to identify P2 dietary treatment effects.

There were no differences in P2 criteria due to blocking by P1 diet (Table 4), but in considering this blocking factor, we are considering the use of 24 pigs as the block. However, when considering the P1 × P2 interaction, which in essence considers the use of 8 pigs as a blocking factor, there were 2 statistically significant effects, indicating that the response of pigs to the P2 diet was dependent on the P1 diet. In contrast, when the individual pig was used as a covariate (a blocking factor of 1), 5 variables were statistically significant out of the 9 variables measured: GE, NDF, N, S, and PUN. Overall, this indicates that controlling experimental variation improved our ability to more accurately compare treatment means. In the current experiment, the group of pigs, which were selected, did not vary substantially in age, genetics, sex, and BW and as such, may have decreased our ability to detect the effects of BW, initial diet, or initial criterion on subsequent treatment means. In addition, our diets differed widely in nutrient composition such that in no case did the difference in data analysis affect the comparisons of individual treatment means. Recently, Waguespack et al. (2011) reported that initial PUN was statistically significant for final PUN in 9 of 14 experiments summarized, but the overall treatment effect changed from nonsignificant to significant only in 1 experiment and the individual treatment mean comparisons changed the level of significance only in 3 experiments. We would speculate, however, that with a greater variation in the source of pigs or with smaller differences expected between dietary treatments, balancing subsequent treatments for the initial diet because of potential diet × diet interactions

would be helpful in data interpretation. Furthermore, if the previous diet consumed by the pigs is not known, then using initial digestibility coefficients or plasma analysis would be effective in reducing the variation associated with subsequently obtained data and should be considered as a covariate in future nutrition research.

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