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Evaluation of NE predictions and the impact of feeding maize distillers dried grains with solubles (DDGS) with variable NE content on growth performance and carcass characteristics of growing-finishing pigs



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ABSTRACT

Growing-finishing pigs (n = 432; initial body weight = 22.0 ± 4.3 kg) were utilized to measure growth performance and carcass characteristics when fed 4 sources of maize distillers dried grains with solubles (DDGS) with predicted net energy (NE) content ranging from 2083 to 2743 kcal/kg. Pigs were blocked by initial body weight, and within blocks, pens were randomly allotted to one of four dietary treatments (nine pigs/pen, 12 replicates/treatment). Dietary treatments consisted of four maize and soybean meal based diets containing 40% DDGS from (1) source A with low NE (LOW; 2083 kcal/kg), (2) source B with medium-low NE (ML: 2255 kcal/kg), (3) source C with medium-high NE (MH: 2469 kcal/kg). and (4) source D with high NE (HIGH; 2743 kcal/kg), based on NE estimates provided by a commercial service using a proprietary equation-based system. Experimental diets were formulated to meet or exceed nutrient requirements and contained similar standardized ileal digestible lysine: NE within phases. Overall, ADFI of pigs fed ML was greater (P < 0.05) than for pigs fed MH and HIGH, but not different from LOW, and no differences were observed among LOW, MH, and HIGH. Pigs fed ML had similar ADG with LOW and HIGH, but less (P < 0.05) than that of pigs fed MH, and no differences were observed among LOW, MH, and HIGH. Gain: feed was reduced (P < 0.02) in pigs fed ML compared with other dietary treatments. No treatment differences (P>0.19) were observed in hot carcass weight, carcass yield, backfat depth, loin muscle area, and percentage of carcass fat-free lean among dietary treatments. The NRC (2012) model was used to estimate NE content of diets by matching the model-predicted G:F with the observed G:F. Using NRC (2012) NE content values for maize and soybean meal, NE content was calculated for DDGS sources LOW, ML, MH, and HIGH to be 2377, 1924, 2612, and 2513 kcal/kg, respectively. Predicted NE values from eight identified equations were calculated and compared with model-determined NE

Abbreviations: AA, amino acids; ADF, acid detergent fiber; ADFI, average daily feed intake; ADG, average daily gain; BF, backfat; BW, body weight; CP, crude protein; DDGS, maize distillers dried grains with solubles; DE, digestible energy; DM, dry matter; EE, ether extract; FFL, carcass fat-free lean; GE, gross energy; GF, gain to feed ratio; HCW, hot carcass weight; LMA, loin muscle area; Lys, lysine; ME, metabolizable energy; NDF, neutral detergent fiber; NE, net energy; Pd, protein deposition; PE, prediction error; SID, standardized ileal digestible; STTD, standardized total tract digestible; TDF, total dietary fiber.

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content of the 4 DDGS sources. Gain:feed responses of pigs indicated that the NE content estimates provided by the commercial service were overestimated for sources B and D, and underestimated for the sources A and C. Feeding 40% DDGS with less NE content increased ADFI and reduced ADG and G:F, but carcass traits were not affected when the difference of NE content was less than 700 kcal/kg among DDGS sources or less than 275 kcal/kg among dietary treatments.

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1. Introduction

Maize distillers dried grains with solubles (DDGS) is a co-product of ethanol production that has been used widely in swine diets as a cost effective source of energy and amino acids (AA). Variability in chemical composition, digestible energy (DE) and metabolizable energy (ME) content, and nutrient digestibility has been reported among DDGS sources (Stein and Shurson, 2009; Anderson et al., 2012), and the use of oil extraction procedures by most ethanol plants has further increased the variability in energy and nutrient content among sources (Kerr et al., 2013). However, limited data are available regarding the impact of feeding DDGS with variable net energy (NE) content on the growth and carcass responses of growing-finishing pigs.

The NE system represents the energy requirements of pigs fed high-fiber diets better than the ME system (Noblet et al., 1994). As a result, the NE system is being adopted increasingly in the U.S. to facilitate more efficient use of high-fiber ingredients, such as DDGS, in commercial swine diet formulations. Traditionally, NE of feedstuffs has been determined using comparative slaughter or indirect calorimetry, which are labor intensive and require expensive equipment. Therefore, a relatively low cost, fast, and accurate method is needed to determine the NE content of DDGS sources. Empirical NE equations (Noblet et al., 1994), based on analyzed chemical composition, have been developed for use in complete feed, but have not been validated for use with individual ingredients. More recently, a NE prediction equation (Graham et al., 2014) and a commercial service (ILLUMINATE®; Nutriquest, Mason City, IA) have been developed for rapid and low cost estimation of NE content of DDGS sources, but the accuracy of these methods has not been evaluated. The objectives of this experiment were to determine the growth performance and carcass traits of growing-finishing pigs fed four DDGS sources with variable predicted NE content, and to evaluate the accuracy and precision of using published NE equations and ILLUMINATE® to predict NE content of DDGS sources with variable chemical composition.

2. Materials and methods

All experimental procedures in this study were approved by the University of Minnesota Institutional Animal Care and Use Committee (St. Paul, MN).

2.1. Animals and housing

Barrows (n = 432) were blocked by initial body weight (BW; $22.0\pm4.3\,\mathrm{kg}$) and allotted to 12 blocks (four pens/block; nine pigs/pen). Pigs were housed in an environmentally-controlled growing-finishing facility at the University of Minnesota West Central Research and Outreach Center (Morris, MN). Each pen ($1.60\times4.5\,\mathrm{m}$) consisted of completely slatted, concrete floors, and was equipped with one nipple waterer and one single-sided self-feeder with four feeding spaces. Room temperature of the facility was maintained at about 20 °C. Pigs were allowed ad libitum access to feed and water throughout the experiment. Pigs that showed signs of poor health were treated individually with appropriate medication or removed from the experiment.

2.2. Diets and experimental design

ILLUMINATE® is a proprietary commercial service that uses chemical composition of DDGS sources and equations to estimate DE, ME, NE, and standardized ileal digestible (SID) AA content of the majority of DDGS sources produced by ethanol plants in the U.S. Net energy estimates for DDGS sources provided by ILLUMINATE® were used as the basis for selecting four sources of DDGS with increasing concentrations of predicted NE (as-fed) for this study. The four DDGS sources contained: (1) 2083 kcal NE/kg for source A, (2) 2255 kcal NE/kg for source B, (3) 2469 kcal NE/kg for source C, and (4) 2743 kcal NE/kg for source D. Each source of DDGS and one source of maize were obtained in single lots, and samples were collected and analyzed for chemical composition, which was used in formulating the experimental diets (Table 1). Soybean meal was obtained in multiple lots from the same supplier, and analyzed nutrient composition of a sample obtained from the first lot was used to formulate diets throughout the experiment.

Pens of pigs were allotted randomly to one of four dietary treatments (Tables 2 and 3) in a four-phase feeding program (22–50 kg, 50-75 kg, 75-100 kg, and 100-115 kg BW). Phases were switched on weigh days when average BW of pigs in the pen reached the targeted starting BW ± 2.3 kg of the subsequent phase. Dietary treatments consisted of maize-soybean

Table 1Analyzed nutrient composition and physical characteristics of feed ingredients (as-fed basis).

Item, g/kg	DDGS ^a				Maize	Soybean mea
	A	В	С	D		
DM	874.4	881.8	896.0	890.0	874.3	881.8
CP	258.2	281.7	268.4	270.4	72.5	477.6
Ether extract	107.0	56.1	141.9	159.8	29.0	2.6
Crude fiber	81.5	88.1	85.4	92.9	24.6	35.2
Ash	43.8	52.6	39.1	45.6	11.1	63.5
ADF	162.3	97.0	137.2	117.3	37.5	64.0
NDF	260.3	229.9	281.1	229.8	85.1	71.5
Ca	0.3	0.9	0.2	0.2	0.2	3.4
P	7.5	8.1	6.6	8.1	1.8	5.8
Starch	33.0	75.4	34.6	22.5	619.0	8.8
Particle size, µm	580	390	620	380	-	-
Essential AA, g/kg						
Arg	11.7	12.9	12.4	12.4	3.3	34.6
His	7.7	8.5	8.2	7.8	2.2	13.3
Ile	10.5	11.0	10.8	11.1	2.4	21.9
Leu	29.5	31.6	31.6	32.1	8.2	37.4
Lys	8.4	9.8	9.8	9.0	2.5	31.7
Met	5.2	5.9	5.2	5.0	1.7	6.8
Phe	12.6	13.0	13.2	13.5	3.3	24.1
Thr	10.8	11.3	11.1	11.1	2.7	18.9
Trp	1.8	1.9	1.8	1.9	0.5	6.7
Val	13.3	13.8	13.7	14.0	3.4	22.5
Non-essential AA, g/kg						
Ala	16.9	19.0	18.5	18.6	5.1	20.6
Asp	15.9	18.1	17.1	16.9	5.4	54.5
Cys	4.8	6.0	5.2	5.1	1.6	6.9
Glu	28.5	39.3	34.6	33.1	12.6	85.3
Gly	10.0	11.2	10.6	10.5	2.9	20.2
Hyl	2.6	1.5	2.0	2.5	0.2	0.6
Нур	2.1	0.8	1.0	1.5	0.3	0.4
Orn	0.6	0.6	0.6	0.6	0.1	0.6
Pro	17.1	21.3	19.0	18.7	6.1	24.6
Ser	11.8	12.4	12.2	12.3	3.4	20.7
Tau	0.6	0.7	0.7	0.7	1.1	1.2
Tyr	8.6	9.4	9.1	8.9	1.8	16.7
NE ^b , kcal/kg	2083	2255	2469	2743	2672	2087

^a Selected sources of distillers dried grains with solubles with increasing concentrations of predicted NE estimated by a commercial service (ILLUMINATE®; Nutriquest, Mason City, IA).

meal based diets containing: (1) 40% DDGS source A with low predicted NE (LOW), (2) 40% DDGS source B with medium-low predicted NE (ML), (3) 40% DDGS source C with medium-high predicted NE (MH), and (4) 40% DDGS source D with high predicted NE (HIGH). The predicted NE values of the diets are shown in Tables 2 and 3. Diets were balanced for SID AA and standardized total tract digestible (STTD) P, and were calculated to contain the same SID lysine: NE within phases. The coefficients of AA digestibility for DDGS sources were obtained from equations reported by Almeida et al. (2013) based on analyzed AA composition. Energy values and coefficients for SID AA and STTD P of maize and soybean meal and the coefficient for STTD P of DDGS used in diet formulation were obtained from NRC (2012). All diets met or exceeded the nutrient requirements of growing-finishing pigs, which were estimated using the NRC (2012) model. Model inputs were based on growth performance and lean growth rate of pigs fed maize-soybean meal diets in a similar experiment (Wu et al., 2015) conducted in the same facilities with the same genetic line of pigs. Body weight of individual pigs and feed disappearance in each pen were measured every two weeks (period) to calculate ADG, ADFI, and G:F. Pigs were fed a common maize-soybean meal diet for five days prior to harvest (holding diet; Table 3) in the both harvest groups. Switching to maize-soybean meal diets for this short duration before harvest was done because actual ADFI was greater than predicted ADFI and the supply of each source of DDGS was depleted before the trial concluded. Feed samples were obtained and frozen $(-20 \,^{\circ}\text{C})$ when each batch of feed was mixed, and four samples of each dietary treatment (one sample from each of the four phases) as well as one sample of the holding diet were selected randomly for analysis of nutrient composition.

2.3. Carcass measurements

When the average BW of pigs reached 75 kg and 110 kg, backfat (BF) depth and loin muscle area (LMA) were measured between the 10th and 11th ribs using an ALOKA 500 V real-time ultrasound machine (Corometrics Medical Systems, Walling-

^b Predicted NE values from ILLUMINATE® for DDGS sources and recommended NE values from NRC (2012) for maize and soybean meal (dehulled, solvent extracted).

Table 2 Diet composition, phase 1 and 2 (as-fed basis).

Item	Phase 1 (2)	2-50 kg BW)			Phase 2 (50–75 kg BW)					
	LOW ^a	ML ^a	MHª	HIGHa	LOW	ML	MH	HIGH		
Ingredients, g/kg										
Maize	364.2	364.1	364.0	364.1	441.0	441.0	440.9	441.0		
Soybean meal	205.9	205.9	205.9	205.9	135.2	135.2	135.2	135.2		
DDGS	400.0	400.0	400.0	400.0	400.0	400.0	400.0	400.0		
Limestone	16.2	16.0	15.5	16.2	14.0	14.5	13.0	13.9		
Monocalcium P (210 g/kg P)	5.1	4.8	5.7	3.7	1.7	1.7	2.8	0.8		
Salt	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0		
VTM premix ^b	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5		
L-Lys HCl	2.1	1.7	2.2	3.3	1.5	1.0	1.4	2.4		
DL-Met	_	_	_	0.1	_	_	_	_		
L-Thr	_	0.9	_	_	_	_	_	_		
L-Trp	_	0.1	0.2	0.2	0.1	0.1	0.2	0.2		
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0		
Calculated composition, g/kg										
NE ^c , kcal/kg	2176	2246	2331	2445	2236	2303	2390	2503		
CP	230.1	239.8	234.3	236.3	201.4	210.4	205.4	207.2		
Ca	7.9	8.0	7.7	7.6	6.2	6.7	6.0	6.0		
Total P	5.9	6.1	5.7	5.9	4.9	5.2	4.8	5.0		
STTD ^d P	3.6	3.7	3.5	3.5	2.8	3.0	2.8	2.8		
Ca: STTD P	21.4	21.1	21.4	21.1	21.4	21.6	20.7	20.7		
Total Lys SID ^e AA	12.5	12.7	13.1	13.7	10.0	10.1	10.4	10.9		
Lys	10.4	10.7	11.1	11.6	8.1	8.4	8.7	9.0		
Met	3.4	3.8	3.4	3.4	3.0	3.5	3.1	2.9		
Met + Cys	6.3	7.4	6.7	6.6	5.7	6.8	6.0	5.9		
Thr	7.3	8.6	7.5	7.6	6.3	6.8	6.6	6.6		
Trp	1.8	1.8	1.9	2.0	1.4	1.5	1.5	1.6		
SID Lys/NE, g/kcal	47.8	47.6	47.6	47.4	36.2	36.5	36.4	36.0		
Analyzed composition, g/kg										
DM	873.8	875.9	876.7	879.6	870.6	869.4	873.5	875.6		
CP	234.2	239.8	234.0	240.7	198.9	210.0	207.6	207.7		
Ether extract	47.6	28.4	58.4	62.6	50.4	30.4	59.3	67.3		
Crude fiber	48.6	47.2	48.1	43.7	48.9	45.6	49.7	47.0		
ADF	85.0	60.9	76.3	74.0	83.3	60.4	67.3	68.3		
NDF	142.2	139.7	159.2	144.0	149.6	141.6	162.8	141.7		
Ca	8.5	7.1	7.3	7.9	7.5	5.6	5.7	5.5		
P	6.7	6.1	5.7	5.6	5.0	05.1	4.8	5.1		
AA	12.0	11.2	12.7	12.4	0.7	10.0	0.4	6.0		
Lys	12.9	11.2	12.7	13.4	9.7	10.0	9.4	9.9		
Thr	9.4	9.3	9.4	8.9	8.0	7.9	7.5	7.6		
Trp	2.4	2.7	2.6	2.6	2.1	2.1	2.2	2.3		
Met	4.2	3.8	3.7	4.0	3.6	3.9	3.4	3.1		

^a LOW = diet containing 40% distillers dried grains with solubles (DDGS) source A with low predicted NE (2083 kcal/kg); ML = diet containing 40% DDGS source B with medium-low predicted NE (2255 kcal/kg); MH = diet containing 40% DDGS source C with medium-high predicted NE (2469 kcal/kg); and HIGH = diet containing 40% DDGS source D with high predicted NE (2743 kcal/kg).

ford, CT) by a certified technician. Pigs were slaughtered in two groups, with the heavier pigs from blocks one through six slaughtered first, followed by pigs from blocks seven through 12 slaughtered 11 days later. For each harvest group, after ultrasound measurements were obtained, final BW was determined and pigs were tattooed individually and transported to a commercial abattoir (Hormel Foods; Austin, MN). Hot carcass weight (HCW) was recorded at harvest and was used to calculate carcass yield using the equation: carcass yield, $\% = \text{HCW/final BW} \times 100$. Carcasses of 14 pigs were trimmed during USDA inspection, and as a result, their HCW data were removed from the data set used in the analysis. Percentage of carcass fat free lean (FFL%) was calculated using: FFL% = {[2.620 + (0.456 × sex of pig) - (3.358 × 10th rib BF depth, cm) + (0.306 × 10th rib LMA, cm²) + (0.401 × HCW, kg)]/HCW, kg} × 100, where sex of pig is defined as barrow = 1 and gilt = 2 (NPPC, 2000).

^b VTM premix = vitamin-trace mineral premix, which provided the following nutrients per kg of diet: 8818 IU vitamin A, 1654 IU vitamin D₃, 33 IU vitamin E, 3.3 mg vitamin K, 5.5 mg riboflavin, 33.1 mg niacin, 22.0 mg pantothenic acid, 0.03 mg vitamin B₁₂, 0.3 mg iodine as ethylenediamine dihydroiodide, 0.3 mg selenium as sodium selenite, 55.1 mg zinc as zinc oxide, 33.1 iron as ferrous sulfate, 5.5 mg manganese as manganous oxide, and 3.9 mg copper as copper sulfate.

^c Calculated NE content of diets based on diet formulation; NRC (2012) recommended NE values were used for corn and soybean meal (dehulled, solvent extracted), and NE estimates from ILLUMINATE® (Nutriquest, Mason City, IA) were used for DDGS sources.

d STTD = standardized total tract digestible.

^e SID = standardized ileal digestible. Coefficients for AA digestibility were determined by equations from Almeida et al. (2013) for DDGS, and NRC (2012) recommended coefficients were used for maize and soybean meal.

Table 3 Diet composition, phase 3 and 4 (as-fed basis).

Item	Phase 3 (75-100 kg BW	/)		Phase 4 (100-115 kg BW)					
	LOWa	MLa	MHª	HIGHa	LOW	ML	MH	HIGH	Holding	
Ingredients, g/kg										
Maize	477.7	477.6	477.7	477.6	495.7	495.8	495.8	495.8	807.5	
Soybean meal	100.1	100.1	100.1	100.1	82.5	82.5	82.5	82.5	167.2	
DDGS	400	400	400	400	400	400	400	400	_	
Limestone	13.4	13.9	12.9	13.8	13.4	13.8	12.7	13.8	9.6	
Monocalcium P (210 g/kg P)	1.0	1.2	1.7	_	1.1	1.3	2.1	0.1	9.2	
Salt	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	
VTM premix ^b	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	
L-Lys HCl	1.2	0.6	0.9	1.8	0.7	_	0.3	1.2	_	
L-Trp	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.1	_	
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	
Calculated composition, g/kg										
NE ^c , kcal/kg	2262	2328	2415	2528	2272	2339	2426	2538	2403	
CP	187	195.8	190.8	192.5	179.3	188.2	183.2	184.8	138.4	
Ca	5.7	6.2	5.6	5.7	5.7	6.2	5.6	5.6	5.8	
Total P	4.7	4.9	4.5	4.7	4.6	4.9	4.5	4.6	4.4	
STTD ^d P	2.6	2.8	2.5	2.6	2.6	2.8	2.5	2.5	2.7	
Ca: STTD P	21.6	21.8	22	21.6	21.6	21.8	22.0	22.0	21.5	
Total Lys	8.7	8.8	9.0	9.4	7.7	7.8	8.0	8.4	7.3	
SIDeAA										
Lys	6.9	7.1	7.4	7.7	6.0	6.2	6.4	6.7	6.2	
Met	2.9	3.3	2.9	2.8	2.8	3.2	2.8	2.7	2.1	
Met + Cys	5.4	6.4	5.7	5.5	5.2	6.3	5.6	5.4	4.1	
Thr	5.8	6.3	6.1	6.1	5.6	6	5.8	5.8	4.4	
Trp	1.2	1.3	1.3	1.4	1.1	1.1	1.2	1.2	1.4	
SID Lys/NE, g/kcal	3.05	3.05	3.06	3.05	2.64	2.65	2.64	2.64	2.58	
Analyzed composition, g/kg										
DM	871.2	871.9	876.3	876.0	867.5	876.4	875.1	872.9	874.1	
CP	187.0	192.3	190.0	190.3	170.6	191.5	187.2	180.3	121.3	
Ether extract	49.9	30.4	59.3	66.8	50.9	33.1	60.9	67.6	21.4	
Crude fiber	50.4	45.3	45.8	43.9	47.1	43.7	44.9	43.7	46.3	
ADF	82.6	55.6	72.0	71.9	82.8	58.9	70.3	65.3	34.0	
NDF	164.3	141.9	165.5	150.2	162.1	139.7	162.9	140.8	91.7	
Ca	6.4	8.0	6.3	5.2	7.7	6.6	5.8	6.4	5.6	
P	4.6	4.9	4.7	4.8	4.5	6.0	4.1	5.0	3.7	
AA										
Lys	8.1	8.5	8.2	9.6	7.4	7.8	7.9	8.7	7.2	
Thr	7.1	7.6	7.3	7.6	6.8	7.4	7.1	7.3	5.1	
Trp	2.1	2.1	2.1	2.2	2.0	1.9	1.8	1.9	1.5	
Met	3.3	3.6	3.2	3.6	3.3	3.6	3.3	3.4	2.2	

^a LOW = diet containing 40% distillers dried grains with solubles (DDGS) source A with low predicted NE (2083 kcal/kg); ML = diet containing 40% DDGS source B with medium-low predicted NE (2255 kcal/kg); MH = diet containing 40% DDGS source C with medium-high predicted NE (2469 kcal/kg); HIGH = diet containing 40% DDGS source D with high predicted NE (2743 kcal/kg); and Holding = maize-soybean meal diet fed to pigs 5 days prior to harvest due to depletion of DDGS.

2.4. Chemical analysis

Six feed ingredient samples (four sources of DDGS, one source of maize, one source of soybean meal) and 17 complete diets were analyzed for nutrient composition at University of Missouri Agricultural Experiment Station Chemical Laboratory (Columbia, MO). Standard procedures of AOAC International (2006) were followed for analysis of moisture (Method 934.01), crude protein (CP; Method 990.03), ether extract (EE; Method 920.39), crude fiber (Method 978.10), acid detergent fiber (ADF; Method 973.18), neutral detergent fiber (NDF; Holst, 1973), Ca and P (Method 985.01), AA profile (Method 982.30), and starch content (AACC International, 1995; Approved Methods, No. 76-13).

^b VTM premix = vitamin-trace mineral premix, which provided the following nutrients per kg of diet: 8818 IU vitamin A, 1654 IU vitamin D3, 33 IU vitamin E, 3.3 mg vitamin K, 5.5 mg riboflavin, 33.1 mg niacin, 22.0 mg pantothenic acid, 0.03 mg vitamin B12, 0.3 mg iodine as ethylenediaminedihydroiodide, 0.3 mg selenium as sodium selenite, 55.1 mg zinc as zinc oxide, 33.1 iron as ferrous sulfate, 5.5 mg manganese as manganous oxide, and 3.9 mg copper as copper sulfate.

^c Calculated NE content of diets based on diet formulation; NRC (2012) recommended NE values were used for corn and soybean meal (dehulled, solvent extracted), and NE estimates from ILLUMINATE® (Nutriquest, Mason City, IA) were used for DDGS sources.

^d STTD = standardized total tract digestible.

^e SID = standardized ileal digestible. Coefficients for AA digestibility were determined by equations from Almeida et al. (2013) for DDGS, and NRC (2012) recommended coefficients were used for maize and soybean meal.

2.5. Energy determination of DDGS

Gross energy (GE) of DDGS was determined using bomb calorimetry (Model 1281, Parr Instrument Co., Moline, IL). Digestible energy and ME of each DDGS source were obtained using equations: $DE = -2161 + (1.39 \times GE) - (20.7 \times NDF) - (49.3 \times EE)$ and $ME = -261 + (1.05 \times DE) - (7.89 \times CP) + (2.47 \times NDF) - (4.99 \times EE)$ from Anderson et al. (2012), which were evaluated and validated by Urriola et al. (2014) and Wu et al. (2015).

Estimates of NE concentration for each DDGS source were calculated using overall G:F responses observed and the NRC (2012) growth model as follows:

2.5.1. Step 1

Standard growth potential (growth curve) of pigs used in the present experiment was defined using the "User observed intake as model input" option, which was based on observed overall ADFI (2.721 kg/d) and initial and final BW (39.2 and 122.7 kg, respectively) of 12 pens of pigs (n = 108) fed maize-soybean meal control diets in a previous experiment (Wu et al., 2015). This previous experiment was conducted in the same facility with the same genetics, gender, and a similar feeding program and environment conditions used in the current study. Dietary NE of the control diet from the previous experiment was calculated based on diet formulation and NE values for maize (2672 kcal/kg) and soybean meal (2087 kcal/kg) from NRC (2012).

2.5.2. Step 2

The whole body protein deposition (Pd) was defined using the "Specify mean Pd and gender" option, which was based on observed carcass composition of pigs fed the maize-soybean meal control diets in the previous experiment, conducted in the same facilities and under similar conditions. Mean Pd rate was calculated using the following equations suggested by NPPC (2000):

Initial FFL, $kg = (0.418 \times initial BW, kg) - 1.656$

Final FFL,
$$kg = 2.620 + (0.456 \times sex of pig) - (3.358 \times 10th rib BF depth, cm) + (0.306 \times 10th rib LMA, cm2) + (0.401 \times HCW, kg), where sex of pig is defined as barrow = 1 and gilt = 2$$

Lean gain, kg/d = (final FFL, kg-initial FFL, kg)/days from initial to final

$$Pd, g/day = (lean gain, g/day)/2.55$$

2.5.3. Step 3

For each feeding period (two wk; six periods total) in the present experiment, NE content of a dietary treatment was obtained by adjusting dietary NE inputs until G:F predicted by the model matched the observed G:F. Analyzed least-squares means of BW and G:F of pigs fed each dietary treatment were used in this calculation.

2.5.4. Step 4

Based on the assumption that maize, soybean meal, and DDGS were the only energy-containing ingredients in the diets, NE content of DDGS was determined by subtracting NE of maize and soybean meal derived from NRC (2012) from the dietary NE and adjusting for the percentage (40%) of DDGS in the diet. Finally, the mean NE content of DDGS was determined by calculating the average among the six periods weighted for total feed consumption in each period.

2.6. Evaluation of NE predictions

Predicted NE of each DDGS source was calculated using Eq. (4), (5), (7), (8), (9), (10), and (11) from Noblet et al. (1994; energy expressed as kcal/kg and composition expressed as g/kg DM):

$$NE = (0.703 \times DE) + (1.58 \times EE) + (0.47 \times starch) - (0.97 \times CP) - (0.98 \times crudefiber)$$
 (4)

$$NE = (0.700 \times DE) + (1.61 \times EE) + (0.48 \times starch) - (0.91 \times CP) - (0.87 \times ADF)$$
(5)

$$NE = (0.730 \times ME) + (1.31 \times EE) + (0.37 \times starch) - (0.67 \times CP) - (0.97 \times crudefiber)$$
 (7)

$$NE = (0.726 \times ME) + (1.33 \times EE) + (0.39 \times starch) - (0.62 \times CP) - (0.83 \times ADF)$$
(8)

$$NE = 2796 + (4.15 \times EE) + (0.81 \times starch) - (7.07 \times ash) - (5.38 \times crudefiber)$$
 (9)

$$NE = 2790 + (4.12 \times EE) + (0.81 \times starch) - (6.65 \times ash) - (4.72 \times ADF)$$
(10)

Table 4Effects of dietary treatment and feeding period on growth performance of growing-finishing pigs.

Source of variation, P-value	BW	ADFI	ADG	G:F
Diet Period	0.85	0.05	0.12	< 0.01
Period	< 0.01	< 0.01	<0.01	< 0.01
$Diet \times period^a$	<0.01	<0.01	0.07	0.48

^a Interactive effect of dietary treatment and feeding phase analyzed as repeated measures in phases.

$$NE = 2875 + (4.38 \times EE) + (0.67 \times starch) - (5.50 \times ash) - [2.01 \times (NDF - ADF)] - (4.02 \times ADF), \tag{11}$$

and also the equation from Graham et al. (2014; energy expressed as kcal/kg and composition expressed as% DM):

$$NE = (115.011 \times EE) + 1501.01$$

Net energy estimates from prediction equations and ILLUMINATE® were compared with the estimated NE content of DDGS determined using steps one to four previously described. Prediction error (PE) and bias were calculated using the following equations adapted from Urriola et al. (2014):

$$PE = \sqrt[2]{\frac{1}{n} \sum_{i=1}^{n} (yi - \widehat{y}i)^2}$$

And

$$Bias = \frac{1}{n} \sum_{i=1}^{n} (y_i - \hat{y}_i)$$

where y_i is the predicted value for the ith observation, \hat{y}_i is the determined (model-calculated) value for the ith observation, and n is the total number of observations (Lane et al., 2014). We assumed that the NE estimates derived from the current experiment using steps one to four were the most accurate estimates of actual NE content of the DDGS sources and therefore, served as the basis for the comparison.

2.7. Statistical analysis

All analyses were conducted using the MIXED procedure (SAS Inst. Inc., Cary, NC) in a randomized complete block design. Pen served as the experimental unit for all data analyses. Growth performance data of each period were analyzed, and overall ADFI, ADG, and G:F were generated using a statistical model that included dietary treatment as a fixed effect and block as a random effect with repeated measures in time. For analysis of carcass characteristics, dietary treatment was a fixed effect and block was a random effect. Body weight was measured during the same week when ultrasound measurement was performed, and was used as a covariate to adjust BF depth, LMA, and FFL% if the covariate effect was significant (P < 0.05). Means were reported as least-squares means and were separated by the PDIFF option when P < 0.05, and trends are reported when 0.05 < P < 0.10.

3. Results

3.1. Growth performance and carcass composition

Six pigs (one, three, and two pigs from LOW, ML, and MH treatments, respectively) were removed from the study because of death or poor health. A dietary treatment \times period interaction (P < 0.01) was observed for BW (Table 4). However, no treatment differences were detected for BW in any period or for final BW.

There was also a dietary treatment \times period interaction (P<0.01) for ADFI. In periods one to three, pigs had similar ADFI regardless of dietary treatment. However in period four, ADFI of pigs fed ML was greater (P<0.05) than that of pigs fed MH and HIGH, but not different from LOW, and ADFI of pigs fed LOW was similar to that of pigs fed MH but greater (P<0.05) than pigs fed HIGH (Table 5). Pigs fed ML had a greater (P<0.05) ADFI compared with other dietary treatments in period five and six, and no differences were observed among pigs fed LOW, MH, and HIGH diets. Overall, ADFI of pigs fed ML was greater (P<0.05) than that of pigs fed MH and HIGH, but was not different from pigs fed LOW. No differences in overall ADFI were observed among LOW, MH, and HIGH treatments.

A tendency (P=0.075) for a dietary treatment × period interaction was observed for ADG. In period 1, ADG of pigs fed ML was lower (P<0.05) than that of pigs fed MH, but was not different from LOW and HIGH, and no differences were observed among pigs fed LOW, MH, and HIGH. Pigs fed LOW had a greater (P<0.05) ADG than those fed ML and HIGH, but were not different from MH in period two, and no differences were found among pigs fed ML, MH, and HIGH. In period three, ADG of pigs fed LOW was similar compared with pigs fed MH and HIGH, but was greater (P<0.05) than ML, and no differences

Table 5Effects of feeding growing-finishing pig diets containing 40% distillers dried grains with solubles (DDGS) with variable NE content on growth performance.

Item	40% DDGS	SEM			
	LOW ^d	ML^d	MH ^d	HIGHd	
No. Pens	12	12	12	12	
BW, kg					
Initial	22	22	21.9	21.9	1.98
Period 1	44.5	43.3	44.8	44.5	2.07
Period 2	58.5	56.4	58.7	57.6	2.15
Period 3	72.8	70	72.9	71.5	2.19
Period 4	87.2	83.5	86.7	85.2	2.28
Period 5	99	95.3	98.8	97.2	2.31
Final	112.2	109.6	112.7	110.8	2.2
ADFI, kg/d					
Period 1	1.54	1.54	1.49	1.49	0.06
Period 2	2.27	2.31	2.23	2.17	0.06
Period 3	2.69	2.7	2.56	2.52	0.06
Period 4	2.82 ^{a,b}	2.87ª	2.68 ^{b,c}	2.63 ^c	0.06
Period 5	2.78 ^b	2.97ª	2.71 ^b	2.67 ^b	0.06
Period 6	3.06 ^b	3.38ª	3.03 ^b	2.98 ^b	0.07
Overall	2.53 ^{a,b}	2.63ª	2.45 ^b	2.41 ^b	0.06
ADG, kg/d					
Period 1	0.81 ^{a,b}	0.76 ^b	0.82^{a}	0.81 ^{a,b}	0.02
Period 2	0.99ª	0.94 ^b	0.99 ^{a,b}	0.94 ^b	0.02
Period 3	1.04ª	0.97 ^b	1.01 ^{a,b}	0.99 ^{a,b}	0.02
Period 4	1.03ª	0.97 ^b	0.99 ^{a,b}	0.97 ^b	0.02
Period 5	0.87	0.84	0.86	0.86	0.02
Period 6	0.88€	0.95 ^{a,b}	0.97ª	0.89 ^{b,c}	0.03
Overall	0.93 ^{a,b}	0.90 ^b	0,94ª	0.91 ^{a,b}	0.01
G:F					
Period 1	0.524 ^b	0.498 ^c	0.552^{a}	0.545 ^{a,b}	0.008
Period 2	0.440 ^a	0.410 ^b	0.448ª	0.437ª	0.008
Period 3	0.388ª	0.362 ^b	0,401 ^a	0.397ª	0.008
Period 4	0.365ª	0.337 ^b	0.368 ^a	0.370 ^a	0.008
Period 5	0.305ª	0.282 ^b	0,319 ^a	0.323ª	0.008
Period 6	0.28	0.277	0.303	0.294	0.01
Overall	0.384 ^a	0.361 ^b	0.398 ^a	0.394 ^a	0.006

- ^a Means with different superscripts within a row differ (P < 0.05).
- ^b Means with different superscripts within a row differ (P < 0.05).
- ^c Means with different superscripts within a row differ (P < 0.05).

were observed among ML, MH, and HIGH. In period four, the results followed the same pattern as that for period two. No treatment differences in ADG were observed in period five. In period six, ADG of pigs fed MH was greater (P < 0.05) than LOW and HIGH but not different from ML, and ADG of pigs fed ML was similar with those fed MH and HIGH, but greater (P < 0.05) than LOW. No difference was observed between pigs fed LOW and HIGH in period six. Overall, ADG of pigs fed ML was less (P < 0.05) than for pigs fed MH, and tended (P < 0.09) to be less than that of pigs fed LOW, but was not different from pigs fed HIGH. No differences in ADG were observed among pigs fed LOW, MH, and HIGH over the entire growing-finishing period. No dietary treatment \times period interaction (P = 0.48) was observed for G:F. The overall G:F of pigs fed LOW, MH, and HIGH were not different, but were higher (P < 0.05) than that of pigs fed ML.

Hot carcass weight, carcass yield, and FFL% were not different among all dietary treatments (Table 6). No treatment differences were observed for BF depth or LMA at the end of the growing phase (average BW of 75 kg) or the end of the finishing phase (average BW of 110 kg). Pigs had a similar amount of increased BF depth and LMA between the two ultrasonic measurements.

3.2. Model calculations and equation evaluation

Based on the NRC (2012) model calculations using observed G:F responses in this experiment, NE concentration was lower in DDGS source B compared with other DDGS sources (Table 7). In contrast, DDGS source C contained the greatest NE content among the four sources, which was 688 kcal/kg higher than the DDGS source B, while sources D and A had the second and third highest NE values, which were 589 and 453 kcal/kg, respectively, greater than that of the DDGS source B.

Prediction of NE content from ILLUMINATE® resulted in the least bias and a moderate PE (>200 and <300 kcal/kg, respectively). Among the Noblet et al. (1994) equations, estimates from Eq. (9) had the least PE with a moderate bias (>100 and <200 kcal/kg, respectively), while using Eq. (4), (5), (7), and (8) resulted in relatively low PE and biases compared with esti-

d LOW = diet containing 40% distillers dried grains with solubles (DDGS) source A with low predicted NE (2083 kcal/kg); ML = diet containing 40% DDGS source B with medium-low predicted NE (2,255 kcal/kg); MH = diet containing 40% DDGS source C with medium-high predicted NE (2469 kcal/kg); and HIGH = diet containing 40% DDGS source D with high predicted NE (2743 kcal/kg).

Table 6Effects of feeding growing-finishing pig diets containing 40% distillers dried grains with solubles (DDGS) with variable NE content on carcass characteristics.

Item	40% DDGS		SEM	<i>P</i> -value		
	LOW ^a	ML ^a	MH ^a	HIGHa		
HCW, kg	76.7	75.05	76.82	75.56	1.08	0.59
Carcass yield, %	69.57	69.51	69.77	69.43	0.19	0.63
BF depth ^b , (75 kg), mm	12.03	11.79	12.12	12.49	0.3	0.19
BF depth ^c (109 kg), mm	16.48	16.34	16.71	17.06	0.35	0.34
LM area ^b (75kg), cm ²	30.2	30.05	30.69	30.8	0.84	0.35
LM area ^c (109 kg), cm ²	43.35	44.32	44.26	44.47	0.47	0.31
Fat-free lean ⁵ , %	54.35	54.8	54.52	54.54	0.27	0.65

^a LOW = diet containing 40% distillers dried grains with solubles (DDGS) source A with low predicted NE (2083 kcal/kg); ML = diet containing 40% DDGS source B with medium-low predicted NE (2255 kcal/kg); MH = diet containing 40% DDGS source C with medium-high predicted NE (2469 kcal/kg); and HIGH = diet containing 40% DDGS source D with high predicted NE (2743 kcal/kg).

mates using Eq. (10) and (11). The Graham et al. (2014) equation generated NE estimates that had the greatest PE and bias compared with other predictions.

4. Discussion

4.1. Growth performance and carcass characteristics

Several studies have been conducted to investigate growth responses of pigs fed diets containing variable energy concentrations. Beaulieu et al. (2009) observed a linear decrease in ADFI and improved G:F of growing pigs when increasing the DE density of diets through changes in dietary composition, but inconsistent responses (increased in Exp. 1, but not changed in Exp. 2) in ADG were observed. Quiniou and Noblet (2012) reported a linear reduction in ADFI and increased ADG and G:F in pigs fed diets with increased concentration of dietary NE from 1935 to 2651 kcal/kg. In the present study, ADFI, ADG, and G:F did not differ among pigs fed LOW, MH, and HIGH diets with a maximum calculated difference of 94 kcal/kg in dietary NE content (maximum difference of 235 kcal/kg for NE among DDGS sources A, C, and D; Table 7). It appears that feeding DDGS sources with NE content greater than 2300 kcal/kg, may result in similar growth performance of growing-finishing pigs, but reduced G:F can be expected when DDGS contains less than 2000 kcal/kg NE. In addition, dietary fiber concentration may have also affected ADFI, because increased bulkiness of fiber may limit the physical gut capacity of pigs to consume more feed. However, the ability of pigs to maintain energy intake from fiber-rich diets appears to be related to their physiological age (Kennelly and Aherne, 1980). Studies have reported reduced ADFI of pigs fed diets containing 40% or greater levels of DDGS in early grower feeding phases (Hardman, 2013; Wu et al., 2015). Therefore, it is likely that the "gut fill" effect of dietary DDGS may have prevented pigs fed ML to overcome the negative impact of low NE on ADG by increasing feed intake in periods one to three. However, pigs fed ML were able to maintain greater ADFI and ADG than pigs fed other DDGS diets in the late finisher feeding periods when gut capacity of pigs was increased with increased BW. This finding may explain the dietary treatment × period interactions observed for ADFI and ADG.

Studies have shown less prominent effects of variable dietary NE content on carcass characteristics compared with growth performance criteria. Kerr et al. (2003) reported that pigs had similar HCW, LMA, 10th rib BF thickness, and FFL% when fed diets with a difference of about 100 kcal/kg (as-fed) in dietary NE content. Quiniou and Noblet (2012) also reported that HCW, BF thickness, and carcass yield were not affected when differences in dietary NE were less than 286 kcal/kg. In the present study, although overall ADG responses varied among treatments, pigs fed DDGS sources with up to a 688 kcal/kg difference in NE content, resulting in about a 275 kcal/kg difference in dietary NE, had no discernible differences in HCW, carcass yield, BF depth, LMA, and FFL%. The use of the NRC (2012) model to calculate dietary NE using observed G:F responses was based on the assumption that the effect of variable NE intake on G:F was not affected by the differences in deposition of energy in carcass fat or lean. This assumption was tested by comparing the two sets of body composition data measured using ultrasound at the end of the grower and finisher periods, which showed similar increases in BF depth and LMA among dietary treatments.

4.2. Net energy content of DDGS

To achieve our goal of obtaining DDGS sources with variable NE content, we chose to use the predicted NE content of DDGS sources provided by ILLUMINATE® (Table 7). According to the ILLUMINATE® estimates, NE content of DDGS sources A, B, C, and D gradually increased with an interval of about 220 kcal/kg. When included in diets at 40%, final dietary NE concentration increased by about 90 kcal/kg with each DDGS source in the progression of increasing NE content estimates. We hypothesized that if the NE values were predicted precisely, pigs would respond with a linear decrease in ADFI and

b Backfat depth or LM area measured by real-time ultrasound at the end of growing phase (average BW = 75 kg). Body weight measured at the end of growing phase was used as a covariate in the statistical analysis.

^c Backfat depth or LM area measured by real-time ultrasound at the end of finishing phase (average BW = 109 kg). Final BW was used as a covariate in the statistical analysis.

 Table 7

 Calculation and evaluation of predicted energy content for 4 sources of dietary distillers dried grains with solubles (DDGS; as-fed basis).

Item	Equation	Aª	Ba	Ca	Da	PEb	Bias
GE ^c , kcal/kg	-	4578	4406	4814	4809	-	-
DEd, kcal/kg	$-2161 + (1.39 \times GE) - (20.7 \times NDF) - (49.3 \times etherextract)$	3408	3466	3473	3498	_	_
ME ^d , kcal/kg	$-261 + (1.05 \times DE) - (7.89 \times CP) + (2.47 \times NDF) - (4.99 \times etherextract)$	3157	3215	3200	3204	-	-
NE, kcal/kg							
Model calculation ^e	-	2377	1924	2612	2513	_	_
ILLUMINATE®	-	2083	2255	2469	2743	259.2	31.2
Noblet et al. (1994) ^f							
Equation 4	$(0.703 \times DE) + (1.58 \times ether extract) + (0.47 \times starch) - (0.97 \times CP) - (0.98 \times crude fiber)$	2246	2193	2335	2366	216.7	-71.1
Equation 5	$(0.700 \times DE) + (1.61 \times ether \ extract + (0.48 \times starch) - (0.91 \times CP) - (0.87 \times ADF)$	2194	2204	2309	2366	237.2	-88.1
Equation 7	$(0.730 \times ME) + (1.31 \times ether \ extract) + (0.37 \times starch) - (0.67 \times CP) - (0.97 \times crude \ fiber)$	2202	2168	2269	2284	255.0	-125.4
Equation 8	$(0.726 \times ME) + (1.33 \times ether extract) + (0.39 \times starch) - (0.62 \times CP) - (0.83 \times ADF)$	2149	2177	2242	2281	276.7	-144.0
Equation 9	$2796 + (4.15 \times \text{ether extract}) + (0.81 \times \text{starch}) - (7.07 \times \text{ash}) - (5.38 \times \text{crude fiber})$	2161	1900	2381	2344	179.5	-159.8
Equation 10	$2790 + (4.12 \times \text{ether extract}) + (0.81 \times \text{starch}) - (6.65 \times \text{ash}) - (4.72 \times \text{ADF})$	1844	1931	2199	2299	353.6	-288.0
Equation 11	$2875 + (4.38 \times \text{ether extract}) + (0.67 \times \text{starch}) - (5.50 \times \text{ash}) - [2.01 \times (\text{NDF} - \text{ADF})] - (4.02 \times \text{ADF})$	1909	1874	2160	2322	339.6	-290.0
Graham et al. (2014g							
NE equation	(115.011 × ether extract) + 1501.01	2543	1969	2977	3174	387.0	309.3

^a Sources of DDGS selected based on NE estimates from a commercial service (ILLUMINATE®; Nutriquest, Mason City, IA).

^b Prediction error.

^c Determined GE using bomb calorimetry.

d Anderson et al. (2012); Energy values expressed as kcal/kg and composition expressed as % DM.

^e Back-calculated NE using the NRC (2012) growth model based on observed G:F.

f Energy values expressed as kcal/kg and composition expressed as g/kg DM.

g Energy values expressed as kcal/kg and composition expressed as % DM.

linear increase in G:F when fed LOW, ML, MH, and HIGH treatments, respectively. However, we observed increased ADFI and reduced G:F from pigs fed ML, but similar growth responses among pigs fed LOW, MH, and HIGH. Based on these results, our NRC (2012) model calculations suggest that the ILLUMINATE® NE estimates for DDGS sources B and D were overestimated slightly by 331 and 230 kcal/kg, respectively, and NE estimates of DDGS sources A and C were underestimated slightly by 294 and 143 kcal/kg, respectively. Nevertheless, the ILLUMINATE® NE prediction still resulted in the lowest prediction bias and a moderate prediction error compared with other approaches to estimate NE (Table 7).

Compared with published values from NRC (2012), NE content of DDGS sources C and D determined by the NRC (2012) model calculation in this experiment, were greater than the value (2384 kcal/kg) for DDGS with >100 g/kg oil, and the NE content of DDGS source B was lower compared with the value (2009 kcal/kg) for DDGS with <40 g/kg oil. Gutierrez et al. (2014) determined NE content of two DDGS sources using the comparative slaughter method, and reported that the low-oil DDGS source with 26 g/kg EE contained less NE content (1860 kcal/kg) than DDGS source B, and NE concentration of their conventional DDGS source (2187 kcal/kg) with 130 g/kg EE was also lower compared with DDGS sources A, C, and D evaluated in the present study. Similarly, NE content of six DDGS sources (ranging from 2012 to 2298 kcal/kg) determined by Kerr et al. (2015) using the dual energy X-ray absorptiometry method were lower than the NE values for DDGS sources A, C, and D, but was slightly greater than source B. In addition, Graham et al. (2014) also reported a large variation in NE values among five DDGS sources (ranging from 2122 to 2893 kcal/kg), which were slightly greater than the range in NE content among DDGS sources evaluated in the current study. These observations indicate that there is considerable variability of NE content among DDGS sources, and use of different methodologies may also contribute to this variation. However, there is increased risk of inaccurate diet formulation if static NE loading values are used when formulating diets containing DDGS.

To compare the NE estimations, we determined precision (measured by PE) which refers to the repeatability of an equation for different observations, and accuracy (measured by prediction bias) which refers to the proximity of predicted estimates to the true or observed values. Among the Noblet et al. (1994) equations, precision and accuracy were improved when using DE content (Eq. (4) and (5)) as a predictor variable compared with using ME content (Eq. (7) and (8)). This result is mainly explained by the accumulation of error associated with using predicted DE value in the calculation of ME content. In addition, if predicting NE of DDGS directly from chemical composition, crude fiber (Eq. (9)) may be a better predictor variable than ADF and NDF content (Eq. (10) and (11), respectively). However, the Noblet et al. (1994) equations were derived from complete feeds consisting of ingredients with high starch and low fiber concentrations. Therefore, these equations may not sufficiently consider the variable characteristics of dietary fiber and composition of lipids (Noblet et al., 1994), which may have caused the underestimation of NE for DDGS sources C and D. Finally, although the equation developed by Graham et al. (2014) is designed for use in DDGS sources, it is based on the EE concentration of DDGS and results in substantially large PE and bias, which suggests that using EE as the only predictor variable does not adequately estimate NE content of DDGS.

5. Conclusions

Growing-finishing pigs fed diets containing 40% DDGS with lower NE content are likely to have increased ADFI and reduced ADG and G:F, but differences in carcass characteristics cannot be detected when the difference of NE content is less than 700 kcal/kg among DDGS sources (approximately 275 kcal/kg difference in dietary NE). In addition, current NE prediction equations and commercial estimates from ILLUMINATE® resulted in suboptimal prediction of NE content among DDGS sources evaluated in this study. Refinements in these calculations are needed to achieve better NE predictions among DDGS sources that contain relatively low oil concentrations.

Conflict of interest

All authors declare that we have no actual or potential conflict of interest including any financial, personal, or other relationships that could inappropriately influence, or be perceived to influence this work.

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