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In vitro digestibility techniques to predict apparent total tract energy digestibility of wheat in grower pigs[1,2]

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ABSTRACT: In vitro digestibility techniques have been developed to predict the apparent total tract digestibility (ATTD) of energy and DE content of mixed diets and feedstuffs including barley grain in swine. However, the techniques have not been tested properly for their accuracy in predicting the variation in ATTD of energy and DE content within wheat grain. The objectives were 1) to compare two 3-step in vitro digestibility techniques with either cellulase (IVD-CEL) or Viscozyme (a multienzyme complex to digest fiber; Novozymes, Bagsvaerd, Denmark; IVD-VIS) as the third step, and differing in the amount of enzymes used and the duration of digestion, for their accuracy in predicting ATTD of energy and DM of wheat in grower pigs; and 2) to develop equations to predict ATTD of energy of different batches of wheat. Wheat grain samples (n = 20) with a wide range in quality were collected; the ADF and CP content ranged from 3.3 to 6.2% and from 11.2 to 20.8% (DM basis), respectively. The ATTD of energy was determined using barrows (n = 60, 30.7 ± 4.7 kg of initial BW) in 2 periods with 6 observations per sample, and ranged from 73.3 to 84.5%. In IVD-CEL, 1 g of ground wheat was digested sequentially in digestion solutions containing pepsin (10 mg/36.5 mL) for 6 h, pancreatin (150 mg/54.5 mL) for 18 h, and cellulase (75 mg/55.5 mL) for 24 h. In IVD-VIS, 0.5 g of ground wheat sample was digested sequentially in solutions containing pepsin (25 mg/36.5 mL) for 2 h, 3 mL of pancreatin (100 mg/54.5 mL) for 6 h, and Viscozyme (0.5 mL/65.3 mL) for 18 h. The in vitro energy and DM digestibility ranged from 79.8 to 91.0% and from 82.0 to 91.5% for IVD-CEL, and ranged from 76.2 to 87.0% and from 79.1 to 89.4% for IVD-VIS, respectively. The R^2 between ATTD of energy and in vitro DM and energy digestibility for IVD-VIS (0.82 and 0.73, respectively) was greater than for IVD-CEL (0.55 and 0.54, respectively). The equation $y = 1.05x - 8.85$ using the in vitro DM digestibility value from IVD-VIS can predict the ATTD of the energy of wheat in swine with an SE of prediction of 1.2. The relationship between in vitro DM digestibility and grain characteristics such as ADF was stronger for the IVD-VIS than for the IVD-CEL technique (R^2 = 0.89 vs. 0.70). In conclusion, the IVD-VIS, but not the IVD-CEL, technique can accurately (R^2 = 0.82) predict the ATTD of energy in wheat in grower pigs. Therefore, the IVD-VIS technique might be useful as the reference analysis to calibrate analytical equipment to predict the ATTD of energy rapidly in wheat.

Key words: digestibility, energy, in vitro, pig, wheat

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INTRODUCTION

Wheat is used in swine diets predominantly as a source of energy. The DE content among wheat samples can vary by more than 20% (Kim et al., 2005) because of changes in the apparent total tract digestibility (**ATTD**) of energy (Zijlstra, 2006). Therefore, before mixing a batch of wheat into a swine diet, prediction of the ATTD of energy, and thus DE content of the wheat, is important to ensure that the proper dietary DE is achieved.

Existing approaches for the prediction of ATTD or DE content of the energy in wheat, such as equations based on chemical characteristics, may not be applicable or dependable in practice because of various constraints. For example, the time required for laboratory analyses or in vivo measurement interferes with making rapid feed formulation decisions, and errors associated with analyses among laboratories may reduce the accu-

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racy of prediction (Zijlstra, 2006). In vitro digestibility techniques using enzymes and lengths of incubations that mimic in vivo digestion may predict the ATTD of energy among feedstuffs and compound feeds (Boisen and Fernández, 1997; Noblet and Jaguelin-Peyraud, 2007) and within a feedstuff, such as barley (Regmi et al., 2008), with greater accuracy and repeatability. The accuracy of 2 existing in vitro digestibility techniques (Boisen and Fernández, 1997; Huang et al., 2003) to predict the ATTD of energy within wheat has not been tested. The 2 techniques mainly differ in the enzymes used to mimic digestion in the large intestine: Viscozyme, a multienzyme complex to digest fiber, was used by Boisen and Fernández (1997), whereas solely cellulase was used by Huang et al. (2003). The hypothesis was that the 2 in vitro techniques might predict with the same accuracy the ATTD of energy of wheat in swine.

The objectives of the present study were to compare the 2 in vitro digestibility techniques to predict the ATTD of energy in wheat fed to grower pigs, and to develop equations to predict the ATTD of energy of different batches of wheat.

MATERIALS AND METHODS

The animal use protocol was approved by the University of Saskatchewan Committee on Animal Care and Supply, and followed established principles (Canadian Council on Animal Care, 1993). The animal experiment was conducted at the Prairie Swine Centre Inc. (Saskatoon, Saskatchewan, Canada).

Sample Collection and Diet Formulation

To test the hypothesis above, 20 hard red spring-type wheat samples with an expectedly wide range in physical, chemical, and nutritional characteristics owing to an array of growing and harvesting conditions were collected across Saskatchewan, Canada. The samples were grown and collected individually from different geographical locations and were not created via blending of multiple wheat samples. The physical characteristics of the wheat samples were measured by the Canadian Grain Commission (Saskatoon, Saskatchewan, Canada). Briefly, the field test weight was measured using a 0.5-L measure, followed by conversion to kilograms per hectoliter. Subsequently, dockage (i.e., any material intermixed with the wheat, such as chaff, straw, weed and other grain seed, and dirt) was removed from the wheat sample with a Carter-Day dockage tester (Seedburo Equipment Co., Chicago, IL) and dockage was expressed as a percentage of field test weight. Finally, clean test weight was determined using an approach similar to that used to measure field test weight.

Wheat samples were milled through a 3.2-mm screen in a hammer mill (Model 160-D, Jacobsen Machine Works, Minneapolis, MN) and subsequently mixed into experimental diets (Table 1). The individual wheat sample was presumed to be the sole source of energy in the diets, and the small contribution of energy from the vitamin and mineral premixes was assumed to be negligible. Diets were processed as a mash and were fortified to exceed the vitamin and mineral requirements of 20- to 50-kg grower pigs (NRC, 1998). Chromic oxide was used as an indigestible marker.

Table 1. Composition of the experimental diets for in vivo determination of apparent total tract energy digestibility of wheat

Ingredient	Amount, % (as-fed basis)
Wheat	96.36
Limestone	1.03
Dicalcium phosphate	0.81
Vitamin premix[1]	0.50
Mineral premix[2]	0.50
Salt	0.40
Chromic oxide	0.40

[1]Provided per kilogram of diet: vitamin A, 8,250 IU; vitamin D_3, 825 IU; vitamin E, 40 IU; niacin, 35 mg; D-pantothenic acid, 15 mg; riboflavin, 5 mg; menadione, 4 mg; folic acid, 2 mg; thiamine, 1 mg; D-biotin, 0.2 mg; and vitamin B_{12}, 0.025 mg.

[2]Provided per kilogram of diet: zinc, 100 mg as zinc sulfate; iron, 80 mg as ferrous sulfate; copper, 50 mg as copper sulfate; manganese, 25 mg as manganous sulfate; iodine, 0.5 mg as calcium iodate; and selenium, 0.1 mg as sodium selenite.

Apparent Total Tract Energy Digestibility

The ATTD of energy of the 20 wheat samples was measured using 60 crossbred barrows (Camborough-22 × Line 65, PIC Canada Ltd., Airdrie, Alberta, Canada; 30.7 ± 4.7 kg of initial BW) in a crossover design. Three replications of 20 pigs were used in each of 2 consecutive periods, for a total of 120 observations, to obtain 6 measurements per sample. Each pig within a replication was assigned randomly to 1 of the 20 test diets, and pigs were fed different test diets in each period. Each of the 3 pigs eating the same diet in period 1 received different diets from the others in period 2. Pigs were housed in individual pens that allowed freedom of movement for 30 d (a 10-d acclimation to a 96.36% wheat diet, followed by 2 consecutive 10-d experimental periods). Each experimental period comprised a 5-d adaptation to a specific experimental diet, followed by a 5-d collection of feces. Daily feed allowance was adjusted to 3 times the maintenance requirement for energy (3×110 kcal of DE/kg of $BW^{0.75}$; NRC, 1998). Pigs received an equal amount of mash feed twice daily at 0830 and 1530 h. Pigs had free access to water throughout the experiment.

Freshly voided feces were collected hourly from 0800 to 1530 h by using the grab method. Feces were pooled for each pig and frozen at −20°C. Before analyses, feces were thawed, homogenized, subsampled, and freeze-dried.

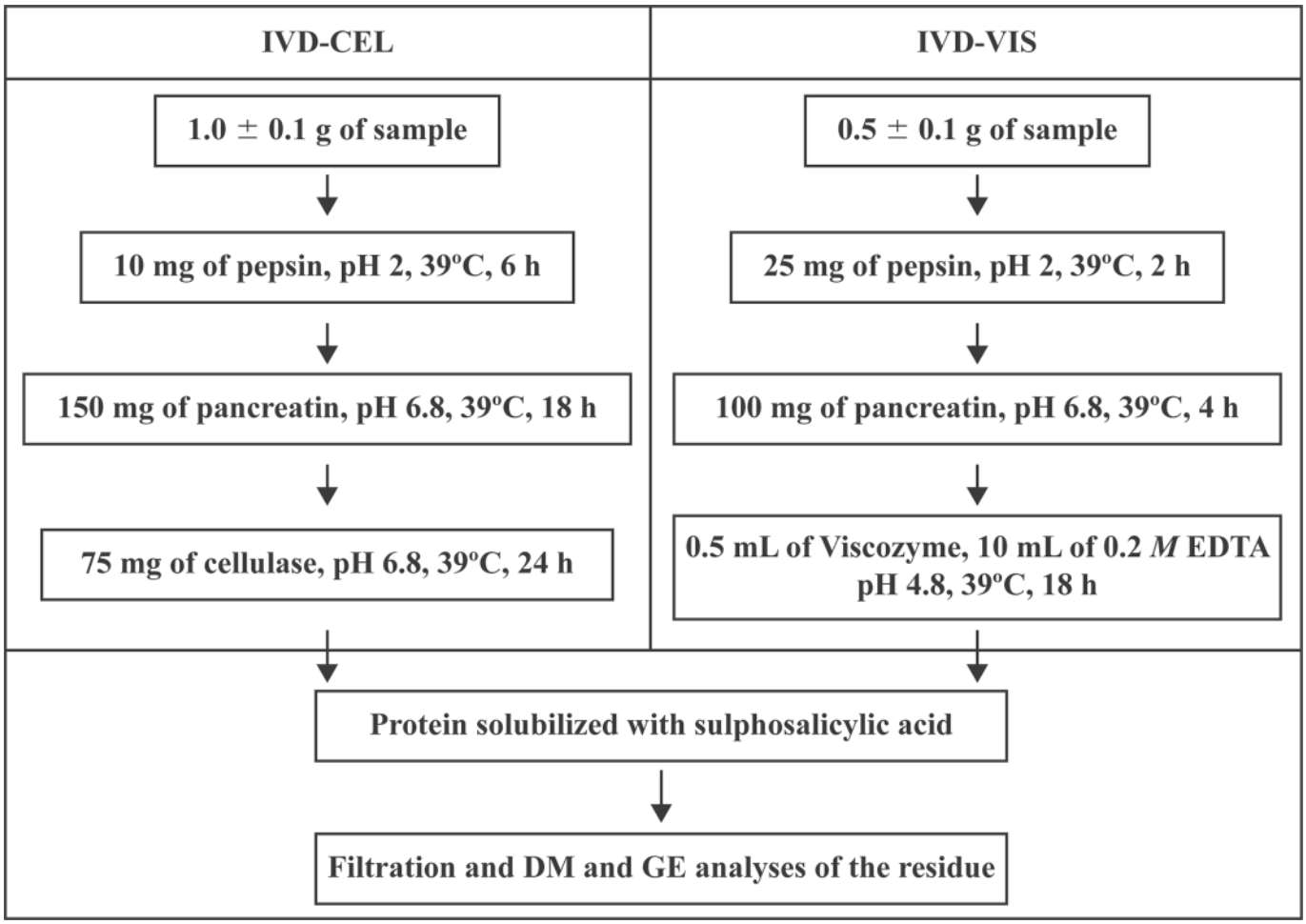

Figure 1. In vitro digestibility of 20 wheat samples with IVD-CEL and IVD-VIS techniques. IVD-CEL = in vitro digestibility technique using cellulase as the third step (Huang et al., 2003); IVD-VIS = in vitro digestibility technique using Viscozyme (Novozymes, Bagsvaerd, Denmark) as the third step (Boisen and Fernández, 1997).

Chemical Analyses and Calculations

Wheat, feed, and freeze-dried fecal samples were ground finely in a Retsch mill (model ZM1, Brinkman Instruments, Rexdale, Ontario, Canada) through a 1-mm screen and analyzed for DM by drying at 135°C in an airflow-type oven for 2 h (method 930.15; AOAC, 2006). The GE of wheat, feed, and feces was analyzed by an adiabatic bomb calorimeter (Model 5003, IKA-Werke GmbH and Co. KG, Staufen, Germany); benzoic acid was used as a standard. Chromic oxide in feed and feces was analyzed by a spectrophotometer (LKB-Ultraspec III model 80-2097-62, Pharmacia, Cambridge, UK) at 440 nm after ashing overnight at 450°C (Fenton and Fenton, 1979). Furthermore, the wheat samples were analyzed for the content of CP (Kjeldahl N; method 990.03; AOAC, 2006), ADF and acid detergent lignin (method 973.18; AOAC, 2006), NDF (Van Soest et al., 1991), ether extract (method 920.39; AOAC, 2006), ash (method 942.05; AOAC, 2006), and Lys (method 999.13; AOAC, 2006).

Based on the results of the chemical analyses, the ATTD of energy for each diet was determined using the indicator method (Jørgensen et al., 1984). The ATTD of energy of the diet was assumed to be identical to the ATTD of energy for the specific wheat sample. The DE content of each wheat sample was calculated by multiplying the ATTD of energy by the GE content of the specific wheat sample.

In Vitro DM and Energy Digestibility

The in vitro DM and energy digestibility of the 20 wheat samples was determined using 2 in vitro digestibility techniques differing in types and amount of enzymes and digestion periods (Figure 1). The first technique (**IVD-CEL**) was originally developed in our laboratory (Huang et al., 2003) and was used to predict the ATTD of energy for barley (Regmi et al., 2008). The second technique (**IVD-VIS**) was originally described by Boisen and Fernández (1997) to predict ATTD of energy among feedstuffs and diet samples in swine. The wheat samples were finely ground in a Retsch mill (model ZM1, Brinkman Instruments, Rexdale, Ontario, Canada) through a 1-mm screen and used for digestion with IVD-CEL and IVD-VIS, as described below.

IVD-CEL. A sample (1.0 ± 0.1 g) was weighed into a 125-mL conical flask. Phosphate buffer (25 mL,

0.1 *N*, and pH 6) solution was added to the flask and stirred with a small magnetic rod. After 10 mL of 0.2 *N* HCl solution was added to the flask, the pH of the solution was adjusted to 2 using a 1 *N* HCl or 1 *N* NaOH solution. Solutions of 1 mL (10 mg/mL) of freshly prepared pepsin (P-7000, 800 to 2,500 units/mg of protein, from porcine gastric mucosa, Sigma-Aldrich, Oakville, Ontario, Canada) and 0.5 mL of chloramphenicol (0.5 g/100 mL of ethanol) were then added to the flask. The flask was incubated in a water bath at 39°C for 6 h. After the incubation, solutions of 10 mL of 0.2 *N* phosphate buffer (pH 6.8) and 5 mL of 0.6 *N* NaOH were added to the flask, and the pH of the solution was adjusted to 6.8 with a 1 *N* HCl or 1 *N* NaOH solution. Thereafter, 3 mL (50 mg/mL) of freshly prepared pancreatin (P-1750, Sigma-Aldrich; contains 113 units of amylase, 20.8 units of lipase, and 110 units of protease per milligram of solids from porcine pancreas) solution was added to the flask. The flask was incubated in a water bath at 39°C for 18 h. After the second incubation, 1 mL (75 mg/mL) of freshly prepared cellulase solution (C-9422, Sigma-Aldrich; 3 to 10 units/mg of solids; from *Trichoderma viridae*) was added, and the flask was incubated for 24 h at 39°C.

IVD-VIS. A sample (0.5 ± 0.1 g) was weighed into a 125-mL conical flask. Phosphate buffer (25 mL, 0.1 *N*, and pH 6) solution was added to the flask and stirred with a small magnetic rod. After 10 mL of 0.2 *N* HCl solution was added to the flask, the pH of the solution was adjusted to 2 using a 1 *N* HCl or 1 *N* NaOH solution. Solutions of 1 mL (25 mg/mL) of freshly prepared pepsin (P-7000, Sigma-Aldrich) and 0.5 mL of chloramphenicol (0.5 g/100 mL of ethanol) were then added to the flask. The flask was incubated in a water bath at 39°C for 2 h. After the incubation, solutions of 10 mL of 0.2 *N* phosphate buffer (pH 6.8) and 5 mL of 0.6 *N* NaOH were added to the flask, and the pH of the solution was adjusted to 6.8 with a 1 *N* HCl or 1 *N* NaOH solution. Thereafter, 3 mL (100 mg/3 mL) of freshly prepared pancreatin (P-1750, Sigma-Aldrich) solution was added to the flask. The flask was incubated in a water bath at 39°C for 4 h. After the second incubation, 10 mL of a 0.2 *M* EDTA solution was added to the flask, and the pH was adjusted to 4.8 with 30% acetic acid solution. Then 0.5 mL of Viscozyme (multienzyme complex from *Aspergillus aculeatus* containing cellulase, β-glucanase, arabinase, xylanase, mannanase, and pectinase; Novozymes, Bagsvaerd, Denmark) was added, and the flask was incubated at 39°C for 18 h.

The enzymatic digestion in both the techniques was terminated by addition of 5 mL of 20% sulfosalicylic acid, and the flask was kept at room temperature for 30 min to facilitate precipitation of undigested soluble proteins. The undigested residue was then collected in a filtration unit using a porcelain filtration funnel lined with preweighed filter paper (Whatman no. 54; Whatman Inc., Florham Park, NJ). The residue, along with the filter paper, was dried overnight at 80°C. In vitro DM digestibility was calculated by deducting the residue DM from the sample DM, followed by division by the sample DM. The in vitro energy digestibility was calculated using the following formula: In vitro energy digestibility = [(sample DM × sample GE) − (residue DM × residue GE)]/(sample DM × sample GE).

Statistical Analyses

Wheat sample was considered the experimental unit for linear regression analyses. With the REG procedure (SAS Inst. Inc., Cary, NC), the R^2 between the ATTD of energy and chemical and physical characteristics was determined. Furthermore, the REG procedure was used to develop regression equations to predict the ATTD of energy based on in vitro DM and energy digestibility, using the R^2 value as an indicator of quality of the prediction equation. The SE of prediction (**SEP**) was calculated by the following formula:

$$\text{SEP} = \sqrt{\frac{\sum(\text{Y} - \text{Y}')}{\text{N}}},$$

where Y is the actual value, Y′ is the predicted value, and N is the total number of wheat samples.

RESULTS

Physical and Chemical Characteristics

The physical characteristics of the 20 wheat samples, field test weight, clean test weight, and dockage, ranging from least to greatest, were 24.2 kg/hL, 21.3 kg/hL, and 3.8%, respectively (Table 2). Of the characteristics, dockage had the greatest CV.

Among the chemical characteristics, the range for NDF was 12.8 percentage units, with the greatest CV (Table 2). The ranges in content of CP, ADF, ether extract, ash, lignin, and Lys were 8.6, 2.9, 1.3, 1.2, 1.0, and 0.25 percentage units, respectively. The range for GE was 0.19 Mcal/kg of DM, with the least CV.

The R^2 between ATTD of energy and chemical and physical characteristics was greatest for ADF (0.79), followed by clean test weight (0.73), field test weight (0.70), Lys (0.63), NDF (0.42), and GE (0.40; Table 2). The R^2 between ATTD of energy and other listed chemical and physical characteristics were not more than 0.30.

Apparent Total Tract Energy Digestibility

The ATTD of energy ranged from 73.3 to 84.5% for the 20 wheat samples (Table 2). The relative error ranged from 0.66 to 2.57 and averaged 1.55 (data not shown). The range in ATTD of energy corresponded (R^2 = 0.93) to the range in DE content, from 3.36 to 3.81 Mcal/kg of DM.

Table 2. Physical, chemical, and energy characteristics of the 20 wheat samples and their R^2 with apparent total tract energy digestibility[1]

Characteristic	Mean	SD	CV	Least	Greatest	R^2
Physical characteristic						
Field test weight, kg/hL	69.3	5.9	8.5	53.1	77.3	0.70
Clean test weight, kg/hL	70.2	5.4	7.7	56.0	77.3	0.73
Dockage, %	1.72	1.1	64.4	0.70	4.50	0.30
Chemical characteristic, DM basis						
Moisture, %	13.0	1.7	12.6	11.5	19.3	0.05
GE, Mcal/kg	4.54	0.1	1.07	4.42	4.61	0.40
CP, %	17.5	2.5	14.4	11.2	20.8	0.24
ADF, %	4.5	0.8	18.2	3.3	6.2	0.79
NDF, %	17.0	4.0	23.2	11.1	23.9	0.42
Ether extract, %	2.2	0.3	13.5	1.6	2.9	0.07
Ash, %	2.1	0.3	14.2	1.6	2.8	0.29
Lignin, %	1.3	0.3	23.0	0.8	1.8	0.21
Lys, %	0.46	0.1	15.1	0.32	0.57	0.63
In vivo swine energy characteristic						
Apparent total tract digestibility, %	80.6	2.9	3.7	73.3	84.5	—
DE content, Mcal/kg of DM	3.65	0.1	3.0	3.36	3.81	0.93

[1]R^2 = between in vivo apparent total tract digestibility of energy and the specific characteristic.

In Vitro Digestibility

For IVD-CEL, in vitro energy digestibility ranged from 79.8 to 91.0% and DM digestibility ranged from 82.0 to 91.5% (Table 3). For IVD-VIS, in vitro energy digestibility ranged from 76.2 to 87.0% and DM digestibility ranged from 79.1 to 89.4%. The relative errors of in vitro energy and DM digestibility in IVD-CEL ranged from 0.01 to 2.22 and averaged 0.70, whereas that in IVD-VIS ranged from 0.03 to 1.87 and averaged 0.67. Similarly, in vitro DE content ranged from 3.65 to 4.12 Mcal/kg of DM for IVD-CEL and ranged from 3.50 to 3.94 Mcal/kg of DM for IVD-VIS.

The R^2 between ATTD of energy and in vitro energy digestibility was 0.54 ($y = 0.71x + 19.45$; SEP = 1.89) for IVD-CEL and was 0.73 ($y = 0.85x + 10.39$; SEP = 1.46) for IVD-VIS (Figure 2). The R^2 between ATTD of energy and in vitro DM digestibility was 0.55 ($y = 0.84x + 7.08$; SEP = 1.89) for IVD-CEL and was 0.82 ($y = 1.05x - 8.85$; SEP = 1.20) for IVD-VIS (Figure 3). The regression equation to predict the ATTD of energy using in vitro DM digestibility in IVD-VIS, $y = 1.05x - 8.85$, had the greatest R^2 and the least SEP among the equations. The R^2 between in vivo DE content and in vitro DM digestibility was 0.47 for IVD-CEL and was 0.77 for IVD-VIS (Table 4). Similarly, the R^2 between in vitro energy digestibility and in vitro DM digestibility was 0.91 for IVD-CEL and was 0.94 for IVD-VIS. The R^2 between in vivo and in vitro DE content was 0.40 for IVD-CEL and was 0.65 for IVD-VIS.

The R^2 between in vitro DM digestibility and chemical and physical characteristics was greatest for ADF for both techniques (0.70 for IVD-CEL and 0.89 for IVD-VIS; Table 5). The R^2 between in vitro DM digestibility and clean test weight, Lys, and GE content were 0.46, 0.46, and 0.39 for IVD-CEL and were 0.81, 0.62, and 0.44 for IVD-VIS, respectively. The R^2 between in vitro DM digestibility in IVD-CEL and IVD-VIS was 0.62 ($y = 0.77x + 17.99$) and between in vitro energy digestibility in IVD-CEL and IVD-VIS was 0.54 ($y = 0.72x + 20.86$; Figure 4). The R^2 between in vitro DE content in IVD-CEL and IVD-VIS was 0.45 ($y = 0.65x + 1.26$; Figure 5).

Table 3. In vitro DE content and energy and DM digestibility of 20 wheat samples using 2 in vitro digestibility techniques

Characteristic	Mean	SD	CV	Least	Greatest	Relative error, %
IVD-CEL[1]						
In vitro DE, Mcal/kg of DM	3.88	0.12	3.18	3.65	4.12	—
In vitro energy digestibility, %	85.9	0.03	3.63	79.8	91.0	0.01 to 2.21
In vitro DM digestibility, %	87.2	0.03	3.04	82.0	91.5	0.01 to 2.22
IVD-VIS[2]						
In vitro DE, Mcal/kg of DM	3.76	0.12	3.07	3.50	3.94	—
In vitro energy digestibility, %	82.7	0.03	3.61	76.2	87.0	0.03 to 1.67
In vitro DM digestibility, %	84.9	0.03	3.00	79.1	89.4	0.03 to 1.87

[1]IVD-CEL = in vitro digestibility technique using cellulase as the third step (Huang et al., 2003).
[2]IVD-VIS = in vitro digestibility technique using Viscozyme (Novozymes, Bagsvaerd, Denmark) as the third step (Boisen and Fernández, 1997).

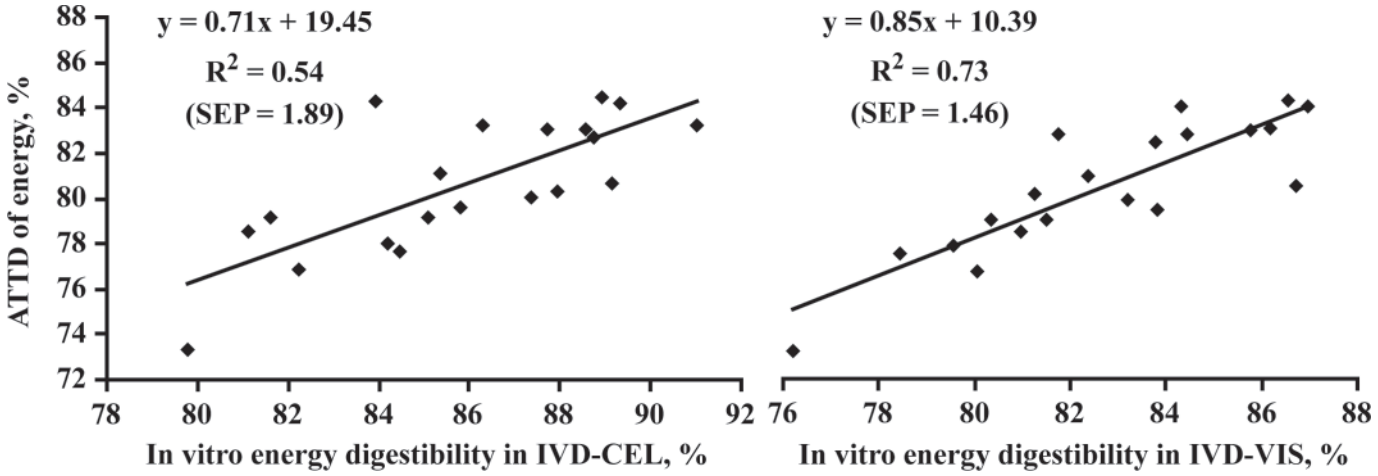

Figure 2. Relationship between ATTD of energy and in vitro energy digestibility of 20 wheat samples using IVD-CEL and IVD-VIS techniques. ATTD = apparent total tract digestibility; SEP = SE of prediction; IVD-CEL = in vitro digestibility technique using cellulase as the third step (Huang et al., 2003); IVD-VIS = in vitro digestibility technique using Viscozyme (Novozymes, Bagsvaerd, Denmark) as the third step (Boisen and Fernández, 1997).

DISCUSSION

Wheat is used as an important energy-rich feedstuff in swine diets. The reported DE content among batches of wheat varies considerably, ranging from 3.18 to 4.06 Mcal/kg of DM among studies (Fuller et al., 1989; Kopinski, 1997). The variation in DE content is mostly due to changes in the ATTD of energy (Zijlstra, 2006), which are mainly caused by differences in varieties and growing conditions (Anderson and Bell, 1983; Van Barneveld, 1999). In addition, variation in the ATTD of energy is the major factor affecting the NE content of feedstuffs (Noblet, 2006). Because energy is the most expensive component of swine diets, accurate prediction of the ATTD of energy in wheat on a routine basis is necessary to formulate diets with proper energy content.

The 3-step in vitro digestibility techniques use enzymes and length of incubations that mimic in vivo digestion. These techniques can accurately and repeatably predict the ATTD of energy among feedstuffs (Boisen and Fernández, 1997; Noblet and Jaguelin-Peyraud, 2007) and within different batches of an individual feedstuff such as barley in swine (Huang et al., 2003; Regmi et al., 2008). The techniques are less expensive and time-consuming than animal experiments and can potentially be used as the reference analyses to calibrate rapid feed quality evaluation equipment, such as near-infrared reflectance spectroscopy, to predict the ATTD of energy and DE content of feedstuffs in real time (Zijlstra, 2006).

The 2 in vitro digestibility techniques studied in the present study, IVD-CEL and IVD-VIS, used different approaches to mimic in vivo digestion, especially the

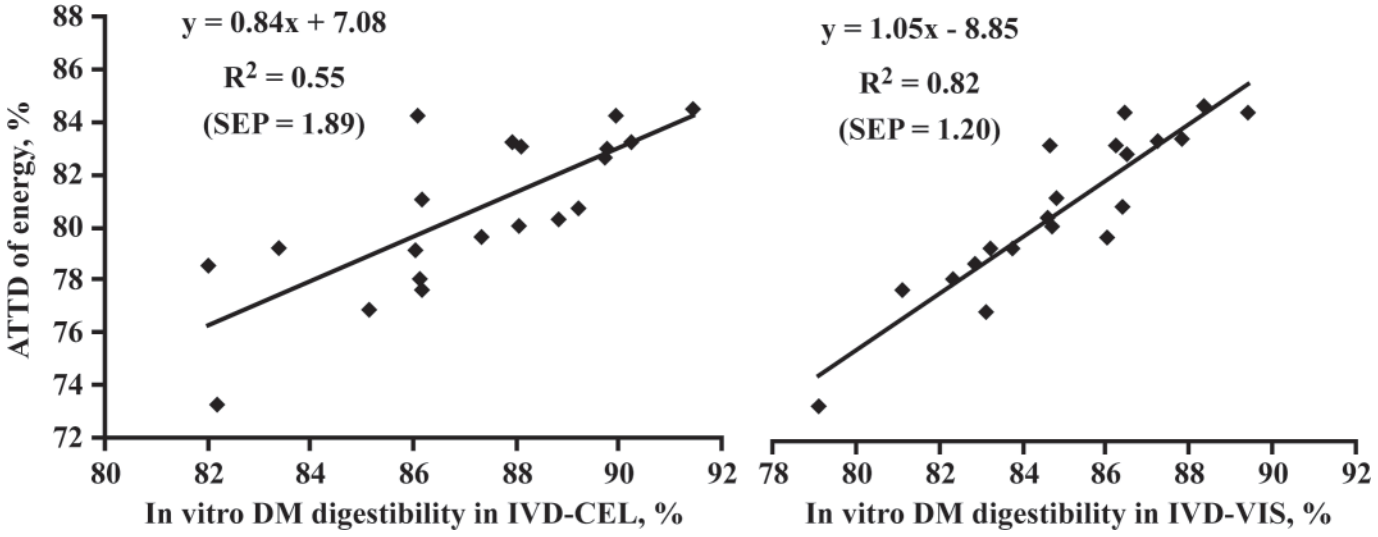

Figure 3. Relationship between ATTD of energy and in vitro DM digestibility of 20 wheat samples using IVD-CEL and IVD-VIS techniques. ATTD = apparent total tract digestibility; SEP = SE of prediction; IVD-CEL = in vitro digestibility technique using cellulase as the third step (Huang et al., 2003); IVD-VIS = in vitro digestibility technique using Viscozyme (Novozymes, Bagsvaerd, Denmark) as the third step (Boisen and Fernández, 1997).

Table 4. Relationship (R^2) between in vivo and in vitro characteristics using 2 in vitro digestibility techniques

Characteristic	IVD-CEL[1]	IVD-VIS[2]
In vivo DE vs. in vitro DE	0.40	0.65
In vivo DE vs. in vitro DM digestibility	0.47	0.77
In vitro energy digestibility vs. in vitro DM digestibility	0.91	0.94

[1]IVD-CEL = in vitro digestibility technique using cellulase as the third step (Huang et al., 2003).
[2]IVD-VIS = in vitro digestibility technique using Viscozyme (Novozymes, Bagsvaerd, Denmark) as the third step (Boisen and Fernández, 1997).

types and amounts of enzyme used and the duration of digestion. The most important difference between the 2 techniques is the final step of the 3-step digestion to mimic digestion in the large intestine: Viscozyme (0.5 mL/65.3 mL of digestion solution), containing a range of carbohydrases such as cellulase, β-glucanase, arabinase, xylanase, mannanase, and pectinase, is used in IVD-VIS, whereas solely cellulase (75 mg/55.5 mL of digestion solution) is used in IVD-CEL. Furthermore, the amount of pepsin was 150% greater and pancreatin was 25% greater in IVD-VIS than in IVD-CEL. Finally, the duration of sample incubation with pepsin, pancreatin, and fiber-digesting enzyme was 4, 14, and 6 h shorter, respectively, for IVD-VIS than IVD-CEL. Hence, the accuracy of these 2 techniques to predict the ATTD of energy in wheat may be different. The present study was designed to compare the 2 in vitro digestibility techniques (Boisen and Fernández, 1997; Huang et al., 2003) for the error of predicting the ATTD of energy of wheat varying in quality and to recommend equations to predict the ATTD of energy in swine accurately. Theoretically, the third step mimicking hindgut fermentation can also be conducted with a fecal inoculum containing an active microflora (Löwgren et al., 1989; Wang et al., 2004). However, whereas enzymes can be transported, a fecal inoculum will differ greatly among pigs globally. Because this interferes with our long-term goal of developing reference analyses, we selected against such an approach. To our knowledge, in vitro techniques based on enzymes to accurately predict the ATTD of energy or DE content of wheat in swine have not been developed.

For the development of equations to predict the ATTD of energy within a feedstuff accurately, a wide range in physical and chemical characteristics, ATTD of energy, and DE content of the feedstuff samples is critical. In the present study, the range in test weight of wheat samples was wide. The range extended below the lower end of the range measured for multiple wheat samples grown and harvested in normal conditions (70 to 84 kg/hL; Garnsworthy et al., 2000; Kim et al., 2003) and was comparable to the range of wheat grown and harvested in poor to optimal conditions (57.8 to 77.6 kg/hL; Zijlstra et al., 1999). The GE content was similar to values reported in previous studies (4.38 to 4.47 Mcal/kg of DM; Kim et al., 2003; Pedersen et. al., 2007). Similarly, the chemical composition of wheat in the present study had a range as wide as has been reported in previous studies (CP, 7.3 to 19.1; ADF, 3.0 to 4.4, NDF, 13.0 to 18.9, and ether extract, 1.3 to 2.2%;

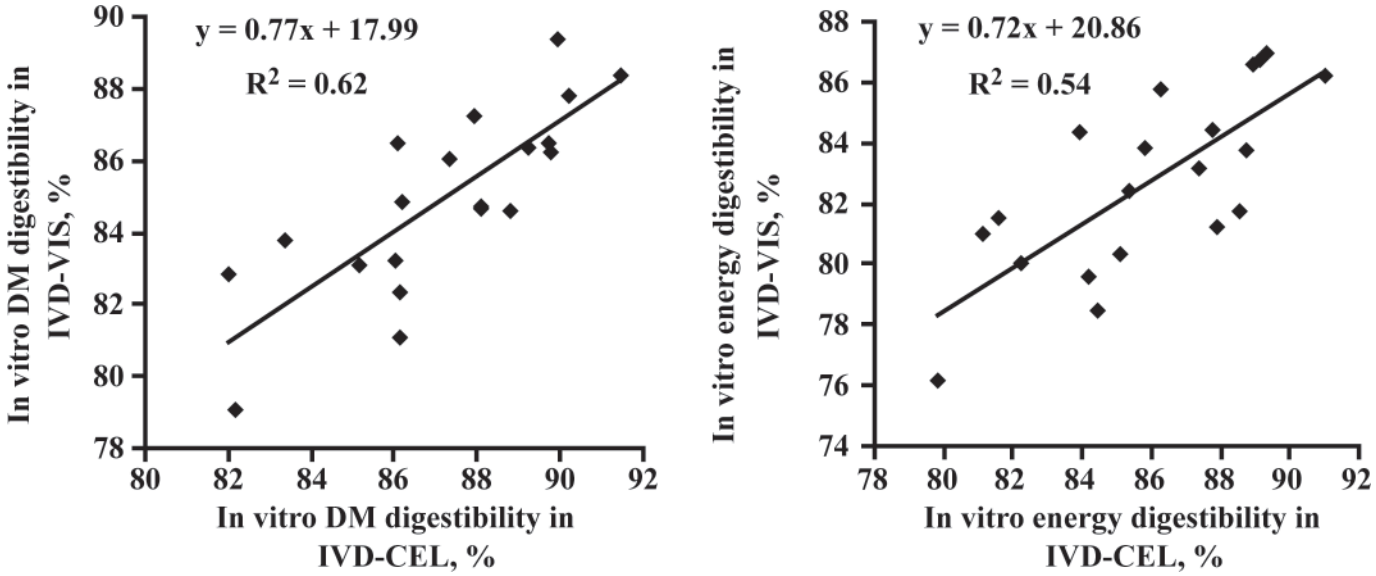

Figure 4. Relationship of in vitro DM and energy digestibility in IVD-CEL and IVD-VIS techniques in 20 wheat samples. IVD-CEL = in vitro digestibility technique using cellulase as the third step (Huang et al., 2003); IVD-VIS = in vitro digestibility technique using Viscozyme (Novozymes, Bagsvaerd, Denmark) as the third step (Boisen and Fernández, 1997).

Table 5. The R^2 between in vitro DM digestibility and chemical and physical characteristics in the 2 in vitro digestibility techniques

Characteristic	IVD-CEL[1]	IVD-VIS[2]
CP	0.15	0.19
ADF	0.70	0.89
NDF	0.19	0.28
Ether extract	0.16	0.13
Ash	0.06	0.21
Moisture	0.01	0.03
Lignin	0.27	0.26
Lys	0.46	0.62
GE	0.39	0.44
Field test weight	0.46	0.80
Clean test weight	0.46	0.81
Dockage	0.55	0.32

[1]IVD-CEL = in vitro digestibility technique using cellulase as the third step (Huang et al., 2003).

[2]IVD-VIS = In vitro digestibility technique using Viscozyme (Novozymes, Bagsvaerd, Denmark) as the third step (Boisen and Fernández, 1997).

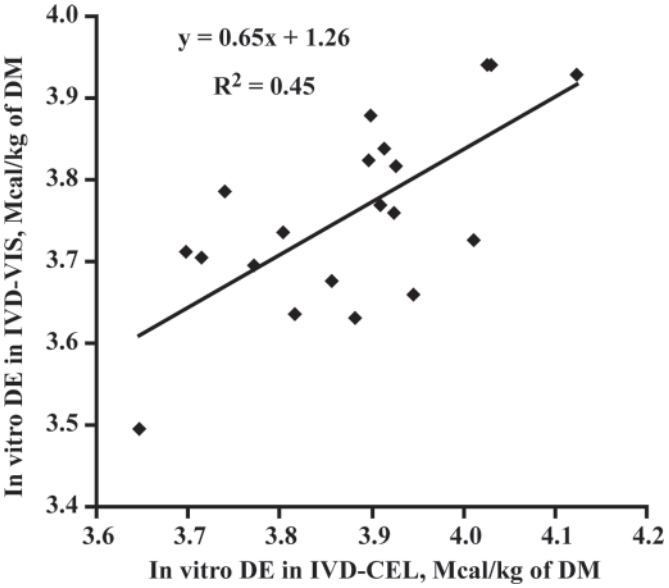

Figure 5. Relationship between in vitro DE content using IVD-CEL and IVD-VIS techniques in 20 wheat samples. IVD-CEL = in vitro digestibility technique using cellulase as the third step (Huang et al., 2003); IVD-VIS = in vitro digestibility technique using Viscozyme (Novozymes, Bagsvaerd, Denmark) as the third step (Boisen and Fernández, 1997).

Bell and Keith, 1989; Garnsworthy et al., 2000; Kim et al., 2003; Pedersen et al., 2007). The DE content of wheat samples in the present study was comparable with the range of 3.49 to 3.82 Mcal/kg of DM reported by Garnsworthy et al. (2000) but was slightly less than the range of 3.70 to 4.05 Mcal/kg of DM reported by Zijlstra et al. (1999). Hence, the physical, chemical, and energy characteristics of the wheat samples used in the present study had a range sufficiently wide to establish whether the ATTD of energy had a strong relationship with physical, chemical, and in vitro digestibility characteristics. Similarly, the range in ATTD of energy for the 20 wheat samples in the present study was greater than that reported previously (80.3 to 88.0%, Zijlstra et al., 1999; 84.8 to 89.0%, Wiseman, 2000) in grower pigs. However, the absolute value of the ATTD of energy of wheat in the present study was less than in these earlier studies. The greater ADF content and smaller test weight of the wheat samples in the present study might have contributed to less ATTD of energy, because ADF content and the ATTD of energy have an inverse relation in wheat samples (Shi and Noblet, 1993; Kim et al., 2005).

The R^2 between ATTD of energy and in vitro digestibility and physical and chemical characteristics were analyzed in the present study to determine and compare the accuracy of prediction equations. In vitro DM digestion using IVD-VIS predicted the ATTD of energy in wheat better ($R^2 = 0.82$; $y = 1.05x - 8.85$) than IVD-CEL and analyses of chemical and physical characteristics. Previously, xylose had the greatest R^2 (0.61) with DE content of wheat among the chemical and physical characteristics, followed by total nonstarch polysaccharide (0.54), NDF (0.49; Zijlstra et al., 1999), and test weight (0.45; Wiseman, 2000). The relationship of chemical and physical characteristics with the ATTD of energy was stronger in the present study than in previous studies, probably because of a wider range of specific characteristics in the present study. Previously, the IVD-VIS allowed simulation of ATTD of OM among feedstuffs, including wheat in swine (Wilfart et al., 2007), and IVD-CEL accurately predicted the ATTD of energy of different batches of barley samples (Regmi et al., 2008). To our knowledge, the scientific literature directly comparing in vitro techniques to predict ATTD of energy in feedstuffs or diets is not available.

Improved prediction of ATTD of energy with IVD-VIS in wheat as opposed to IVD-CEL could be due to various factors, including grain composition and contributions of the different enzymes to in vitro digestion (Wilfart et al., 2008). The greater R^2 between in vitro DM digestibility and grain characteristics, such as ADF, NDF, Lys, and test weight, in IVD-VIS than in IVD-CEL indicated a stronger relation between grain composition and in vitro digestion (i.e., substrate and enzyme). Wheat grain contains different fiber fractions, such as arabinose (2.6 to 4.1%), xylose (4.3 to 6.5%), mannose (0.08 to 0.22%; Zijlstra et al., 1999), β-glucan (0.5 to 1%; Wood, 2002), and cellulose (2.7%; Boyacioglu and Hettiarachchy, 1995). The content of cellulose in wheat is less than in barley (3.1 to 4.4%; Fairbairn et al., 1999), indicating that Viscozyme, containing less cellulase but a broader range of carbohydrases, might more closely mimic the in vivo digestion of fiber fractions in wheat samples than cellulase alone. Furthermore, the content of starch and CP is greater in wheat than in barley (Fairbairn et al., 1999; Zijlstra et al., 1999). The use of EDTA (Csiszr, 2005) and the greater activity of pepsin and pancreatin per unit sample in

IVD-VIS might have accelerated the digestion of starch and protein, thus more closely mimicking in vivo digestion, even during a shorter incubation period.

The 2 in vitro digestibility techniques used in the present study generated significantly different in vitro DM and energy digestibility values and in vitro DE content of wheat samples. Even though the amount of pepsin and pancreatin used was less in IVD-CEL, the in vitro DM digestibility and in vitro DE content were greater in IVD-CEL than in IVD-VIS. The longer duration of incubation in IVD-CEL might have contributed to greater digestibility values of wheat samples. The SEP of equations to predict the ATTD of energy were also less in IVD-VIS, indicating a greater accuracy of IVD-VIS than IVD-CEL to predict ATTD of energy. Finally, the relative error of in vitro digestibility was less for IVD-VIS than IVD-CEL, indicating greater precision or repeatability of the IVD-VIS technique. The weak relationship between IVD-VIS and IVD-CEL ($R^2 \leq 0.62$) for in vitro DM and energy digestibility and DE content further highlight that the different approaches to digestion indeed resulted in different in vitro digestibility values.

The relationship between ATTD of energy and in vitro DM digestibility within wheat samples in the present study was stronger than between ATTD of energy and in vitro OM digestibility among compounded pig diets, including diets containing 61 to 96% wheat ($R^2 = 0.77$; Noblet and Jaguelin-Peyraud, 2007). In contrast, the R^2 of ATTD of energy and in vitro DM digestibility within barley samples ($R^2 = 0.97$; Regmi et al., 2008) was greater than that within wheat samples in the present study. One reason for the smaller R^2 in the present study could be the smaller range in ATTD of energy of wheat compared with barley samples (11.2 vs. 26.6 percentage units; Regmi et al., 2008). The R^2 between ATTD of energy and DE content of the wheat samples in the present study was less than that of barley samples (0.99; Regmi et al., 2008), pointing to a comparatively greater contribution of GE content on changes in DE content in wheat than in barley (R^2 of ATTD of energy and GE content of barley = 0.17; Regmi et al., 2008). Furthermore, the relationship between in vitro DM and in vitro energy digestibility was not perfect in wheat, unlike in barley ($R^2 = 1.00$; Regmi et al., 2008), indicating that factors not related to in vitro DM digestibility may affect in vitro energy digestibility in wheat samples. The difference in content of ash between wheat and barley might have contributed to the change in the relationship between in vitro DM and energy digestibility. Finally, the R^2 between ATTD of energy and in vitro energy digestibility had a greater SEP compared with that between ATTD of energy and in vitro DM digestibility, indicating that in vitro DM digestibility provided a better prediction of ATTD of energy than in vitro energy digestibility.

In conclusion, the in vitro digestibility technique with Viscozyme in the final step of the 3-step digestion (Boisen and Fernández, 1997) can predict the ATTD of energy of wheat samples with greater accuracy ($R^2 = 0.82$ vs. 0.55) than the technique with cellulase (Huang et al., 2003). In particular, in vitro DM digestibility provides an accurate prediction. The regression equation developed in the present study with wheat samples having a wide range of chemical and physical characteristics can be used to estimate the ATTD of energy of batches of wheat in grower pigs. The in vitro digestibility technique with Viscozyme may also be useful as the reference analysis to calibrate rapid analytical equipment (e.g., near-infrared reflectance spectroscopy) to predict the energy digestibility of wheat samples on a routine basis (Zijlstra, 2006). However, improvements in the technique will be useful to further enhance the predictive power of the technique. Further research is warranted to validate the in vitro technique to predict the ATTD of energy within feedstuffs other than wheat.

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