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Prediction of in vivo apparent total tract energy digestibility of barley in grower pigs using an in vitro digestibility technique[1]

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ABSTRACT: The DE content within cereal grains can vary 25% mainly due to changes in apparent total tract digestibility (ATTD) of energy. In vitro digestibility techniques have been developed to predict the DE value among feedstuffs. However, these techniques have not been tested properly for their suitability to predict the variation in energy digestibility and DE content within a cereal grain. The objective of the present study was to establish and evaluate an in vitro digestibility technique to predict in vivo ATTD of energy of barley in grower pigs. Barley grain samples (hulled, n = 21) with a large range in quality were collected; the ADF and CP content ranged from 4.5 to 11.4% and 10.0 to 16.4% (DM basis), respectively. The ATTD of energy was determined using barrows (n = 63, 33 ± 2.1 kg of initial BW) in 2 periods with 6 observations per sample and ranged from 51.9 to 78.5%, with relative errors between 0.4 and 5.0%. A preliminary study, comparing a 2- and a 3-step in vitro digestibility technique using 3 barley samples, indicated that R^2 between in vivo and in vitro energy digestibility was greater using the 3- than the 2-step technique (0.92 vs. 0.76). Therefore, the 3-step in vitro digestibility technique was used solely in subsequent analyses. Briefly, ground barley was subsequently incubated with pepsin for 6 h, pancreatin for 18 h, and cellulase for 24 h. The DM and GE content of samples and residues were measured to calculate digestibility. The in vitro energy digestibility of the 21 barley samples with duplicate measurements ranged from 63.7 to 82.2%, with relative errors between 0.1 and 2.6%. In vitro energy digestibility was strongly related ($y = 1.25 x - 25.22$; $R^2 = 0.81$) to in vivo energy digestibility. Finally, a subset of 7 barley samples was analyzed in quadruplicate using the 3-step in vitro technique. The relationship between in vitro and in vivo energy digestibility was very strong ($y = 1.23 x - 25.33$; $R^2 = 0.97$) with relative errors between 0.5 and 2.7%. In vitro DE and energy digestibility were perfectly related ($R^2 = 1.00$). In summary, the 3-step in vitro energy digestibility technique can accurately predict the ATTD of energy in barley in grower pigs. The 3-step in vitro digestibility technique, thus, might be useful as the reference laboratory procedure to calibrate analytical equipment to rapidly predict the ATTD of energy in barley.

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

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INTRODUCTION

Cereal grains are used as feedstuffs in swine diets, mainly as an energy source. The DE content varies 20% within barley and wheat (Fairbairn et al., 1999; Zijlstra et al., 1999) due to variation in the apparent total tract digestibility (**ATTD**) of energy that is caused mostly by a wide array of growing and harvesting conditions and genetic variation (Molina-Cano et al., 1997). Therefore, before mixing a batch of cereal grain into a swine diet, prediction of the ATTD of energy and DE content of cereal grains is important to ensure that the proper dietary DE content is achieved.

Existing approaches for the determination of DE content, such as the ATTD of energy and the equations to predict DE content from chemical characteristics, may not be applicable or dependable in practice because of various constraints such as time required for in vivo measurements or laboratory analyses and errors associated with analyses among laboratories (Zijlstra, 2006). In vitro digestibility techniques using enzymes and length of incubations that mimic in vivo digestion

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can be used to predict the ATTD of energy among feedstuffs and compound feeds in swine with reasonable accuracy (Boisen and Fernández, 1997; Noblet and Jaguelin-Peyraud, 2007) and might also be useful to evaluate the variation in the ATTD of energy within a cereal grain (Huang et al., 2003). The hypothesis that in vitro digestibility techniques can predict the variation in the ATTD of energy and DE content within barley has, to date, not been tested properly and rigorously using samples collected from independent sources. In vitro digestibility techniques might be used to develop procedures to accurately predict the DE content of specific batches of cereal grains for swine.

The objectives of the present study were to determine and establish an in vitro digestibility technique to predict the ATTD of energy in barley fed to grower pigs and to develop equations to predict the ATTD of energy.

MATERIALS AND METHODS

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

Sample Collection and Diet Formulation

To test the hypothesis, 21 hulled barley samples with an expected wide range in physical, chemical, and nutritional characteristics due to an array of growing and harvesting conditions were collected across Saskatchewan, Canada. Samples of hull-less barley were excluded from the collection to ensure that changes in barley characteristics, and not barley type, were studied. The samples were grown and collected from different geographical locations and were not created by blending of multiple barley samples. The physical characteristics of the barley samples were measured by the Canadian Grain Commission (Saskatoon, Saskatchewan, Canada). Briefly, 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 barley such as chaff, straw, weed and other grain seed, and dirt) was removed from the barley sample using a Carter-Day dockage tester (Seedburo Equipment Co., Chicago, IL) and expressed as a percentage of field test weight. Finally, clean test weight was determined using a similar approach that was used to measure field test weight.

Barley 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, in which the individual barley sample was presumed to be the sole source of energy (Table 1); the small contribution of energy from the vitamin and mineral premixes was assumed to be negligible. Diets were processed as mash and fortified to exceed the vitamin and mineral requirements of 20- to 50-kg grower pigs (NRC, 1998). Chromic oxide was included as an indigestible marker.

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

Ingredient	%, as-fed basis
Barley	96.2
Limestone	1.1
Dicalcium phosphate	0.8
Vitamin premix[1]	0.5
Mineral premix[2]	0.5
Chromic oxide	0.5
Salt	0.4

[1]Provided per kg 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 kg of diet: Zn, 100 mg as zinc sulfate; Fe, 80 mg as ferrous sulfate; Cu, 50 mg a copper sulfate; Mn, 25 mg as manganous sulfate; I, 0.5 mg as calcium iodate; and Se, 0.1 mg as sodium selenite.

In Vivo Total Tract Energy Digestibility

The ATTD of energy was measured using 63 crossbred barrows (Camborough-22 × Line 65; PIC Canada Ltd., Airdrie, Alberta, Canada; 33 ± 2 kg of initial BW) to obtain 6 measurements per barley sample. Three replications of 21 pigs were used in each of 2 consecutive periods for a total of 126 observations; each pig was randomly fed 1 diet per period. Pigs were housed in individual pens that allowed freedom of movement for 30 d: a 10-d acclimation to a 96%-barley diet followed by 2 consecutive 10-d experimental periods, feeding 2 different experimental diets. 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). Equal amounts of the diets were fed at 0800 and 1600 h. Diets were fed as a wet mash; dry mash was added to the feeder, followed immediately by water (approximately 1:1). Pigs had free access to water throughout the experiment.

Feces were collected for a minimum of 2 times per day at 0800 and 1600 h using plastic bags attached to the skin around the anus (Van Kleef et al., 1994). Feces were pooled for each pig and frozen at –20°C. Before analyses, feces were thawed, homogenized, subsampled, and freeze-dried.

Chemical Analyses

Barley, feed, and freeze-dried feces 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, 1990). The GE of barley, feed, and feces was analyzed by an adiabatic bomb calorimeter (model 5003, Ika-Werke GMBH & Co. KG, Staufen, Germany); benzoic acid was used as 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 barley samples were analyzed for CP (Kjeldahl N; method 990.03; AOAC, 1995), ADF and acid detergent lignin (method 973.18; AOAC, 1990), NDF (Van Soest et al., 1991), ether extract (method 920.39; AOAC, 1990), ash (method 9420.5; AOAC, 1990), and Lys (method 15:982.30; AOAC, 1990) contents.

Based on the results of the chemical analyses, the ATTD of energy for each diet was calculated 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 barley sample. The DE content of each barley sample was calculated by multiplying the ATTD of energy with the GE content of the specific barley sample.

In Vitro Energy Digestibility

In a preliminary study, 3 of the 21 barley samples with a wide range in ATTD of energy were selected. The 3-step technique (described later) was compared with a 2-step technique. In the 2-step technique, the final step of the 3-step technique was omitted (i.e., the cellulase incubation).

The 21 barley samples were then analyzed in duplicate using the 3-step technique for in vitro energy digestibility. Finally, 7 of the 21 samples were selected, ensuring that the entire ranges of ATTD and fiber characteristics were covered and analyzed using the 3-step technique for in vitro energy digestibility in quadruplicate to decrease analytical errors.

The 3-step in vitro energy digestibility technique developed in our laboratory was used (Huang et al., 2003). Briefly, the barley samples were finely ground through a 1-mm mesh size screen in a Retsch mill (model ZM1, Brinkman Instruments, Rexdale, Ontario, Canada). A sample (1.0 ± 0.1 mg) 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 using a small magnetic rod. After adding 10 mL of 0.2 *N* HCl solution to the flask, the pH of the solution was adjusted to 2 using 1 *N* HCl or 1 *N* NaOH solutions. Then, 1 mL of freshly prepared pepsin (P-7000, Sigma-Aldrich, Oakville, Ontario, Canada; 800 to 2,500 units/mg of protein, from porcine gastric mucosa) and 0.5 mL of chloramphenicol solutions (0.5 g/100 mL of ethanol) were added to the flask and incubated in a water bath at 39°C for 6 h. After the incubation, 10 mL of 0.2 *N* phosphate buffer (pH 6.8) and 5 mL of 0.6 *N* NaOH solutions were added to the flask, and the pH of the solution was adjusted to 6.8 with 1 *N* HCl or 1 *N* NaOH solutions. Thereafter, 3 mL of freshly prepared pancreatin (P-1750; activity equivalent to 8 × USP specification; from porcine pancreas; Sigma-Aldrich) solution was added to the flask. The flask was incubated in a water bath at 39°C for 18 h. Then, 20 mL of freshly prepared cellulase solution (C-9422; 3 to 10 units/mg of solid; from *Trichoderma viridae,* Sigma-Aldrich) was added, and the flask was incubated for 24 h at 39°C. The enzymatic digestion was terminated by addition of 5 mL of 20% sulphosalicylic acid, and the flask was kept at room temperature for 30 min to facilitate precipitation of undigested soluble proteins. The undigested residues were then collected in a filtration unit using porcelain filtration funnel lined with preweighed filter paper (Whatman no. 54, Whatman Inc., Florham Park, NJ). The residues along with the filter paper were 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

The barley sample was considered as the experimental unit for linear regression analyses. Using 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 energy digestibility, using the R^2 value as an indicator of quality of the prediction equation. For the final set of 7 barley samples, in vitro DM digestibility was regressed to in vitro energy digestibility and the absolute difference between in vitro and in vivo energy digestibility.

RESULTS

Physical and Chemical Characteristics

The physical characteristics of the 21 barley samples are presented in Table 2. The ranges (from least to greatest) for field and clean test weight and dockage were 34.6 kg/hL, 31.7 kg/hL, and 3.8%, respectively. Of all the characteristics, dockage had the greatest CV.

Among the chemical characteristics, the range for GE was 0.12 Mcal/kg DM with the lowest CV (Table 2). The ranges in the contents of CP, ADF, NDF, ether extract, ash, and lignin of the barley samples were 6.4, 6.9, 13.2, 1.2, 2.5, and 1.8 percentage units, respectively. Lignin and ADF had the greatest CV among the chemical characteristics.

The R^2 between in vivo energy digestibility and chemical and physical characteristics was greatest for ADF (0.74), followed by field test weight (0.64), NDF (0.60), clean test weight (0.59), and lignin (0.49; Table

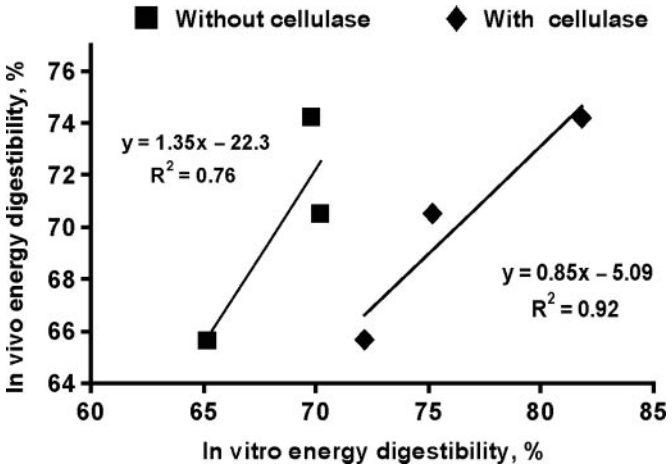

Figure 1. Relationship between in vivo apparent total tract energy digestibility in grower pigs and in vitro energy digestibility of 3 barley samples using a 2-step (without cellulase) and 3-step (with cellulase) in vitro digestibility technique (preliminary study).

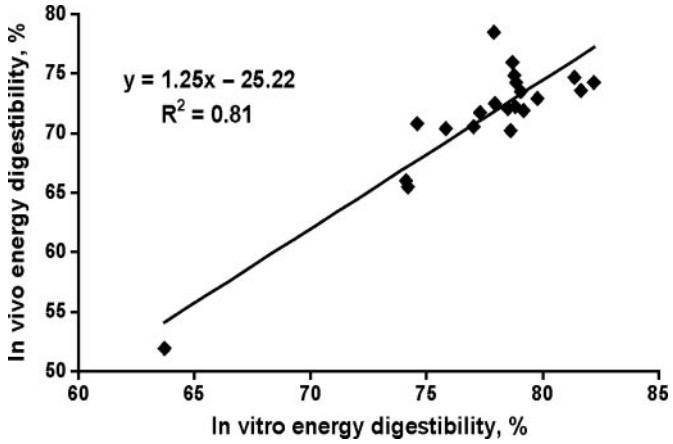

Figure 2. Relationship between in vivo apparent total tract energy digestibility in grower pigs and initial 3-step in vitro energy digestibility of 21 barley samples in duplicate.

2). The R^2 between in vivo ATTD of energy and other listed chemical and physical characteristics was less than 0.20.

Apparent Total Tract Digestibility of Energy

The ATTD of energy ranged from 51.9 to 78.5% for the 21 barley samples (Table 2). The range in the ATTD of energy corresponded strongly (R^2 = 0.99) with the range in DE content from 2.32 to 3.43 Mcal/kg of DM.

In Vitro Energy Digestibility

In the preliminary study with the 3 barley samples, the relationship between in vivo and in vitro energy digestibility was stronger using the 3- than the 2-step in vitro digestibility technique (R^2 = 0.92 vs. 0.76; Figure 1). The 3-step technique resulted in greater in vitro energy digestibility values and in a wider range (10 vs. 5%) in energy digestibility values. Hence, the 3-step technique was selected for the remainder of the in vitro digestibility analyses.

Using the 3-step technique initially in duplicate for the 21 barley samples, in vitro energy digestibility values ranged from 63.7 to 82.2% (Figure 2) and were strongly related (R^2 = 0.81) to the ATTD of energy. The regression equation to predict in vivo from in vitro energy digestibility was y = 1.25 x − 25.22. The in vitro energy digestibility values were greater than the ATTD values of energy.

With the 3-step in vitro digestibility technique in quadruplicate for the 7 selected samples, energy digestibility ranged from 63.5 to 82.8% (Figure 3). The

Table 2. Physical, chemical, and energy characteristics of the 21 hulled barley samples and their R^2 with in vivo apparent total tract energy digestibility[1]

Characteristic	Mean	SD	CV	Least	Greatest	R^2
Physical characteristics						
Field test weight, kg/hL	55.0	7.2	13.1	31.5	66.1	0.64
Clean test weight, kg/hL	56.1	6.6	11.7	35.1	66.8	0.59
Dockage, %	1.72	1.1	65.2	0.26	4.10	0.02
Chemical characteristics, DM basis						
Moisture, %	11.8	1.6	11.5	8.8	14.1	0.11
GE, Mcal/kg	4.39	0.03	0.01	4.34	4.46	0.17
CP, %	13.7	1.4	10.5	10.0	16.4	0.01
ADF, %	6.8	1.5	22.4	4.5	11.4	0.74
NDF, %	26.2	2.9	11.1	21.9	35.1	0.60
Ether extract, %	2.3	0.3	13.3	1.8	3.0	0.00
Ash, %	2.8	0.6	19.9	2.2	4.7	0.04
Lignin, %	1.3	0.4	29.8	0.9	2.7	0.49
Lys, %	0.52	0.04	8.6	0.38	0.57	0.13
In vivo swine energy characteristics						
Apparent total tract digestibility, %	71.4	5.4	7.5	51.9	78.5	—
DE content, Mcal/kg of DM	3.14	0.23	7.3	2.32	3.43	0.99

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

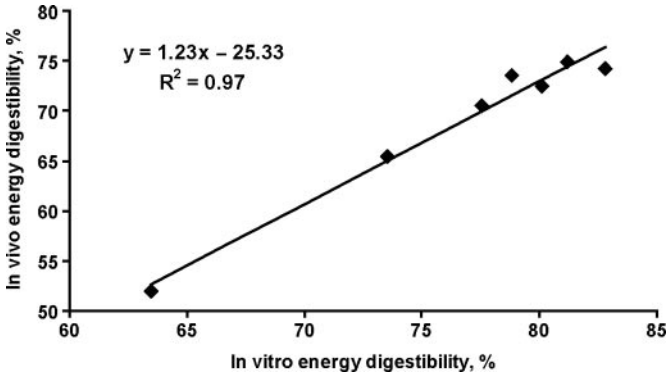

Figure 3. Relationship between in vivo apparent total tract energy digestibility and the final 3-step in vitro energy digestibility of 7 barley samples in grower pigs.

R^2 between in vitro and in vivo energy digestibility was very high (R^2 = 0.97). The regression equation to predict in vivo from in vitro energy digestibility was y = 1.23 x − 25.33. The in vitro energy digestibility values were 6.3 to 11.6% units greater than the in vivo ATTD values of energy.

The difference between in vivo and in vitro energy digestibility was inversely related to in vivo energy digestibility (R^2 = 0.72; Figure 4). In vitro DM digestibility was perfectly related (R^2 = 1.00; Figure 5) to in vitro energy digestibility values. The R^2 between in vitro DM digestibility and in vivo ATTD values of energy was very high (R^2 = 0.97; Figure 6).

The analytical error for the determination of the ATTD values for energy ranged from 0.5 to 5% for the 21 barley samples, whereas the error for the initial duplicate in vitro analysis of the 21 barley samples ranged from 0.2 to 2.7% (Figure 7). The error of the final quadruplicate in vitro analysis ranged from 0.2 to 2.6% for the 7 samples. Specifically, the error was 2.6% for the barley sample with the lowest energy digestibility and ranged from 0.2 to 1.1% for the other 6 barley samples.

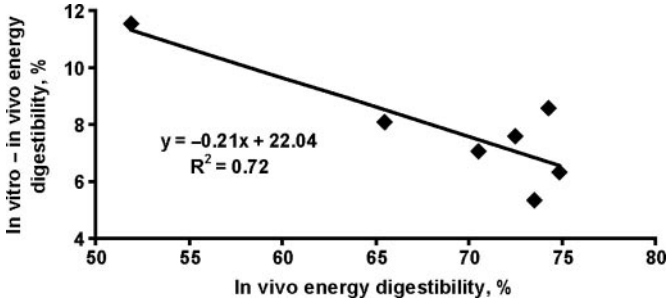

Figure 4. Relationship between the absolute difference between in vitro and in vivo energy digestibility and in vivo energy digestibility of 7 barley samples in grower pigs.

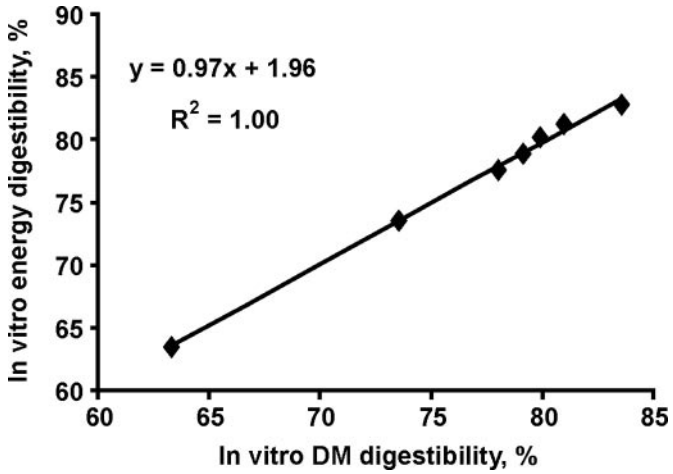

Figure 5. Relationship between in vitro energy digestibility and in vitro DM digestibility of 7 barley samples in growing pigs.

DISCUSSION

Barley is a major feedstuff providing energy to pigs. The variation in DE content among samples of barley grain is considerable. The reported DE content ranges from 2.80 to 3.59 Mcal/kg of DM among studies (Anderson and Bell, 1983; Noblet et al., 1993; Fairbairn et al., 1999; Van Barneveld, 1999), and this range is largely dependent on changes in the ATTD of energy and not changes in GE content (Zijlstra, 2006). Similarly, changes in the ATTD of energy are the largest cause of the variation in NE content of feedstuffs (Noblet, 2006). Energy is the most expensive component in diets for pigs; hence, accurate prediction of the ATTD of energy in barley on a routine basis is important so that diets can be formulated and processed with the proper energy content.

In vitro digestibility techniques can predict the energy digestibility of diets for pigs (Boisen and Fernán-

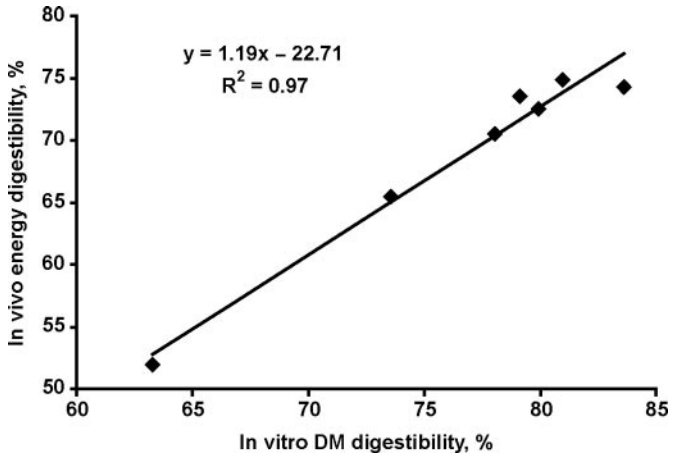

Figure 6. Relationship between in vivo energy digestibility and in vitro DM digestibility of 7 barley samples in growing pigs.

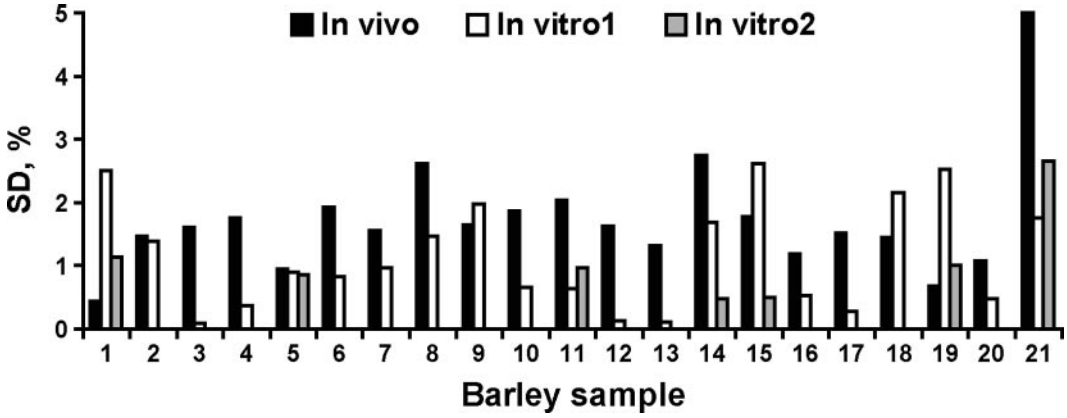

Figure 7. Analytical relative error (SD) of the in vivo and in vitro energy digestibility measurements of barley samples; (black boxes), % error in the apparent total tract energy digestibility of 21 barley samples; (white boxes), % error in the initial 3-step in vitro energy digestibility of 21 barley samples analyzed in duplicate; and (gray boxes), % error in the final 3-step in vitro energy digestibility of 7 barley samples analyzed in quadruplicate.

dez, 1997; Huang et al., 2003) and are less expensive and time-consuming than animal experiments. These techniques have been validated for compound feeds and feedstuffs for pigs (Noblet and Jaguelin-Peyraud, 2007). However, in vitro techniques may not be suitable for predicting the variability of in vivo digestibility of DM, and for that matter, also likely for energy within cereal grains such as barley (Pujol and Torrallardona, 2007). Therefore, the present study focused on establishing and validating the in vitro energy digestibility technique developed in our laboratory (Huang et al., 2003) to predict the ATTD of energy of barley varying in quality. For example, the comparison of the 2-step and 3-step techniques in the preliminary study indicated that a better separation of barley samples that differ in quality (i.e., DE content) can be achieved using the 3-step technique.

Previously, the R^2 between in vivo and in vitro energy digestibility was determined using 6 barley samples including 2 hulled, 2 hull-less, and 2 samples of mixtures of both barley types, and the R^2 was 0.93 (Huang et al., 2003). However, limitations in the study by Huang et al. (2003) existed: 1) the barley samples had a narrow range in ADF, from 2.2 to 5.1%, in contrast to a range from 4.5 to 11.4% in the present study, 2) 2 barley types, thus, 2 feedstuffs were included in the study (hulled and hull-less barley), thereby creating an artificial range in sample quality, and 3) the sample set included mixed samples (i.e., half each of the hulled and hull-less barley samples so that the ability to properly mix samples was determined but not the ability to predict in vivo energy digestibility). Similarly, a sample set of barley that was created by including both hulled and hull-less barley types and separating barley samples into several fractions by gravity (Beames et al., 1996) will overestimate the quality of in vitro techniques to predict ATTD of energy. Independent samples (i.e., samples collected separately with a large range in quality characteristics) are required to properly determine if an in vitro energy digestibility assay can properly predict the in vivo ATTD values of energy.

The 21 samples of hulled barley in the present study had a wide range in physical and chemical characteristics, ATTD values of energy, and DE content. Thus, these samples were a fair representation of the large range in characteristics and DE content in barley reported previously. For example, the DE and ADF content in the present study ranged from 2.32 to 3.43 Mcal/kg of DM and 4.5 to 11.4%, respectively, whereas Fairbairn et al. (1999) reported ranges from 3.00 to 3.50 Mcal/kg of DM and from 5.0 to 10.2% for ADF. A wide range in characteristics of interest is critical for the development of accurate predictions of the main component of interest (i.e., in vivo ATTD values of energy). Interestingly, among all characteristics measured on the 21 barley samples in relation to in vivo ATTD values of energy, the R^2 was greatest for in vitro energy digestibility ($R^2 = 0.81$), and none of the other specific physical and chemical characteristics had a R^2 greater than 0.74. Previously, among chemical characteristics, the ADF content had been suggested as the best predictor of DE content (Fairbairn et al., 1999). Thus, mimicking energy digestion in vitro will result in a better prediction of in vivo ATTD values of energy than analyses of chemical constituents or descriptions of physical appearance.

Results from the present study indicated that some improvement must be made in the in vitro digestion technique or its execution so that predictions of the ATTD of barley are more accurate. The in vitro technique used in the present study can predict quality differences within barley accurately and differs from other techniques. For example, a cellulase enzyme was used as the third step to mimic hindgut fermentation, instead of a fiber-digesting, multi-enzyme complex in other studies (e.g., Boisen and Fernández, 1997). The enzymatic activity of the multi-enzyme complex might not be sufficient to properly digest barley samples that

are of a very low quality (Noblet and Jaguelin-Peyraud, 2007). The sample with the lowest in vivo ATTD value of energy had the largest gap between in vitro and in vivo energy digestibility in the present study. Thus, for low quality barley samples that are high in fiber, either the enzyme activity is not sufficient to hydrolyze the greater amount of fiber, or the sample has a different composition of fiber or other components that can reduce energy digestibility.

Apart from accuracy, repeatability of in vitro analyses is important. The relative error of the in vitro analysis was less than for in vivo ATTD values of energy. However, the high relative error of the initial duplicate in vitro analyses (up to 2.7%) indicated that further improvement in precision can be achieved via increased experience of the operator or increased number of analyses per sample. Indeed, with quadruplicate in vitro analysis of 7 selected barley samples that covered a wide range in the ATTD of energy and ADF content, the relative error of the in vitro analysis decreased to less than 1.2%, except for 1 sample with a very low ATTD of energy and a high fiber content that had a relative error of 2.6%. As a combined result, the R^2 of the in vitro technique increased to 0.97. The high R^2 and low relative error of the analysis indicate that the present in vitro digestibility technique can serve as a reference analysis for developing equations or calibration of technologies to rapidly predict the ATTD of energy and DE content of barley (Zijlstra, 2006; Pujol et al., 2007).

In the present study, the perfect relationship (R^2 = 1.00) between in vitro DM digestibility and in vitro energy digestibility measurements was observed, indicating that both measurements were equally good in predicting the ATTD of energy of barley samples. The relationship between DM and energy digestibility seems logical, in part because GE is a direct indicator of the OM, which is the main component of DM. Therefore, in vitro DM digestibility values have been used to predict the ATTD of energy and DE content of compound feeds and individual feedstuffs (Boisen and Fernández, 1997; Noblet and Jaguelin-Peyraud, 2007). Equations have been developed using a combined array of feedstuffs, including barley, to predict energy quality of all feedstuffs (Noblet and Jaguelin-Peyraud, 2007). However, the R^2 of these equations (Noblet and Jaguelin-Peyraud, 2007) was less than that observed in the present study (0.77 versus 0.81 to 0.97). Thirty-three different feedstuffs with different sample sizes were used to develop in vivo energy digestibility prediction equations by Boisen and Fernández (1997). The R^2 between in vitro OM digestibility and in vivo ATTD values of energy using many different feedstuffs was less than using 1 specific category of feedstuffs (0.69 versus 0.94 to 0.98). Therefore, the approach of using 1 regression equation developed using multiple feedstuffs might not be suitable for the accurate prediction of the ATTD of energy and DE content of different batches of an individual feedstuff, and in vitro digestibility techniques or prediction equations should be developed for individual feedstuffs or feedstuff categories based on the macronutrient profile.

In vitro digestibility techniques do not directly give an indication of the absolute values of the ATTD of energy. The in vitro energy digestibility values were consistently greater than in vivo ATTD values of energy digestibility of barley samples in the present and previous (Huang et al., 2003) studies. The difference could be due to the observation that the in vivo digestibility represents apparent digestibility and the in vitro digestibility represents true digestibility because endogenous losses (e.g., mucin and sloughed villi) are not included in the in vitro techniques (Boisen and Fernández, 1995). Furthermore, this difference might be partly due to difference in grinding procedures between the 2 procedures; the barley samples were ground more finely for the in vitro technique than for the determination of ATTD in pigs. Increased particle size reduced the ATTD of barley-based diets in pigs (Oryschak et al., 2002) and reduced particle size increased digestibility values in vitro techniques (Heaton et al., 1988). Finally, incomplete precipitation of soluble proteins by sulfosalicylic acid might underestimate in vitro digestion residues; thus, digestibility of the protein fraction is overestimated (Wilfart et al., 2008). The difference between in vitro and in vivo digestibility values was greater for hulled barley samples (6.3 to 11.6% units; present study) compared with hulled and hull-less samples (3.5 to 6.6% units; Huang et al., 2003). The largest difference between in vitro and in vivo digestibility values was observed for the barley sample with the greatest ADF content and the least ATTD values of energy; whereas, this difference was consistent for the rest of the samples. Larger differences between in vivo and in vitro energy digestibility were also observed for compound feeds with low energy digestibility in pigs (Noblet and Jaguelin-Peyraud, 2007). This difference indicates that the in vitro energy digestibility measurements probably underestimate the negative effects of fiber on energy digestibility or that a better technique needs to be developed for barley samples with a very low quality.

In conclusion, the in vitro digestibility technique using pepsin, pancreatin, and cellulase sequentially can predict the ATTD of energy of barley samples in grower pigs with high accuracy. As such, the regression equation developed in the present study with barley samples having a wide range in quality attributes can be used to estimate energy digestibility of barley. The in vitro digestibility technique can be useful as the core analytical procedure to calibrate rapid analytical equipments (e.g., near infrared reflectance spectroscopy, to predict energy digestibility on a routine basis; Zijlstra, 2006). However, improvement in the technique is necessary to further enhance the predictive power of the technique, especially in very low quality barley samples. Further

research is warranted to validate the in vitro technique to predict the ATTD of energy in feedstuffs other than barley.

LITERATURE CITED

Anderson, D. M., and J. M. Bell. 1983. The digestibility by pigs of dry matter, energy, protein and amino acids in wheat cultivars. II. Fifteen cultivars grown in two years, compared with Bonanza and Fergus barleys, and 3CW-grade hard red spring wheat. Can. J. Plant Sci. 63:393–406.

AOAC. 1990. Official Methods of Analysis. 14th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 1995. Official Methods of Analysis. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Beames, R. M., J. H. Helm, B. O. Eggum, S. Boisen, K. E. Bach Knudsen, and M. L. Swift. 1996. A comparison of methods for measuring the nutritive value for pigs of a range of hulled and hulless barley cultivars. Anim. Feed Sci. Technol. 62:189–201.

Boisen, S., and J. A. Fernández. 1995. Prediction of the apparent ileal digestibility of protein and amino acids in feedstuffs and feed mixtures for pigs by in vitro analyses. Anim. Feed Sci. Technol. 51:29–43.

Boisen, S., and J. A. Fernández. 1997. Prediction of the total tract digestibility of energy in feedstuffs and pig diets by in vitro analyses. Anim. Feed Sci. Technol. 68:277–286.

CCAC. 1993. Guide to the care and use of experimental animals. Vol. 1. 2nd ed. Canadian Council on Animal Care, Ottawa, Ontario, Canada.

Fairbairn, S. L., J. F. Patience, H. L. Classen, and R. T. Zijlstra. 1999. The energy content of barley fed to growing pigs: Characterizing the nature of its variability and developing prediction equations for its estimation. J. Anim. Sci. 77:1502–1512.

Fenton, T. W., and M. Fenton. 1979. An improved procedure for the determination of chromic oxide in feed and faeces. Can. J. Anim. Sci. 59:631–634.

Heaton, K. W., S. N. Marcus, P. M. Emmett, and C. H. Bolton. 1988. Particle size of wheat, maize, and oat test meals: effects on plasma glucose and insulin responses and on the rate of starch digestion in vitro. Am. J. Clin. Nutr. 47:675–682.

Huang, G., W. C. Sauer, J. He, J. Hwangbo, and X. Wang. 2003. The nutritive value of hulled and hulless barley for growing pigs. 1. Determination of energy and protein digestibility with the in vivo and in vitro method. J. Anim. Feed Sci. 12:759–769.

Jørgensen, H., W. C. Sauer, and P. A. Thacker. 1984. Amino acid availabilities in soybean meal, sunflower meal, fish meal and meat and bone meal fed to growing pigs. J. Anim. Sci. 58:926–934.

Molina-Cano, J. L., M. Francesch, A. M. Perez-Vendrell, T. Ramo, J. Voltas, and J. Brufau. 1997. Genetic and environmental variation in malting and feed quality of barley. J. Cereal Sci. 25:37–47.

Noblet, J. 2006. Energy systems for pig feeds. Pages 30–39 in Proc. Net Energy Systems for Growing and Fattening Pigs, Pre-Symp. 10th Int. Symp. Digestive Physiol. in Pigs. May 24, Vejle, Denmark. CVB, Lelystad, the Netherlands.

Noblet, J., H. Fortune, C. Dupire, and S. Dubois. 1993. Digestible, metabolizable and net energy values of 13 feedstuffs for growing pigs: Effect of energy system. Anim. Feed Sci. Technol. 42:132–149.

Noblet, J., and Y. Jaguelin-Peyraud. 2007. Prediction of digestibility of organic matter and energy in the growing pig from an in vitro method. Anim. Feed Sci. Technol. 134:211–222.

NRC. 1998. Nutrient Requirements of Swine. 10th rev. ed. Natl. Acad. Press, Washington, DC.

Oryschak, M. A., P. H. Simmins, and R. T. Zijlstra. 2002. Effect of dietary particle size and carbohydrase and/or phytase supplementation on nitrogen and phosphorus excretion of grower pigs. Can. J. Anim. Sci. 82:533–540.

Pujol, S., A. M. Pérez-Vendrell, and D. Torrallardona. 2007. Evaluation of prediction of barley digestible nutrient content with near-infrared reflectance spectroscopy (NIRS). Livest. Sci. 109:189–192.

Pujol, S., and D. Torrallardona. 2007. Evaluation of in vitro methods to estimate the in vivo nutrient digestibility of barley in pigs. Livest. Sci. 109:186–188.

Van Barneveld, R. J. 1999. Chemical and physical characteristics of grains related to variability in energy and amino acids availability in pigs: A review. Aust. J. Agric. Res. 50:667–687.

Van Kleef, D. J., K. Deuring, and P. van Leeuwen. 1994. A new method of faeces collection in the pig. Lab. Anim. 28:78–79.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fibre, neutral detergent fibre, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597.

Wilfart, A., Y. Jaguelin-Peyraud, H. Simmins, J. Noblet, J. van Milgen, and L. Montagne. 2008. Kinetics of enzymatic digestion of feeds as estimated by a stepwise in vitro method. Anim. Feed Sci. Technol. 141:171–183.

Zijlstra, R. T. 2006. Rapid methods for estimation of energy values of feedstuffs for pigs. Pages 74–80 in Proc. Net Energy Systems for Growing and Fattening Pigs, Pre-Symp. 10th Int. Symp. Digestive Physiol. in Pigs. May 24, Vejle, Denmark. CVB, Lelystad, the Netherlands.

Zijlstra, R. T., C. F. M. de Lange, and J. F. Patience. 1999. Nutritional value of wheat for growing pigs: Chemical composition and digestible energy content. Can. J. Anim. Sci. 79:187–194.