RESEARCHREPORT

ANIMAL SCIENCE — Nutritional Efficiency Consortium

Title: In vitro estimation and in vivo determination of metabolizable energy in corn co-products - **NPB - #08-107**

Investigator: Brian J. Kerr, Ph.D.

Institution: USDA-ARS-NSTL, Ames, IA

Date Submitted: May 21, 2009

Industry Summary: Energetics will continue to be of great importance to the swine industry because energy contributes a significant expense to feed which contributes more than 60% of pig production costs. Changes in the corn milling industry are improving the quality and increasing the variety of co-products that can be fed to pigs. Accurate energy values must be established on these new co-products as they are developed to provide nutritionists more options in generating balanced diets. The twenty co-products evaluated in this study had unique nutrient profiles and resulted in a wide range of organic matter digestibility and apparent metabolizable energy values. The *in vitro* organic matter (OM) digestibility ranged from 33.3 to 93.5% for corn bran and dried solubles, while metabolizable energy (ME) ranged from 2,334 to 8,755 kcal/kg for corn gluten feed and corn oil, respectively. Although *in vitro* OM digestibility was correlated to *in vivo* ME ($r = 0.62$, $P < 0.01$), it did not improve the prediction of ME from ingredient analysis. Stepwise regression resulted in the equation: ME, kcal/kg = (0.949 × gross energy) – (32.238 × total dietary fiber) – (40.175 × ash) ($r^2 = 0.95$, SE = 306, $P < 0.01$), thereby providing a reliable estimate of ME in corn co-products commonly fed to growing swine. For further information, contact Dr. Brian Kerr, USDA-ARS, Ames, IA, by phone (515-294-0224) or email (brian.kerr@ars.usda.gov).

Scientific Abstract: Twenty co-products from various ethanol plants were fed to finishing pigs to determine ME and to generate an equation to predict ME based upon each ingredient's chemical analysis. Additionally, a 3-step enzymatic assay was used to determine if in vitro OM digestibility would predict in vivo ME or improve the prediction estimate of ME for corn co-products. Co-products included: DDGS (7), HP-DDG (3), bran (2), germ (2), gluten meal and feed, dehulled degermed corn, dried solubles, starch, and corn oil. The in vitro OM digestibility for each co-product was determined in triplicate using procedures as described by Boisen and Fernandez (1997). For the in vivo study, the control diet was based on corn (97.1%), limestone, salt, vitamins, and trace minerals. All but two test diets were formulated by mixing the control diet with 30% of a co-product. Dried solubles and oil were included at 20% and 10%, respectively. Eight groups of 24 gilts (n=192, 112.7 final

BW ± 7.9 kg) were randomly assigned to a test diet and each diet was fed to a total of 8 pigs. Gilts were placed in metabolism crates and fed an amount equivalent to 3% BW daily for 9 d followed by collecting feces and urine separately for 4 d. Ingredients were analyzed for GE, CP, moisture, crude fat, crude fiber, ash, total dietary fiber (TDF), NDF, and ADF. Gross energy was determined on the feed, feces, and urine to calculate ME for each ingredient. The in vitro OM digestibility ranged from 33.3 to 93.5% for corn bran and dried solubles, while ME ranged from 2,334 to 8,755 kcal/kg for corn gluten feed and corn oil, respectively. Although in vitro OM digestibility was correlated to in vivo ME ($r = 0.62$, $P < 0.01$), it did not improve the prediction of ME from ingredient analysis. Stepwise regression resulted in the equation: ME, kcal/kg = (0.949 × GE) – (32.238 × TDF) – (40.175 × ash) ($r^2 = 0.95$, SE = 306, $P < 0.01$). These results indicate that OM digestibility and ME vary substantially between corn co-products and the best predictors of ME are GE, TDF, and ash.

Introduction: Dietary energy is the most expensive component of swine diets. Corn has been the cereal grain of choice for swine diets because it is easily grown in the United States and has high available energy content (starch). For these same reasons, the biofuels industry use corn for the production of ethanol. Currently 170 wet and dry-grind plants are operational in the United States with only 9 not using corn as the major feedstuff for fuel production. The majority (67%) of ethanol produced in the United States is produced by dry-grind milling which only generates one co-product, dried distillers grains with solubles (DDGS). Dried distillers grains with solubles is a moderately high fiber product that has been used widely in cattle diets but has had limited inclusion in swine diets due to their decreased capacity for fiber utilization. Current developments in corn milling technologies are increasing the efficiency of starch and oil extraction generating "new" marketable co-products that have potential use in the swine industry. Energy values for these "new" corn co-products are lacking in the literature and further research in this area is warranted. Prediction equations from chemical analysis are a useful tool and currently are only available for complete diets and DDGS.

Objectives: The objectives of the proposed research are to: 1-determine the ability of an *in vitro* organic matter dry digestibility technique to predict digestible energy content of various corn co-products in finishing pigs, 2-determine the apparent digestible and metabolizable energy content of these feedstuffs in finishing pigs, and 3-compare these two methods of digestible energy determination.

Materials & Methods-Energy Determination

General Pig Management. The Iowa State University Animal Care and Use Committee approved all experimental protocols, #12-07-6480-S. Twenty co-products from the corn wet- and dry-milling industry were obtained from vendors located throughout the U.S. (Table 1). Table 2 describes the basal diet while Table 3 describes ingredient composition as determined by a commercial laboratory (University of Missouri Agriculture Experiment Station Chemical Laboratories, Columbia, MO), GE (isoperibol bomb calorimeter, model 1281, Parr Instrument Co., Moline, IL), particle size (Mashoffs, 13-sieve RoTap), and bulk density (USDA, 1953). For the *in vivo* study, the control diet was based on corn (97.1%), limestone, salt, vitamins, and trace minerals. This basal diet was used to independently determine the ME of corn compared to book values and was used as a covariate between groups of pigs. Test diets were formulated by mixing the control diet (70%) with 30% of a co-product, except for test diets containing dried solubles or corn oil which were included at 20% and 10% of the total diet, respectively. Inclusion of corn starch and corn oil were used to validate our methodology as outlined by Adeola (2001). A moderate inclusion level for all products used as there was concern about the effect of inclusion level on feed intake and subsequently on ME determination.

Eight groups of 24 gilts (Cambrough 22 females × L337 sires, n=192, 112.7 final BW ± 7.9 kg) that had previously been fed a standard corn-soybean meal finisher diet were randomly assigned to a test diet such that each diet was fed to a total of 8 pigs. Each group of 24 gilts consisted of feeding 5 test diets to 4 gilts in addition to the common basal diet fed to 4 gilts. Consequently, two groups of 24 gilts were required to obtain a total of 8 pigs fed each test diet. Gilts were placed into stainless steel metabolism crates (1.1 × 2.3 m) and fed an amount equivalent to 3% BW daily for 9 d followed by total collection of feces and urine for an additional 4 d. During the collection period, urine was collected once daily into stainless steel buckets containing 30 mL of 6 N HCl,

and stored at 0°C until the end of the collection period. At the end of the collection period, urine was thawed, weighed, and a subsample collected and stored at 0°C until subsequent analysis. Likewise, feces were collected daily and stored at 0°C until subsequent processing. Feed consumption and refusal was recorded at the end of the experimental period. Water was available from a nipple waterer at all times.

Additionally, a 3-step enzymatic assay was used to determine if *in vitro* OM digestibility would predict *in vivo* ME or improve the prediction estimate of ME for corn co-products. The *in vitro* OM digestibility for each co-product was determined in triplicate using procedures as described by Boisen and Fernandez (1997), using corn as the control feedstuff.

Chemical Analyses. Feedstuff samples were ground through a 1-mm screen before energy determination. Fecal samples were thawed, dried at 70°C for 48 h, and weighed to determine the DM content. Fecal samples were ground through a 1-mm screen in preparation for energy determination. For urine energy determination, 3 mL of urine was added to 0.5 g of dried cellulose and subsequently dried at 50°C for 24 h before energy determination. The GE of feed, feces, and urine plus cellulose was determined using an isoperibol bomb calorimeter (model number 1281, Parr Instrument Co., Moline, IL), with benzoic acid used as a standard. Duplicate analyses were performed on all diets and fecal samples from each pig, whereas triplicate analyses were performed on urine plus cellulose from each pig. Urinary energy was determined by subtracting the energy contained in cellulose from the combined urine plus cellulose energy.

Calculations and Statistical Analysis. Energy intake was calculated by multiplying the GE value of the diet fed by feed intake over the 4-d collection period. Apparent DE values were calculated by subtracting fecal energy from intake energy and apparent ME values calculated by subtracting urinary energy from apparent DE. The apparent DE and ME values of the test ingredient fed to the pigs was estimated by difference from the basal diet as described by Adeola (2001). Using the individual pig as the experimental unit, data from each experiment were subjected to ANOVA using the basal ME as a covariate and group and treatment in the model (SAS Inst. Inc., Cary, NC). Differences between means were tested using the PDIFF option. In addition, a stepwise regression model was used to equate the effect of feedstuff composition on apparent ME with variables having P values < 0.15 maintained in the model.

Materials and Methods-*In vitro* Method

A slightly modified 3-step enzymatic assay previously described by Boisen and Fernandez (1997) was used. Twenty corn co-products and diet subsamples were collected from a previous study which determined the DE and ME of these co-products in finishing pigs. All subsamples were stored frozen over a six month period prior to this analysis. Feed samples were done in triplicate in a series of 24. A blank and a control (corn) were also done in triplicate in each series. Prior to conducting the *in vitro* assay, all samples were ground to 1 mm and weighed out to 0.5 g (± 0.1 g) per flask.

Preliminary Incubation. Samples were placed into a 125 mL Erlenmeyer flask with a magnetic stir bar and 25 mL of phosphate buffer (0.1 M, pH = 6.0). Flasks were gently stirred at room temperature for 1-2 minutes. To lower the pH, 10 mL of 0.2 M hydrochloric acid was added to the solution of each flask and the pH is adjusted to 2.0 (±0.05) with 1 M hydrochloric acid. One mL of pepsin solution (porcine, reference Sigma P-7012) containing 25 mg of pepsin per mL of nanopure water was then added to the flask. To minimize fermentation in further incubation steps, 0.5 mL chloramphenicol solution (Sigma C0378, 0.5 g in 100 mL ethanol) was also added. Flasks were then capped with a glass stopper and placed into an incubated shaker (150 rpm) at 39˚C for 2 hours (± 1 min).

Secondary Incubation. After the preliminary incubation period, the flasks were removed from the shaker and 10 mL of phosphate buffer (0.2 M, pH 6.8) added. To increase the pH of the solution, 3.6 mL of 0.6 M NaOH is added. The final pH was adjusted to 6.8 (±0.05) with 1 M hydrochloric acid or 1 M NaOH. One milliliter of pancreatin solution (porcine, reference Sigma P-1750) containing 100 mg pancreatin per mL nanopure water was then added to the flasks. The flasks were subsequently capped and placed back in a 39˚C incubated shaker for 4 hours (± 1 min).

Final Incubation. After the secondary incubation period, the flasks were removed from the shaker and 10 mL of 0.2 M ethylenediaminetetraacetic acid (EDTA) added to the flasks. The pH of the solution was lowered to

4.8 (± 0.05) using 30% acetic acid solution and 0.5 mL of a multi enzyme complex, Viscozyme (reference Sigma V-2010) is added. The flasks were then capped and placed back in the incubated shaker at 39˚C for 18 hours. At the end of this final incubation, the flasks were removed from the shaker and the enzymatic reaction blocked by adding 1 mL of phenylmethylsulfonyl fluoride (reference Sigma P-7626) solution containing 10 mg/mL of methanol. The flasks were gently stirred at room temperature for 2 minutes.
Filtration. Before filtration, 0.400 g (± 0.050) of diatomaceous earth was added to each Gooch crucible as a filtration aid and heated in a muffle oven at 550˚C for 4 hours to what?. The crucible was cooled in a desiccator and weighed. The remaining residue from the incubation flasks was collected in a corresponding Gooch crucible through filtration after several rinses with nanopure water. The residue is further rinsed twice with 10 mL ethanol, once with 10 mL acetone, and once with nanopure water. After filtration and rinsing was complete, all Gooch crucibles were heated in a forced air oven at 103˚C for 4 hours, cooled in a desiccator until reaching room temperature, and weighed. Crucibles were then heated in a muffle oven at 550˚C for 4 hours. Crucibles were cooled in a desiccator and are weighed.
Calcualations. Organic matter digestibility (OMD) was determined by calculating the difference between the organic matter (OM) of the sample and the undigested residue (ash) of the sample after converting it to a DM basis, and then correcting for the OM in the blank. Percent of OMD was determined by dividing the digested OM weight by the weight of the OM of the sample.
Statistical Analysis. Using the average sample OMD as the dependent variable and ME as the independent variable, the relationship between *in vitro* OMD and *in vivo* ME data were analyzed with PROC REG and PROC CORR procedures of SAS (SAS Inst. Inc., Cary, NC).

Results and Discussion

Corn starch and corn oil were included to determine DE and ME as a basis to compare to the NRC (1998) and to provide a validation of using this methodology for energy determination. In addition, they provided a highly digestible, low fiber extreme of corn co-products. They were not, however, included in chemical analysis due to their purity and lack of other nutrients. As expected, nutrient composition of the corn co-products varied substantially (Table 3). The concentration of CP ranged from 8.3% to 66.3% for dehulled, degermed corn and corn gluten meal, respectively. Starch content ranged from 0.5% to 100% for HP-DDG (MOR) and starch, respectively. Crude fiber ranged from 0.08% to 11.5% for dried solubles and bran (without solubles), respectively. Total dietary fiber (TDF) ranged from 2.6% to 53.6% for dehulled, degermed corn and bran (without solubles), respectively. Neutral detergent fiber (NDF) ranged from 2.3% to 61.1% for dried solubles and corn germ meal, respectively. Acid detergent fiber (ADF) ranged from 0.5% (dehulled, degermed corn and dried solubles) to 25.4% (HP-DDG MOR). Cellulose ranged from 0.8% to 22.6% for dehulled, degermed corn and HP-DDG (MOR), respectively. Lignin ranged from 0.3% to 3.5% for dried solubles and RO-DDGS, respectively. Crude fat (ether extract) ranged from 0.2% to 18.5% for dehulled, degermed corn and corn germ dehydrated, respectively. Ash ranged from 0.5% to 14.08% for dehulled degermed corn and dried solubles, respectively.

With the wide range in corn co-product composition, ME varied substantially ($P < 0.01$, Table 4). Because it was not our goal to compare these feedstuffs against each other, but rather to establish needed values for these co-products, individual comparisions will not be made, but rather the ranges will be described. The low fiber co-products (starch, oil, dried solubles and dehulled, degermed corn) had a range in ME from 4,080 to 8,755 kcal/kg DM, respectively. The seven DDGS samples had a range in ME from 3,414 to 4,141 kcal/kg DM, respectively. The high protein co-products (corn gluten meal and three sources of HP-DDG) ranged in ME from 3,676 to 4,606 kcal/kg DM for HP-DDG (ICM) and HP-DDG (MOR), respectively. The remaining fibrous feedstuffs (two sources of bran, and one source of corn gluten feed and corn germ meal) ranged in ME from 2,334 to 3,692 kcal/kg DM.

All co-products were included in the diet at a level of 30% with exception of dried solubles and corn oil which were included in the diet at 20% and 10%, respectively. Dried solubles were initially included in the diet at 30%, however, within a few days of adapting to this treatment, most pigs developed scours. The decision was made to reduce the inclusion of dried solubles to 20% of the diet for an additional 9 d of adaptation

whereupon no further problems were noted. Corn oil was included in the diet at 10% due to the high energy concentration of the feedstuff. Pigs were fed once daily at 3% BW for the entire duration of the metabolism trial. Although this might be a concern in diets containing crystalline AA, this amount was essentially *ad libidum* because almost all pigs had residual feed in the feeders each morning they were fed. Feed intake in energy balance experiments varies widely as reviewed by Kerr et al. (2009), but we felt it important to reflect the *ad libidum* feeding situation commonly found in the U.S. Treatments were acceptable to the pigs and very minimal feed refusal across all treatments was noticed, thereby confirming our full feeding desire. Only two pigs fed the DDGS-WI treatment refused greater than 20% of total feed offered and were subsequently removed from the study. In all, a total of 7 pigs were not included in statistical analysis for reasons including: greater than 20% total feed refused, lost fecal collections, or contaminated urine samples. Most treatments had eight observations with exception for DDGS-WI (6 observations), RO-DDGS (6 observations), corn germ (7 observations), and corn basal (30 observations) (Table 4).

Using stepwise regression and chemical analysis a prediction equation for ME was generated. Initially a y-intercept was included in the model but because the y-intercept was not significant, it was removed and the equation redefined. The equation was significant ($P < 0.01$) and provided a good estimate ($r^2 = 0.95$, SE 306 kcal/kg DM) for predicting the ME of the corn co-products evaluated in this study. Gross energy had a positive effect on the estimate for ME while TDF and ash had negative effects on the estimate for ME.

The corn co-products selected for this study varied substantially in the nutrient profiles. Ingredients included in this study were: low in fiber (starch, oil, dried solubles, and dehulled, degermed corn), moderate in protein and fiber (DDGS, 7 samples), high in protein (corn gluten meal; HP-DDG, 3 samples), and high in fiber (bran, 2 samples; corn germ meal, 2 samples; corn gluten feed). Most ingredients were obtained from various dry-grind ethanol plants with exception of corn gluten meal, corn gluten feed, and one source of corn germ meal which were obtained from various corn wet milling plants. One feedstuff, dehulled, degermed corn was a co-product from corn dry milling while corn starch and corn oil were food-grade products.

Although it was not the intention of this study to specifically evaluate the energy content of DDGS, this co-product has a reputation of nutritional inconsistencies which is largely dependent upon the plant source. Therefore the DDGS products we selected for this study included differences among quality as well as processing techniques. The cost of drying distillers grain is an expensive process and cylindrical drum drying which is traditionally used has some potential to cause overheating, burning, and unwanted Malliard reactions. The latter points have a negative effect on the palatability as well as the availability of nutrients and therefore energy to the animal. A burnt product can generate a bitter taste that is undesirable to the animal and over heating can lead to Maillard reactions that leave the total protein of the ingredient in a bound fraction (Powers et al., 1995). To partially address this situation an alternative drying method using microwave technology for DDGS was also included in our sample collection relative to the same product that was drum dried. Deoiling distillers is occasionally occurring in the dry milling industry such that we also obtained a DDGS that was reduced in oil where oil was removed using hexane extraction after fermentation resulting in DDGS with 3.2% crude fat compared to traditional DDGS that ranges 8 to11% (Spiehs, 2002).

Only a few ME values for the corn co-products evaluated in this study were available for comparison through published literature. The NRC (1998) lists ME values for corn (3,843 kcal/kg DM), starch (4,205 kcal/kg DM), and corn oil (8,405 kcal/kg DM). In comparison, the ME values determined in this study were 3,771, 4,080, and 8755 kcal/kg DM for corn, starch, and corn oil, respectively. These values are basically equal given our experimental pooled SD of 413 kcal/kg DM. Using these established co-products as an internal controls, this finding validated our animal and laboratory techniques, and the difference method for ingredient energy determination.

The ME for corn gluten meal published in the NRC (1998) is 4,255 kcal/kg DM and compares favorably to our determined ME value of 4,598 kcal/kg DM. Likewise, the NRC (1998) value for corn gluten feed is 2,894 kcal/kg DM and which is slightly higher than our obtained value of 2,334 kcal.kg DM. The seven DDGS selected for this study varied substantially in composition and processing technique. Metabolizable energy values for DDGS ranged from 3,414 kcal.kg DM (SD-BPX) to 4,141 kcal/kg DM (WI) with an average of 3,770 kcal/kg DM. On average, our results compared favorably to Pedersen et al. (2007) where the ME for 10

sources of DDGS in growing pigs that ranged from 3,674 to 4,336 kcal/g DM with an average of 3,897 kcal/kg DM. In contrast, Moeser et al. (2002) determined the ME for dehulled, degermed corn in growing pigs to be 3,517 kcal/kg (SE = 69.7) which is lower than our value of 4,316 kcal/kg DM (SE = 413). Differences in these obtained values may be due to experimental design (Kerr et al., 2009). Moeser et al. (2002) used 27 kg growing barrows as compared to the 112.7 kg finishing gilts used in this study and included the product at 96.4% of the diet compared to our level of 30% of the diet (70% corn). The difference in ME determined is suprising given that dehulled, degermed corn is a highly digestible product generated from flaking grits in the dry milling process and it has been suggested that products of a highly digestible nature are little affected by BW or inclusion level (Kerr et al., 2009).

In the current stydy, the basal diet contained 97.1% corn and was not balanced for amino acids. It is well known that AA contribute to the energy in a diet and imbalances in AA can lead to a reduced feed intake as well as poor growth and performance (Lewis, 2001). Realizing this relationship, the ME values in the current study could have been under estimated because N excretion in the urine is increased. Our experimental design, however, is similar to that used by Windmer et al. (2007). Another factor with N balance experiments is that urinary N is excreted as urea and can quickly volatilize as ammonia if the nitrogen is not stabilized in a container, with acid, or at cold temperatures such that unaccounted N loss can lead to inaccurate and inflated ME values(van Kempen et al., 2003). In our study, 6N HCl was used to stabilize N excretion in stainless steel buckets placed below the metabolism crates. Lastly, the addition of high fiber co-products used in this study have additional implications on N loss. Nitrogen in the form of urea in the blood is destined for urinary excretion. However, in the presence of high dietary fiber, the loss of N in the urine shifts to losses in the feces as microbes in the hindgut utilize urea for fermentation (van Kempen et al., 2003). The overall effect would be a decreased urinary N loss and an increased fecal N loss, thereby reducing the DE value relative to the ME value.

Metabolizable energy is often acquired from tables or calculated from equations and is not determined due to the expense, time, and labor required to achieve such results. Prediction equations are a useful tool in generating energy values however, care must be used to ensure the application is being used within its designed limits (Stein et al., 2009 Midwest ASAS abstract). The prediction equation generated from out data indicates that for the corn co-products evaluated in this study, GE, TDF, and ash provide the best prediction estimate for ME in finishing pigs [ME, kcal/kg DM = $(0.949 \times GE) - (32.238 \times TDF) - (40.175 \times Ash)$; $R^2 = 0.95$, SD = 306, $P < 0.01$)]. Although Noblet and Perez (1993) evaluated a variety of feedstuffs and Windmer et al. (2007) evaluated DDGS, to our knowledge no such an equation has not been generated for this group of corn co-products. Although we are confident in our methods further studies need to be conducted to validate these findings. Caution must be used when implementing any prediction equation. Prediction equations are only as good as their intended use, and misuse will lead to inaccurate energy estimates. Our equation evaluated the parameters within corn co-products and attempts to use it for other feedstuffs are beyond the limits of the equation at this present time.

Although there is a vast array of *in vitro* digestibility assays, the *in vitro* OMD assay selected for this study was established by Boisen and Fernandez (1997) and later repeated by Noblet and Jaguelin-Peyraud (2007). It is an approved method in Denmark and is used routinely for feed energy evaluation (Noblet and Jaguelin-Peyraud, 2007). The corn co-products used in this study varied substantially in their composition, ranging in products that were low in energy and digestibility to products that were higher in energy and digestibility. Not surprisingly, the *in vitro* OMD varied substantially among corn co-products, ranging from 32.3 to 100% for corn bran (without solubles) and corn oil, respectively (Table 5).

At first glance there did appear to be a qualitative relationship between as the correlation coefficient describing the relationship between *in vivo* ME and *in vitro* OMD was 0.62 (Figure 1). Bran (without solubles) had the lowest (32.3%) organic matter digestibility of all the samples which was not surprising due to the fiber content. On the other hand, corn oil was interpreted to have 100% OMD in our analysis. Overall, the linear regression equation relating ME to OMD was: ME (kcal/kg DM) = $(48.557 \times \%\ OMD) + 628.240$; $R^2 = 0.38$.

It was quickly realized that this assay cannot accurately predict the digestibility of a liquid, especially oil. Although it is suspected that enzymatic activity did hydrolyze the corn oil to a degree, it is difficult to asses

the capacity to which this occurred. The density differences in the oil and liquid portion of the assay led to oil floating on the surface of the enzymatic liquid whereas the rest of the feedstuffs remained in suspension. An additional problem was encountered when filtering the digested contents after the 24 hour incubation period. Corn oil was essentially filtered through the crucible, and an expected increase in weight after the drying and ashing periods was not detected for these samples. We initially included corn oil in our regression analysis (Figure 1), but with the noted problems cited above, we removed it from the model whereupon the correlation coefficient decreased to 0.57 (Figure 2). We suspect that by including a data point that is not only extremely high in energy but also high in OMD, the rest of the data points become skewed and not as significant. Removing corn oil from our data set may more accurately describe the relationship *in vitro* OMD has on predicting *in vivo* ME for the remaining corn co-products, albeit low in this experiment.

Other studies using the described procedures from Boisen and Fernandez (1997) showed good correlation between OMD and apparent total tract digestibility of various diets and common feedstuffs (van der Meer and Perez, 1992; Noblet and Jaguelin-Peyraud, 2007). Prediction equations using OMD and chemical analysis were generated with high significance.

In reviewing our slightly modified procedures from the procedures described by Boisen and Fernandez (1997), potential sources of error may explain our relatively low OMD values and poor correlation. The pepsin product described in their procedure by Boisen and Fernandez (1997) was characterized as: porcine, 2000 FIP-U/g (Merck No 7190) and is a product that is readily accessible in Europe, but is not available in the United States or Canada. Therefore, we obtained a pepsin product that closely resembled the activity level indicated in their publication (porcine, 2,500 to 3,500 units/mg protein, Sigma 7012). However, the activity level of the pepsin products were not expressed in the same units and conversions were needed in order for a comparison to be made. These conversions were not easy to find and in retrospect, we suspect that our pepsin activity may have been lower that that described by Boisen and Fernandez (1997). Because the addition of pepsin to the assay was the first enzymatic step, a discrepancy in enzyme activity at this stage of the assay could have had dramatic effects on the digestibility outcome. In addition, our study used an incubated orbital shaker instead of a shaking water bath as described by Boisen and Fernandez (1997). Incubated orbital shakers are commonly used in microbiology labs and we were confident that there would be no difference in the two shakers as long as the desired temperature remained constant throughout incubation. Lastly, our study evaluated the OM digestibility relative to ME determined in 112.7 kg finishing pigs, while the procedure by Boisen and Fernandez (1997) was compared to DE values determined in pigs weighing 40 to 60 kg. It is possible the enzyme activities may need to be altered to accurately reflect the increased digestibility (especially fiber fermentation) in pigs of greater BW.

In conclusion, this study showed that there was a poor relationship between *in vitro* OMD and *in vivo* ME. Previous authors have indicated a strong relationship between *in vitro* OMD and apparent total tract digestibility in growing pigs (Boisen and Fernandez, 1997; Noblet and Jaguelin-Peyraud, 2007). The inability of our lab to reproduce such results is potentially due to procedural differences as well as the assay may need to be modified to accurately reflect the higher digestion of fibous feedstuffs commonly reported in larger pigs.

Table 1. Source of corn co-products

Feedstuff	Vendor
Corn gluten feed	Tate & Lyle, Ft. Dodge, IA
Corn bran	ICM/Lifeline Foods, St. Joseph, MO
Corn bran w/solubles	Poet Biorefining, Glenville, MN
DDGS	Ace Ethanol, Racene, WI
DDGS – drum dry	Cellencor, Heron Lake, MN
DDGS – microwave dry	Cellencor, Heron Lake, MN
DDGS	Hawkeye Renewables, Iowa Falls, IA
DDGS- Dakota Gold BPX	Poet Biorefining, Groton, SD
DDGS	VeraSun Energy Corportation, Aurora, SD
DDGS – oil extracted	VeraSun Energy Corporation, Aurora, SD
Corn gluten meal	Archer Daniels Midland, Cedar Rapids, IA
HP-DDG	ICM/Lifeline Foods, St. Joseph, MO
HP-DDG	MOR Technology, Cape Girardeau, MO
HP-DDG	Poet Biorefining, Coon Rapids, IA
Corn germ, dehydrated	Poet, Coon Rapids, IA
Corn germ meal	Cargill, Eddyville, IA
Corn dried distillers solubles	Pulse Combustion Systems, Payson, AZ
Dehulled, degermed corn	Bunge North America, Atchison, KS
Corn starch	Archer Daniels Midland, Clinton, IA
Corn oil	Mazola, ACH Food Co., Memphis, TN

Table 2. Ingredients of corn basal diet for finishing pigs (as fed basis)

Ingredient	Concentration (%)
Corn	97.05
Dicalcium phosphate	1.22
Limestone	0.73
Sodium chloride	0.40
Vitamin mix[1]	0.35
Trace mineral mix[2]	0.20
ISU Se premix[3]	0.05

[1]Provided the following per kilogram of diet: vitamin A, 7,716 IU; vitamin D3, 1,929 IU; vitamin E, 39 IU; vitamin B_{12}, 0.04 mg; riboflavin, 12 mg; niacin, 58 mg; pantothenic acid, 31 mg.

[2]Provided the following per kilogram of diet: copper, 35 mg; iron, 350 mg; iodine, 4 mg; manganese 120 mg; zinc, 300 mg.

[3]Provided the following per kilogram of diet: selenium, 0.3 mg

Table 3. Composition of corn co-products[1]

DM BASIS	DDGS (WI)	DDGS (IA)	DDGS (Verasun)	RO-DDGS (Verasun)	DDGS (BPX)	DDGS (MNdm)	DDGS (MNmc)	Dried solubles	Corn gluten feed
Bulk density, g/cm^3		0.470	0.487	0.494	0.467	0.530	0.396	0.330	0.499
Particle size, microns	1054	784	579	480	330	568	866	WNP	571
Moisture	6.82	9.75	13.41	12.64	10.87	11.43	12.95	22.3	4.14
OM digestibility	74.22	62.25	64.7	57.14	65.43	63.85	62.97	93.48	60.99
Gross energy	5314	5375	5434	5076	5547	5550	5502	54.76	4539
Crude protein	29.62	29.65	31.94	34.74	29.49	32.69	34.12	23.75	24.29
Alanine	2.07	2.09	2.38	2.48	2.09	2.38	2.47	1.47	1.52
Arginine	1.33	1.46	1.49	1.44	1.37	1.47	1.55	1.20	1.13
Aspartic acid	1.87	1.96	2.11	2.19	1.93	2.24	2.22	1.48	1.45
Cysteine	0.53	0.57	0.60	0.61	0.59	0.64	0.61	0.39	0.52
Glutamic acid	4.41	4.50	5.20	5.43	4.70	5.11	5.33	2.79	3.70
Glycine	1.18	1.24	1.34	1.39	1.22	1.38	1.38	1.26	1.03
Histidine	0.77	0.83	0.90	0.89	0.82	0.90	0.94	0.60	0.72
Isoleucine	1.06	1.14	1.19	1.25	1.11	1.23	1.29	0.68	0.70
Leucine	3.47	3.45	3.90	4.12	3.37	3.88	4.08	1.58	2.03
Lysine	1.03	1.21	1.19	1.00	1.10	1.20	1.29	1.09	0.67
Methionine	0.56	0.58	0.65	0.64	0.54	0.64	0.65	0.32	0.30
Phenylalanine	1.29	1.61	1.48	1.51	1.31	1.48	1.55	0.53	0.77
Proline	2.08	2.23	2.52	2.54	2.29	2.44	2.57	1.29	1.87
Serine	1.37	1.32	1.52	1.58	1.30	1.47	1.53	0.90	0.88
Threonine	1.11	1.10	1.22	1.26	1.09	1.25	1.26	0.81	0.78
Tryptophan	0.21	0.19	0.20	0.18	0.21	0.23	0.23	0.21	0.13
Tyrosine	1.04	1.17	1.19	1.22	1.05	1.16	1.22	0.62	0.65
Valine	1.49	1.57	1.69	1.76	1.53	1.73	1.80	1.08	1.11
Starch	7.85	3.47	6.24	3.04	4.94	2.12	1.05	6.34	12.57
Crude fiber	7.05	7.76	7.56	8.69	7.95	7.93	8.35	0.08	8.56
Total dietary fiber	30.34	38.14	35.69	37.20	35.90	35.38	43.18	16.07	40.07
NDF	34.61	40.13	40.12	50.96	33.41	44.87	49.12	2.33	42.66
ADF	11.25	10.55	14.42	15.82	8.62	13.16	14.66	0.49	9.90
Cellulose	10.64	10.12	11.72	12.72	8.21	11.95	13.37	0.79	9.17
Lignin	1.21	1.06	3.16	3.49	1.00	1.72	1.92	0.31	1.05
Crude fat	11.45	10.89	10.16	3.15	11.71	12.10	11.98	11.81	2.70
Ash	4.16	4.43	4.46	5.16	5.41	4.55	4.04	14.08	6.81
Calcium (mg/kg)	204	248	475	652	663	240	230	1699	683
Copper (mg/kg)	6	6	5	8	6	5	5	9	8
Iron (mg/kg)	81	72	125	288	90	104	132	129	125
Magnesium (mg/kg)	3485	3023	3456	3986	3710	3736	3125	11389	5192
Manganese (mg/kg)	21	13	16	23	15	20	18	40	34
Phosphorus (mg/kg)	7913	8582	7527	8373	9613	8377	7394	24356	11979
Potassium (mg/kg)	11465	10974	10069	11232	13140	11758	10172	38597	19862
Selenium (mg/kg)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Sodium (mg/kg)	172	1287	2414	3776	2659	1361	1324	4259	364
Sulfur (mg/kg)	8475	7940	7616	9772	11087	7288	6982	18069	4907
Zinc (mg/kg)	63	55	59	67	89	82	75	95	120

[1] Identity of individual feedstuffs described in Table 1. BDL = below detection limit and WNP = would not pass. All values based on DM basis except particle size and bulk densities which are based on as-is basis. Values on a percentage basis unless listed otherwise.

Table 3. Composition of corn co-products

DM BASIS	DHDG corn	Corn germ dehydrated	corn germ meal	corn bran (ICM)	corn bran (Poet)	corn gluten meal	HP-DDG (MOR)	HP-DDG (Poet)	HP-DDG (ICM)
Bulk density, g/cm^3		0.435			0.346			0.576	0.604
Particle size, microns	477	1175	483		2166	577	471	587	783
Moisture	12.78	9.44	10.87	12.62	9.18	8.51	8.3	5.95	12.31
OM digestibility	93.15	75.54	56.98	32.32	73.32	79.95	61.46	71.54	54.36
Gross energy	4397	5224	4767	4847	4982	5467	58.11	53.21	5464
Crude protein	8.28	17.54	23.64	10.94	15.17	66.30	57.45	43.83	39.98
Alanine	0.66	1.05	1.41	0.78	1.04	5.54	4.65	3.49	2.92
Arginine	0.28	1.31	1.67	0.65	0.77	2.38	2.26	1.63	1.68
Aspartic acid	0.48	1.35	1.68	0.81	1.02	4.23	3.75	2.82	2.44
Cysteine	0.17	0.34	0.37	0.22	0.30	1.08	1.13	0.81	0.74
Glutamic acid	1.74	2.47	3.22	1.67	1.95	13.51	10.88	7.88	6.84
Glycine	0.25	0.91	1.31	0.55	0.77	1.93	1.93	1.51	1.46
Histidine	0.22	0.51	0.72	0.31	0.44	1.41	1.36	1.17	1.07
Isoleucine	0.31	0.53	0.84	0.38	0.50	2.83	2.33	1.86	1.53
Leucine	1.25	1.27	1.91	1.10	1.30	10.67	8.57	6.37	5.12
Lysine	0.17	0.97	1.17	0.58	0.62	1.39	1.58	1.33	1.20
Methionine	0.16	0.28	0.42	0.18	0.23	1.41	1.44	0.94	0.81
Phenylalanine	0.45	0.66	1.02	0.50	0.55	4.14	3.13	2.37	1.96
Proline	0.77	1.07	1.20	0.82	1.08	5.59	4.77	3.79	3.06
Serine	0.39	0.68	1.00	0.53	0.65	2.91	2.86	2.02	1.68
Threonine	0.26	0.57	0.88	0.50	0.61	2.12	2.14	1.61	1.33
Tryptophan	0.06	0.17	0.20	0.06	0.09	0.24	0.29	0.14	0.19
Tyrosine	0.25	0.53	0.71	0.37	0.41	3.16	2.61	1.77	1.46
Valine	0.38	0.86	1.37	0.56	0.76	3.18	2.88	2.32	2.02
Starch	87.96	25.00	15.29	23.25	25.73	11.08	0.51	7.30	5.10
Crude fiber	0.60	4.87	10.69	11.54	4.80	1.44	8.14	9.42	7.87
Total dietary fiber	2.61	24.78	47.76	53.60	26.65	9.24	28.80	31.28	36.75
NDF	4.27	27.37	61.05	56.86	25.21	12.25	43.52	32.00	51.09
ADF	0.49	6.13	12.49	13.14	5.35	7.57	25.42	12.61	15.11
Cellulose	0.77	5.21	11.71	12.78	5.38	5.95	22.55	12.05	14.25
Lignin	0.33	1.28	1.22	0.89	0.55	2.24	3.40	0.95	1.44
Crude fat	0.17	18.45	2.38	5.14	9.68	1.34	4.12	2.86	6.97
Ash	0.49	6.46	2.70	2.33	5.31	3.99	1.10	2.05	2.09
Calcium (mg/kg)	13	159	359	164	314	6408	173	114	78
Copper (mg/kg)	1	7	36	5	5	18	6	4	4
Iron (mg/kg)	15	90	122	54	98	242	102	53	61
Magnesium (mg/kg)	268	5626	1905	1675	3277	1039	456	1110	936
Manganese (mg/kg)	1	22	11	15	17	25	17	6	5
Phosphorus (mg/kg)	879	15187	6496	4379	7578	6318	2486	4185	5029
Potassium (mg/kg)	1449	16593	4093	6464	13682	4596	1700	4389	3028
Selenium (mg/kg)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Sodium (mg/kg)	115	83	839	63	4270	1029	231	1260	563
Sulfur (mg/kg)	1048	2141	3274	1460	9506	9051	7178	9034	7002
Zinc (mg/kg)	5	85	77	39	195	42	71	28	37

[1] Identity of individual feedstuffs described in Table 1. BDL = below detection limit. All values based on DM basis except particle size and bulk densities which are based on as-is basis. Values on a percentage basis unless listed otherwise.

Table 4. Determined energy of corn co-products in finishing pigs[1]

Ingredient	n	GE	DE[2]	ME[2]
DDGS (HK)	8	5375	3841	3659
DDGS (VS)	8	5434	4164	3937
DDGS (Poet)	8	5347	3705	3414
DDGS (drum)	8	5550	4116	3876
DDGS (micro)	8	5502	4016	3713
DDGS (WI)	6	5314	4332	4141
DDGS (RO)	6	5076	3868	3650
HP-DDG (MOR)	8	5811	4955	4606
HP-DDG (ICM)	8	5464	3994	3676
HP-DDG (Poet)	8	5321	4210	3823
Bran (ICM)	8	4847	3004	2957
Bran (Poet)	8	4982	3282	3031
Germ meal	7	4767	3521	3417
Germ DH	8	5224	3889	3692
Gluten feed	8	4539	2517	2334
Gluten meal	8	5467	5047	4598
DHDG corn	8	4397	4401	4316
Dried solubles	8	5476	4762	4525
Starch	8	3952	4082	4080
Corn oil	8	9323	8988	8755
Standard deviation	-	-	363	413

[1]Gross energy (GE), digestible energy (DE), and metabolizable energy (ME) are expressed as kcal/kg DM. Pigs were collected for 4 d following a 9 d adaptation period. The basal diet contained 97.1% corn with the remaining 2.9% consisting of minerals and vitamins. All data reported on a DM basis.

[2] Digestible energy adjusted using a basal DE of 3,772 kcal/kg DM (actual as-is average = 3,287, SD 65.9), and ME adjusted using a basal ME of 3,695 kcal/kg DM (actual as-is average = 3,221, SE 69.4) obtained from 8 groups of control pigs (4 pigs per ingredient grouping).

Table 5. Metabolizable energy and OM digestibility of various corn co-products

Ingredient	ME[1]	OMD[2]
DDGS (HK)	3659	62.25
DDGS (VS)	3937	64.70
DDGS (Poet)	3414	65.43
DDGS (drum)	3876	63.85
DDGS (micro)	3713	62.97
DDGS (WI)[3]	4141	74.22
DDGS (RO)[4]	3650	57.14
HP-DDG (MOR)	4606	61.46
HP-DDG (ICM)	3676	54.36
HP-DDG (Poet)	3823	71.54
Bran (ICM)	2957	32.32
Bran (Poet)	3031	73.32
Germ meal[5]	3417	56.98
Germ DH	3692	75.54
Gluten feed	2334	60.99
Gluten meal	4598	79.95
DHDG corn	4316	93.15
Dried soluble	4525	93.48
Starch	4080	90.24
Corn oil	8755	99.00

[1]Data obtained from 8 (unless otherwise noted) individually fed finishing gilts (112.7 ± 7.9 kg final BW) collected for 4 d following a 9 d collection period. Ingredients were included in the diet at 30% (70% corn basal) with the basal diet containing 97.1% corn. ME = kcal/kg DM.

[2]Organic matter digestibilities (OMD) adjusted using an average corn OM digestibility of 89.41 (S.D 1.12). The basal diets used in the *in vivo* experiments had an average OM digestibility of 89.72 (SD 0.82).

[3]ME determination was based on 6 individually fed pigs.

[4]ME determination was based on 6 individually fed pigs.

[5]ME determination was based on 7 individually fed pigs.

FIGURE 1.

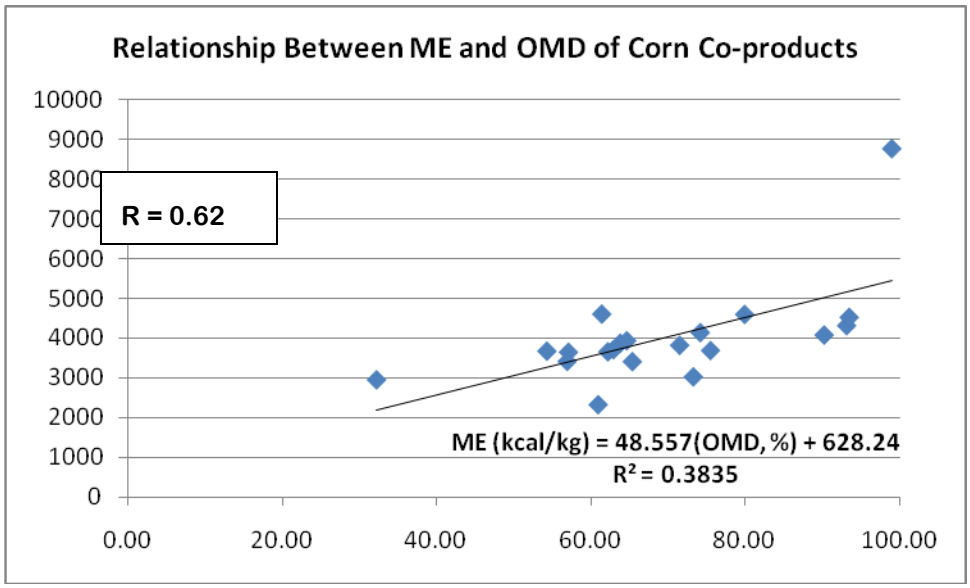

FIGURE 2.