RESEARCH REPORT
ANIMAL SCIENCE

Title: Development of "Nutritional Tools" to Estimate ME Content in Rendered Animal Protein By-products for Swine - Revised with Expanded Objectives, **NPB #12-132 and #14-245**

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Date submitted: December 6, 2016

Industry Summary:

Rendering is a process of grinding, heating, partial separation of fat, and drying of a wide array of inedible animal and carcass tissues, including blood, feathers, muscle, bones, fat, and offal. In the U.S., the rendering industry processes over 20 million tonnes of raw animal components into various protein, fat, and mineral products. Of the 4 million tonnes of animal derived protein products produced annually, about 85% are utilized as animal feed ingredients, and is an essential role that the rendering industry plays for achieving environmental and economic sustainability of animal agriculture Animal protein by-products are concentrated sources of energy, amino acids, and minerals which have been used in swine feeding programs depending upon price relative to competing ingredients. Although several studies have determined the digestible and metabolizable energy and the digestible AA and P content of animal protein by-products, the types and number of sources of these by-products evaluated in these studies has been limited.

The objectives of this study were to first contact rendering companies, feed ingredient suppliers, feed companies, and commercial poultry and swine production operations to obtain a variety of animal protein by-products. Geographic locations and sources of these by-products were selected over an extended period of time to represent the inherent variability in chemical composition of a wide variety of products among and within rendering plants to create a comprehensive and robust database. From this data base, 13 samples of animal protein by-products were then selected to conduct a digestibility experiment in growing pigs to determine DE and ME of these feedstuffs, and then develop DE and ME prediction equations based upon animal protein by-products chemical composition.

The results of the survey indicate there is substantial variation in the energy and nutrient composition among animal protein by-products, and there is also substantial variability within an animal protein by-product. These differences are not however, unique to animal protein by-products as similar variation is noted in other alternative feedstuffs utilized in swine feed formulations, such as corn co-products, which also have a moderate amount of variation in nutrient content. The results of the animal metabolism experiment showed that there was also substantial variation in the DE and ME of these animal protein by-products, with a range in DE from 5,367 to 2,567 kcal DE/kg of DM, and a range in ME from 4,783 to 2,340 kcal ME/kg DM. Prediction of DE and ME from the analyzed energy and nutrient components was possible, with the GE or ash content of the animal protein by-product accounting for approximately 85% of the DE and ME variability. The use of typical nutrient components commonly used in rendering plants and feed manufacturing for quality control (CP, EE, and ash), may also be used to predict DE and ME content of animal protein by-products, but they resulted in less accuracy than using GE or ash.

Key Findings:

Of the 220 samples received, the greatest concentration of CP was observed in blood meal (BM) and least in meat and bone meal (MBM), while the greatest concentration of ether extract (EE) was in meat meal and least in BM, and ash was greatest in MBM and least in BM. Calcium and P levels represented 36.1 and 16.3% of the ash content, respectively

The DE of the animal protein by-products ranged from 5,367 to 2,567 kcal DE/kg of DM, and ME ranged from 4,783 to 2,340 kcal ME/kg DM; indicating that DE and ME can vary substantially among the animal protein by-products tested.

Using all the animal protein by-products, the best fit equations were as follows: DE, kcal/kg DM = -2,468 + (1.26 × GE, kcal/kg DM), with R^2 of 0.84, SE = 390, and $P < 0.01$; ME, kcal/kg DM = -2,331 + (1.15 × GE, kcal/kg DM), with R^2 of 0.86, SE = 327, and $P < 0.01$); indicating that various nutritional components can be used to accurately predict DE and ME for growing pigs.

The average ATTD of Ca and P for the animal protein by-products, excluding BM and FM, was 27.1 and 39.1%, respectively. However, it appears that testing high levels of animal protein by-products may give low ATTD values of Ca and P, likely due to high levels of total Ca and P affecting digestibility

Keywords: animal protein by-products, energy, growing pigs, minerals, prediction

Scientific Abstract (1 page)

An industry survey and an animal experiment were conducted to evaluate compositional variability and DE and ME in animal protein by-products, and to generate equations predicting DE and ME based on chemical analysis. Of the 220 samples received, the greatest concentration of CP was observed in blood meal (BM) and least in meat and bone meal (MBM), while the greatest concentration of ether extract (EE) was in meat meal and least in BM, and ash was greatest in MBM and least in BM. Calcium and P levels represented 36.1 and 16.3% of the ash content, respectively. For the balance experiment, a corn-soybean meal basal diet was used, with test diets formulated by mixing the basal diet with 20% of the animal protein by-product, except for BM which was tested at 10 and 20%. There were there were 10 groups of 24 gilts (final BW = 92.5 ± 7.4 kg. Within each group, gilts were randomly assigned to the test or the basal diet for a total of 16 replications per animal protein by-product or basal diet, except for DM which had 20 replications at each test level. The experiment was conducted as a completely randomized design. Gilts were placed in metabolism crates and offered 2.4 kg daily of their assigned diet for 13 d, with total collection of feces and urine during the last 4 d. The GE was determined in the diets, feces, and urine to calculate DE and ME for each ingredient by the difference procedure with the DE and ME of the basal diet ere used as covariates among groups of pigs. The DE of the animal protein by-products ranged from 5,367 to 2,567 kcal DE/kg of DM, and ME ranged from 4,783 to 2,340 kcal ME/kg DM. Using all the animal protein by-products, the best fit equations were as follows: DE, kcal/kg DM = -2,468 + (1.26 × GE, kcal/kg DM), with R^2 of 0.84, SE = 390, and $P < 0.01$; ME, kcal/kg DM = -2,331 + (1.15 × GE, kcal/kg DM), with R^2 of 0.86, SE = 327, and $P < 0.01$). The ATTD of Ca and P were also determined using the difference procedure, with the average ATTD of Ca and P for the animal protein by-products, excluding BM and FM, was 27.1 and 39.1%, respectively. These data indicate that DE and ME varied substantially among the animal protein by-products, and that various nutritional components can be used to accurately predict DE and ME for growing pigs. In addition, it appears that testing high levels of animal protein by-products may give low ATTD values of Ca and P, likely due to high levels of total Ca and P affecting digestibility.

Introduction

Rendering is a process of grinding, heating, partial separation of fat, and drying of a wide array of inedible animal and carcass tissues, including blood, feathers, muscle, bones, fat, and offal (Garcia et al., 2006; NRA, 2006). In the U.S., the rendering industry processes over 20 million tonnes of raw animal components into various protein, fat, and mineral products. Of the 4 million tonnes of animal derived protein products produced annually (ERS, 2011), about 85% are utilized as animal feed ingredients, and is an essential role that the rendering industry plays for achieving environmental and economic sustainability of animal agriculture (Informa, 2011; Gooding, 2012). Animal protein by-products are concentrated sources of energy, amino acids, and minerals which have been used in swine feeding programs (NRC, 2012) depending upon price relative to competing ingredients. Although several studies have determined the digestible and metabolizable energy and the digestible AA and P content of animal protein by-products (Adedokun and Adeola, 2005; Rojas and Stein, 2013; Castilho et al., 2015), the types and number of sources of these by-products evaluated in these studies has been limited. Furthermore, the wide range in chemical composition within and among animal protein sources, the relative subjectiveness of classification of various by-products, and continual changes in composition of raw materials warrants a more current and comprehensive evaluation of the nutritional value of animal protein by-products for use in swine diets. Therefore, the objectives of this study were to obtain sources of animal protein by-products varying in chemical composition to determine DE and ME content, and to develop DE and ME prediction equations based upon animal protein by-products chemical composition.

Materials & Methods

The Institutional Animal Care and Use Committee at Iowa State University (Ames, IA) approved the animal experimental protocol.

Survey of Animal Protein By-Products

Rendering companies, feed ingredient suppliers, feed companies, and commercial poultry and swine production operations were contacted to obtain a variety of animal protein by-products. Geographic locations and sources of these by-products were selected over an extended period of time (December 2012 to January 2014) to represent the inherent variability in chemical composition of a wide variety of products among and within rendering plants to create a comprehensive and robust database which from which to develop DE and ME prediction equations. Samples were shipped to the University of Minnesota where they were recorded and subdivided for GE and proximate analysis determination using the methods described in Table 1.

Animal Management

The study was conducted over an 8-mo period (November 2014 through June 2015) at the Iowa State University Swine Nutrition Farm (Ames). In total, there were 10 groups of 24 gilts (final BW = 92.5 ± 7.4 kg) obtained from PIC Camborough 22 sows × L337 boars (Pig Improvement Company, Hendersonville, TN). Gilts were housed individually in metabolism crates (0.7 × 1.5 m) that allowed for separate but total collection of feces and urine. Crates were equipped with a stainless steel feeder and a nipple waterer which provided ad libitum access to feed and water.

Diets

Gilts were fed a standard corn–soybean meal-based diet prior to being randomly assigned to their experimental diets, and were weighed at the beginning and end of each trial. The composition of the basal diet fed to all groups of pigs is shown in Table 2. Pigs were fed either 100% of the basal diet or a test diet that contained 80% of the basal diet and 20% of a specific animal protein by-product sample, except for pigs fed the 2 blood meal (BM) samples which were included in the diet at 2 different levels (10% test ingredient with 90%

basal or 20% test ingredient with 80% basal), due to concerns about diet palatability and feed intake when pigs were fed diets containing 20% BM. All diets were fed in a meal form.

Sample Collection

During the time-based 4-d total fecal and urine collection period, stainless steel screens were placed under each metabolism crate for total fecal collection and stainless steel buckets containing 25 mL of 6 N HCl were placed under each crate for the total urine collection. Feces and urine were collected twice daily and stored at 0°C until the end of the collection period. Feces were pooled by pig over the 4-d period, dried in a 70°C forced-air oven, weighed, ground through a 1-mm screen, and subsampled for subsequent analysis. Likewise, urine samples were pooled by pig over the 4-d period, thawed at the end of the collection period, weighed, and subsampled for analysis.

Chemical Analysis and Calculations

The basal diet and all animal protein by-products were ground through a 1-mm screen before chemical analysis performed as listed in Table 1. To determine DE and ME content, GE of the animal by-products, diets, feces, and urine samples were determined using an isoperibol bomb calorimeter (Model 1282, Parr Instrument Company, Moline, IL) with benzoic acid used as a standard. For urine, 1 mL of filtered subsample urine was added to 0.5 g of dried cellulose and subsequently dried at 50°C for 24 h. Urine addition and subsequent drying was repeated 3 times, for a total of 3 mL of filtered urine, over a 72-h period before urinary GE determination. Gross energy in cellulose was also determined and urinary GE was calculated by subtracting the GE in cellulose from the GE in the samples containing both urine and cellulose.

Gross energy intake was calculated as the product of GE content of the treatment diet and the actual feed intake over the 4-d collection period. Within a specific treatment diet, the DE and ME content of each test ingredient was calculated by subtracting the DE or ME contributed by the basal diet from the DE or ME of the diet containing a particular animal protein by-product source. All energy values are reported on a DM basis. Similar to the calculations for energy, apparent total tract digestibility (ATTD) of DM, Ca, CP, ether extract (EE), and P of each test ingredient were calculated by subtracting the respective component contributed by the basal diet from the similar component of the diet containing that particular animal protein source. Digestibility coefficients were then determined by dividing grams of component digested by the grams of component consumed and values are reported on a percentage basis (Adeola, 2001).

Statistical Analysis

The experiment was conducted as a completely randomized design with the individual pig as the experimental unit. The DE and ME content of the basal diet within each group of gilts was used as a covariate to determine DE and ME content values for each test ingredient, respectively. Data were then summarized using Proc MEANS (SAS Inst. Inc., Cary, NC), with means for DE and ME content and their respective SD reported individually for each animal protein by-product. Using Proc REG, stepwise regression was used to determine the effect of nutrient composition among animal protein sources on DE, ME, and DE:GE, and ME:DE, with variables that had P-values ≤ 0.15 being retained in the model. In all cases the y-intercept remained in the model, regardless of its P-value. The R2, the SE of the estimate, and the Mallows statistic [C(p)] were used to define the best fit equation. The adjusted R2 was calculated and used to compare equations with more than 1 predictor variable. Similar to the analysis for DE, ME and energy ratios, the digestibility of each component in the basal diet within each group of pigs was used as a covariate to determine the digestibility of each component in the animal protein source, and the mean digestibility coefficient along with its respective SD are reported individually for each animal protein by-product. industry

Results and Discussion

Chemical Composition of Animal Protein By-Product Sources

The greatest concentration of CP was observed in BM and least in meat and bone meal (MBM), while the greatest concentration of EE was in meat meal (MM) and least in BM, and ash was greatest in MBM and least in BM (Table 3). As expected Ca and P levels were highly related to ash content. When all samples were evaluated (n = 220), Ca and P represented 36.1 and 16.3% of the ash content, respectively, on a DM basis (Figure 1a). If FM and BM are excluded from the evaluation (n = 167), Ca and P represented 40.7 and 15.8% of the ash content, respectively, on a DM basis (Figure 1b). The types of animal protein by-products collected in this survey had similar composition and variation compared with nutrient content of animal protein by-products reported by others (Dozier et al., 2003; Adedokun and Adeola, 2005; Hua et al., 2005; Olukosi and Adeola, 2009; Almeida and Stein, 2011; Rochell et al., 2013; Rojas and Stein, 2013; Sulabo et al., 2013; Castilho et al., 2015). In addition, the mean and standard deviation data are comparable those reported in a survey conducted by Knabe (1995). Variation in animal protein by-products among and with sources is typical of other by-product feed ingredients used in animal feeds, such as corn co-products, which also have a moderate amount of variation in nutrient content (Kerr et al., 2009, 2016; Anderson et al., 2012). Although there are specific classifications of animal proteins by-products based on their CP, EE, Ca, P, and Ca:P ratio (AAFCO, 2015; NRC, 1994, 2012), in many cases these general classifications seemed ambiguous for some of the samples we collected in this survey, as evident by the range in composition denoted for some of the animal by-products listed in Table 3. This observation reinforces the prerequisite to have good communications with ingredient suppliers regarding desired or expected nutrient composition of by-products, as well as the need to analyze sources of feedstuffs being used to insure proper diet formulation.

From this data set of 220 samples, we determined which classification and source of animal protein by-products we would use to determine DE and ME content and subsequent development of DE and ME prediction equations based upon their GE and nutrient composition. Our selection of specific animal by-product sources was based on our previous experience of developing accurate DE and ME prediction equations for corn co-products (Rochell et al., 2011; Anderson et al., 2012; Kerr et al., 2013, 2015; Meloche et al., 2013, 2014), where including a wide variety of compositionally diverse samples is necessary to generate robust prediction equations. As a result, we collected a broader range of animal protein by-products than others for our DE and ME evaluation (Adedokun and Adeola, 2005; Olukosi and Adeola, 2009; Rojas and Stein, 2013; Sulabo et al., 2013; Castilho et al., 2015), with the goal of generating accurate prediction equations that would be applicable to a wide range of animal protein by-products. Therefore, the 13 samples we selected represent a wide range of animal protein by-products that provided a wide range of GE and nutrient composition (Table 4) from which to determine DE and ME content and develop robust equations. We chose not to report the nutrient composition (GE, CP, EE, ash, Ca, and P) for each of the 220 surveyed (Table 3) because of the cumbersomeness of very large data tables, but have instead provided the full listing of these data on a web link (http://www.extension.umn.edu/agriculture/swine/components/nutrition.htm) for readers to use as they desire.

In the current experiment, we elected to use higher dietary inclusion rates of animal protein by-products (20%) than typically used in practical feed formulations because our goal was to improve accuracy of DE and ME estimates and to reduce experimental error. In comparison, Adedokun and Adeola (2005) and Olukosi and Adeola (2009) fed up to 10% MBM to 32 to 35 kg pigs, Rojas and Stein (2013) fed 11 to 17% of various animal by-products to 12 to 14 kg pigs, Sulabo et al. (2013) fed 25% feather meal (FM) to 13 to 24 kg pig, and Castilho et al. (2015) fed 20% MBM to 27 kg pigs. We were also cognizant, however, of potential palatability issues and reductions in feed intake due to our high inclusion levels as some animal protein by-products (e.g. BM) have an AA balance that may affect feed intake (Wahlstrom and Libal, 1977; Ilori et al, 1984; Hansen et al., 1993; Kats et al., 1994, DeRouchey et al., 2002; Kerr et al., 2004a,b; Parr et al., 2004).

In the current experiment, the initial BW of pigs was expected to be about 80 kg within each group of pigs, and the amount of experimental diets was offered was equivalent to 3% of initial BW (2.4 kg/d) during the 9-d adaption and 4-d collection periods. Averaged across all animal protein by-product treatments, ADFI was

2,383 g/d with an average pig final BW of 92.5 kg, which equates to 2.6% of BW (Table 5). Although ADFI was less than expected, there was little difference between the amount of feed consumed (2,383 g/d) and the amount of feed offered (2,400 g/d) among all groups of pigs, and ADFI did not differ among the animal protein by-products fed. However, we did observe that the amount of feed consumed relative to the amount offered during the adaptation period was relatively low and erratic the first 2 d, which may have been due to adaptation stress from placing the pigs into the metabolism crates or the need to adapt to the animal protein containing diets. Consequently, we used a 9 d adaptation period to stabilize feed intake to at or near ad libitum consumption prior to the 4 d collection period.

Concentration of Energy and Nutrients and DE and ME Content Prediction of Animal Protein By-Products

Comparisons of DE and ME content among each of the 13 animal protein by-products (Table 5) with other published reports (Adedokun and Adeola, 2005; Olukosi and Adeola, 2009; Rojas and Stein, 2013; Sulabo and Stein, 2013; Sulabo et al., 2013; Castilho et al., 2015; NRC, 2012) are useful for updating nutrient composition values in feed formulation databases and establish safety margins. As previously described, our intent was to select animal protein by-products to represent a wide range of composition from which to generate accurate DE and ME prediction equations based on chemical composition. Development and use of prediction equations to estimate energy and nutrient content is not a new concept, but the majority of these equations are based on composition of complete feeds (Just et al., 1984; Noblet and Perez, 1993; Bulang and Rodehutscord, 2009) and not specific ingredients. In contrast, our approach has been to develop prediction equations based on the chemical composition of specific feedstuffs (Anderson et al., 2012; Kerr et al., 2013, 2015; Urriola et al., 2014) in an effort to improve accuracy and reduce prediction error and bias.

Several issues were considered when developing prediction equations in this study. First, because total Ca and P in animal protein by-products are highly correlated (Dozier et al., 2003; Hua et al., 2005; Adedokun and Adeola, 2005; Olukosi and Adeola, 2009; Rochell et al.,. 2013; Rojas and Stein, 2013; Sulabo et al., 2013a,b; Castilho et al., 2015), and because the concentrations of total Ca and P in ash are relatively high (about 36% Ca and 16% P, Figure 1a), we did not allow either Ca or P to be variables in our prediction equations. Second, we first allowed all variables (GE, CP, EE, and ash) to be offered into the initial model that included all of the animal protein by-products, but have reported only the final best-fit equations (series-1 equations). Third, after this initial best-fit process, we elected to not offer GE in to the model prediction, because it is not a common analysis conducted by commercial laboratories; and because CP, EE, and ash are nutrient components commonly evaluated for quality control in rendering plants we forced these 3 variables into a prediction equation as a practical means of predicting its caloric value (series-2 equations). Forth, because BM and FM are unique animal protein by-products (typically high in CP and low in EE and ash content) compared with most other animal protein by-products, we developed alternative DE and ME prediction models by excluding BM and FM. In this scenario, we included GE and all nutrient variables (series-3 equations) or excluded GE, but forced CP, EE, and ash variables in the model (series-4 equations).

The best-fit equation for DE for all animal protein by-products evaluated used only GE in the model (DE-1; Table 6). Eliminating GE and forcing CP, EE and ash into the equation resulted in a slightly higher SE of the estimate and lower adjusted R2 (DE-2). Removing BM and FM data resulted in GE no longer being in the model, and was replaced by ash (DE-3). Subsequently, eliminating GE and forcing CP, EE and ash into the model when BM and FM were not included, had little effect on the SE of the estimate and the adjusted R2 (DE-4). Equations to predict ME are shown in Table 7.

Prediction equations for ME closely resembled those for DE, with the best-fit equation of ME content for all animal protein by-products resulting in only GE being included in the model (ME-1). When BM and FM data were removed, GE was no longer included in the model, and was replaced by ash (ME-3). Likewise, when GE was removed from the model, and CP, EE, and ash were forced into the model, the SE of the estimate increased and the adjusted R2 decreased when data from all animal protein by-products were included (ME-2). However, there was little change in the SE of the estimate and the adjusted R2 when BM and FM data were not included in the model (ME-4). Our data are similar to that reported by others (Adedokun and Adeola, 2005; Olukosi and

Adeola, 2009; Castilho et al., 2015) where GE was one of the first variables included in models for predicting ME content of MBM sources, and depending upon the model selected, GE often remained in the predictive model. When GE did not appear in predictive models reported by Adedokun and Adeola (2005), Olukosi and Adeola (2009) and Castilho et al. (2015), a combination of CP, EE, or ash was typically included. Because of this observation, and because GE determinations are not commonly available from commercial laboratories while CP, EE, and ash are commonly measured, we elected to develop a prediction equation with only these variables. Although these equations did not reduce the SE of the estimate or its accompanying adjusted R2, we believe that they would be more readily adopted for practical use because these nutrients are commonly guaranteed by rendering plants when marketing these by-products to the feed industry.

When predicting DE as a percentage of GE (DE:GE series of equations, Table 6), CP was the only variable used in the models using data for all animal protein by-products, as well as when BM and FM data were excluded (DE:GE-1 and DE:GE-3, respectively). This was somewhat surprising because we expected that ash content would have a greater effect on GE digestibility than CP content, as shown in predictive models reported by Adedokun and Adeola (2005), Olukosi and Adeola (2009) and Castilho et al. (2015). Similarly, for the DE and ME predictive equations, removing GE and forcing CP, EE, and ash as fixed inputs in the model did not improve the predictive power (as measured by SE of the estimate or the adjusted R2). Attempts to predict ME as a percentage of DE (ME:DE series of equations, Table 7) resulted in no variable being used in the model when all data from all animal protein by-products were included (ME:DE-1), and when all data for BM and FM were removed, ash was the only variable included in the model (ME:DE-3). This observation was also surprising given that in a complete diet, CP is considered an important factor in this relationship (Noblet and Perez, 1993; Anderson et al., 2012; Urriola et al., 2014). As for DE, ME, and DE:GE prediction equations, removing GE and forcing CP, EE, and ash as fixed inputs did not improve the predictive power (as measured by SE of the estimate or the adjusted R2) for ME:DE.

ATTD of Nutritional Components

Apparent total tract digestibility of Ca, CP, DM, EE, and P for pigs fed the basal diet and each animal protein product are shown in Table 5. While other researchers have developed prediction equations for DE and ME using total tract digestibility coefficients of nutrients (Noblet and Perez, 1993; 1994; Blok, 2006), this methodology was beyond the scope of this study as it requires conducting animal experiments, which is one of the objectives in predicting DE, ME, or various energy ratios from compositional data. However, some discussion of ATTD coefficients obtained in this study are worthy of discussion. On average, the ATTD of GE for all animal proteins, except BM and FM was 72.2%, which is similar to values reported for MBM (74.1% from Olukosi and Adeola, 2009; 62.5% from Castilho et al., 2015), but less than that reported for 3 poultry by-product meals (89.9%; Rojas and Stein, 2013) and 4 FM (89.5%; Sulabo et al. (2013). Excluding BM and FM, the ATTD of CP was relatively high (83.9%), and was slightly greater than that the ATTD of DM and EE (60.1 and 73.4%, respectively) for these same animal protein by-products.

The ATTD values of Ca and P for the animal protein by-products evaluated in the current experiment were substantially different than in other previous reports warrants further discussion. The ATTD of Ca (27.1%) and P (39.1%) reported in the current experiment for the animal protein by-products, excluding BM and FM, are dramatically less than the 86% relative bioavailability of P in MBM compared to monosodium phosphate of (Traylor et al., 2005a,b), the ATTD of Ca (63.9%) and P (65.9%) in MBM (Sulabo and Stein, 2013), the ATTD of P (82.6%) in FM (Sulabo et al., 2013), and the ATTD of P (67.1%) in BM (Almeida and Stein, 2011). It is not known if this may be due to differences in length of adaptation period when feeding experimental diets, feeding level, pig BW, or basal diet composition (Almeida and Stein, 2011; Sulabo et al., 2013; Sulabo and Stein, 2013) compared to the current experiment. However, based on data by Dungelhoef et al (1994), Kemme et al. (1997), and Chastanet et al. (2007), it seems unlikely that adaptation length, BW, or ADFI are major contributing factors toward these differences. Because our experimental methods, collection techniques, and laboratory analysis resulted in an ATTD of Ca (58.0%) and P (50.3%) for our basal diet, which are similar to other reported values (Kemme et al., 1997; Stein et al., 2011; Kerr et al., 2015), experimental methodology does

not help explain our Ca and P ATTD differences. Likewise our basal diet and our test ingredient plus basal diet combinations had a dietary Ca:P ratio of 1.8:1 or less, which is similar to those reported by others (Fernandez, 1995; Selle et al., 2009; Almeida and Stein, 2011; Stein et al., 2011), and should not have affected the ATTD of Ca and P in the animal protein by-products we evaluated. Even the use of a P-free basal diet with a Ca:P ratio of 35:1 wat not shown to affect the ATTD values of Ca and P when testing 8% inclusion levels of MBM (Sulabo and Stein, 2013). Thus our use of a corn-soybean meal basal diet in the current experiment likely had no effect on our Ca and P digestibility coefficients.

In contrast to similar Ca:P ratios among experiments, the total dietary Ca and P level in the final test diets we evaluated were much higher than reported by others. For example, the final test diets evaluated by Sulabo and Stein (2013), 92% of a P-free basal diet in combination with 8% MBM, had a total test-diet Ca and P level being at or below 0.85 and 0.42%, respectively. In the current experiment, when the animal protein by-products were added at the 20% inclusion level with 80% of the corn-soybean meal basal diet, the total test-diet Ca and P approached 2.6 and 1.5% P, respectively. Thus, while the final dietary Ca:P ratio in the text diets was not a concern, the high levels of dietary Ca and P likely influenced the digestibility of Ca and P as suggested by others (Combs et al., 1966; Gutierrez et al., 2015). Therefore, it is highly possible that our use of relatively high dietary inclusion levels of the animal protein by-products (20%) in combination with 80% of a basal diet that provided an additional 0.664% Ca and 0.448% P may have resulted in interactions with other nutritional components to reduce our ATTD Ca and P digestibility values. The ATTD values for Ca and P in BM and FM reported herein (Table 5) compared to others also warrants discussion. It is important to note that differences in ATTD of Ca and P estimates can be a result of very low Ca and P levels in the feedstuffs being evaluated when using the difference procedure, because a corn-soybean meal basal diet will provide a moderate amount of Ca and P (Table 5) compared with using a purified diet which commonly provides very low amounts of Ca and P (Almeida and Stein, 2011; Sulabo et al., 2013). This is supported by others (Wilfart et al., 2007; Adeola and Ilelega, 2009; Urriola and Stein, 2012; Li et al., 2016) who reported that the composition of the basal diet may have small, but different effects on the ATTD of GE. We are not aware of similar studies that have shown this relationship for ATTD of minerals. From this discussion above, it is apparent that differences in ATTD of Ca and P, and likely other dietary components, due to seemingly minor differences in experimental methodology need to be further evaluated.

Given the time, expense, of in vivo determination of DE and ME content of feed ingredients, as well as the applicability for dynamic estimation of ME content of the sources being fed, development and use of prediction equations to estimate energy content of feed ingredient based upon nutrient composition is needed, especially for feed ingredients with substantial variability among sources. Animal protein by-products not only provide a valuable source of energy (2,300 to 4,800 kcal ME/kg DM in the current experiment), but are also a valuable source of digestible AA Ca, and P in growing pig diets. Depending upon which animal protein by-products are included in energy prediction models, GE or ash are the most important predictors of DE or ME. The use of typical nutrient components commonly used in rendering plants and feed manufacturing for quality control (CP, EE, and ash), may also be used to predict DE and ME content, but they result in less accuracy than using GE or ash. Caution should be used when evaluating ATTD of Ca and P values among studies based on diet inclusion rates of test ingredients, type of basal diet, and diet Ca and P content.

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Table 1. Methods of analysis

Parameter	Method
GE[1]	Isoperibol bomb calorimeter (Model 1281, Parr Instrument Co., Moline, IL)
Ash[2]	AOAC (2005) official method 942.05
CP[2]	AOAC (2005) official method 990.03
DM[2]	AOAC (2005) official method 934.01
Ether extract[2]	AOAC (2005) official method 920.39 (A), petroleum ether
Minerals[2]	AOAC (2005) official method 985.01 (A–D)

[1]Analyzed by USDA-ARS, Ames, IA.

[2]Analyzed at the Agricultural Experimental Station Chemistry Laboratory of the University of Missouri, Columbia, MO.

Table 2. Ingredient composition of basal diet, as-fed basis[1]

Ingredient	Concentration, %
Corn	75.28
Soybean meal (46.5% CP)	22.50
Monocalcium phosphate (21% P)	0.51
Limestone	0.96
Sodium chloride	0.35
Vitamin mix[2]	0.20
Trace mineral mix[3]	0.20

[1]Formulated to contain 0.52% total Ca (0.74% analyzed Ca), 0.47% total P (0.50% analyzed P), and 0.24% standardized total tract digestible P.

[2]Provided the following per kg of diet: vitamin A, 6,125 IU; vitamin D_3, 700 IU; vitamin E, 50 IU; vitamin K, 30 mg; vitamin B_{12}, 0.05 mg; riboflavin, 11 mg; niacin, 56 mg; and pantothenic acid, 27 mg.

[3]Provided the following per kg of diet: Cu (as $CuSO_4$), 22 mg; Fe (as $FeSO_4$), 220 mg; I (as $Ca(IO_3)_2$), 0.4 mg; Mn (as $MnSO_4$), 52 mg; Zn (as $ZnSO_4$), 220 mg; and Se (Na_2SeO_3), 0.4 mg.

Table 3. Proximate analysis of animal protein by-products, DM basis[1]

	DM	CP	EE	Ash	Ca	P	GE
Blood meal							
Average (n = 30)	90.28	95.48	0.93	2.41	0.05	0.21	5,878
SD	2.14	2.72	1.14	0.65	0.04	0.07	115
High	95.01	99.92	3.34	5.47	0.11	0.35	6,185
Low	86.19	87.96	0.00	1.85	0.00	0.06	5,545
Chicken by-product meal							
Average (n = 19)	96.16	68.33	14.82	13.59	3.58	2.14	5,338
SD	0.72	8.12	2.99	4.79	2.07	0.54	254
High	97.31	75.08	24.17	27.74	10.25	3.20	5,599
Low	94.91	47.66	9.39	8.44	1.60	1.42	4,592
Chicken meal							
Average (n = 9)	96.07	68.23	13.22	18.72	6.11	3.36	5,083
SD	1.94	3.37	2.07	2.32	1.06	0.44	265
High	98.17	74.89	16.34	21.52	7.39	3.94	5,633
Low	92.18	64.69	9.16	14.68	4.34	2.66	4,791
Feather meal							
Average (n = 23)	92.05	92.11	6.55	2.52	0.52	0.29	5,877
SD	1.41	3.23	2.59	1.32	0.30	0.14	117
High	94.90	98.44	10.16	7.42	1.41	0.71	6,073
Low	89.54	87.70	2.67	1.33	0.24	0.11	5,726
Meat and bone meal							
Average (n = 98)	95.51	55.60	11.82	28.84	9.46	4.54	4,349
SD	1.52	3.29	2.01	3.29	1.76	0.67	244
High	99.67	64.80	18.62	38.35	15.56	6.73	4,911
Low	90.15	48.38	9.05	22.43	5.71	3.16	3,708
Meat meal							
Average (n = 17)	97.09	56.79	14.95	24.90	8.02	3.94	4,672
SD	0.59	3.44	2.32	3.55	1.73	0.78	217
High	98.25	61.61	18.79	30.41	10.87	5.16	5,223
Low	96.17	51.06	10.36	16.79	4.44	2.39	4,405
Poultry by-product meal							
Average (n = 18)	97.45	62.81	12.86	23.23	7.54	3.68	4,686
SD	0.68	5.94	1.86	7.06	3.32	1.44	458
High	98.90	71.66	16.85	32.73	11.20	5.39	5,431
Low	96.72	54.93	9.29	12.62	2.44	1.62	4,158
Poultry meal							
Average (n = 6)	96.98	67.55	13.31	17.69	5.21	2.86	5,099
SD	0.50	2.45	0.94	3.92	1.86	0.68	186
High	97.55	69.83	14.29	25.48	8.84	4.18	5,275
Low	96.17	62.97	11.89	14.82	3.72	2.25	4,742

[1]All values are reported on a percent basis except for GE which is reported as kcal/kg.

Table 4. Composition of the animal protein by-products and the basal diet, DM basis

Source identification	DM, %	GE, kcal/kg	CP, %	EE, %	Ash, %	Ca, %	P, %
Blood meal	90.25	5,748	98.83	0.55	1.77	0.06	0.07
Blood meal	93.01	5,830	95.35	3.02	1.99	0.10	0.32
Chicken meal	96.24	5,015	69.52	13.95	17.10	5.44	3.26
Chicken byproduct meal	98.41	5,521	69.20	16.55	10.45	2.25	1.84
Feather meal	90.43	5,656	91.36	5.43	2.44	0.27	0.32
Feather meal	94.35	5,962	86.35	11.00	1.84	0.44	0.31
Meat meal	97.92	4,360	60.29	10.35	25.52	8.01	4.39
Meat meal	97.97	5,104	55.65	17.12	17.43	4.97	2.59
Meat and bone meal	98.48	4,735	54.04	16.73	25.50	7.37	3.98
Meat and bone meal	95.70	4,077	54.54	10.73	29.63	9.54	5.06
Meat and bone meal	97.56	4,596	59.83	13.00	25.40	7.79	4.33
Poultry meal	98.48	4,183	49.26	13.35	31.11	8.84	4.51
Poultry byproduct meal	98.03	4,381	58.04	12.46	27.05	8.82	4.67
Basal	88.96	4,242	17.90	3.18	4.48	0.83	0.56

Table 5. Energy content and digestibility coefficients of pigs fed animal protein by-products and the basal diet, DM basis

					Apparent total tract digestibility				
Identification	BW, kg	ADFI, g	DE, kcal/kg	ME, kcal/kg	Ca, %	CP, %	DM, %	EE, %	GE, %	P, %
Blood meal	93.2	2,591	5,367	4,783	399.24	94.99	94.10	177.17	93.38	275.13
SD	9.5	31	576	584	301.40	4.16	11.74	42.87	10.03	216.25
Blood meal	91.4	2,538	5,150	4,453	289.20	91.54	86.48	128.39	88.33	98.70
SD	9.7	161	601	731	150.69	5.00	9.97	30.29	10.31	56.92
Chicken meal	94.6	2,383	4,120	3,719	27.67	91.22	73.40	81.69	82.15	41.68
SD	7.7	25.3	441	271	10.66	3.67	9.88	20.23	8.79	12.16
Chicken byproduct meal	92.1	2,360	4,605	4,204	46.14	86.68	78.89	93.54	83.40	62.68
SD	6.7	114	383	431	14.69	3.71	9.08	22.17	6.93	21.91
Feather meal	92.0	2,321	3,870	3,502	132.76	74.43	67.62	55.31	68.43	59.17
SD	8.1	155	483	492	143.15	3.49	10.32	36.85	8.54	38.62
Feather meal	91.4	2,288	4,900	4,560	135.4	86.14	80.94	84.92	82.19	118.19
SD	7.3	229	504	386	65.14	3.35	9.74	24.98	8.45	73.04
Meat meal	90.8	2,279	3,185	2,798	23.48	82.89	55.34	80.95	73.06	31.70
SD	7.1	232	259	276	8.68	5.54	12.88	32.21	5.95	10.10
Meat meal	90.8	2,322	3,556	3,270	32.30	80.04	62.77	71.01	69.67	45.52
SD	8.8	184	370	367	15.67	3.73	7.72	17.32	7.25	16.74
Meat and bone meal	92.9	2,354	3,149	2,828	16.53	85.14	55.61	61.35	66.49	31.97
SD	5.9	152	484	487	4.29	4.38	11.45	19.67	10.22	7.64
Meat and bone meal	92.8	2,313	2,567	2,340	28.43	78.84	50.36	60.75	62.96	36.32
SD	5.3	157	524	396	11.66	4.02	11.21	22.28	12.85	8.28
Meat and bone meal	93.6	2,384	3,034	2,691	18.33	82.48	53.44	70.30	66.01	30.94
SD	5.9	28.7	403	429	12.61	4.12	9.47	26.64	8.76	15.85
Poultry meal	93.3	2,383	2,865	2,508	26.71	80.41	49.55	68.19	68.50	36.85
SD	7.4	48	279	426	10.04	5.06	10.49	26.42	6.66	13.45
Poultry byproduct meal	93.2	2,361	3,394	3,038	24.59	87.42	61.54	80.91	77.48	34.38
SD	8.2	78	371	464	9.89	3.68	10.27	24.43	8.47	10.67
Basal	92.2	2,411	3,812	3,704	57.98	89.19	90.09	41.15	89.87	50.26
SD	6.4	189	48	52	5.31	1.31	1.04	5.61	1.13	5.88

Table 6. Stepwise regression equations for DE and DE:GE in animal protein meals, DM basis

	Regression coefficient parameter[1]					Statistical parameter[2]		
Item	Intercept	GE	CP	EE	Ash	SE	R^2	C(p)
DE								
Eq. 1: all animal protein meals; STEPWISE regression								
Coefficient	-2,468	1.26	-	-	-	390	0.84	-0.66
SE[3]	847	0.17	-	-	-	-	-	-
P-value[3]	0.01	0.01	-	-	-	-	-	-
Eq. 2: all animal protein meals; GE removed; CP, EE, and ash as fixed inputs								
Coefficient	-669	-	56.1	73.4	-12.5	417	0.85 (0.80)	-
SE[3]	5,435	-	53.4	74.4	58.9	-	-	-
P-value[3]	0.90	-	0.32	0.35	0.84	-	-	-
Eq. 3: excluding blood meal and fish meal; STEPWISE regression								
Coefficient	5,420	-	-	-	-87.5	240	0.87	0.62
SE[3]	302	-	-	-	12.5	-	-	-
P-value[3]	0.01	-	-	-	0.01	-	-	-
Eq. 4: excluding blood meal and fish meal; GE removed; CP, EE, and ash as fixed inputs								
Coefficient	665	-	46.7	59.2	-36.5	234	0.92 (0.86)	-
SE[3]	3,520	-	31.3	66.4	40.1	-	-	-
P-value[3]	0.86	-	0.20	0.41	0.40	-	-	-
DE:GE								
Eq. 5: all animal protein meals; STEPWISE regression								
Coefficient	48.03	-	0.40	-	-	6.95	0.52	-1.71
SE[3]	8.20	-	0.11	-	-	-	-	-
P-value[3]	0.01	-	0.01	-	-	-	-	-
Eq. 6: all animal protein meals; GE removed; CP, EE, and ash as fixed inputs								
Coefficient	-19.44	-	1.05	1.09	0.62	7.38	0.56 (0.41)	-
SE[3]	96.35	-	0.95	1.32	1.04	-	-	-
P-value[3]	0.84	-	0.30	0.43	0.57	-	-	-
Eq. 7: excluding blood meal and fish meal; STEPWISE regression								
Coefficient	20.39	-	0.88	-	-	4.55	0.66	-0.92
SE[3]	14.03	-	0.24	-	-	-	-	-
P-value[3]	0.19	-	0.01	-	-	-	-	-
Eq. 8: excluding blood meal and fish meal; GE removed; CP, EE, and ash as fixed inputs								
Coefficient	24.99	-	0.79	0.19	-0.09	5.27	0.68 (0.48)	-
SE[3]	79.48	-	0.71	1.50	0.91	-	-	-
P-value[3]	0.77	-	0.31	0.91	0.92	-	-	-

[1]Equations based on analyzed nutrient content expressed on a DM basis. Units are kcal/kg of DM for GE and DE and percent for CP, EE, and ash.

[2]SE = standard error of the regression estimate defined as the root of the mean square error, R^2 = coefficient of determination, with the adjusted R^2 based upon the number of parameters in the model represented within the parenthesis, C(p) = the Mallows statistic.

[3]SE and *P*-values of the corresponding regression coefficient parameter.

Table 7. Stepwise regression equations for ME and ME:DE in animal protein meals, DM basis

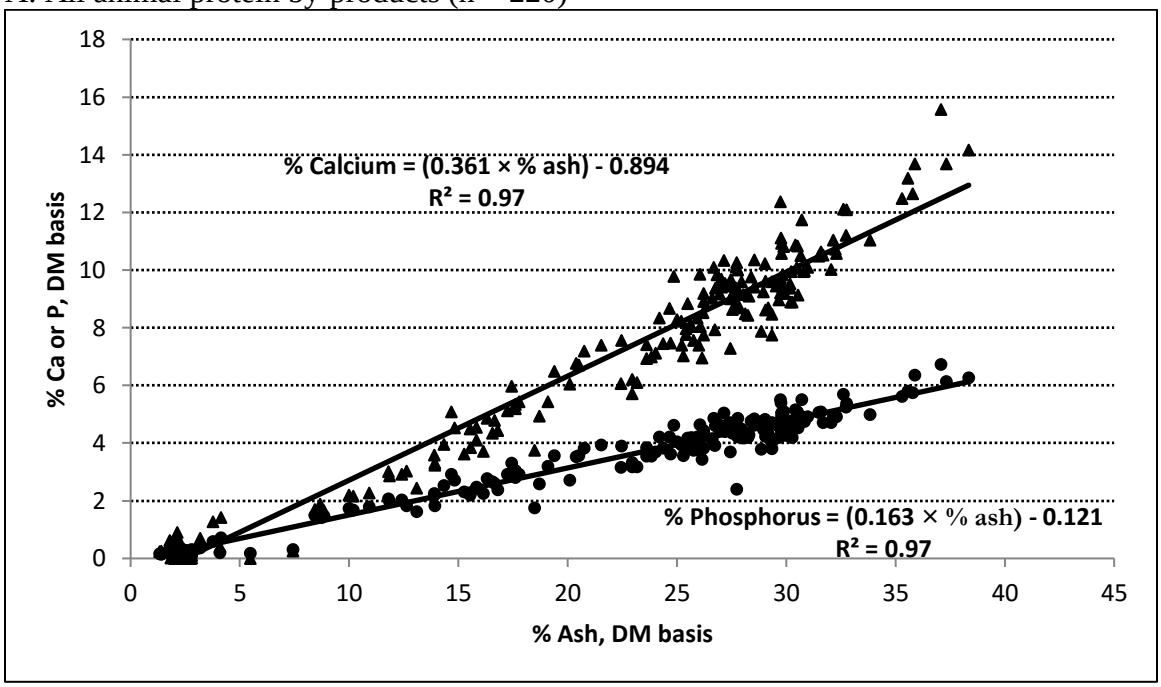
	Regression coefficient parameter[1]					Statistical parameter[2]		
Item	Intercept	GE	CP	EE	Ash	SE	R^2	C(p)
ME								
Eq. 9: all animal protein meals; STEPWISE regression								
Coefficient	-2,331	1.15	-	-	-	327	0.86	-1.80
SE[3]	708	0.14	-	-	-	-	-	-
P-value[3]	0.01	0.01	-	-	-	-	-	-
Eq. 10: all animal protein meals; GE removed; CP, EE, and ash as fixed inputs								
Coefficient	-443	-	48.1	75.9	-18.0	357	0.86 (0.82)	-
SE[3]	4,657	-	45.8	63.8	50.5	-	-	-
P-value[3]	0.93	-	0.32	0.26	0.73	-	-	-
Eq. 11: excluding blood meal and fish meal; STEPWISE regression								
Coefficient	4,996	-	-	-	-84.0	199	0.90	0.06
SE[3]	250	-	-	-	10.4	-	-	-
P-value[3]	0.01	-	-	-	0.01	-	-	-
Eq. 12: excluding blood meal and fish meal; GE removed; CP, EE, and ash as fixed inputs								
Coefficient	1,192	-	36.8	49.7	-43.1	199	0.93 (0.89)	-
SE[3]	3,000	-	26.7	56.6	34.2	-	-	-
P-value[3]	0.71	-	0.23	0.42	0.27	-	-	-
ME:DE								
Eq. 13: all animal protein meals; STEPWISE regression								
Coefficient	-	-	-	-	-	-	-	-
SE[3]	-	-	-	-	-	-	-	-
P-value[3]	-	-	-	-	-	-	-	-
Eq. 14: all animal protein meals; GE removed; CP, EE, and ash as fixed inputs								
Coefficient	97.89	-	-0.09	0.16	-0.21	1.59	0.45 (0.27)	-
SE[3]	20.81	-	0.20	0.28	0.23	-	-	-
P-value[3]	0.01	-	0.66	0.58	0.38	-	-	-
Eq. 15: excluding blood meal and fish meal; STEPWISE regression								
Coefficient	93.05	-	-	-	-0.14	1.31	0.29	23.01
SE[3]	1.64	-	-	-	0.07	-	-	-
P-value[3]	0.01	-	-	-	0.08	-	-	-
Eq. 16: excluding blood meal and fish meal; GE removed; CP, EE, and ash as fixed inputs								
Coefficient	107.12	-	-0.15	-0.12	-0.29	1.42	0.47 (0.16)	-
SE[3]	21.42	-	0.19	0.40	0.24	-	-	-
P-value[3]	0.01	-	0.46	0.78	0.29	-	-	-

[1]Equations based on analyzed nutrient content expressed on a DM basis. Units are kcal/kg of DM for GE and ME and percent for CP, EE, and ash.

[2]SE = standard error of the regression estimate defined as the root of the mean square error, R^2 = coefficient of determination, with the adjusted R^2 based upon the number of parameters in the model represented within the parenthesis, C(p) = the Mallows statistic.

[3]SE and *P*-values of the corresponding regression coefficient parameter.

A: All animal protein by-products (n = 220)



B: Animal protein by-products excluding blood meal and feather meal (n = 167).

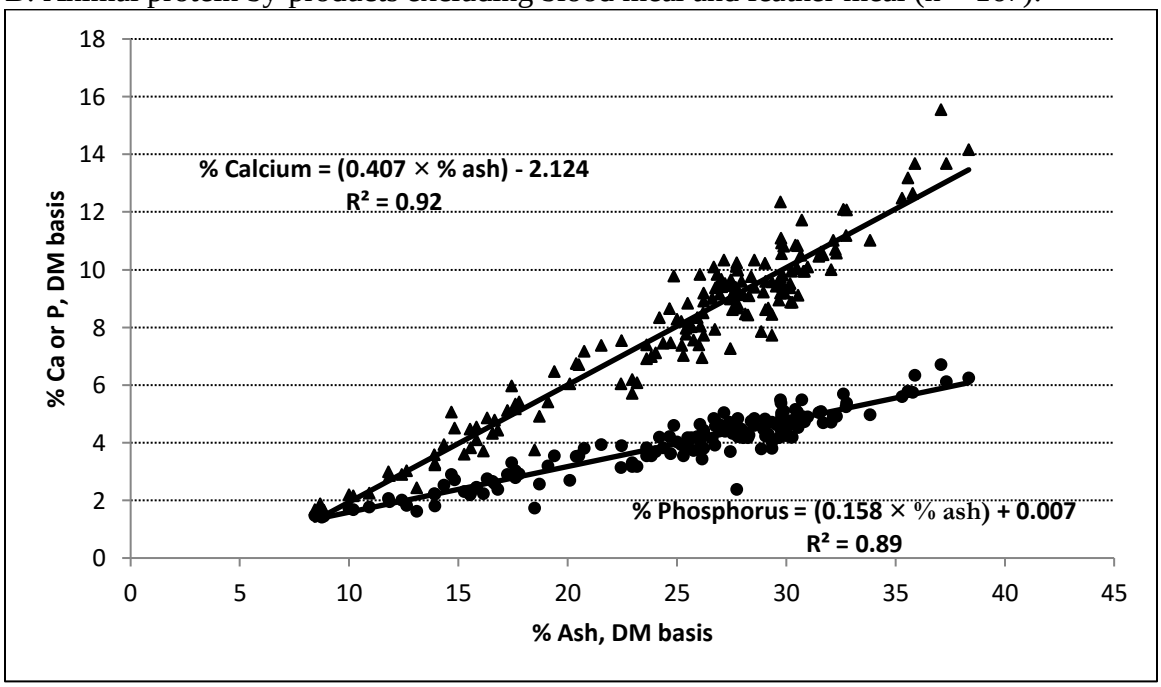


Figure 1. Prediction of the concentration of calcium (▲) and P (●) among types and sources of animal protein meals. A: All animal protein by-products (n = 220). B. Animal protein by-products excluding blood meal and feather meal (n = 167).