RESEARCHREPORT

ANIMAL SCIENCE — Nutritional Efficiency Consortium

Title: Comprehensive evaluation of lysine digestibility in DDGS – **NPB #09-142**

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Industry Summary

Lysine is the most variable of the indispensable amino acids in dried distiller's grains with solubles (DDGS), and it has the lowest digestibility in swine feed. In order to search for an accurate indicator for lysine digestibility of DDGS in swine diet, we examined five analytical methods for DDGS testing including; color, immobilized digestible enzyme assay (IDEA), enzymatic pepsin-pancreatin procedure, acid detergent insoluble nitrogen (ADIN) and the front face fluorescence method. The values for DDGS obtained from each procedure were compared with the standardized ileal digestibility (SID) of lysine of DDGS in a swine diet obtained from a previous animal study conducted at the University of Illinois urban Champaign. Results showed

1. Neither color nor IDEA value of DDGS accurately predicted the SID value of lysine in DDGS (both correlation coefficients lower than 0.3).

2. Neither enzymatic pepsin-pancreatin procedure nor acid detergent insoluble nitrogen of DDGS predicted the SID value of crude protein or lysine in DDGS fed to pigs (both correlation coefficients lower than 0.2).

3. When using the front face fluorescence method accurate SID lysine prediction models can be developed to provide good estimates within a defined data set but do not accurately predict SID lysine from samples not included in the model.

Therefore, more research is needed to identify methods that can adequately predict lysine digestibility of DDGS in swine diet.

Keywords DDGS, lysine digestibility, color of DDGS, IDEA value, pepsin-pancreatin procedure, acid detergent insoluble nitrogen (ADIN), values for front face fluorescence

Scientific Abstract

The variability of lysine digestibility in DDGS has made it difficult to formulate swine diets with DDGS. Therefore searching for an accurate in vitro indicator for lysine digestibility of DDGS in swine becomes imperative in order for swine producers to better utilize DDGS without observing variation in performance of pigs. W**e** examined five in vitro methods for DDGS testing including the measurement of color, the immobilized digestible enzyme assay (IDEA), the enzymatic pepsin-pancreatin procedure, acid detergent insoluble nitrogen (ADIN) measurement, and front face fluorescence method. The values for DDGS obtained from each procedure were compared with the standardized ileal digestibility (SID) of lysine of DDGS in swine diet obtained from a previous study, and the results showed that, (1) no linear correlation has been observed between the color of DDGS or the the IDEA value of DDGS and the SID of lysine of DDGS (both correlation coefficients lower than 0.3); (2) the in vitro enzymatic assay or ADIN did not predict in vivo standardized ileal digestibility of CP and Lys in DDGS fed to pigs (both correlation coefficients lower than 0.2); (3) accurate SID Lys prediction models can be developed to provide good estimates within a defined data set but does not accurately predict SID Lys using data from samples not included in the model. We concluded in this study that more research is needed to identify methods that can adequately predict lysine digestibility of DDGS in swine diet.

Final Reports

Part A from the National Corn-to-Ethanol Research Center

Evaluation of color and IDEA of DDGS as predictors of standardized ileal digestibility of crude protein and lysine in distillers dried grains with solubles fed to growing pigs

Introduction

The growth in the fuel-ethanol industry has been accompanied by growth in the production of distillers dried grains with solubles (DDGS) and the potential for increased use of DDGS in diets fed to swine is great. However, the variability of lysine digestibility in DDGS (Fastinger and Mahan, 2006; Stein et al., 2006; Pahm et al., 2008a) has made it difficult to formulate swine diets with DDGS and growth performance of pigs may sometimes be reduced if DDGS with a low lysine digestibility is used. As a consequence, animal growth performance is reduced in approximately one third of the experiments in which DDGS is included in the diets (Stein and Shurson, 2008). It is likely that one of the major reasons for the reduced growth performance of pigs fed diets containing DDGS is that some of the sources of DDGS have a low concentration of digestible lysine, but producers and feed companies are currently not able to identify these sources of DDGS. It is, therefore, desirable that an in vitro procedure that can rapidly predict the digestibility of lysine in DDGS be developed.

Measurement of reactive lysine in DDGS can be used to predict lysine digestibility in DDGS reasonably accurately (Pahm et al., 2008b), but this procedure is relatively time consuming and requires access to sophisticated analytical equipment. The Immobilized Digestible Enzyme Assay (IDEA) has also been used to predict the digestibility of lysine in diets fed to chickens (Schasteen et al., 2005), but data for swine have not been generated. The proprietary immobilized digestible enzyme assay (IDEA) was developed by Novus International, Inc. in order to quantify lysine digestibility value of animal feed fast and inexpensively. It was the interest of Novus to verify the application of the IDEA in swine feed with this study.

In this study, we used samples that were generated in a separately funded research project that involves measurement of reactive lysine in 100 sources of DDGS. From the other project, 19 samples with high, medium, or low concentration of reactive lysine were selected, and in vivo ileal lysine digestibility were measured using ileally cannulated pigs. The IDEA values of the 19 DDGS samples were measured at NCERC with the assistance of Novus. Since color is a simple way to characterize DDGS, we included color measurement too in this study.

Methods and Results

IDEA test

According to the proposal, Novus International provided NCERC their pre-manufactured kit containing immobilized enzymes and a DDGS standard for the assay. An experienced chemist from Novus conducted the training on how to use the kit and how to process the data. A DDGS sample was ground to pass through a 1 mm screen using a wiley mill. 2.4 g of ground DDGS sample was mixed with 40 ml of solubilization solution (0.1% NaN_3 in pH of 7.7) for about an hour. Then, 0.25 mL of the slurry solution was pipetted into a digestor tube containing the immobilized enzyme from Novus, and the mixture was incubated at 37°C for 18 hr. Afterwards, 10 ul of the supernant from the digestor tube was mixed with 1 ml of freshly made o-phthaldialdehyde solution, and the mixture was scanned on a Shimadzu spectrophotometer at 340 nm for 4 min. The maximum absorbance (around 2 min. during the scan) was used for calculating the IEDA value, and that value was corrected based on the IDEA value of the DDGS standard and became the final IDEA value. Duplicates were run for each DDGS sample and the data are shown in Table 1.

Since the calculation of IDEA value of DDGS relies on crude protein content in the sample, the crude protein measurement was performed based on the American Feed Industry Association recommended

method (AOAC 990.03). A DDGS sample goes under catalytic tube combustion in an oxygenated atmosphere at 800^{o}C in a Rapid N Nitrogen Analyzer (Elementar), and the reduced form of nitrogen is detected with a thermal conductivity detector. The total protein content per sample is based on the calculated percent of nitrogen within that sample multiplied by the Kjeldahl factor of 6.25. Two check standards, a synthetic compound and an in-house DDGS check sample, were used for quality control for each batch of sample run. Triplicates were run for each DDGS sample and the results for the 19 DDGS samples are listed in Table 1.

Color test

A spectrophotometer, ColorFlex from the HunterLab, was used for the color measurement. Duplicate samples were run and the mean of spectra and color scale values were obtained for each DDGS sample. Based on the Opponent-Colors theory, three color scales, "L" for lightness, "a" for redness and "b" for yellowness, were obtained during each measurement. Here, "L" value ranges from 0 to 100 with 0 for black and 100 for white; positive "a" value represents red, negative "a" value represents green and 0 value represents neutral; and positive "b" value represents yellow, negative "b" value represents blue and 0 value represents neutral. For DDGS study, we use the values of "L" and "b" and data for the 19 DDGS are shown in Table 1.

Discussion

Based on the data of standardized ileal digestibility (SID) of lysine in DDGS from the University of Illinois, we plotted the color measurement, the brightness and yellowness, against the SID, and no linear correlation ($r2 < 0.2$) has been observed between the color and SID (Fig. 1). We also plotted the IDEA value against the SID, and no linear correlation ($r2 < 0.2$) has been observed between the IDEA value and SID (Fig. 2).

Table 1. Data for the Study of Indicators for In Vitro Lysine Digestibility

Univ. of Illinois Sample Label	DDGS Source	Color Scale "L"	Color Scale "b"	Crude Protein (%)*	IDEA value	SID
19031	1	50.3	39.7	27.4	1.2	49.6
32	2	54.7	41.4	25.4	1.2	69.7
33	3	58.4	49.0	25.9	1.4	71.1
34	4	45.4	34.4	27.0	1.4	62.1
35	5	54.6	44.0	24.3	1.2	61.8
36	6	47.4	42.7	25.1	1.1	60.5
37	7	58.7	43.9	31.5	0.6	55.5
39	8	56.2	45.6	25.4	1.1	60.0
40	9	59.2	46.4	25.0	1.1	68.6
41	10	57.6	47.1	25.7	1.0	62.7
42	11	53.2	41.3	25.2	1.1	68.8
43	12	57.7	42.0	30.6	0.7	68.1
44	13	60.8	44.1	25.7	1.0	68.6
45	14	51.0	41.4	24.4	1.1	71.4
46	15	53.8	47.0	23.4	1.0	65.8
47	16	54.2	46.3	25.8	0.9	74.1
48	17	59.7	46.7	25.5	0.9	68.3
49	18	50.8	42.7	25.4	0.9	46.5
50	19	57.5	43.0	25.7	1.0	64.2

* %, wt/wt, on as-received basis

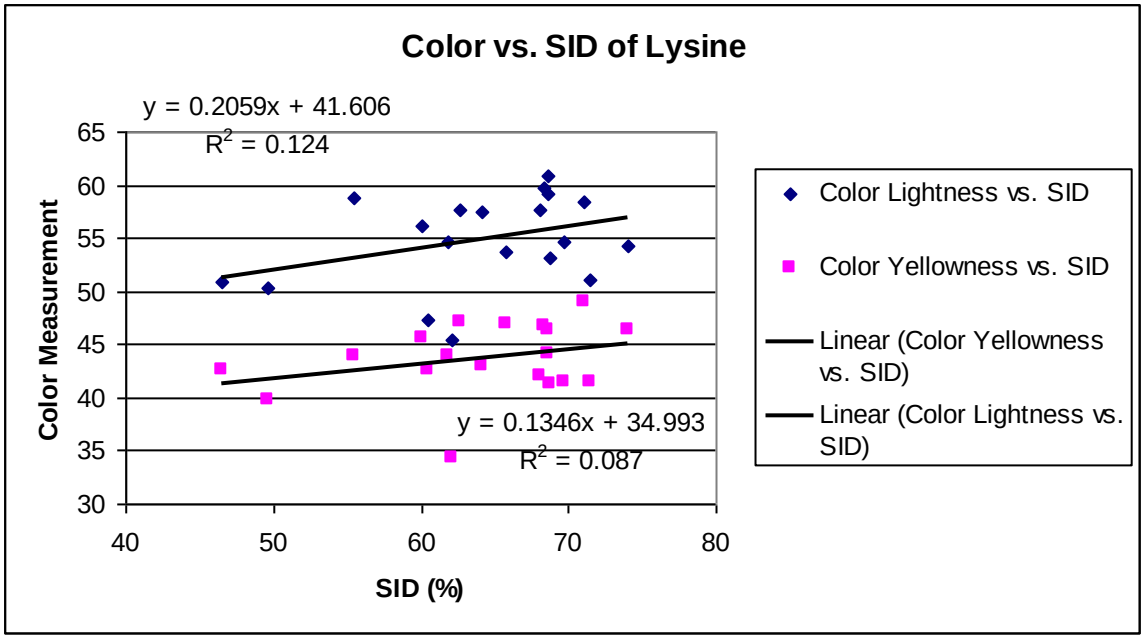

Fig. 1. The color measurement of DDGS against the SID of lysine in DDGS.

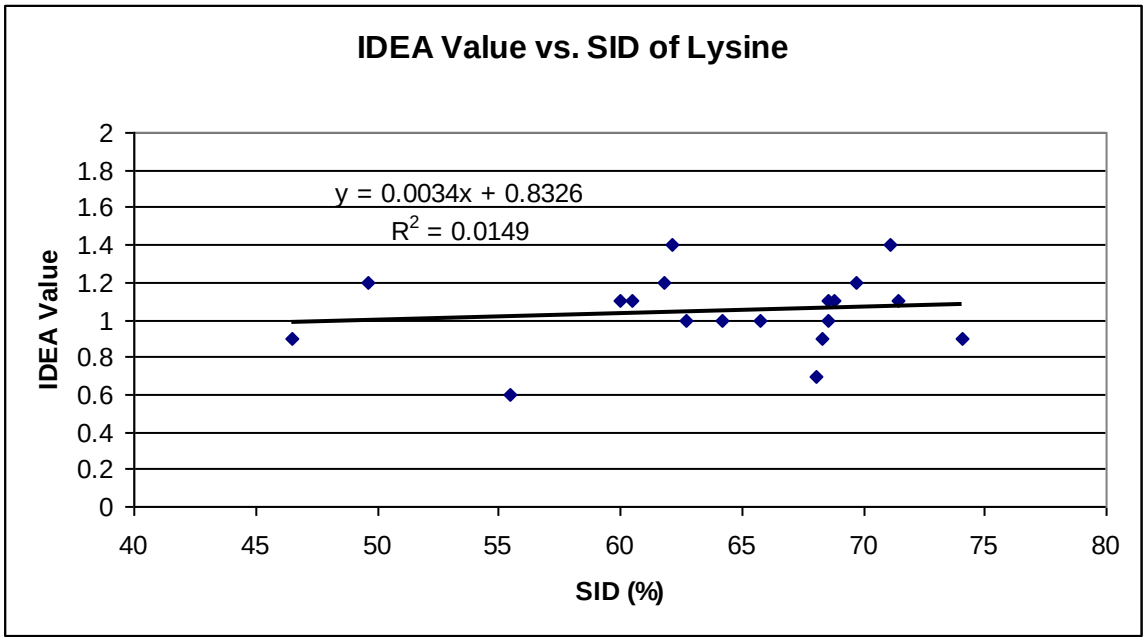

Fig. 2. The IDEA value of DDGS against the SID of lysine in DDGS.

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Part B from the University of Illinois Urbana-Champaign

Evaluation of an *in vitro* enzymatic assay and acid detergent insoluble nitrogen as predictors of standardized ileal digestibility of crude protein and lysine in distillers dried grains with solubles fed to growing pigs

Introduction

Ileal digestibility studies have been performed to determine the nutritional value of DDGS fed to pigs (Stein et al., 2006; Pedersen et al., 2007; Urriola et al., 2009); however, in vivo methods are often tedious, time-consuming, require large amounts of feedstuffs, and involve surgery of pigs. Digestibility coefficients may be predicted using in vitro methods (Boisen and Eggum, 1991), which involve incubation of the feedstuff with specific enzymes that attempt to simulate in vivo digestion of nutrients. The Boisen and Fernandez (1995) method is the most popular in vitro assay in predicting CP and AA digestibility of feedstuffs and diets, and a number of studies has validated this procedure (Boisen, 2007; Jiezerny et al., 2010). However, there has been no research that evaluated this assay in predicting in vivo standardized ileal digestibility (**SID**) of CP and AA in DDGS fed to growing pigs.

Among all indispensable AA, the digestibility of Lys in DDGS has the greatest variability, which indicates that varying degrees of heat damage during ethanol production largely affect its nutritive value (Stein et al., 2006; Pahm et al., 2008). Acid detergent insoluble nitrogen (**ADIN**) is the N remaining in the ADF residue (Firkins et al., 1984) and, while some occurs naturally in all plant material, it is considered to be an estimate of heat damage occurring during processing or storage. Research in forages fed to cattle has previously demonstrated a one-to-one reduction in N digestibility as ADIN increased (Goering et al., 1972; Yu and Thomas, 1976). In addition, Cromwell et al. (1993) observed that darker-colored DDGS, which is suggestive of greater heat damage, had higher ADIN values. Thus, ADIN may be an adequate predictor of CP and Lys digestibility in DDGS fed to pigs; however, this hypothesis has not been tested.

Therefore, the first objective of this study was to determine the relationship between in vitro values derived from the Boisen and Fernandez (1995) assay and in vivo SID of CP and Lys in DDGS. The second objective was to evaluate ADIN as a predictor of SID of CP and Lys in DDGS fed to pigs.

Material and Methods

Chemical and in vivo digestibility values

The in vivo values for standardized ileal digestibility of CP and Lys were obtained from 21 different sources of DDGS that were used in a previous experiment (Kim et al., 2010). Each DDGS source was analyzed for DM, CP, and AA using standard procedures (AOAC, 2006). Representative samples were taken from the same batch of DDGS used in the digestibility studies and were subsequently used for the *in vitro* assays.

In vitro enzymatic assay

The 2-step pepsin/pancreatin procedure described by Boisen and Fernandez (1995) was used for determining the in vitro digestibility of CP and Lys in DDGS. Briefly, a 1 g sample of DDGS (finely-ground; <1 mm) were mixed with 10 ml 0.2 M HCl and 1 ml of freshly prepared pepsin solution containing 10 mg pepsin (from pig stomach, 2000 U/mg, Sigma-Aldrich #P7000) in a 100 ml conical flask. A blank was included in each series. The sample was incubated in a thermostatic-controlled heating chamber at 39°C at pH 2 for 6 h. Afterwards, pH was adjusted to pH 6.8 by adding phosphate buffers and NaOH solution. The slurry was mixed with 1 ml of freshly prepared pancreatin solution containing 50 mg pancreatin (from pig pancreas, Sigma-Aldrich #P7545). The flasks were then incubated at 39°C for 18 h. The undigested residues were obtained by filtration, their CP content was analyzed, and the in vitro CP digestibility was calculated.

Boisen and Moughan (1996a,b) indicated that in vitro N digestibility is an estimate of true ileal digestibility (TID) of dietary N, which discounts both basal and specific ileal endogenous N losses. Specific endogenous CP (N × 6.25) losses correspond linearly to indigestible DM content (Boisen and Fernandez, 1995; Boisen, 1998). Thus, in vivo SID of CP can be predicted from in vitro CP digestibility when it is

corrected for specific endogenous CP losses of the assay feed ingredient using the following equations by Boisen (2007):

$$TD_{CP} = CP_A \times DN/1000 \quad [1]$$

where TD_{CP} = true digestible CP (g/kg DM); CP_A = CP content of the assay feed ingredient (g/kg DM), DN = in vitro digested N (g/kg DM).

$$SEL_{CP} = 0.066 \times UDM \quad [2]$$

where SEL_{CP} = specific endogenous losses of CP (g/kg DMI), UDM = in vitro undigested DM (g/kg).

$$CP_{SID} = TD_{CP} - SEL_{CP} \quad [3]$$

where CP_{SID} = standardised ileal digestible CP (g/kg DM).

$$SID_{CP} = CP_{SID}/CP_A \times 100\% \quad [4]$$

where SID_{CP} = standardized ileal digestibility of CP (%).

The in vitro digestibility of Lys can be estimated by assuming that the AA composition of endogenous CP is constant (Boisen and Moughan, 1996b). Boisen (1998) also suggested that specific endogenous losses of an individual AA can be calculated by using conversion factors from N to individual AA based on a standardized composition of endogenous CP.

$$TD_{Lys} = AA_{Lys} \times DN/1000 \quad [5]$$

where TD_{Lys} = true digestible Lys (g/kg DM), AA_{Lys} = Lys content in the assay feed ingredient (g/kg DM).

$$SEL_{Lys} = CF \times SEL_{CP}/6{:}25 \quad [6]$$

where SEL_{Lys} = specific endogenous loss of Lys (g/kg DMI); CF = conversion factor from N to Lys is 0.188 (Boisen, 1998).

$$Lys_{SID} = TD_{Lys} - SEL_{Lys} \quad [7]$$

where Lys_{SID} = standardized digestible Lys (g/kg DM).

$$SID_{Lys} = AA_{SID}/AA_{Lys} \times 100\% \quad [8]$$

where SID_{Lys} = standardized digestibility of Lys (%).

Acid detergent insoluble N (ADIN) analysis

The ADF and ADIN concentrations were determined using the method of Goering and Van Soest (1970). Acid detergent insoluble N (%) of each DDGS source was calculated from the protein analysis of the ADF residue by using the equation:

$$ADIN\ (\%) = \%N_{ADF} \times \%ADF/100 \quad [9]$$

where $\%N_{ADF}$ = percent N of ADF residue (on DM basis); and %ADF = percent ADF (on DM basis). Acid detergent insoluble N was then expressed as a percentage of total N.

Statistical analyses

Simple linear regression analyses were performed to determine the relationship between in vitro values and ADIN (as % of total N) with SID of CP and Lys in DDGS using the REG procedure (SAS Inst. Inc., Cary, NC). In vitro and in vivo values for SID of CP and Lys were also compared using the GLM procedure. Statistical significance and tendencies were set at $P \leq 0.05$ and $P < 0.10$, respectively, for all statistical tests.

Results

In vitro predicted values for all DDGS sources were consistently lower than their corresponding in vivo values for SID of CP (Table 1). Overall, the average in vitro SID of CP was lower (58.3 vs. 74.9%; $P < 0.0001$) than the in vivo SID of CP in DDGS. The mean residual was 16.6 percentage units. Likewise, there was no ($r^2 = 0.06$; $P = 0.27$) linear relationship between in vitro and in vivo values of SID of CP in DDGS (Figure 1). For the SID of Lys, most of the in vitro values, except for 5 DDGS sources, underestimated in vivo values. Overall, the average in vitro SID of Lys tended to be lower (59.6 vs. 63.3%; $P < 0.08$) than in vivo SID of Lys in DDGS. The mean residual was 3.6 percentage units. There was also no ($r^2 = 0.03$; $P = 0.42$) linear relationship between in vitro and in vivo SID of Lys in DDGS (Figure 2). Greater variation was observed for in vivo SID of Lys (CV = 12.8%) than SID of CP (CV = 3.6%); however, in vitro SID of CP and Lys did not reflect the same difference in variability (CV = 7.3 and 6.8%, respectively) among the DDGS sources.

The ADF concentration of the DDGS sources was between 5.67 to 14.82%. There was a positive (r^2 = 0.61; $P < 0.0001$) linear relationship between ADF and ADIN (as a percentage of total N) in the DDGS samples (Figure 3). The N concentration of ADF ranged between 0.05 and 0.23%, whereas the ADIN as a percentage of total N ranged from 1.11 to 5.30%. However, ADIN had no linear relationship with in vivo SID of CP (r^2 = 0.003; P = 0.82) or Lys (r^2 = 0.002; P = 0.86) of DDGS (Figures 4 and 5, respectively).

Discussion

In vitro vs. in vivo CP and Lys digestibility of DDGS

The accuracy of the 2-step pepsin/pancreatin assay in predicting the digestibility of CP and AA in feedstuffs and feed mixtures has been investigated previously (Moughan et al., 1989; Cone and van der Poel, 1993; Boisen and Fernandez, 1995); however, results have been variable. In some experiments (Moughan et al., 1989; Cone and van der Poel, 1993; Beames et al., 1996), no relationship between in vitro and in vivo CP or AA digestibility of feed ingredients such as meat and bone meal, barley, beans, peas, rapeseed products, and soybean products were observed, which is in agreement with data from the present experiment. In contrast, Boisen and Fernández (1995) modified the procedure and found better agreement between in vitro and in vivo apparent ileal digestibility of CP and AA in feed ingredients and feed mixtures. Boisen (2007) and Jezierny et al. (2010) were also able to validate the accuracy of this assay in predicting SID of CP and AA of different feedstuffs; however, inconsistent results were observed when barley was evaluated (Pujol and Torrallardona, 2007). In a review, Moughan (1999) concluded that the 2-step pepsin/pancreatin assay lacks accuracy and requires further refinement, especially when specific feedstuffs are evaluated. Assay factors such as enzymes used, enzyme:substrate ratio, use of co-enzymes and co-factors, pH, temperature, incubation time, sample size, and particle size need further evaluation. Another limitation of this in vitro assay is its inability to mimic the effects of dietary fiber and antinutritional factors on protein and AA digestibility (Moughan, 1999). Considering that DDGS is very high in fiber (31 to 46%; Stein and Shurson, 2008), its effects on digestibility of nutrients are not replicated in the current in vitro assay. Until improvements can be made, the current in vitro assay cannot be used to predict in vivo SID of CP and Lys in DDGS fed to pigs.

ADIN vs. CP and Lys digestibility of DDGS

Heat processing is often employed to reduce the moisture content of wet distillers grains; however, this process also may lead to heat damage that negatively affects the utilization of heat-sensitive AA such as Lys (Pahm et al., 2009). Therefore, developing methods that can predict Lys digestibility may have value in assessing different sources of DDGS for use in swine diets. In cattle diets, the concentration of ADIN is determined in feedstuffs as a measure of heat damage (Machacek and Kononoff, 2009). It is also assumed that ADIN is completely indigestible, and thus, it represents the portion of protein in feedstuffs that is unavailable for use by the animal (Sniffen et al., 1992). In DDGS, Cromwell et al. (1993) observed that increasing darkness of samples, which is suggestive of greater heat damage, was positively related to increased ADF and ADIN concentrations. It was also observed that ADIN concentrations were highly correlated with both weight gain and feed efficiency of chicks.

The positive relationship observed between ADF and ADIN concentration indicates that a greater percentage of total N is bound to ADF as ADF concentration in DDGS increases. If ADIN is indigestible, then DDGS with higher ADF have greater proportions of unavailable protein. However, there was no linear association between ADIN concentrations and in vivo SID of CP and Lys in DDGS fed to pigs. This conclusion agrees with the observations by Nakamura et al. (1994) in wethers fed corn and milo DDG. More importantly, their study in non-forage protein sources also demonstrated that ADIN is 58% digestible. It is not known if ADIN is partially digestible in pigs; however, this may explain the lack of relationship between ADIN and in vivo SID of CP and Lys. Therefore, ADIN may be related to heat damage in feed ingredients, but it is not a good predictor of CP and Lys digestibility in DDGS fed to pigs.

In conclusion, the in vitro enzymatic assay and ADIN did not predict in vivo standardized ileal digestibility of CP and Lys in DDGS fed to pigs. More research is needed to identify methods that can adequately predict protein quality for use in assessing the nutritive value of specific feedstuffs, such as DDGS.

Table 1. In vivo and in vitro standardized ileal digestibility (SID, %) of CP in 21 different sources of corn DDGS.

DDGS source	SID of CP (%)		
	In vivo	In vitro	Residual[1]
1	71.8	59.6	-12.2
2	77.5	67.2	-10.3
3	78.3	56.8	-21.5
4	71.9	66.9	-5.0
5	75.3	60.1	-15.2
6	69.8	56.4	-13.4
7	77.7	51.4	-26.3
8	72.1	62.7	-9.4
9	75.7	59.9	-15.8
10	74.1	55.0	-19.1
11	75.2	58.3	-16.9
12	74.0	51.7	-22.3
13	77.2	57.3	-19.9
14	78.5	57.3	-21.2
15	76.4	57.2	-19.2
16	79.6	57.3	-22.3
17	75.0	59.2	-15.8
18	74.3	56.2	-18.1
19	75.3	52.0	-23.3
20	70.5	61.9	- 8.6
21	73.0	60.8	-12.2

Average[2]	74.9	58.3	-16.6
S.D.	2.7	4.2	5.6
CV, %	3.6	7.3	-

[1]Residual = in vitro value - in vivo value

[2]Average in vitro value was lower than in vivo value ($P <$ 0.0001, SEM = 0.8).

Table 2. In vivo and in vitro standardized ileal digestibility (SID, %) of Lys in 21 different sources of corn DDGS.

DDGS source	SID of Lys (%)		
	In vivo	In vitro	Residual[1]
1	49.6	58.3	8.7
2	69.7	66.7	-3.0
3	71.1	58.6	-12.5
4	62.1	67.5	5.4
5	61.8	61.1	-0.7
6	60.5	58.2	-2.3
7	55.5	50.7	-4.8
8	60.0	64.1	4.1
9	68.6	62.1	-6.5
10	62.7	56.9	-5.8
11	68.8	60.8	-8.0
12	68.1	53.6	-14.5
13	68.6	59.5	-9.1
14	71.4	60.3	-11.1
15	65.8	59.6	-6.2
16	74.1	59.1	-15.0
17	68.3	60.9	-7.4
18	46.5	56.2	9.7
19	64.2	54.2	-10.0
20	45.3	61.8	16.5
21	65.6	62.3	-3.3

Average[2]	63.3	59.6	-3.6
S.D.	8.1	4.0	8.4
CV, %	12.8	6.8	-

[1]Residual = in vitro value - in vivo value

[2]Average in vitro value tended to be lower than in vivo value ($P < 0.08$, SEM =1.4).

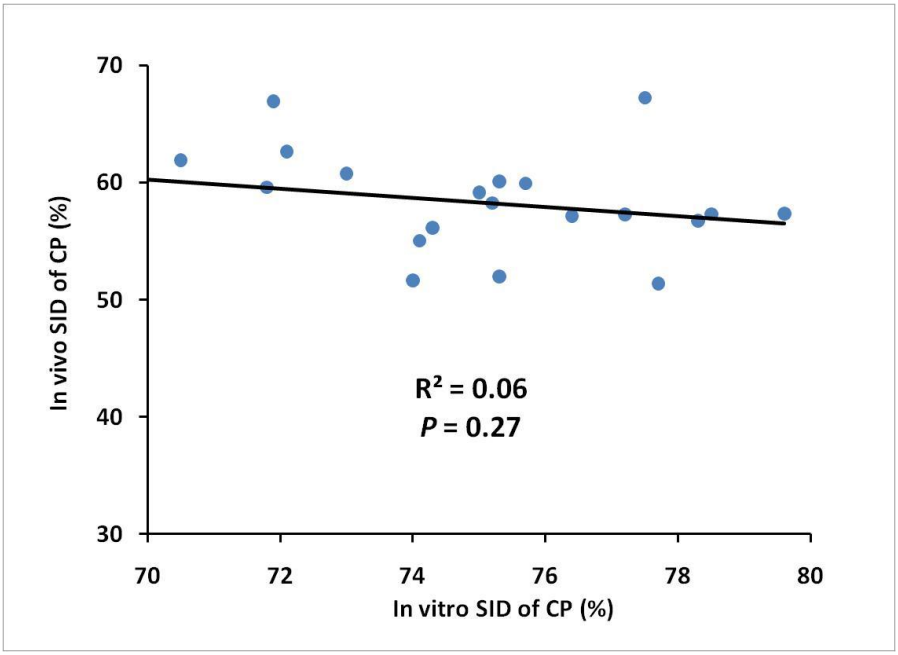

Figure 1. Relationship of in vitro and in vivo standardized ileal digestibility (SID, %) of CP in 21 different corn DDGS sources.

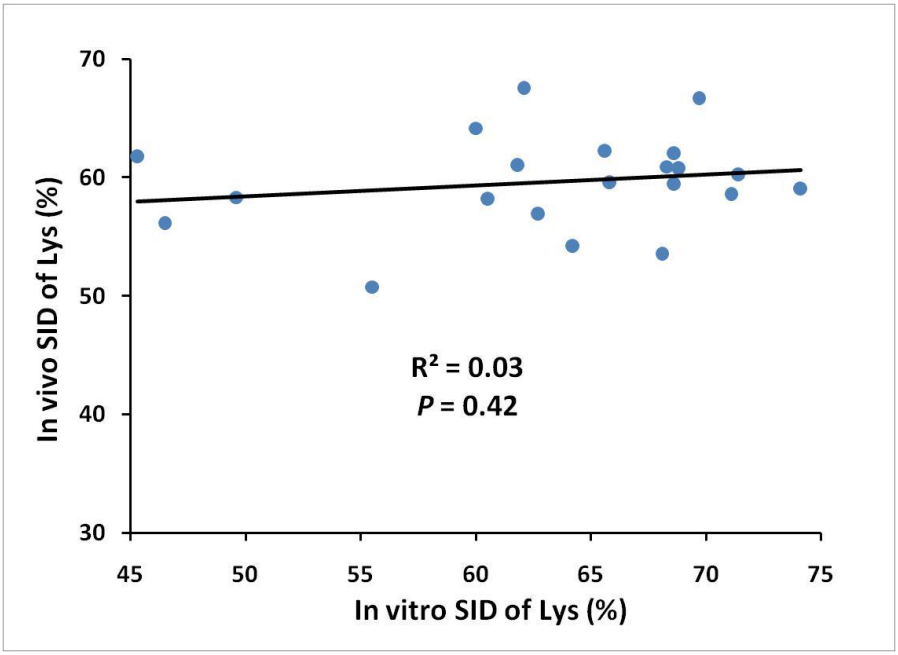

Figure 2. Relationship of in vitro and in vivo standardized ileal digestibility (SID, %) of Lys in 21 different corn DDGS sources.

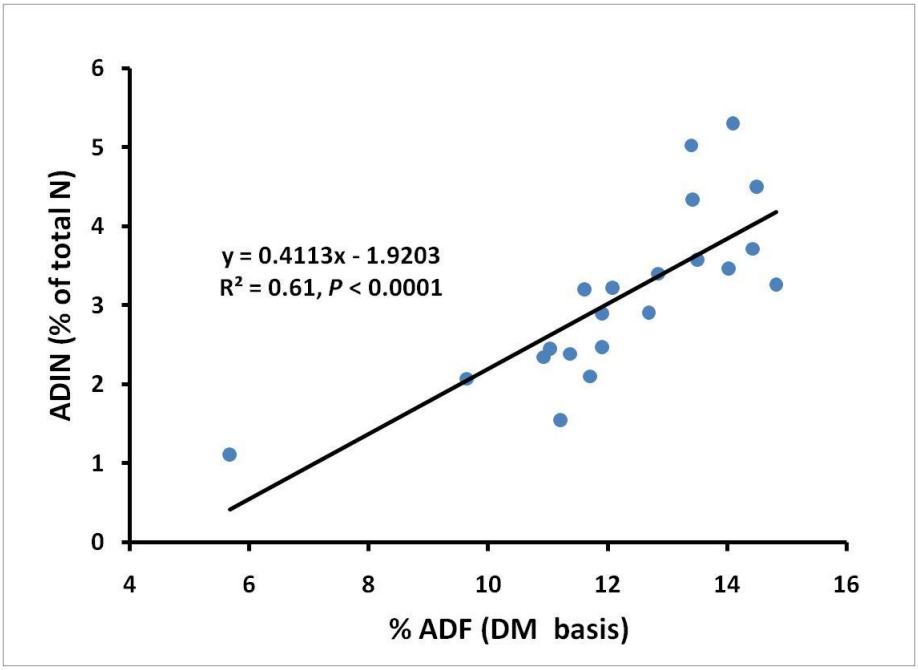

Figure 3. Relationship of ADF (%, DM basis) and acid detergent insoluble nitrogen (ADIN, as a percentage of total N) in 21 different corn DDGS sources.

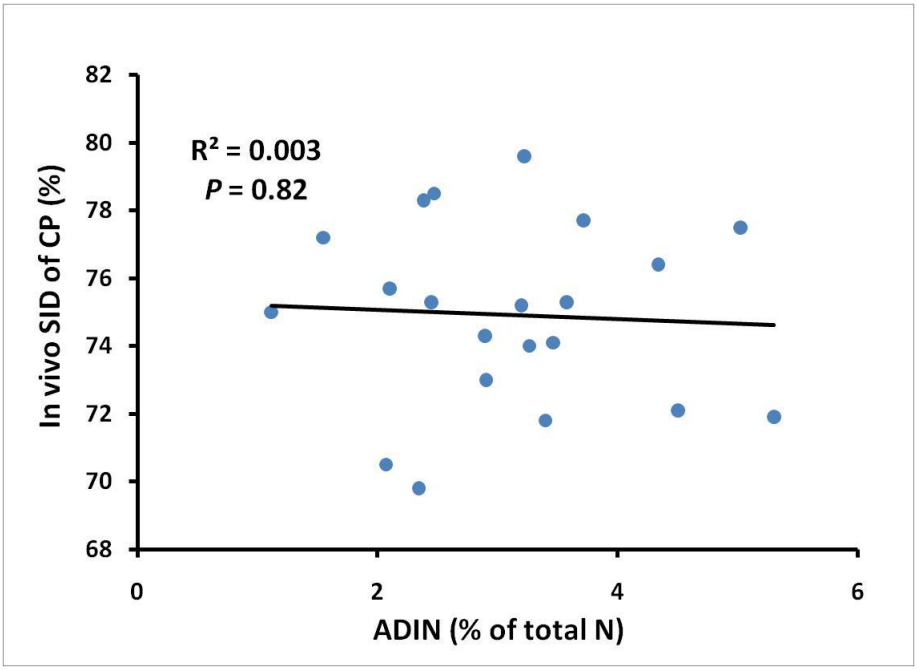

Figure 4. Relationship of acid detergent insoluble nitrogen (ADIN, as a percentage of total N) and in vivo standardized ileal digestibility (SID, %) of CP in 21 different corn DDGS sources.

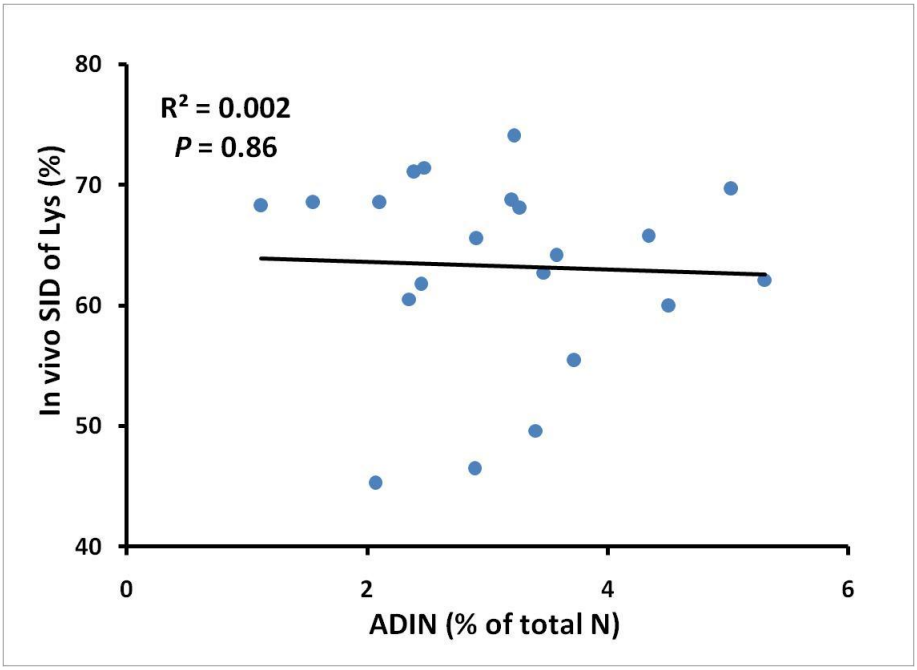

Figure 5. Relationship of acid detergent insoluble nitrogen (ADIN, as a percentage of total N) and in vivo standardized ileal digestibility (SID, %) of Lys in 21 different corn DDGS sources.

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Part C from the University of Minnesota

Use of Front Face Fluorescence for Predicting SID Lysine Digestibility in DDGS

Introduction

Research on the use of fluorescence was first conducted in 1942 to measure deterioration of dried egg extracts and several studies have been conducted since that time to measure heat damaged proteins in foods (Davies and Labuza, 2000). When proteins and peptides are heated, they become fluorescent to varying degrees based on temperature and pH, and are precursors to the brown melanoidins in food ingredients (Davies and Labuza, 2000; Matiacevich et al., 2005). Birlouez-Aragón et al. (1998, 2001, 2002) determined heat and storage damage in milk and concluded that fluorescence was a reasonable indicator of intermediate and advanced Maillard stages.

The application of fluorescence spectroscopy, which is highly sensitive and specific, in food research has the advantage of being a rapid and non-invasive analytical technique (Liu and Metzger, 2007). Diamandis (1993) reported that fluorometry is 10 to 1,000 fold more sensitive than spectrophotometry. This sensitivity is due to the combination of excitation and emission wavelengths, as well as fluorescence life span and polarization. This method has been successfully used to detect structural changes in proteins (Dufour and Riaublanc, 1997), oxidative changes in vitamins (Andersen et al, 2005), and development of Maillard reaction products (Kulmyrzaev and Dufour, 2002).

Two methods have been used to measure fluorescence in food. The traditional right angle method was used by Birlouez-Aragón et al. (1998, 2001, 2002), but has limitations of light scattering of particles, the size of proteins, and the size of the absorption surface. Front face fluorescence (FFF) spectroscopy has the advantage over the traditional right angle method of measuring the molecular structure of Maillard products in turbid and solid samples (Urriola, 2007). Schamberger and Labuza (2006) and Kulmyrzaev and Dufour (2002) demonstrated that FFF is capable of quantifying heat damage in milk, and Birlouez-Aragón et al. (2005) showed it can be used to quantify heat damage in infant formulas. However, FFF has not been evaluated for use in quantifying heat damage and Maillard products in dry feed ingredients until Urriola (2007) conducted a study to evaluate its application in corn dried distillers grains with solubles (DDGS).

Principal component analysis (PCA) is a mathematical procedure used to identify patterns in large data sets and derive a smaller number of linear combinations (principal components) that retain as much information from the original variables as possible (Smith, 2002; SAS user's guide, 1990). Principal components extract differences among factors and the new set of variables (principal components) are independent. Principal components analysis is the basis or initial step for principal components regression (PCR) and partial least squares regression (PLSR) (CAMO, 2006). In PCR, the first step is to obtain the eigenvectors and Eigen values from a square symmetric covariance matrix of the original data. The eigenvector determines the orientation and the Eigen value measures the magnitude of the response. The first principal component (PC) explains the greatest portion of the variation while the second PC is the next most important independent component explaining the variation in the original data.

Sequential PCs are generated cumulatively until nearly 100% of the variation is explained. As a result the dimension of the data can be reduced by selecting the number of PCs that account for most of the variation, with minimal loss of information. Since principal components are orthogonal or independent among each other, the problem of multicollinearity is minimized. That allows finding significant variables that explain patterns in the data, and improves the accuracy of the analysis (Spicer 2005).

Urriola (2007) adapted FFF and PCR technologies to develop prediction equations for digestible amino acids for swine in DDGS using a data set comprised of SID amino acids representing 34 ethanol plants in the U.S.. The goal of this research was to provide an accurate and reliable tool to predict standardized ileal digestible (SID) amino acids in DDGS samples while reducing the cost and time of using *in vivo* experiments and *in vitro* laboratory procedures. Urriola (2007) was the first to report that the use of FFF and PCA allowed fast and accurate prediction (R^2 > 0.93) of digestible crude protein, lysine,

methionine, threonine, and tryptophan in DDGS samples, and was superior compared to using color and optical density.

Because of the excellent results obtained by Urriola (2007), the objective of this study was to validate the use of FFF and PCA and regression on the development of prediction equations for digestible Lys content in DDGS using a new data set.

Material and Methods

SID lysine data

Two Standardized Ileal Digestible Lysine (SID Lys) data sets were used in this study. The first data set consisted of SID Lys data (as-is and DM basis) from 21 DDGS samples obtained from different ethanol plants (Table1). Growing pigs fitted with ileal cannulas we used to determine SID amino acid content of these samples at the University of Illinois. A subsample of each DDGS was sent to the University of Minnesota to obtain Front Faced Fluorescence (FFF) readings used to develop the SID Lys prediction equations. The second data set included SID Lys (as-is and DM basis) data (Table 2) and FFF readings from a group of 37 DDGS samples from a previous study (Urriola, 2007). Appendix A contains the FFF and SID Lys values for each group of DDGS samples in data sets 1 and 2.

Determination of front face fluorescence (FFF) measurements

Samples were ground to a fine particle size using in a Wiley mill with a 1 mm screen mounted in a sample cell holder designed for powder. Fluorescence intensity was recorded from each sample twice using a spectrometer (model Aminco Bowman II, Thermo Electron Corporation Waltham, MA) at excitation wavelength of 360 nm and emission spectra of 380 to 600 nm measuring each 5 nm within the emission spectra range (Schamberger and Labuza, 2006). Slit widths of the spectrometer were set at 2 and 4 nm for excitation and emission, respectively. All DDGS samples were analyzed for FFF in triplicate.

Statistical methods

Two models were tested for the development of the prediction equations. Model A used SID Lys data and FFF readings from only data set 1 (21 samples) to develop the SAS input for Principal Component Analysis (PROC PRINCOMP), Stepwise Regression (PROC STEPWISE) and Regression (PROC REG) from SAS 9.1 (SAS Inst., Inc., Cary, NC). Analysis of SID Lys on an as-is and DM basis was conducted separately using PROC PRINCOMP followed by PROC STEPWISE resulting in two different sets of selected principal components and fluorescence wavelengths (one for SID Lys as-is basis and one for SID Lys DM basis), which were used to validate the prediction model.

Model B used SID Lys information and FFF readings from data set 2 (37 samples) and FFF readings from data set 1 to develop the prediction equations for the each of the 21 samples under investigation (cross-validation). For data set 2, Urriola (2007) used multiple linear regressions (Bowerman and O'Connell, 1990) to analyze and correlate SID Lys and FFF readings. Linear regression using stepwise elimination of non- significant factors was performed with the PROC REG from SAS 9.1. Using this approach, the multiple coefficient of determination (r^2) determined the proportion of variation of the dependent variable explained by the overall regression model. Many of the chemical and physical characteristics of DDGS analyzed by Urriola (2007) were correlated among each other (i.e., not independent). Linear regression models that include correlated explanatory variables suffer from a multicollinearity problem and can only be used to predict the dependent variable because they produce incorrect least squares estimates (Bowerman and O'Connell, 1990). Therefore, principal components analysis from PROC PRINCOMP of SAS was used to extract differences among explanatory variables to create a new smaller set of variables (i.e. principal components) that were orthogonal or truly independent of each other (Spicer, 2005). The newly created principal components were used in multiple linear regression analysis to study the relationship of physical and chemical properties to digestible amino acid content in all DDGS sources.

The model developed by Urriola (2007) did not include all FFF data when performing the principal component analysis which was subsequently used to perform the stepwise regression analysis, as done for developing model A. As a result, the predicted SID Lys for each DDGS sample was obtained by running one regression for each particular row of observations, omitting the observed Lys content. Principal Components Analysis was performed for combinations of fluorescence variables from X380 to X600 for Model A and from X389 to X594 for Model B. The PROC PRINCOMP and PROC REG of each model resulted in:

a) Observed and predicted values for SID Lys on an as-is (SIDLys) and on a DM basis (SIDLysDM)
b) Graphs for observed and predicted SIDLys and SIDLysDM
c) Regression equations for SIDLys and SIDLysDM
d) Coefficient of determination for observed and predicted values
e) Residual Mean Square Error.

Results and Discussion

Model A

Observed and predicted values for SID Lys (as-is basis) were similar and highly correlated (adjusted R^2 = 0.9964) in the validation of Model A (Table 3 and Figure 1). Residual variance component values (Table 3) showed that the variance observed in variables, not accounted for by the factors included in the model used to predict the SID Lys content and develop the prediction equations, were very low or non-significant. The closer the residuals are to zero, the more confidence the researcher has in the number of factors selected for use in the model. The SEM predicted values are a measure of the accuracy of the prediction, or the mean standard deviation of the observed value relative to the predicted value. The observed SEM predicted values using this model suggest that the predicted SID Lys values (as-is basis) from FFF can represent the actual SID Lys values within this data set.

Likewise, SID Lys (DM basis) observed and predicted values (Table 4 and Figure 2) were similar and the coefficient of determination (adjusted R^2 = 0.9978) indicated that a high proportion of variability in the data set was accounted for by the statistical model. The residual variance component and SEM of predicted values indicate the ability of the model to explain the variation in this data set and indicate that the predicted SID Lys values (DM basis) represent the observed values accurately within this data set. These results were similar to those reported by Urriola (2007).

The resulting prediction equations for SID Lys on an as-is and DM basis using Model A are as follows:

Prediction equation for SID Lys (as-is basis)

SID Lys (as-is basis) = 0.5941 -0.0085*Prin1 +0.0409*Prin2 -0.0303*Prin3 -0.0728*Prin4 +0.0330*Prin5 -0.2039*Prin6 -1.2746*Prin7 -0.1597*Prin8 +1.5705*Prin9 -2.0959*Prin10 +0.3957*Prin11 +0.1196*Prin12 -1.1194*Prin13 -1.3945*Prin14 +13578*Prin15 -0.9528*Prin16 +2.7344*Prin17 -0.7619*Prin18 -1.1391*Prin19

Prediction equation for SID Lys (DM basis)

SID Lys (DM basis) = 0.6985 -0.0085*Prin1 +0.0308*Prin2 +0.0333*Prin3 -0.2667*Prin4 +0.0435*Prin5 +0.9804*Prin6 +0.2286*Prin7 +1.3183*Prin8 -2.0695*Prin9 +2.1711*Prin10 -0.5045*Prin11 +3.2793*Prin12 +0.7263*Prin13 -0.3839*Prin14 +2.1148*Prin15 -2.2280*Prin16 -1.5291*Prin18 -2.7087*Prin19 +1.5333*Prin2

Model B

When the FFF readings from the 21 DDGS samples (data set 1) were included in model B using SID Lys and FFF data from data set 2 (Urriola, 1007), the variation between observed and predicted SID Lys (as-is and DM basis) values was dramatically increased as indicated by SEM Predicted values in Tables 5 and 6. Coefficients of determination (R^2 and adjusted R^2) for each of the 21 samples used in the prediction model were included in Tables 5 and 6 because each sample required one regression analysis to generate one graph, one prediction equation and one coefficient of determination (graphs and equations not shown). Although the R^2 values were all greater than 0.74, indicating that there is a high correlation between the actual and predicted SID Lys values, the adjusted R^2 values were very low and negative. Using the adjusted R^2 values as an indicator of the model adequacy is necessary because this coefficient is adjusted for the number of independent variables included in the regression model. In this case, the regression yielded negative values which indicate that the predicted values being compared to the corresponding observed values have not been derived from a model-fitting procedure using those data. The residual variance component was not included in Tables 5 and 6 for Model B because the observed value for each sample from data set 1 is not included in the model, thus, the statistical model cannot calculate this value.

These results show that highly accurate predictions for SID Lys can be achieved using FFF and PCA within a defined data set of actual SID Lys values. However, when predicting SID Lys values for one data set (e.g. data set 1) using the prediction model developed for another data set (e.g. data set 2; Urriola, 2007), the capability of the model used to predicted SID Lys values is poor. Only 37 DDGS samples were used to develop the prediction equations in Model B. Perhaps a larger data set would improve the ability of this model to more accurately predict SID Lys using data from different set of samples not used on its development.

Conclusion

Accurate SID Lys prediction models can be developed to provide good estimates within a defined data set but does not accurately predict SID Lys using data from samples not included in the model. Investigations related to the development of a larger data set for generating more accurate SID Lys prediction equations is warranted.

Table 1. Observed Standardized Ileal Digestible Lys (as-is and DM basis) in 21 DDGS samples from different corn ethanol plants (data set 1)

DDGS ID	SID Lys, % (as-is basis)	SID Lys, % (DM basis)
19031	0.343	0.382
19032	0.516	0.588
19033	0.718	0.842
19034	0.559	0.660
19035	0.519	0.621
19036	0.563	0.651
19037	0.533	0.607
19039	0.576	0.663
19040	0.680	0.814
19041	0.627	0.721
19042	0.675	0.807
19043	0.797	0.895
19044	0.720	0.827
19045	0.693	0.847
19046	0.599	0.734
19047	0.727	0.890
19048	0.655	0.792
19049	0.363	0.443
19050	0.635	0.752
19051	0.335	0.398
19052	0.643	0.735

Table 2. Observed Standardized Ileal Digestible Lys (as-is and DM basis) in 37 DDGS samples from different corn ethanol plants (data set 2)

DDGS ID	SID Lys, % (as is)	SID Lys, % (DM)
1526	0.393	0.447
1527	0.467	0.520
1528	0.690	0.772
1529	0.666	0.752
2702	0.478	0.545
2703	0.475	0.544
2704	0.518	0.566
2705	0.437	0.487
2706	0.304	0.335
2707	0.442	0.505
2708	0.484	0.550
2709	0.323	0.380
2710	0.520	0.600
2711	0.474	0.553
7402	0.548	0.625
7403	0.482	0.541
7404	0.547	0.622
7405	0.534	0.598
7406	0.449	0.519
7407	0.471	0.527
7408	0.426	0.480
7409	0.550	0.595
7410	0.597	0.672

7411	0.405	0.468
7602	0.555	0.611
7603	0.634	0.695
7604	0.625	0.694
7605	0.652	0.744
7606	0.569	0.675
7702	0.766	0.854
7703	0.594	0.668
7704	0.829	0.937
7705	0.544	0.625
7706	0.674	0.762
7707	0.539	0.608
7708	0.702	0.808
7709	0.623	0.710

Table 3. Model A observed and predicted values of SID Lys (%, as-is basis) in 21 DDGS samples using FFF and PCA

SAMPLE ID	Observed SID Lys, %	Predicted SID Lys, %	Residual Variance Component	SEM Predicted
19031	0.343	0.3443	-0.001289	0.007480
19032	0.516	0.5142	0.001773	0.007380
19033	0.718	0.7162	0.001839	0.007364
19034	0.559	0.5577	0.001257	0.007485
19035	0.519	0.5185	0.000543	0.007570
19036	0.563	0.5615	0.001494	0.007441
19037	0.533	0.5335	-0.000535	0.007571
19039	0.576	0.5781	-0.002051	0.007308
19040	0.680	0.6793	0.000678	0.007560
19041	0.627	0.6255	0.001475	0.007445
19042	0.675	0.6717	0.003343	0.006814
19043	0.797	0.7985	-0.001516	0.007437
19044	0.720	0.7186	0.001388	0.007462
19045	0.693	0.6946	-0.001583	0.007423
19046	0.599	0.6003	-0.001277	0.007482
19047	0.727	0.7294	-0.002352	0.007216
19048	0.655	0.6574	-0.002389	0.007204
19049	0.363	0.3613	0.001747	0.007386
19050	0.635	0.6357	-0.000688	0.007559
19051	0.335	0.3370	-0.001966	0.007331
19052	0.643	0.6429	0.000108	0.007589

Table 4. Model A observed and predicted values of SID Lysine (%, DM basis) in 21 DDGS samples using FFF and PCA

SAMPLE ID	Observed SID Lys, %	Predicted SID Lys, %	Residual Variance Component	SEM Predicted
19031	0.382	0.3833	-0.001343	0.006987
19032	0.588	0.5881	-0.000068	0.007114
19033	0.842	0.8405	0.001451	0.006965
19034	0.660	0.6591	0.000884	0.007059
19035	0.621	0.6201	0.000924	0.007054
19036	0.651	0.6500	0.001027	0.007040
19037	0.607	0.6106	-0.003560	0.006160
19039	0.663	0.6627	0.000287	0.007109
19040	0.814	0.8137	0.000342	0.007106
19041	0.721	0.7191	0.001936	0.006846
19042	0.807	0.8091	-0.002070	0.006807
19043	0.895	0.8958	-0.000821	0.007067
19044	0.827	0.8257	0.001279	0.006999
19045	0.847	0.8491	-0.002112	0.006794
19046	0.734	0.7354	-0.001417	0.006972
19047	0.890	0.8910	-0.000954	0.007050
19048	0.792	0.7932	-0.001226	0.007008
19049	0.443	0.4423	0.000681	0.007082
19050	0.752	0.7495	0.002494	0.006663
19051	0.398	0.3980	-0.000019	0.007115
19052	0.735	0.7327	0.002286	0.006737

Table 5. Observed and predicted SID Lys values (%, as-is basis) and coefficients of determination using FFF values from 21 DDGS samples (data set 1) in Model B (data set 2)					
SAMPLE ID	**Observed SID Lys, %**	**Predicted SID Lys, %**	**SEM Predicted**	**R^2**	**Adjusted R^2**
19031	0.343	0.8560	0.6534	0.7576	-0.1281
19032	0.516	0.4257	0.2542	0.7842	-0.1099
19033	0.718	0.5578	0.4087	0.7572	-0.2489
19034	0.559	0.4448	0.2658	0.7604	-0.2325
19035	0.519	0.4559	0.3028	0.7657	-0.2047
19036	0.563	0.4839	0.3325	0.7544	-0.2628
19037	0.533	0.4962	0.2930	0.7692	-0.1870
19039	0.576	0.5478	0.3300	0.7544	-0.2629
19040	0.68	0.5514	0.3734	0.7795	-0.1340
19041	0.627	0.5221	0.3463	0.7696	-0.1852
19042	0.675	0.5219	0.2881	0.7607	-0.2308
19043	0.797	0.6380	0.3371	0.7440	-0.3166
19044	0.72	0.6325	0.4152	0.7708	-0.1787
19045	0.693	0.5067	0.3505	0.7696	-0.1849
19046	0.599	0.6438	0.3091	0.7809	-0.1266
19047	0.727	0.4843	0.3087	0.7587	-0.2412
19048	0.655	0.5375	0.3425	0.7752	-0.1563
19049	0.363	0.4398	0.3122	0.7470	-0.3012
19050	0.635	0.6196	0.3058	0.7722	-0.1713
19051	0.335	0.4989	0.3339	0.7441	-0.3159
19052	0.643	0.5102	0.2751	0.7636	-0.2157

Table 6. Observed and predicted SID Lys values (%, DM basis) and coefficients of determination in 21 DDGS samples (data set 1) using Model B (data set 2)

SAMPLE ID	Observed SID Lys, %	Predicted SID Lys, %	SEM Predicted	R^2	Adjusted R^2
19031	0.382	0.9603	0.7729	0.7291	-0.3932
19032	0.588	0.4939	0.2960	0.7663	-0.2020
19033	0.842	0.6639	0.4752	0.7378	-0.3482
19034	0.66	0.5113	0.3082	0.7427	-0.3232
19035	0.621	0.5394	0.3504	0.7495	-0.2882
19036	0.651	0.5752	0.3851	0.7369	-0.3532
19037	0.607	0.5950	0.3410	0.7503	-0.2840
19039	0.663	0.6535	0.3836	0.7351	-0.3626
19040	0.814	0.6488	0.4371	0.7586	-0.2416
19041	0.721	0.6136	0.4023	0.7515	-0.2779
19042	0.807	0.6270	0.3329	0.7448	-0.3125
19043	0.895	0.7255	0.3929	0.7221	-0.4294
19044	0.827	0.7311	0.4850	0.7501	-0.2850
19045	0.847	0.5838	0.4105	0.7475	-0.2983
19046	0.734	0.7491	0.3583	0.7648	-0.2097
19047	0.89	0.5753	0.3559	0.7437	-0.3180
19048	0.792	0.6425	0.3975	0.7580	-0.2444
19049	0.443	0.4952	0.3608	0.7302	-0.3876
19050	0.752	0.7378	0.3558	0.7536	-0.2670
19051	0.398	0.5951	0.3888	0.7229	-0.4253
19052	0.735	0.6139	0.3200	0.7445	-0.3139

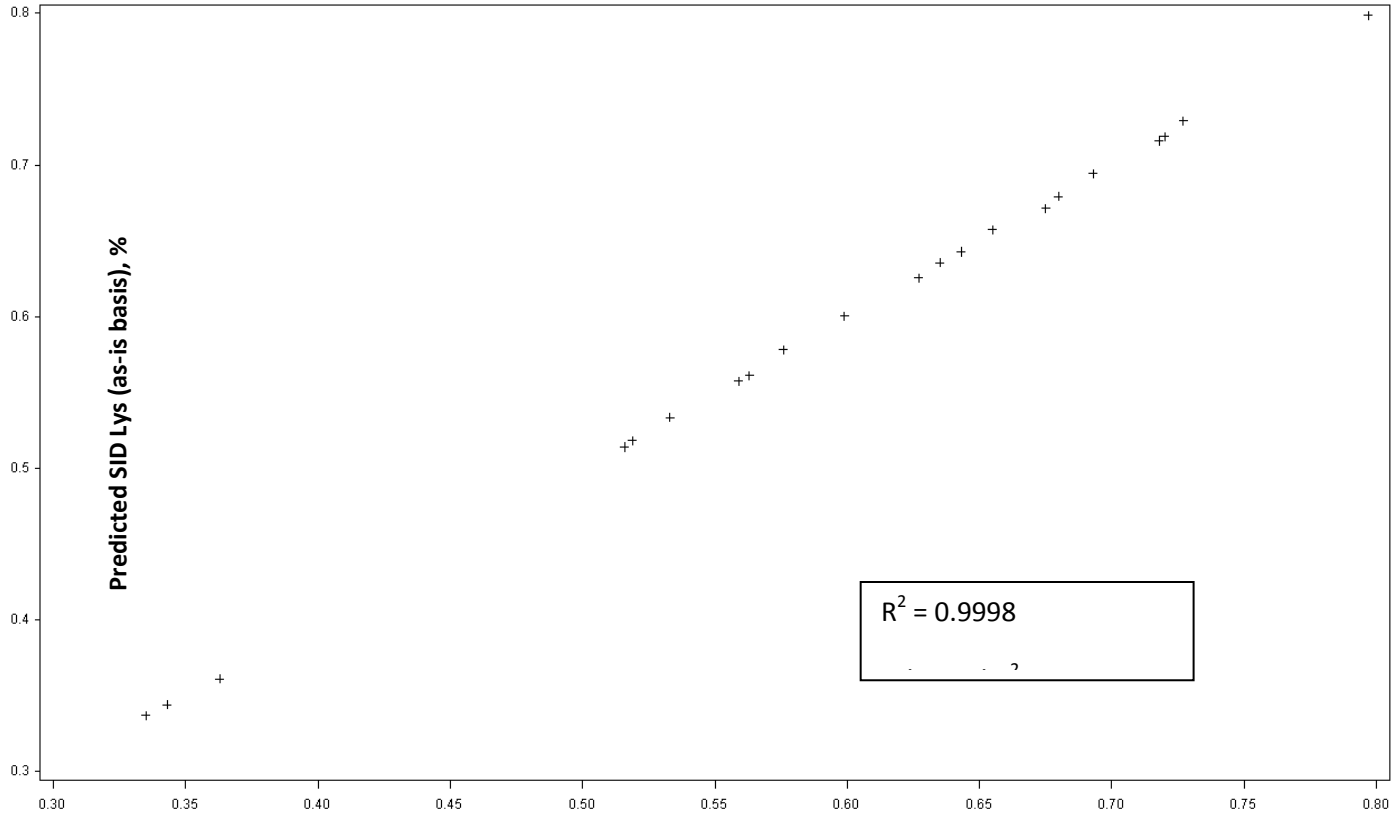

Figure 1. Correlation between determined and predicted values of SID Lysine (as-is basis) in 21 DDGS samples using FFF and PCA (Model A).

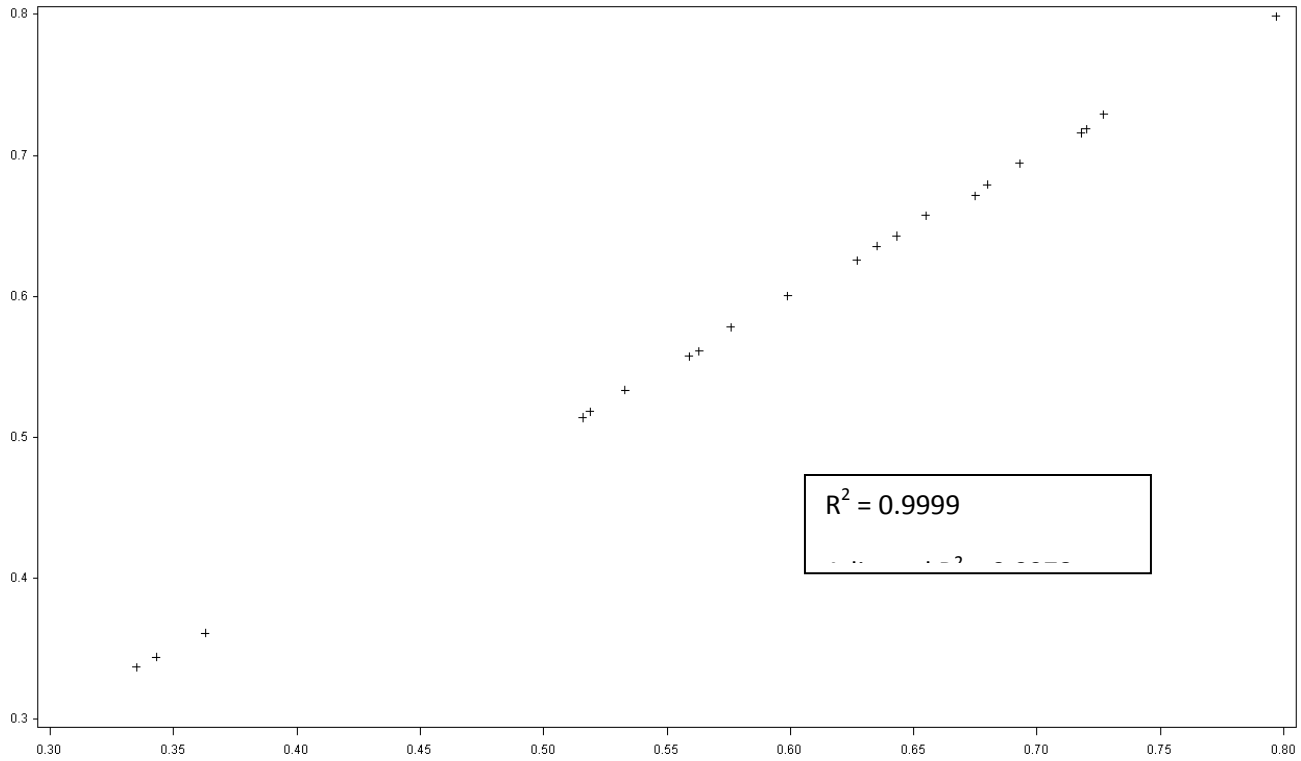

Figure 2. Correlation between determined and predicted values of SID Lysine (DM basis) in 21 DDGS samples using FFF and PCA (Model A).

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