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Prediction of the concentration of standardized ileal digestible amino acids in distillers dried grains with solubles[1]

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ABSTRACT: Values for the concentration of standardized ileal digestible (SID) CP, Lys, Met, Thr, and Trp from 34 sources of distillers dried grains with solubles (DDGS) were obtained from a series of 5 experiments with the objective of predicting the concentration of SID AA from physical and chemical assays. The concentration of NDF, ADF, hemicellulose, acid detergent insoluble CP (ADICP), and KOH soluble protein (SolCP) were measured and calculated in all DDGS sources. Likewise, particle size was measured and color of each source of DDGS was determined with a Minolta colorimeter and HunterLab spectrometer and was expressed as lightness (L*), redness (a*), and yellowness (b*). The HunterLab spectrometer also provided optical density that was recorded between 400 and 700 nm. Front face fluorescence was measured at 360 nm excitation and recorded from 380 to 600 nm. Multiple linear regression and principal components analyses were performed to predict the concentration of SID AA among DDGS sources, and predicted means as well as predicted residual sums of squares (PRESS) were calculated to estimate accuracy and precision of the model. Some correlations ($P < 0.05$) were observed between ADF, hemicellulose, ADICP, and SolCP with SID CP and AA but were generally low ($r < 0.51$). There was a greater association ($R^2 = 0.40$; $P < 0.05$) between L* and SID Lys among DDGS sources when L* was less than 50 than when samples had L* values greater than 50. In addition, a* was negatively correlated ($P < 0.05$) with SID CP ($r = –0.41$), Lys ($r = –0.59$), and Met ($r = –0.50$) whereas b* tended to be positively correlated ($P < 0.10$) with SID Lys ($r = 0.31$) and Trp ($r = 0.30$) and was correlated ($P = 0.05$) with SID Met ($r = 0.43$) and Thr ($r = 0.36$). There were no correlations between NDF or particle size with SID CP and AA. Optical density, along with CP, was highly predictive of SID Lys ($R^2 = 0.97$; PRESS = 0.05), Thr ($R^2 = 0.94$; PRESS = 0.06), and Trp ($R^2 = 0.93$; PRESS = 0.004) but not SID Met ($R^2 = 0.39$; PRESS = 0.12). Front face fluorescence was also highly predictive of SID Lys ($R^2 = 0.99$; PRESS = 0.07), Met ($R^2 = 0.95$; PRESS = 0.05), Thr ($R^2 = 0.99$; PRESS = 0.008), and Trp ($R^2 = 0.99$; PRESS = 0.006). In conclusion, correlations between ADICP, SolCP, NDF, particle size, and color measurements with SID AA concentrations were poor, but optical density and front face fluorescence methods appear to provide good predictions of SID AA concentrations in DDGS. However, these prediction equations need to be validated using samples of DDGS from a separate data set.

Key words: amino acid digestibility, color, distillers dried grains with solubles, fluorescence, optical density, swine

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INTRODUCTION

Total content and standardized ileal digestibility (**SID**) of Lys are highly variable among sources of corn distillers dried grains with solubles (**DDGS**; Goodson and Fontaine, 2004; Stein and Shurson, 2009) and are a challenge when determining appropriate values to use in diet formulation. The second challenge when using

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heat treated feed ingredients is that SID values may not be predictive of the bioavailability of AA due to the formation of compounds of the Maillard reaction (Rérat et al., 2002).

High temperatures (40 to 120°C) used during DDGS drying process cause heat damage of about 15% of Lys resulting from Maillard reactions (58 g/kg as reactive Lys from 77 g/kg of total Lys; Pahm et al., 2009b; Mosqueda and Tabil, 2011, 2012). Browning has been associated with the extent of heat damage and subsequent AA use in severely heated DDGS in chickens and pigs, but these relationships were established using a limited number of samples (Cromwell et al., 1993; Fastinger and Mahan, 2006). It appears that AA digestibility may be predicted by measuring the accumulation of Maillard reaction products, but it is unclear if color, optical density, or front face fluorescence are predictive of SID Lys and AA.

Acid detergent insoluble CP (**ADICP**) and KOH soluble protein (**SolCP**) are common measurements for determining the extent of heat damage in forages and soybean meal (Parsons et al., 1991; Van Soest et al., 1991), but no data have been published to indicate if they are predictive of SID Lys in DDGS. Furthermore, NDF content also varies among DDGS sources (Urriola et al., 2010) and may reduce AA digestibility (Farrell, 1973; Souffrant, 2001), but it is unknown if NDF content is predictive of the SID AA concentration in DDGS. Therefore, the objective of this study was to evaluate if selected in vitro procedures are useful for predicting the SID AA concentration in DDGS using in vivo SID CP, Lys, Met, Thr, and Trp data from multiple sources of DDGS (Stein et al., 2006; Pahm et al., 2008a; Urriola et al., 2009).

MATERIAL AND METHODS

Sources of Distillers Dried Grains with Solubles and Ileal Digestibility Experiments

Standardized ileal digestibility data for indispensable AA from 34 different sources of corn DDGS were measured in a series of 5 ileal digestibility experiments conducted at South Dakota State University. However, only SID CP, Lys, Met, Thr, and Trp data were used in this study. Details for cannulation, sample collection, chemical analyses, and calculation of digestibility have been reported (Stein et al., 2006; Pahm et al., 2008b; Urriola et al., 2009). A summary of the nutritional composition of these DDGS sources has been published (Stein and Shurson, 2009).

Chemical Analyses and Particle Size

The NDF concentration in DDGS samples (Van Soest et al., 1991) was analyzed using heat stable α-amylase and sodium sulfite. The concentration of ADF and NDF were determined using a modified procedure using a 200 Fiber Analyzer and fiber bags rather than reflux beakers and vacuum filtration (Ankom Technologies, Macedon, NY). Hemicellulose was calculated by subtracting the ADF fraction from NDF for each sample. Acid detergent insoluble nitrogen was measured by analyzing the nitrogen residue in the ADF fraction and then multiplying by 6.25 to express it on a CP basis (ADICP; Licitra et al., 1996). The concentration of KOH soluble CP was measured using procedures described by Licitra et al. (1996). Particle size was measured by shaking 100 g of each source of DDGS in a stack of sieves using the method (S319.4) described by American Society of Agricultural and Biological Engineers (ANSI/ASAE, 2008). All measurements were conducted in the swine nutrition laboratory at the University of Minnesota in duplicate and repeated when difference between replicates was greater than 5%.

Measurement of Color, Optical Density, and Fluorescence

Color of each DDGS sample was measured as optical reflectance from a range of wavelengths between 400 and 700 nm using a Minolta colorimeter (Model CR-310; Konica Minolta Sensing Americas, Inc. Ramsey, NJ) and a HunterLab spectrometer (Model Colorflex 45/0; Hunter Associates Laboratory, Inc., Reston, VA). When color was recorded with the Minolta colorimeter, the sample was placed in a 250 mL glass beaker, the surface was leveled, and color data from all samples were collected in triplicate at the same time by gently placing a 50 mm wide probe over the sample, mixing, and leveling again before the next measurement. With the HunterLab spectrometer, all samples were analyzed in the same batch and color data were measured 3 consecutive times by placing each sample in a 2.54 cm wide glass container. In both cases, color was expressed using 3 parameters developed by the Commission Internationale d'Eclairage (**CIE Lab**) in Vienna, Austria. In the 3 parameter color scale, L* indicates lightness of color (L* = 0 represents black and L* = 100 represents white), a* indicates color position between red and green (negative values indicate green and positive values indicate red), and b* indicates color position between yellow and blue (negative values indicate blue and positive values indicate yellow). In addition to CIE Lab information, the HunterLab spectrometer provided optical density data from 400 to 700 nm.

In addition to CIE Lab information, the HunterLab spectrometer provided optical density data from 400 to 700 nm. Samples were ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) through a 1 mm screen and then mounted in a sample cell holder designed for

Table 1. Pearson's correlation coefficients (*r*) between NDF, ADF, hemicellulose, acid detergent insoluble CP (ADICP), KOH soluble CP (SolCP), particle size (PS), and color scales (L*, a*, and b*[1]) in distillers dried grains with solubles vs. the concentration of standardized ileal digestible CP, Lys, Met, Thr, and Trp

	Standardized ileal digestible nutrient					Range in measured values		
Item	CP	Lys	Met	Thr	Trp	Least	Avg	Greatest
NDF, %	0.24	–0.01	–0.26	0.14	0.13	26	32	42
ADF, %	–0.33[a]	–0.01	–0.33[a]	0.13	0.49	9	12	21
Hemicellulose, %	–0.07	–0.01	0.06	0.03	–0.34[a]	15	20	25
ADICP, %	0.31	–0.08	–0.30[a]	0.09	0.50[a]	2	3	7
SolCP, %	0.51	0.08	–0.08	0.15	–0.05	18	26	35
PS, μm	0.23	0.21	–0.23	0.14	0.17	114	685	1,356
Color scale								
L*	0.11	0.33[a]	0.30[b]	0.01	0.01	36.5	52.7	62.5
a*	–0.41[a]	–0.59[a]	–0.50[a]	–0.27	–0.18	8.0	10.1	12.2
b*	0.01	0.31[b]	0.43[a]	0.36[a]	0.30[b]	21.3	36.4	47.0

[a]Correlation effect ($P < 0.05$).

[b]Correlation effect ($P < 0.1$).

[1]L* indicates lightness of color (L* = 0 represents black and L* = 100 represents white), a* indicates color position between red and green (negative values indicate green and positive values indicate red), and b* indicates color position between yellow and blue (negative values indicate blue and positive values indicate yellow).

powder. Front face fluorescence measurements were recorded from duplicate samples using a spectrometer (Model Aminco Browman 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. Samples were analyzed in duplicate and read twice to reduce variability.

Statistical Analysis

Pearson's correlation coefficients (*r*) were calculated for variables using the CORR procedure (SAS Inst. Inc., Cary, NC), and associations between variables were considered significant at $P < 0.05$ (Table 1). The UNIVARIATE procedure of SAS was used to test homogeneity of the residual from the fitted model. The residual vs. the predicted value plot was used to check normality and outliers of the data set. All data had a normal distribution, constant variance, and no outliers. However, several chemical and physical characteristics analyzed in DDGS were correlated among each other (i.e., not independent). Linear regression models that include correlated explanatory variables suffer from a problem of multicollinearity 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 created principal components were used in multiple linear regression analysis to determine the relationship between physical and chemical properties of DDGS with the concentration of SID AA in all sources of DDGS using principal components regression (Massy, 1965). The PROC REG procedure of SAS was used for regression analysis of the concentration of SID AA and the newly created principal components. Variables included in the model were determined by backward elimination of nonsignificant factors at $P < 0.05$. The multiple coefficient of determination (R^2), adjusted R^2, residual sum of squares error, and predicted residual error sum of squares (**PRESS**) were reported and used to assess precision and accuracy of the model (Allen, 1971). The CP concentration of DDGS samples was included in the models used to predict SID AA concentrations from optical density whereas the front face fluoresce spectra was used without including CP concentration in the models to predict SID AA.

RESULTS AND DISCUSSION

Prediction of Standardized Ileal Digestible CP and AA from Chemical Analysis and Particle Size

The concentration of CP was moderately correlated with the concentration of SID CP, Lys, Met, Thr, and Trp ($R^2 = 0.87$, 0.70, 0.65, 0.73, and 0.61, respectively; $P < 0.05$) among sources of DDGS. The concentration of SID CP was positively correlated ($P = 0.05$) with the concentration of SID Lys ($R^2 = 0.67$), Met ($R^2 = 0.28$), Thr ($R^2 = 0.83$), and Trp ($R^2 = 0.64$). These observa-

tions suggest that the concentration of CP and SID CP only partially explain the variation in the concentration of SID Lys among DDGS sources (Pahm et al., 2009a; Kim et al., 2012).

The concentrations of NDF, ADF, and hemicellulose were between the range of values observed in other experiments (Stein et al., 2006; Urriola et al., 2010; Anderson et al., 2012). In general, correlations ($P < 0.05$) were observed among several chemical and physical components of DDGS but the coefficients were less than 0.51 (Table 1). There were no correlations between the concentrations of NDF and SID CP, Lys, Met, Thr, or Trp despite a wide range in NDF concentration among DDGS sources. Negative correlations between ADF and SID CP ($r = –0.33$; $P < 0.05$) and Met ($r = –0.33$; $P < 0.05$) were observed but not for SID Lys, Thr, or Trp. Hemicellulose was negatively correlated ($r = –0.34$; $P < 0.05$) with SID Trp. The lack of a strong correlation between these fiber components and SID AA observed in this experiment can be explained by several factors. First, the variation in SID CP and SID Lys concentrations may be more dependent on the initial concentration of CP and Lys because the effect of heat treatment is likely greater than the effect of NDF, ADF, or hemicellulose concentration. Second, dietary fiber from DDGS appears to have minimal effect on SID of AA because adding 30% DDGS to a corn–soybean meal based diet has been shown to reduce apparent ileal digestibility of Lys but not of other AA (Urriola and Stein, 2010). It appears that the reason for this observation is that DDGS is composed primarily of insoluble dietary fiber (Urriola et al., 2010) from which noncellulose polysaccharides (5.8%) and cellulose (5.8%) are the greatest components (Jaworski, 2012). Insoluble dietary fiber (e.g., Solka floc) has less impact than soluble dietary fiber (e.g., pectin) on SID of AA (Zhu et al., 2005; de Lange, 2008).

The concentration of ADICP is commonly used to measure the extent of heat damage in forages and other plant materials in diets for ruminants (Van Soest and Mason, 1991). However, in the current study, there was also no association between the concentrations of ADICP and SID CP, Lys, and Thr among DDGS sources, suggesting that although ADICP may be a useful indicator of severe heat damage in forages, it is not a useful predictor of heat damage in DDGS.

The concentration of CP soluble in potassium hydroxide (KOH) is considered to be a measure of heat damage in soybean meal (Parsons et al., 1991), but there was no correlation between the concentration of SolCP and SID Lys, Met, Thr, and Trp observed among DDGS sources in the current study. The apparent reason for this difference is that the SID Lys concentration is not proportional to the concentration of SID CP among DDGS sources. Therefore, procedures that measure in vitro digestibility of CP have several sources of error: error of the in vitro procedure in predicting SID CP and error of SID CP in predicting SID Lys (Kim et al., 2012).

Grinding feed ingredients and complete diets to a smaller particle size increases the surface area to allow greater access of digestive enzymes to substrates resulting in increased nutrient digestibility (Wondra et al., 1995). Reducing particle size of DDGS increases DE and ME content (Mendoza et al., 2010; Liu et al., 2012), and reducing the particle size of DDGS from 517 to 383 μm also increases SID of Lys from 64.3 to 70.5% (Yáñez et al., 2011). Therefore, it was expected that sources of DDGS with smaller particle size may have greater SID AA concentrations. There was a wide range (605 to 1,356 μm) in mean values of particle size among sources of DDGS used in the current study. This range in mean values of particle size is similar to results reported in previous surveys (Rausch et al., 2005). However, there was no association between the particle size of DDGS and the concentrations of SID, CP, Lys, Met, Thr, Trp observed in the current experiment. This lack of an association is likely due to other confounding factors among the DDGS sources that have greater contributions to the variability of SID AA content, but within a single source of DDGS, the effect of particle appears to increase SID of AA (Mendoza et al., 2010; Yáñez et al., 2011).

Prediction of Standardized Ileal Digestible CP and AA from Color Characteristics

Color of DDGS sources measured with the Minolta colorimeter resulted in decreased ($P < 0.01$) for L* (52.7) and a* (10.1) than color recorded with the HunterLab spectrometer (L* = 55.6, a* = 11.3). However, these differences were small from a practical standpoint, because both methods and visual appearance provided a similar color ranking of the DDGS sources. There was no difference between b* values measured with Minolta or HunterLab instruments. The range in color among DDGS samples appears to vary among experiments and sampling methodologies. The lowest values for L*, b*, and a* for samples evaluated in the current study were greater than the lowest values reported in previous experiments (Cromwell et al., 1993; Fastinger and Mahan, 2006). This observation may reflect changes in DDGS production technologies implemented in various ethanol plants over time. Drying temperatures vary among ethanol plants and impact of drying techniques result in changes in color of DDGS and concentration of Lys, where microwave drying has less impact on Lys concentration than forced air drying (Mosqueda and Tabil, 2011).

The concentration of SID Lys was positively correlated ($r = 0.33$; $P < 0.05$) and SID Met tended to be correlated ($r = 0.30$; $P < 0.10$) with L*. However, the

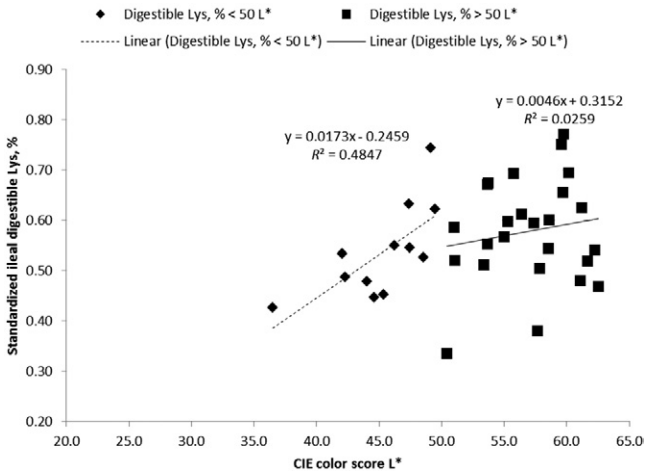

Figure 1. Relationship between Commission Internationale d'Eclairage (CIE) lightness of color (L*) among 34 sources of corn distillers dried grains with solubles and the concentration of standardized ileal digestible Lys.

association between SID Lys and L* was greater (R^2 = 0.48) when L* was less than 50 than when L* values were greater than 50 (R^2 = 0.03; Fig. 1). This observation can be explained by the complex nature of Maillard reactions. During the early to intermediate stages of heating and production of Maillard products, Amadori compounds are formed, which exhibit little or no change in color but have reduced Lys digestibility compared with unheated Lys (van Barneveld et al., 1994; Rérat et al., 2002). As heating progresses or temperature increases, Amadori compounds are converted to melanoidins, which have a dark color, and Lys digestibility is further reduced (Ferrer et al., 2005; Finot, 2005).

Values for a* indicate the color position between red and green (negative values indicate green and positive values indicate red; HunterLab, 2013). Negative correlations ($P < 0.05$) were observed between a* and SID CP (r = –0.41), Lys (r = –0.59), and Met (r = –0.50) but not Thr or Trp. Correlations between a* and SID AA have not been observed in other studies (Fastinger and Mahan, 2006). However, the relatively large number of DDGS samples (n = 34) and the wider range in a* values among samples in the current study may have resulted in these stronger associations than the relatively small number and narrow range in a* values analyzed in previous studies.

Values for b* indicate the color position between yellow and blue (negative values indicate blue and positive values indicate yellow; HunterLab, 2013). In the current study, b* was positively correlated ($P < 0.05$) with SID Met and Thr and tended to be correlated ($P < 0.10$) with SID Lys and Trp. Previous studies have shown similar associations between L* and b* and SID Lys (Cromwell et al., 1993; Fastinger and Mahan, 2006), but these associations were much stronger than observed in the current study. These results indicate that the accuracy of using color measurements to predict SID AA in DDGS is poor but is improved when evaluating heat damaged DDGS sources with L* values less than 50.

Prediction of Standardized Ileal Digestible AA from Optical Density

The HunterLab spectrophotometer provided optical density data each 10 nm over a wavelength range of 400 to 700 nm (Fig. 2). The correlation between SID Lys concentration and each point of the optical density spectra was greatest at 420 nm and then had a lag between 460 and 480 nm before it increased again but was lower than at 420 nm. However, the intensity of optical density at 420 nm was not great enough to predict the concentration of SID Lys. Therefore, the entire wavelength range was used along with CP concentration in the prediction models. The concentration of SID Lys in the DDGS sources was predicted (R^2 = 0.97; Fig. 3) with a relatively small predicted residual sum of squares (PRESS = 0.05). Optical density also accurately predicted the concentration of SID Thr (R^2 = 0.94; PRESS = 0.06) and Trp (R^2 = 0.93; PRESS = 0.007), but the concentration of SID Met was poorly predicted (R^2 = 0.39; PRESS = 0.12; Table 2). Therefore, optical density is a better predictor of SID Lys, Thr, and Trp than using the CIE color parameters. These results are in agreement with those reported by other researchers where color changes in heated liquid skim milk due to Maillard reactions were better explained by optical density at 420 nm than when

Table 2. Comparison of statistical models used to predict the concentration of standardized ileal digestible Lys, Met, Thr, and Trp among sources of corn dried distillers grains with solubles by optical density[1] and front face fluorescence[2]

Item	Pred. Mean[3]	R^2	Adj.[4] R^2	RSSE[5]	PRESS[6]
Optical density					
Lys	0.56	0.97	0.95	0.006	0.05
Met	0.51	0.39	0.31	0.09	0.12
Thr	0.80	0.94	0.88	0.01	0.06
Trp	0.16	0.93	0.85	0.001	0.007
Front face fluorescence					
Lys	0.56	0.99	0.96	0.004	0.07
Met	0.51	0.95	0.88	0.007	0.05
Thr	0.80	0.99	0.99	<0.001	0.008
Trp	0.16	0.99	0.94	<0.001	0.006

[1]Optical density from 400 to 700 nm. Model includes the concentration of CP for each source of distillers dried grains with solubles.

[2]Front face fluorescence excitation 360 nm and emission spectra from 380 to 600 nm.

[3]Predicted mean.

[4]Adjusted R^2.

[5]RSSE = residual sum of squares error.

[6]PRESS = predicted residual error sum of squares.

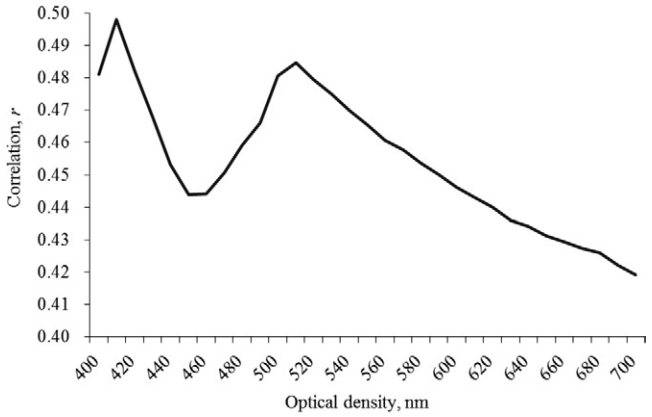

Figure 2. Correlations between standardized ileal digestible Lys among sources of corn distillers dried grains with solubles and each wavelength of the optical density spectra.

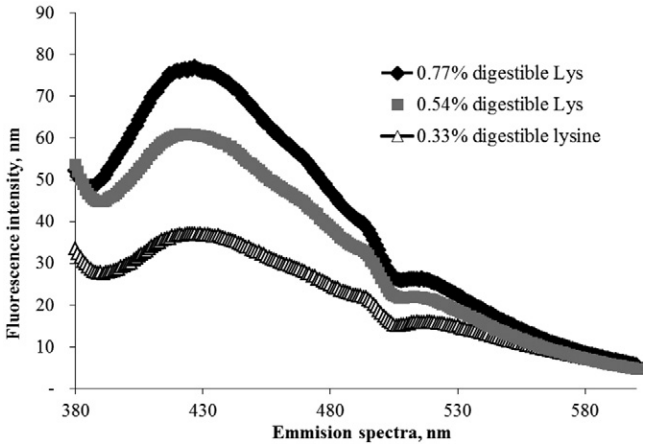

Figure 4. Relationship between front face fluorescence spectra (μ) and the concentration of standardized ileal digestible Lys among sources of corn distillers dried grains with solubles.

color was expressed as L*, b* and a* (Ferrer et al., 2005; Schamberger and Labuza, 2006).

Prediction of Standardized Ileal Digestible AA from Front Face Fluorescence

Front face fluorescence spectra were used to predict SID AA concentrations without any other physical or chemical characteristics. The DDGS source with the greatest SID Lys concentration showed greater fluorescence values and a greater peak of fluorescence intensity than other DDGS sources with lower SID Lys content (Fig. 4). This relationship was especially apparent between 380 and 480 nm. The fluorescence spectra predicted the order of the samples with the least and greatest concentration of SID Lys, but as more samples were recorded, their spectral curves overlapped and it was not possible to select a specific point in the spectra range (380 to 600 nm) that allowed simple regression to be performed. Therefore, the entire spectra (220 predictors) were used in the regression model. Principal components analysis was performed because there was a high correlation between fluorescence intensity at a specific spectra point and its adjacent points (Fig. 4). The concentration of SID Lys in DDGS was predicted (R^2 = 0.99; PRESS = 0.07) by the model with the principal components analysis (Fig. 5). Likewise, the concentration of SID Met (R^2 = 0.95; PRESS = 0.05), Thr (R^2 = 0.99; PRESS = 0.008), and Trp (R^2 = 0.99; PRESS = 0.006) were also predicted by the models.

Maillard reactions have been shown to produce intermediate substances that have fluorescent properties and are precursors of color substances (Ferrer et al., 2005; Matiacevich et al., 2005). Therefore, fluorescent substances that accumulate in heated food ingredients can be used to accurately measure heat damage when exposed to low to mild heat treatment (Birlouez-Aragon et al., 1998, 2001, 2002; Ferrer et al., 2005). Front face fluoresce is a method that measures fluorescence in opaque or solid samples, which classical fluorescence procedures are not able to do, and it has been used to detect heat damage in milk and milk products during processing (Kulmyrzaev and Dufour, 2002; Birlouez-Aragón et al., 2005; Schamberger and Labuza, 2006).

Finally, error associated with the measurement of the nutrient itself will be additive to the error of prediction, thereby reducing the accuracy of the prediction. The

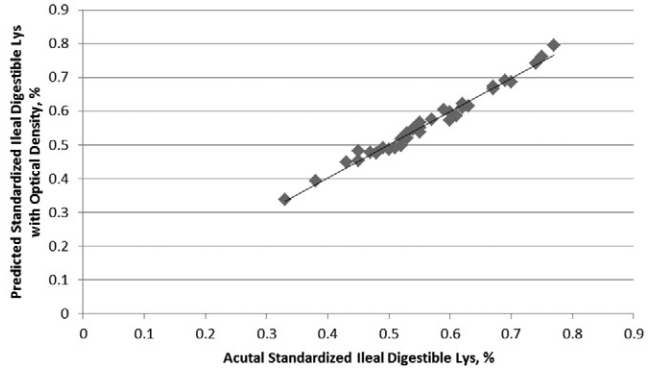

Figure 3. Prediction of the concentration of standardized ileal digestible Lys in 34 sources of corn distillers dried grains with solubles from optical density and CP content using principal components analysis.

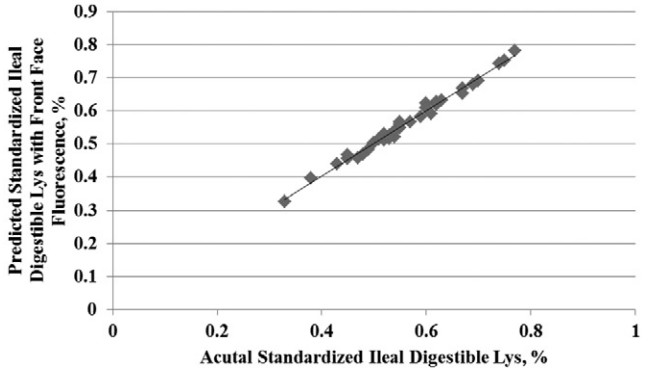

Figure 5. Prediction of the concentration of standardized ileal digestible Lys in 34 sources of corn distillers dried grains with solubles using front face fluorescence and principal components analysis.

greatest analytical errors are observed in the measurement of NDF, ADF, and ADICP. There is also greater repeatability of measurement of optical density than front face fluorescence, and these issues need to be addressed to gain a useful model.

In conclusion, the use of ADICP, SolCP, NDF, ADF, hemicellulose, CP, particle size, and color measurements (L*, a*, and b*) poorly predicts the concentration of SID Lys, Met, Thr, and Trp in DDGS. Optical density data, along with CP concentration, accurately predicted SID Lys, Thr, and Trp concentration among DDGS sources, but it appears that the use of front face fluorescence provides greater precision for predicting SID Lys, Met, Thr, and Trp concentration among DDGS sources. However, use of samples and data outside of the current data set is necessary to validate the optical density and front face fluorescence methods.

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