METHODS TO DETERMINE AMINO ACID DIGESTIBILITY IN CORN BY-PRODUCTS

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l. INTRODUCTION

While corn contains only limited quantities of crude protein and amino acids, corn by-products often contain crude protein and amino acids in appreciable amounts. The reason for this difference is that corn by-products are often produced after processing that primarily removes the nitrogen-free extracts (i.e., the starch) from the corn kernel. Because starch is approximately 70% of the total corn kernel, the quantity of crude protein and other nutrients is concentrated in the product that is left over after processing (Table 1). Corn by-products, therefore, are valuable sources of crude protein and amino acids in diets for monogastric animals. As is the case with most other by-products, variations in the concentrations and the digestibility of the amino acids in corn by-products exist. The main reason for these variations is differences in processing technology and procedures. Currently, the most used corn by-product in the feeding of monogastric animals is distillers dried grain with solubles (DDGS). This product is produced by the ethanol industry and contains mainly the crude protein, fat, fiber and minerals from the corn kernel whereas the majority of the starch has been removed in the fermentation process. In general, the concentrations of crude protein, amino acids, fiber and minerals in DDGS are close to three times that than in corn. Thus, the crude protein concentration in DDGS is usually 26 – 28% and the concentration of amino acids is also approximately three times higher than in corn (Table 1). Because of the relatively high concentration of crude protein and amino acids, DDGS contributes significantly to the dietary concentration of amino acids if included in diets for pigs and poultry. It is, therefore, necessary to estimate the digestibility of these amino acids.

It is the objective of the current contribution to summarize existing knowledge about the methods that are available to measure amino acid digestibility in corn by-products. Because of the importance of DDGS in the feeding of pigs and poultry in the US, the summary will be based on experiences with DDGS. However, the concepts discussed may also be applied to other corn by-products.

2. AMINO ACID AVAILABILITY

Only amino acids that can be incorporated into tissue proteins are bioavailable. Bioavailability is defined as the proportion of dietary amino acids that are absorbed in a chemical form that is suitable for protein synthesis (Batterham, 1992; Lewis, 1992). Bioavailability of AA may be measured using slope ratio techniques in which the response of an animal to increased intake of an amino acid is measured (Batterham, 1992). The diets used for this procedure needs to be deficient in only the amino acid in question and all levels of this amino acid needs to be fed below the requirement of the animal. However, the slope ratio procedure is tedious and costly and the determined values for amino acid availability are unique only to the experimental

procedures used and may not be additive in a mixed diet (Gabert et al., 2001). Therefore, for practical feed formulation, amino acid availability values are not used. In stead, the digestibility coefficients for amino acids are measured and used as an indication of the quantities of dietary amino acids that are available to the animal (Stein and Nyachoti, 2003). The term "amino acid digestibility" does not refer to the digestion of amino acids – it only refers to the digestion of the peptide bonds connecting the amino acids in a dietary protein (Fuller, 2003).

Table 1. Concentration of energy and nutrients in corn and corn by-products[a].

Item Product:	Corn	DDGS	Corn gluten feed	Corn gluten meal
Energy, ME kcal/kg	3,420	2,820	2,605	3,820
Crude protein, %	8.3	27.7	21.5	60.2
Crude fat, %	3.9	8.4	3.0	2.9
NDF, %	9.6	34.6	33.3	8.7
ADF, %	2.8	16.3	10.7	4.6
Calcium, %	0.03	0.20	0.22	0.05
Phosphorus, %	0.28	0.77	0.83	0.44
Amino acids, %				
Arginine	0.37	1.13	1.04	1.93
Histidine	0.23	0.69	0.67	1.28
Isoleucine	0.28	1.03	0.66	2.48
Leucine	0.99	2.57	1.96	10.19
Lysine	0.26	0.62	0.63	1.02
Methionine	0.17	0.50	0.35	1.43
Cysteine	0.19	0.52	0.46	1.09
Phenylalanine	0.39	1.34	0.76	3.84
Tyrosine	0.25	0.83	0.58	3.25
Threonine	0.29	0.94	0.74	2.08
Tryptophan	0.06	0.25	0.07	0.31
Valine	0.39	1.30	1.01	2.79

[a] Values from NRC (1998).

3. AMINO ACID DIGESTIBILITY – GENERAL PRINCIPLES

Because the amino acids of undigested dietary proteins entering the large intestine may be metabolized by hind-gut microbes before they are excreted in the fecal material from the animal, values for total tract digestibility of amino acids are not accurately predicting amino acid absorption by the animal. To avoid the manipulation by hind-gut microbes, the digestibility of amino acids by monogastric animals is most correctly measured at the end of the small intestine and is referred to as ileal digestibility values (Sauer and de Lange, 1992). This creates a need for using techniques that enable researchers to capture ileal fluids at the end of the small intestine. Several techniques have been proposed for this and have been discussed previously (Moughan, 2003b; Stein, 2003). In North America, the installment of a T-cannula in the distal ileum of pigs (10 to 15 cm prior to the ileo-cecal valve) appears to be the procedure of choice. This procedure has proven to be accurate and has a minimal trial to trial variation. Because the T-cannula, like

most other procedures that are used for ileal fluid collections, does not allow for the total collection of the ileal output from the animal, an indigestible marker needs to be included to calculate changes in amino acid concentrations. Chromic oxide is often used for this purpose, but other markers exist. Ileal digestibility values are calculated using Eq. 1.

$$AID = (1 - [(Aad / AAf) \times (Mf / Md)] \times 100\% \quad [\mathbf{1}]$$

where AID is the apparent ileal digestibility of an amino acid, AAd is the amino acid concentration in the ileal digesta DM (g / kg DM), AAf is the amino acid concentration in the feed DM (g / kg DM), Mf is the marker concentration in the feed DM (g / kg DM), and Md is the marker concentration in the ileal digesta DM (g / kg DM).

Amino acid digestibility values calculated using this procedure are called "apparent ileal digestibility values" to reflect the fact that this value is calculated simply by subtracting the ileal amino acid output from the intake of amino acids. The ileal digesta contain not only un-absorbed dietary amino acids, but also amino acids of endogenous origin; i.e., amino acids that were absorbed from the intestines and then re-secreted into the intestinal tract in the form of endogenous proteins such as mucin, sloughed cells, enzymes etc. The value for AID represents, therefore, only the apparent digestibility of the dietary proteins.

The endogenous amino acids that are secreted into the intestinal tract may be divided into basal endogenous secretions and diet-specific endogenous secretions (Jansman et al., 2002). The basal endogenous amino acids consist of amino acids that are secreted into the GI-tract of fasted animals in addition to amino acids that are secreted in response to the DM intake of animals. These losses are usually measured as g per kg DM intake. Recent evidence suggests that the value for endogenous losses (in g per kg DM intake) depends on the DM intake of the animal and declines as DM intake increases because of the relatively lower influence pr kg DM of the fasting endogenous loss (Moter and Stein, 2004). As a consequence, only values for endogenous losses that are measured in animals given free access to feed are applicable to commercial conditions.

In addition to the basal endogenous loss, most feed ingredients also introduce a diet-specific endogenous loss which is mainly caused by fibers and anti-nutritional factors in the ingredient. The diet-specific losses may vary from almost zero in purified ingredients such as casein and lactose to values that exceed the basal endogenous losses in ingredients that are high in fiber and anti-nutritional factors such as canola meal and wheat middlings. Because DDGS contains relatively high quantities of fiber, it is expected that this ingredient will elicit relatively large diet-dependent endogenous losses. Comprehensive reviews of endogenous losses and factors influencing endogenous losses have been published (Taminga et al., 1995, Boisen and Moughan, 1996; Nyachoti et al., 1997). The variation in the total quantities of endogenous proteins that have been reported in the literature also has been published (Jansman, 2002).

Techniques to measure basal endogenous losses include feeding a protein-free diet, the peptide alimentation procedure, and the regression procedure (Stein, 2003). In North America, the protein-free diet is most commonly used. Using this approach, the basal endogenous losses are

calculated from ileal digesta collected from animals that are fed the protein-free diet according to Eq. 2:

$$IAA_{end} = [AAd \times (Mf/Md)] \qquad [\mathbf{2}]$$

where IAA_{end} is the basal endogenous loss of an amino acid at the distal ileum (mg per kg DM intake), AAd is the concentration of that amino acid in the digesta DM, Mf is the marker concentration in the feed DM, and Md is the marker concentration in the ileal digesta DM.

There are no techniques available to measure the diet-specific endogenous losses, but total endogenous losses (basal and diet-specific) may be estimated using the homoarginine technique or the N^{15} isotope dilution technique. Both of these procedures have several limitations and further work to refine these techniques is required before they can be routinely employed in animal nutrition studies (Stein, 2003).

The introduction of endogenous amino acids into the GI-tract of pigs introduces several challenges in accurately estimating amino acid absorption because these secretions are included in the calculations of apparent ileal digestibility values according to Eq. 1. The endogenous amino acids comprise a relatively larger portion of the total amino acid output from pigs fed low-protein feed ingredients (i.e., cereal grains) as compared to pigs fed ingredients with a higher protein concentration such as soybean meal. Therefore, the values for AID that are measured for low-protein feed ingredients are usually under-estimated (Donkoh et al., 1994; Fan et al., 1994). Because of this underestimation, values for AID that are measured in individual feed ingredients usually do not add up in a mixed diet that contains both low-protein feed ingredients and high-protein ingredients (Stein et al., 2005c). However, if the values for ileal digestibility are corrected for the basal endogenous losses of amino acids, then this underestimation may be avoided. By doing that, the standardized ileal digestibility coefficients are calculated according to Eq. 3:

$$SID = [AID + (IAA_{end} / AAf)] \qquad \mathbf{[3]}$$

where SID is the standardized ileal digestibility of an amino acid (%), AID and IAA_{end} are calculated according to Eq. [1] and Eq. [2], respectively, and AAF is the amino acid concentration in the feed DM (g / kg DM). It was recently demonstrated that values for standardized ileal digestibility are additive in mixed diets even if low protein feed ingredients are incorporated in the diets (Stein et al., 2005c). Thus, by using standardized ileal digestibility values, the problems associated with using apparent ileal digestibility values are eliminated. The feed industry is, therefore, moving towards using values for standardized ileal digestibility of amino acids rather than apparent ileal digestibility values. Comprehensive discussions of the principles behind calculations of standardized ileal digestibility values are available (Mosenthin et al., 2000; Jansman et al., 2002).

4. IN VIVO MEASUREMENT OF AMINO ACID DIGESTIBILITY IN CORN BY-PRODUCTS

The amino acid digestibility in corn by-products is usually measured following the procedures outlined above. For DDGS, values for both apparent and standardized ileal digestibility of amino acids have been determined in 14 samples originating from 13 different ethanol plants in Minnesota and South Dakota (Stein et al., 2005a and b). The diets used to measure the apparent ileal digestibility values in these experiments consisted of 67% DDGS, 27% cornstarch, 1% soybean oil, 3% sugar, and vitamins and minerals. The basal endogenous losses were determined using a protein-free diet and the standardized ileal digestibility values were calculated according to Eq. 3. The results of the experiments showed that some variations exist for the amino acid digestibility among different samples of DDGS (Table 2). This is true in particular for lysine that is more variable than all other amino acids in terms of digestibility. The reason for this variation is believed to be that lysine may have been heat-damaged in some of the samples of DDGS which in turn has lowered the calculated digestibility of lysine in these samples. Further work is needed to identify the reasons for this heat damage and to establish procedures for the production that allow ethanol plants to dry the products without heat damaging it. Nonetheless, the amino acids in DDGS have a medium digestibility and, except for lysine, the variability among different samples is within the normal range of variation found in other feed ingredients. Values for apparent and standardized ileal digestibility in five sources of DDGS originating from other parts of the US also have been published (Fastinger and Mahan, 2005). These values confirmed that lysine is the most variable amino acid in DDGS in terms of digestibility.

The digestibility of amino acids in other corn by-products has been less extensively researched than in DDGS, but values for apparent and standardized ileal digestibility of amino acids in corn gluten meal and corn gluten feed have been published (NRC, 1998). The amino acid digestibility in corn gluten feed may be determined as outlined for DDGS and with a similar type of diet. Because of the higher protein concentration in corn gluten meal, only 30 to 35% of corn gluten meal would be needed in the diet used to determine the amino acid digestibility in order to formulate a test diet containing 18 to 20% crude protein. Amino acid digestibility values in other corn by-products may be measured using a similar approach and with similar types of diets.

5. IN VITRO PROCEDURES TO ESTIMATE AMINO ACID DIGESTIBILITY IN CORN BY-PRODUCTS

Because in vivo procedures are expensive and time consuming to conduct, current work at South Dakota State University is focusing on identifying in vitro procedures that are fast and inexpensive and able to predict the digestibility of amino acids in DDGS. A total of six different in vitro procedures have been tested at this point. For each procedure, values obtained using the in vitro procedure have been correlated with the standardized ileal digestibility values that were obtained in vivo for the 14 samples that have been measured (Table 2).

The six in vitro procedures include two enzymatic procedures (i.e., a one-step pepsin procedure, and a two-step pepsin-pancreatin procedure), a colorimetric procedure, and three chemical procedures (i.e., KOH solubility, the furosine procedure, and the reactive lysine procedure).

Table 2. Standardized ileal digestibility (%) of amino acids in 14 samples of DDGS by growing pigs[a].

	Digestibility:	Average	Standard deviation	Lowest value	Highest value
Item	**Crude protein:**	**70.5**	**3.96**	**63.5**	**77.6**
Indispensable amino acids					
Arginine		78.6	3.80	74.1	89.9
Histidine		75.2	4.64	70.0	88.9
Isoleucine		72.7	4.36	67.4	84.2
Leucine		81.9	4.16	75.5	91.3
Lysine		59.0	7.19	43.9	78.4
Methionine		80.3	3.98	73.9	89.2
Phenylalanine		79.0	3.78	73.5	89.4
Threonine		69.9	5.96	62.2	87.2
Valine		71.7	4.30	67.3	87.2
Dispensable amino acids					
Alanine		75.7	4.27	69.7	87.6
Aspartic acid		66.9	4.66	59.4	80.2
Cysteine		72.4	4.46	66.0	85.8
Glutamic acid		76.7	6.11	67.4	91.4
Glycine		57.2	6.36	46.8	76.7
Proline		66.9	9.40	57.8	88.8
Serine		72.1	5.10	59.6	80.9
Tyrosine		80.9	3.74	74.6	89.9

[a] Results reported as the average of 14 samples, the standard deviation, and the range.

5.1. One-Step Pepsin Procedure

The one-step pepsin procedure involves the incubation of a DDGS sample at 37^{o}C in pepsin for 8 or 18 hours at pH 2.5 (procedure 971.09, AOAC, 2000). The sample is filtrated after incubation and the undigestible portion of the protein is believed to be present in the filtrate. By analyzing this portion and relating it to the total protein content in the sample, the disappearance of protein is calculated and assumed to be equal to the digestibility of the protein. Results obtained using this procedure are presented in Figure 1. The correlation between the in vitro digestibility of protein and the value for standardized ileal digestibility obtained in vivo is only 0.52. It is assumed that the digestibility of protein is well correlated to the digestibility of all amino acid which for a protein that may have been heat damaged is somewhat questionable. Based on the results that have been obtained this far, it is questionable if this procedure is accurate enough to predict amino acid digestibility in DDGS.

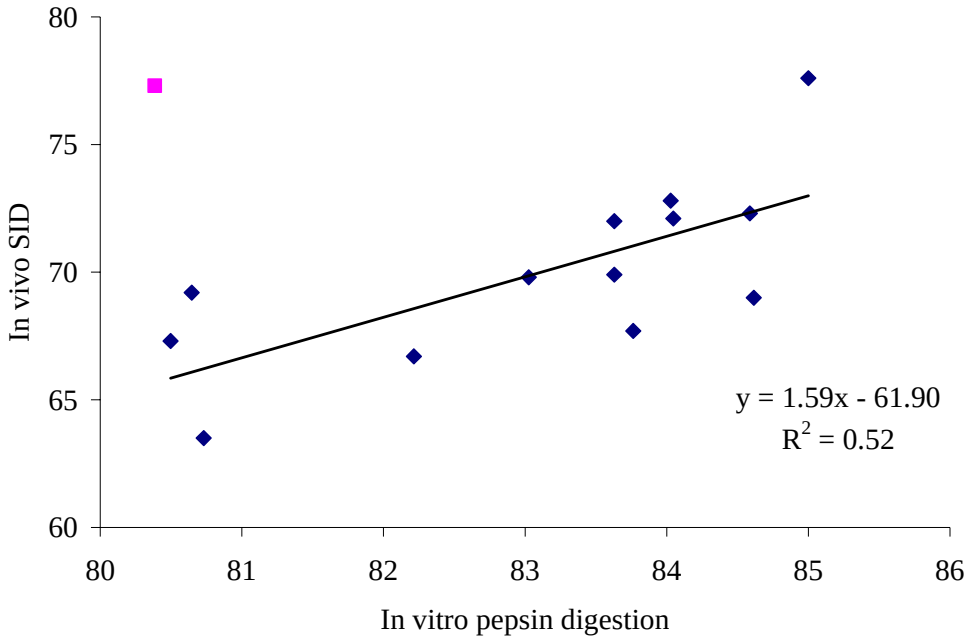

Figure 1. Correlation between the standardized ileal digestibility values obtained in vivo and by the one-step pepsin procedure in 14 samples of DDGS.

5.2. Two-Step Pepsin-Pancreatin Procedure

The two-step pepsin-pancreatin procedure involves sample incubation at 39°C at pH 2 with pepsin followed by the incubation with pancreatin (a mixture of pancreatic proteolytic enzymes) at pH 6.8. Following the incubation, all samples are filtrated and washed in ethanol and acetone and dehydrated. The filtrate is then analyzed for the concentration of crude protein. The procedure was initially developed for predicting amino acid digestibility in mixed diets (Boisen and Fernandez, 1995). Alternatives to this procedure including longer incubation times were later introduced to predict amino acid digestibility in meat and bone meal (Qiao et al., 2004). Based on a series of experiments in our laboratory, it was concluded that to obtain a reasonably high correlation between data obtained using this procedure and standardized ileal digestibility values measured in vivo, it is necessary to incubate the samples with pepsin for 24 hours followed by a 96-hour incubation with pancreatin. An R^2 value of 0.79 has been obtained using this procedure (Figure 2). As was the case with the one-step pepsin procedure, this procedure only estimates the protein digestibility which is assumed to be well correlated to the amino acid digestibility. However, current work at South Dakota State University is focusing on analyzing the filtrate for individual amino acids to investigate the possibility for predicting individual amino acid digestibility with the two-step pepsin-pancreatin procedure.

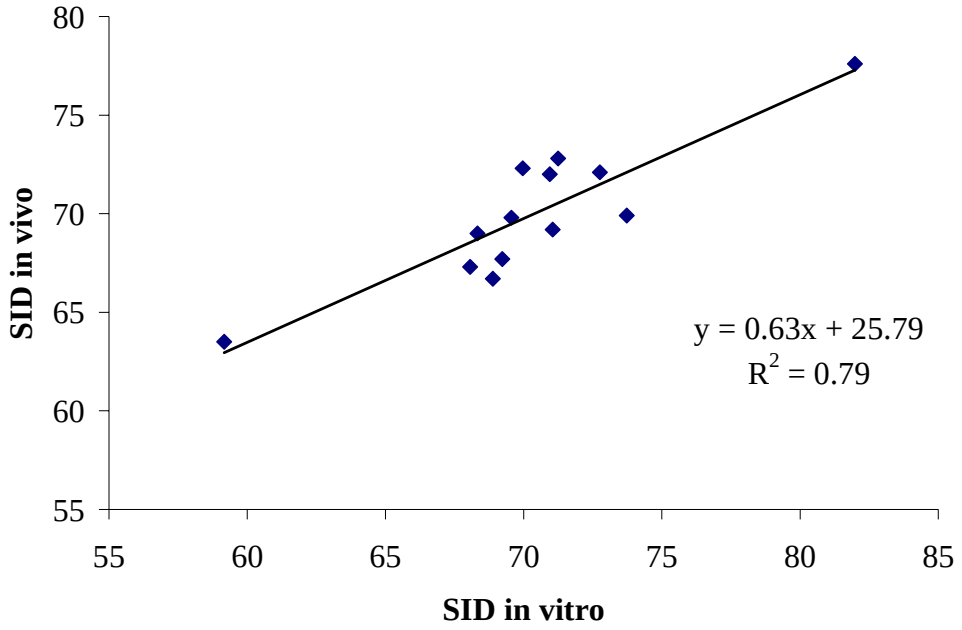

Figure 2. Correlation between the standardized ileal digestibility values obtained in vivo and by the two-step pepsin-pancreatin procedure in 14 samples of DDGS.

5.3. Colorimetric Procedures

Colorimetric assays may be used to predict amino acid digestibility in DDGS because it is believed that processing that is detrimental to amino acid digestibility may result in darker colors of the product. This procedure has previously been correlated to amino acid digestibility in DDGS by chickens (Cromwell et al., 1993). Work at South Dakota State University was completed using two different instruments to measure color in the samples (i.e., Hunterlab and Minolta). It was concluded from the initial work that the Minolta instrument gave a better correlation to in vivo digestibility than did the Hunterlab. Further experiments involved standardizing the distance from the instrument to the sample using a glass plate or measuring the color without the glass plate. Samples were either used as-is or were finely ground prior to the measurements. It was concluded from these experiments that the best correlation between color scores and in vivo amino acid digestibility is obtained if no glass plate is used. However, there is no difference between the correlations obtained in DDGS samples as-is and the finely ground samples (Table 3). The correlations between the color scores and the in vivo amino acid digestibility for the majority of the amino acids have been between 0.60 and 0.75 indicating that although there appears to be some correlation between color and amino acid digestibility this correlation is not particularly strong. Surprisingly, the amino acid with the lowest correlation is lysine. The biggest advantage of the colorimetric procedure is that it is very fast, easy to perform, and inexpensive. Future work at South Dakota State University will focus on refining the procedure to possibly find better correlations.

Table 3. Correlation (%) between standardized ileal digestibility in DDGS by growing pigs and the color in DDGS measured by Minolta in raw and ground samples.

Item **Sample:**	**DDGS, as-is**		**DDGS, ground**	
Glass plate	**-**	**+**	**-**	**+**
Crude protein	0.67	0.60	0.54	0.53
Indispensable amino acids				
Arginine	0.70	0.65	0.76	0.63
Histidine	0.67	0.62	0.76	0.67
Isoleucine	0.60	0.52	0.53	0.49
Leucine	0.76	0.69	0.77	0.71
Lysine	0.49	0.45	0.60	0.53
Methionine	0.71	0.69	0.68	0.55
Phenylalanine	0.70	0.64	0.66	0.59
Threonine	0.65	0.63	0.79	0.61
Valine	0.62	0.55	0.57	0.53
Dispensable amino acids				
Alanine	0.73	0.66	0.73	0.65
Aspartic acid	0.66	0.63	0.44	0.45
Cysteine	0.73	0.71	0.78	0.68
Glutamic acid	0.70	0.66	0.86	0.71
Glycine	0.53	0.46	0.42	0.42
Proline	0.55	0.45	0.51	0.40
Serine	0.52	0.47	0.24	0.30
Tyrosine	0.74	0.73	0.71	0.61

5.4. KOH-Solubility

It has been suggested that the KOH-solubility of a feed stuff is well correlated to the digestibility of protein (Araba and Dale, 1990). The procedure involves the incubation of a sample with a 0.2% KOH solution for 20 min at room temperature. Following this incubation, the sample is centrifuged and the supernatant is analyzed for the nitrogen concentration. The nitrogen that is left in the supernatant is believed to be indigestible to the animal; therefore, the digestible quantity of protein may be calculated as the difference between the protein in the sample and the protein in the supernatant. Results obtained for DDGS using this procedure have not been encouraging (Figure 3) and at this point, it is not recommended that the procedure is used to estimate amino acid digestibility in DDGS.

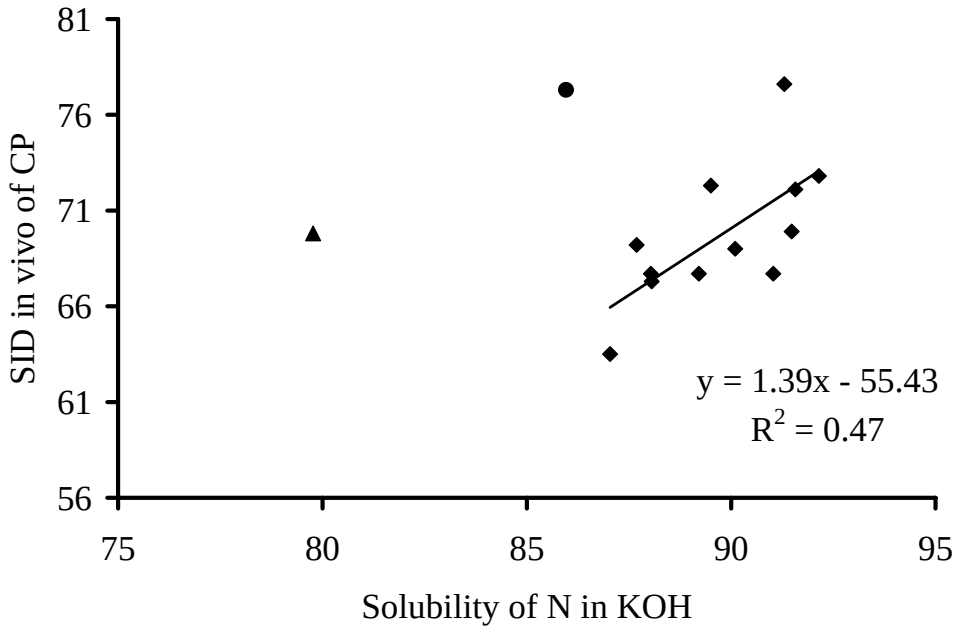

Figure 3. Correlation between the standardized ileal digestibility values obtained in vivo and by the KOH solubility procedure.

5.5. Furosine Concentration Procedure

Heat treatment is used extensively in the production of DDGS to dehydrate the products coming off the fermentation process. During heat treatment of proteins, the epsilon amino group of free lysine and protein-bound lysine may react with reducing sugars in the Maillard reaction (Maillard, 1912, 1916). This reaction generates both early and late Maillard products. The early Maillard products are structurally altered lysine derivatives that are called Amadori compounds, deoxy-ketosyl derivatives, or blocked lysine, while the late Maillard products are called melanoidins. The melanoidins will not be identified in the regular analysis for amino acids and will only result in lower lysine to crude protein ratios in the product. The lysine to crude protein ratio in corn is approximately 0.031. In non-heat damaged DDGS, the ratio is about the same, but in heat damaged DDGS, ratios of less than 0.025 have been detected. The calculation of the lysine to crude protein ratio, therefore, gives an indication of the extent of conversion of lysine to melanoidins, and therefore, of the degree of heat damage that has occurred. The melanoidins do not interfere with the normal analysis for lysine and have no influence on the digestibility values that are calculated – they only result in lower concentrations of lysine in the sample, and therefore, in lower quantities of lysine being absorbed.

The Amadori compounds (early Maillard products), on the other hand, interfere with the amino acid analysis and give inaccurate lysine concentrations in the sample being analyzed. These products are derived from the reaction of lysine with lactose, fructose, aspartate, or glutamate and the resulting derivatives are called lactulosyllysine, fructosyllysine, aspartyllysine and glutamyllysine, respectively (Friedman, 1996). The lysine that is bound in these compounds is called “blocked lysine” and is biologically unavailable because it is resistant to gastrointestinal enzymatic breakdown (Maga, 1981). However, during acid hydrolysis, 40 to 50% of the blocked

lysine is released as lysine (Hurrell and Carpenter, 1981) while the rest is released as furosine and pyridosine. The resulting ileal digestibility values will, therefore, overestimate the digestible lysine content in the sample, but will underestimate the digestibility coefficient of the un-blocked lysine (Moughan, 2003a). Because lysine, furosine and pyridosine are released from the blocked lysine in a constant ratio, the total amount of blocked lysine may be calculated by analyzing either the furosine or the pyridosine concentration in the sample. Work in our laboratory has demonstrated that furosine is easily analyzed using high performance liquid chromatography (HPLC). Subsequently, the concentration of blocked lysine has been calculated assuming that the furosine concentration equals 20% of total blocked lysine. The correlation between the calculated blocked lysine and the in vivo standardized digestibility of lysine in DDGS was calculated at 0.71 (Figure 4). Future work will focus on further developing this procedure which seems to hold some promise as an accurate method to estimate the digestibility of lysine in DDGS. The digestibility of amino acids other than lysine can, however, not be predicted using this procedure.

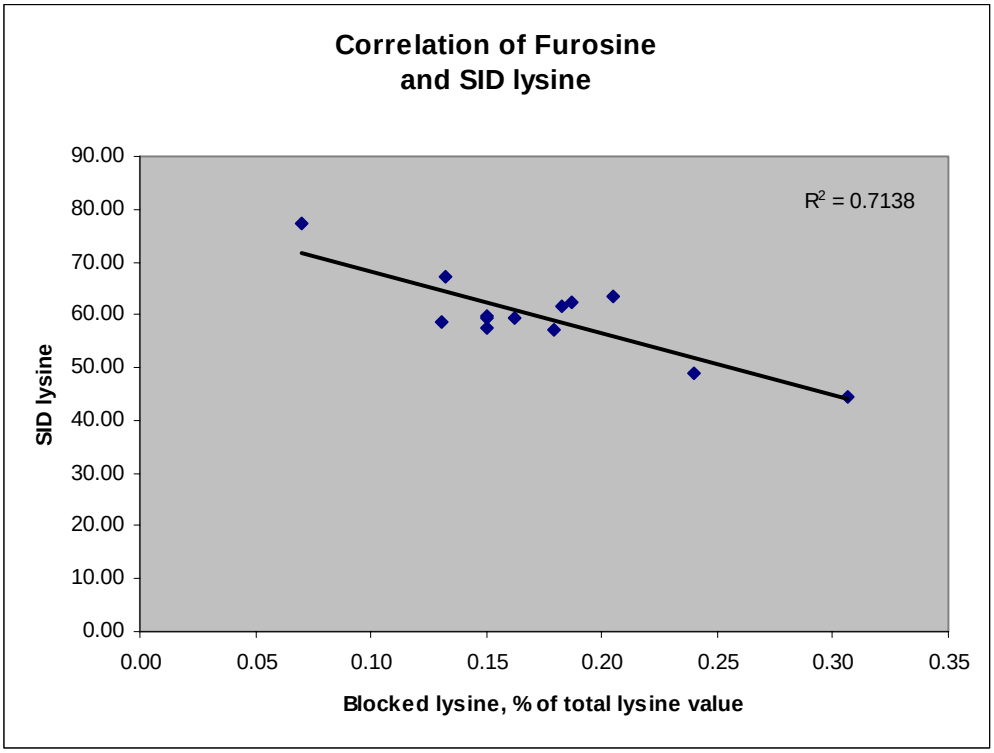

Figure 4. Correlation between blocked lysine (calculated from furosine) and standardized ileal digestibility of lysine in 14 samples of DDGS.

5.6. Reactive Lysine Procedure

While the furosine procedure can be used to estimate the quantity of blocked lysine in heat-damaged feed samples, the reactive lysine method estimates the amount of lysine that is not bound to any food component in a manner that hinders lysine digestibility or availability. This lysine is called the reactive lysine and is available for absorption and utilization by the animal. For feedstuffs that are not heated, the total lysine content in the sample is not contaminated with

Amadori compounds and all the analyzed lysine in the sample is expected to be available for absorption and utilization. In such a feed ingredient the reactive lysine would be expected to be equal to the total analyzed concentration of lysine. However, in heat damaged proteins, a portion of the analyzed lysine may consist of Amadori products and introduce errors in the calculation of digestible lysine as discussed above (Darragh and Hodgkinson, 2003). In the reactive lysine procedure, the quantity of lysine that is not bound in the Amadori compounds is quantified. Because this is the only portion of the analyzed lysine that is available for absorption, a quantification of this portion will be directly related to the digestible quantities of lysine from the animal (Moughan and Rutherfurd, 1996). There are at least three methods available to determine the reactive lysine content of a feed sample. All three methods involve the chemical conversion of the reactive lysine in the feed ingredient to a different chemical compound (i.e., dinitrophenyl lysine, trinitrophenyl lysine, or homoarginine). For samples that may contain starch, the conversion of reactive lysine to homoarginine is believed to be the most accurate. This procedure involves the reaction of the feed sample with O-methyl-isourea which will convert the reactive lysine (but not the blocked lysine) to homoarginine. By measuring the conversion of total lysine to homoarginine, the quantity of reactive lysine can be estimated. Because the quantity of reactive lysine is supposed to be directly related to the digestibility of lysine, the conversion of lysine to homoarginine is believed to be correlated to the digestibility of lysine. Preliminary work in our laboratory has resulted in a medium correlation to the standardized ileal digestibility of lysine in samples of DDGS (Figure 5). However, there are several methodological aspects of this procedure that still need to be researched and it is possible that the reactive lysine procedure may be refined and eventually yield better correlations to in vivo data.

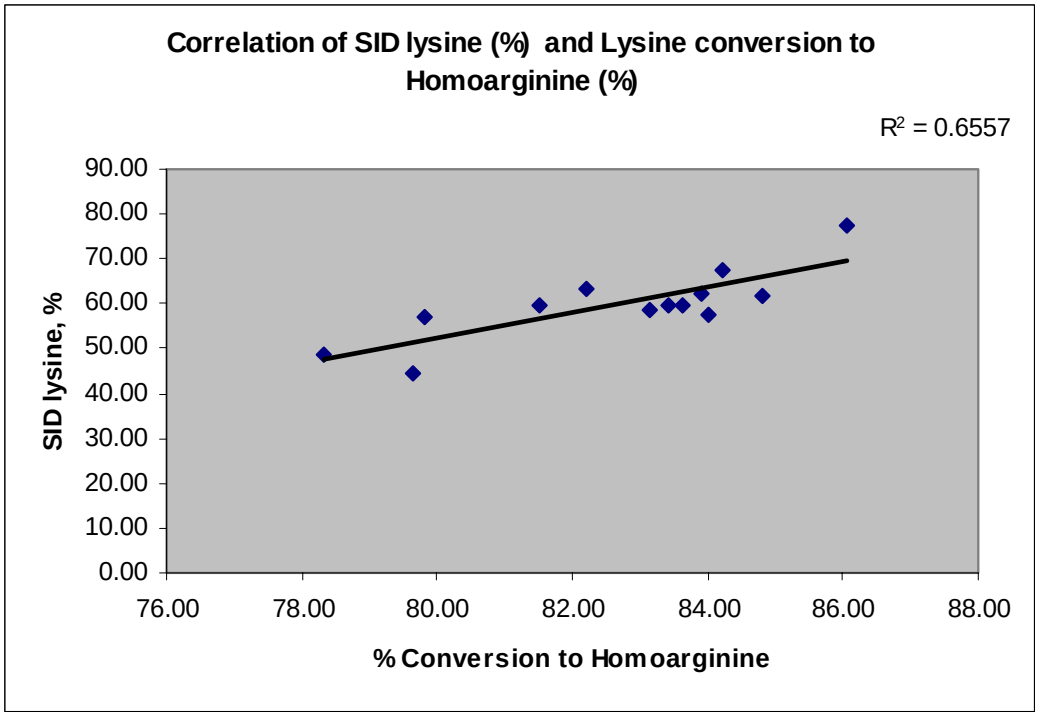

Figure 5. Correlation between the conversion of lysine to homoarginine and standardized ileal digestibility of lysine in 14 samples of DDGS.

Because the furosine procedure and the reactive lysine procedure are measuring the blocked lysine and the reactive lysine, respectively, the values obtained for these procedures theoretically will add up to the total analyzed lysine concentration in the feed ingredient. Based on the limited amount of work that has been conducted in our laboratory at this time it is concluded that for most samples of DDGS, this is also the case. There is, therefore, reason to believe that both of the two procedures may be used in the future to predict the digestibility of lysine in DDGS and other corn by-products that have been heat damaged.

6. CONCLUSIONS

The digestibility of amino acids in corn by-products may be measured in vivo in pigs based on principles for calculating the ileal digestibility. This approach involves a procedure that allows for the collection of ileal digesta at the distal end of the ileum. A T-cannula is most commonly used for this purpose, but other procedures exist and have been shown to be equally effective. Because of the contribution of endogenous amino acids to the ileal output, it is necessary to also estimate the quantities of endogenous amino acids reaching the distal ileum. This is most commonly accomplished by feeding the animals a protein-free diet. By subtracting the endogenous amino acids from the total ileal output, the standardized ileal digestibility of amino acids may be calculated. Values for standardized ileal digestibility have been shown to be additive in mixed diets fed to pigs. Therefore, the values for standardized ileal digestibility should be used in practical feed formulation.

Because of the variation in digestibility of amino acids that has been reported for different samples of DDGS, a need for a rapid and inexpensive in vitro procedure to predict amino acid digestibility has emerged. Several approaches have been researched to identify such a procedure. Based on work with 14 samples of DDGS is has been concluded that a two-step pepsin-pancreatin procedure may yield results that are reasonable well correlated to the in vivo digestibility of protein. However, questions related to how well the protein digestibility is related to the digestibility of individual amino acids still exist. In particular, there seems to be a relatively low correlation between protein and lysine digestibility in a heat treated feed ingredient such as DDGS. Therefore, it is not expected that the data obtained using the pepsin-pancreatin procedure are well correlated with the digestibility of lysine, but this question still remains to be answered. Future work will focus on using the pepsin-pancreatin procedure to predict the digestibility of all individual amino acids including lysine. A one-step pepsin digestibility procedure was shown to be less accurate than the pepsin-pancreatin procedure in predicting protein digestibility in DDGS. Likewise, a procedure based on measuring protein solubility following the incubation of DDGS with KOH seems to be somewhat ineffective in predicting protein digestibility in DDGS.

The furosine procedure and the reactive lysine procedure are similar in that both procedures may be used to calculate the quantity of lysine that is available for absorption in a feed sample. However, both procedures only predict the digestible contents of lysine. If the digestible contents of other amino acids are to be predicted, other procedures are needed. Preliminary work with these two procedures has given results that hold some promise for future research. If it turns out that both procedures are equally effective in predicting the quantities of digestible samples in

DDGS, then the furosine procedure may be the method of choice because it is less tedious and more inexpensive than the reactive lysine procedure.

To get an accurate prediction of the digestibility of all amino acids in a feed ingredient a combination of the pepsin-pancreatin procedure and the furosine procedure may be needed.

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