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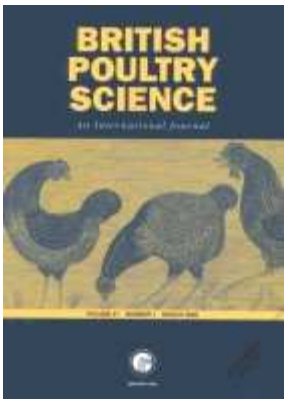

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Standardised amino acid digestibility of wheat distillers' dried grains with solubles in force-fed cockerels

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Abstract 1. In recent years, policies encouraging the production of ethanol from maize or wheat have stimulated an increased production of distillers' dried grains with solubles (DDGS) for which the nutritional value for poultry is poorly described, especially in the case of wheat DDGS.

2. DDGS samples (19) were obtained from 7 plants in Europe from June to September 2007. Each sample was analysed for chemical composition and 10 representative samples were measured for amino acid (AA) content and their standardised digestibility (SDD) in caecectomised cockerels. Lightness score (L) of each DDGS was also measured.

3. Results indicated a rather stable crude protein content (327 to 392 g/kg DM) but the AA profile was variable between samples. Lysine (LYS) was the most affected AA with contents ranging between 0.83 and 3.01 g/100g CP. In addition, only 0.76 of total LYS were free if estimated by the fluoro-dinitro-benzene procedure and 0.85 of total LYS were free if estimated by the furosine procedure.

4. The SDD of LYS was also highly variable (-0.04 to 0.71) with the lowest values observed for DDGS samples with a low LYS content in CP; these latter samples had also a high occurrence of Maillard reactions and low L values (< 50). Consequently, both LYS content in CP (r = 0.63) and SDD of LYS (r = 0.64) values were positively related with L.

5. Our data indicate that LYS SDD can be accurately predicted from LYS content in CP according to a quadratic (R^2 = 0.94) or a linear-plateau model (R^2= 0.90; breakpoint for 1.9 g/100g lysine in CP and a 63% plateau SDD value).

INTRODUCTION

The ethanol industry in Europe is increasing rapidly (+60% between 2007 and 2008; www.ebio.org). Ethanol is currently produced on an industrial scale *via* enzymatic breakdown of starch and yeast fermentation of glucose into ethanol. Mainly produced from wheat in Europe, wheat dried distillers' grains with solubles (DDGS) is the main by-product resulting from the dry milling and fermentation of grains which corresponds to the residual component of the grain kernel after the starch has been fermented by yeast to produce ethanol. Used so far in ruminant diets, this by-product has become available for non ruminants. Rapid adoption of this ingredient as a cost-effective raw material has challenged the poultry nutrition industry. Many publications point out the difference between old and new plants with major concerns on the effects of heat treatment. Required to reduce the moisture content of wet distillers' grains, this treatment may reduce the availability of heat sensitive amino acids (AA) such as lysine (LYS) (Cromwell *et al*., 1993) because of Maillard reactions and the associated production of Amadori compounds. In maize DDGS, Maillard reactions can bind the free ε-NH2 group of LYS to the reducing sugars with subsequent difficulties in dosage of LYS and lowered digestibility in poultry or pigs (Fastinger and Mahan, 2006; Pahm *et al*., 2008*a*; Stein *et al*., 2006). In contrast, the LYS with a free ε-NH2 group is considered to be bio-available. Consequently, total LYS analysis that begins with acid hydrolysis and includes the unavailable fraction of LYS overestimates LYS content for nutritional purposes.

Thus, the objective of this study was (1) to evaluate wheat DDGS amino acid content with a special focus on LYS and (2) to evaluate amino acid digestibility in caecectomised cockerels. Based on a large scale of samples, variability between products was also evaluated and prediction equations were developed.

MATERIAL AND METHODS

Feedstuffs

A total of 19 samples of wheat DDGS from 7 European ethanol plants were collected in July-September 2007. First, 10 of them were chosen as representative of total variability according to their major characteristics (nitrogen, starch, fat, crude fibre, ash) and were measured for AA digestibility in pigs (Cozannet *et al*., 2010) and in cockerels (present study) and for energy digestibility in pigs (Cozannet *et al*., 2010) and in broilers, cockerels, laying hens and turkeys (Cozannet *et al*., 2010). Each plant was represented by at least one sample in the 10 selected samples. In the specific case of AA digestibility in cockerels, the 9 additional samples were also measured in cockerels in the same conditions as those of the 10 samples. These results will be used only for validation of the prediction equations. Experiments on cockerels were carried out at Adisseo CERN, Commentry, France according to the Certificate of Authorization to Experiment on Living Animals No. B0315901 (European Directive 24/11/86 86/609 CEE).

Diets, animals, housing, experimental design and samples collection

A total of 60 adult ISA Brown cockerels, surgically caecectomised according to the technique described previously by Parsons (1985), were used in a factorial block design. The birds, housed in an environmentally controlled room, were kept in individual cages with wire floors that permitted collection of excreta on stainless trays. They had free access to water and were subjected to a daily photoperiod of 16 h. Four birds in adjacent cages were considered as the experimental unit. A maize-based standard maintenance diet was fed between assays. For the present assay, 10 experimental diets were composed with one of the 10 DDGS mixed with wheat starch and 40 g/kg premix in order to achieve a 180 g/kg as-fed CP diet. In addition, a N-free diet (replacement of DDGS by starch) was also used.

The birds were assayed for 3 successive one-week periods. Over the first period, each bird received the N-free diet in order to evaluate unspecific endogenous AA losses. For that purpose, the birds, after a 24-h fasting period, received 100 g of glucose solution (800 g/kg

glucose) in water. Then, 24 h later, 50 g of N-free diet was force fed to each bird and a 4-h collection period was carried out. Over the 2 following periods, each experimental diet was measured on 3 experimental units. For this purpose, fasted birds for 24 h were force fed with approximately 180 g of wet diet (500 g/kg DM) with a subsequent twice-daily total collection of excreta over the following 48 h for each bird. Collected excreta were then immediately frozen and freeze-dried at the end of the experiment. Freeze-dried individual samples from the 2-d collection were pooled and mixed per experimental unit before grinding through a Retsch mill equipped with a 1-mm screen.

Chemical analyses

Samples of raw materials, diets and freeze-dried excreta were analysed for DM, N (according to the methods of AOAC, 1990) and AA contents at Ajinomoto Eurolysine S.A.S., Amiens (France). The AA contents were measured by cation exchange chromatography after acid hydrolysis for 24 h (Directive 98/64/CE, 3/09/99 – Norm NF EN ISO, 2005). Analysis of methionine was performed after initial oxidation of samples with performic acid. Phenylalanine was analysed without oxidation, whereas tryptophan was analysed after hydrolysis in 4 M barium hydroxide at 110°C for 16 h (AFNOR, 1998). The DDGS samples were also analysed according to standard methods (AOAC., 1990) for ash, starch, crude fat and crude fibre. Detergent fibre fractions (NDF, ADF and ADL) were determined by a sequential procedure with prior amylolytic (thermamyl 120L) and proteolytic (extracted from *Streptomyces griseus*) treatments (van Soest and Wine, 1967) . Residual N in NDF and ADF fractions was also measured. Because of incomplete removal of protein from NDF residue, a sequential procedure that used both heat-stable α-amylase and sodium sulphite was developed to measure insoluble dietary fibre in all feeds (values of NDF, ADF and ADL were the average of both values using protease or sulphite). Protein solubilities in KOH and water as defined by Araba and Dale (1990) and the American Oil Chemists' Society (1998) were

measured on DDGS samples in order to evaluate protein quality with high protein solubility corresponding to higher protein quality or less heat damaged products. Colour scores (Luminance, L; red index, a, and yellow index, b) were determined using a CR-410 colorimeter; the L, a and b ranged from 0 (black) to 100 (white), from -60 (green) to 60 (red) and from -60 (blue) to 60 (yellow) respectively.

Total LYS content was dissociated in two categories: free LYS or LYS whose ε-amino groups are bound to other groups. The free LYS (FLYS) estimate was based on free ε-NH_2 conversion to ε-dinitrophenyl-lysine through a reaction using 1-fluoro-2:4-dinitrobenzene (FDNB) before the sample was acid-hydrolysed (Carpenter, 1960) for total AA hydrolysis. The bound LYS (BLYS) content was indirectly evaluated from the furosine measurement. Furosine is one of the compounds that are produced from Amadori products during the acid hydrolysis step in amino acids analysis (Finot *et al*., 1968). Amadori products in milk contain 320 g/kg furosine, 400 g/kg BLYS and 280 g/kg pyridosine (Bujard and Finot, 1978); these ratios are assumed to be constant in any Amadori compound (Guerra-Hernandez *et al*., 1999). Chromatographic determination of furosine in wheat DDGS samples was performed by ion-pair RP-HPLC (hydrolysis with 8 N HCl under inert conditions at 110°C for 24 h. Finally, *in vitro* digestibility of N in wheat DDGS was measured according to a method adapted from Boisen and Fernandez (1995) with a 3 times higher pepsine level including addition of three time pepsine activity.

Calculations and statistical analyses

Total LYS content corresponds to LYS measured after hydrolysis. Assuming constant proportions of the three hydrolysis products of Amadori compounds, the BLYS content was calculated from furosine content as:

$$\text{blys} = \frac{\text{Furosine} \times 40}{32}$$

The standardised digestibility (SDD) of each AA was calculated for each DDGS using the following equation:

$$SDDAA = \frac{AA\,\text{int} + endogenousAAloss \times DM\,\text{int} - AAexc}{AA\,\text{int}} \times 100$$

with AAint and AAexc as the total AA intake and excreted (g DM) respectively, DMint as the dry matter intake (kg) and endogenous AAloss corresponds to the basal endogenous losses (g/kg DMint) as calculated for each experimental unit of birds fed the N-free diet and assumed to be proportional to DM intake.

Data on each replicate over the 2 periods (n = 30) were subjected to an analysis of variance with diet effect (n = 10) (SAS Institute Inc., Cary, NC). Linear regression equations for predicting digestibility coefficients of nitrogen and LYS of wheat DDGS from chemical characteristics were calculated according to a stepwise regression procedure including chemical content, colour indicators or *in vitro* digestibility of nitrogen as explaining variables using SAS regression procedures. Parameters with *P* value below 0.05 were considered in the model. The equations with the lowest residual standard deviations (RSD) are presented.

RESULTS

Chemical composition and physical properties of wheat DDGS

The starch concentration was low in most DDGS samples (41g/kg DM) and varied between 25 and 95g/kg DM (Table 1). However, this variation was mainly due to two products with a high starch content. Concentrations of CP, crude fat and crude fibre in the 10 samples averaged 361, 46 and 83g/kg DM, respectively. One objective in the selection of DDGS to be measured in the digestibility trials was to reduce correlations between chemical characteristics; apart from the inevitable correlations between the different cell wall fractions (Crude fibre, NDF, ADF and ADL), few correlations were significant (data not shown). The N content measured on detergent fibre fractions indicates that a fraction of CP content was included in the fibre fractions as indicated by the high CP content in NDF (91g/kg DM on

average; data not shown) and CP content in ADF (4 to 82; 28 g/kg on average; Table 1) residues. In addition, CP in ADF and ADF content were positively correlated (r = 0.84; Table 2). Protein solubility measured in water and in KOH averaged 0.18 and 0.34 respectively. Nevertheless, no significant correlation between the two criteria was obtained (r = 0.30; data not shown).

Tables 1 and 2 near here

The LYS and arginine levels in CP were highly variable (0.83 to 3.01 and 2.25 to 4.62 g/100g CP respectively), whereas contents in other AA were less variable. It should be noted that all AA, except LYS and arginine, expressed in g/kg DM, were positively and significantly correlated with CP content (g/kg DM) (data not shown). The FLYS content averaged 1.53 g/100g CP and represented 0.76 of LYS in DDGS; this latter ratio varied from 0.52 to 0.89. Unavailable LYS determined from furosine content averaged 0.26 g/100g CP (range: 0.13 to 0.54) and represented 0.16 of LYS (range: 0.05 to 0.37). Consequently, the sum of unavailable and available LYS contents in g/100g of LYS (or recovery) averaged 0.93 with minimum and maximum values of 0.67 and 1.10. Finally, the correlation between LYS and FLYS contents (g/100g CP) was high and positive (r = 0.99), whereas a poor correlation was observed between LYS and BLYS (g/100g CP) content (r = 0.01) in connection with a rather constant BLYS content and highly variable LYS values.

The colour, as estimated from values of L, ranged from 43 to 63. In fact, the L score distribution revealed that, between the 10 DDGS samples, three products could be considered as dark (L < 50) with low LYS levels (1.01 g/100g CP) and 7 as light (L > 50) with higher and rather constant LYS levels (2.29 g/100g CP). Luminance values were correlated with fibre fractions evaluated according to different chemical procedures and especially with ADF content (r = -0.94), CP in ADF residue (r = -0.86), or FLYS content (r = 0.90) expressed in g/100g of LYS (Table 2). In addition, light products might be subdivided in two additional groups in connection with their starch content. Five products had starch content lower than 70

g/kg and two had higher starch content. High starch content products had also higher furosine content (1.4 vs 0.5 g/kg DM); the LYS recovery was also higher for these two high starch samples (1.09 vs 0.92 and 0.82 for the light-low starch category and the dark category respectively).

Digestibility of diets and wheat DDGS

All cockerels remained healthy throughout the duration of the experiment and no animal was removed from the study. Endogenous AA losses are presented in Table 3. Aspartic and glutamic acids represent the most important part of these losses (8.5 and 12.0 g/100g of total losses respectively), while methionine and tryptophan each accounted for less than 2%. The SDD of CP in DDGS varied between 0.55 and 0.85, with an average value of 0.74 (Table 4). It was correlated with colour (r = 0.87) with the lightest samples having the greatest SDD values (Table 2). Except for LYS, SDD, the SDD of each AA and the variability between samples were in agreement with the SDD of CP. The average SDD of LYS was 0.46 and varied between -0.04 and 0.71. Moreover, samples distribution of SDD also suggested a division into two groups, as for chemical composition: a group with rather constant and high SDD (0.61) with L values above 50 and a group with lower and variable SDD (-0.04 to 0.23) and L values lower than 50. This observation could be repeated for all other AA but with smaller differences between the two groups than for LYS. The difference in starch content for the light samples did not affect the SDD of AA, except for LYS with a lower digestibility for the high starch content DDGS (0.52 vs 0.64). These latter samples had also the highest furosine levels (Table 1).

Tables 3, 4, 5 near here

Prediction of nitrogen and LYS digestibility in wheat DDGS

Prediction equations were developed only for N and LYS digestibility whose values were the most variable (Table 5). These equations indicate that N and LYS SDD might be predicted from colour, *in vitro* and chemical characteristics. The strong relationship beteween *in vitro* N

digestibility and N or LYS SDD (r = 0.90 and 0.87, respectively; Table 2) were notable. Nevertheless, the strong relationships previously reported between colour score and chemical characteristics did not allow multivariate models involving both types of criteria. In addition, observations on the darkest samples have a high influence on the coefficients. Equations obtained according to a stepwise regression procedure indicated that nitrogen digestibility can be evaluted from CP in ADF content. However, the only predictor for LYS SDD was LYS concentration (g/100g CP) according to a linear (Table 5, equation 5) or a quadratic regression (Table 5, equation 6) or a linear-plateau regression with a breakpoint for 1.9 g/100g LYS content in CP and a plateau LYS SDD of 0.63 (Table 5, equations 7 and 8). The breakpoint defined for LYS content of 1.9 g/100g CP is close to the previous limit defined between light and dark samples.

DISCUSSION

As reported by Stein *et al*. (2006), DDGS chemical composition is highly related to the process used for ethanol production. In connection with the process, most of the starch is converted to ethanol and only small amounts remain in the DDGS. This was the case in the present trial for wheat DDGS, except for the two highest values that originated from a process with bran removal at the beginning of the process and a reintroduction of the bran at the end. On the other hand, CP, crude fat and dietary fibre are not fermented and their levels in DDGS are about three times higher than in wheat (Sauvant *et al*., 2004). Overall, the average nutrients composition of our DDGS samples was similar to previous reported values for wheat DDGS (Piron *et al*., 2008, Lan *et al*., 2008).

According to the process, most of the AA in the protein are from wheat (0.95) and the rest comes from yeast growth (Ingledew, 1993). Therefore, it is logical to get a rather similar AA profile in DDGS (present trial) and in wheat (Sauvant *et al*., 2004). However, the difference between wheat and wheat DDGS is high for LYS (2.9 vs 1.9 g/100g CP

respectively) with a highly variable LYS content in CP. A similar variability in LYS level of wheat DDGS was measured by Piron *et al*. (2008). This variability for LYS content in CP was also observed in maize DDGS (Cromwell *et al*., 1993; Spiehs *et al*., 2002) but to a smaller extent than in the wheat DDGS of the present trial.

This relative disappearance and high variability of total LYS content in wheat DDGS samples, as compared with LYS in wheat, would be associated with Maillard reactions occurring during the the process of DDGS preparation (Pedersen *et al*., 2007; van Boekel, 1998) and might explain the poor correlation between LYS expressed in g/kg DM and CP content in wheat DDGS. These reactions produce brown-coloured compounds. the present results indicate that samples with low lightness values ($L < 50$; $n = 3$) in connection with high occurrence of these reactions have a specific nutrient profile with a high CP content in ADF, a poor protein solubility in water and a poor LYS content. Based on reversible reaction between the ε-amino group of LYS and reducing sugars under specific *in vitro* conditions, Maillard reactions make LYS nutritionally unavailable for animals (Hurrell and Carpenter, 1981). On the other hand, high starch wheat DDGS had high luminance values ($L > 60$) and would not be subject to Maillard reaction. In fact, these samples come from bio-ethanol plants where wheat bran is removed at the beginning of the process and added back after fermentation. Thus, these products have a specific nutrient profile with low ADF and CP in ADF. Therefore, the colour score appears as a quick and reliable tool for wheat DDGS evaluation. This also means that care must be taken in interpreting the results of LYS content in wheat DDGS. Total LYS is considered to be a relevant chemical indicator of protein quality for animal nutrition. However, complementary analyses indicate that in the case of wheat DDGS, some LYS is potentially unavailable for the animal. Using the homoarginine method, Pahm *et al*. (2008*b*) reported that the average FLYS concentration of 33 maize DDGS samples was 0.75 of LYS. This value is comparable to the average value obtained in the present study

(0.76). Nevertheless, the present study suggests little additional information provided by FLYS evaluation in relation with the high correlation between this parameter and colour score or LYS content as previously reported (Pahm *et al*., 2009). Similarly, BLYS content in our wheat DDGS samples agree with the previous observations on maize DDGS by Pahm *et al*. (2008*b*); 0.16 in both studies. Nevertheless, results for furosine content and BLYS expressed in g/kg of DM content of wheat DDGS appear not to be related with either colour score or LYS content in g/100g CP. In addition, as observed in the present trial, the sum of FLYS and BLYS did not represent the LYS content since the recovery was lower than one, with the lowest value being for the dark products. This suggests that the BLYS content estimated from furosine would underestimate the 'true' BLYS level, with a rapid evolution of furosine into advanced Maillard products and an associated low L value (Leclère and Birlouez-Aragon, 2001).

The SDD of CP and AA (except LYS) in wheat DDGS light samples used in the current study were about 10 percent units lower than the corresponding values in wheat (Sauvant *et al*., 2004) and are in agreement with recent studies on wheat DDGS (Bandegan *et a*l., 2009). This difference with wheat can be partly attributed to the higher dietary fibre content of wheat DDGS (Lenis *et al*., 1996). The difference is more accentuated for LYS. In addition, the SDD of LYS appears highly variable. This result was also observed in maize DDGS but to a smaller extent (Ergul *et al*., 2003; Batal and Dale, 2006) than in our wheat DDGS samples. The presence of Amadori compounds in wheat DDGS may explain the variability in LYS SDD. In addition, low CP and AA digestibility in samples with high Maillard reaction occurence may be also caused by the restricted access of digestive enzymes to proteins including sugar-bonded LYS encapsulated by fibre (Schulze *et al*., 1994; Sève, 1994). As for nutrients profile, the present results indicate that samples with low lightness values ($L < 50$; $n = 3$) in connection with high occurrence of these reactions have a specific

AA SDD profile. In order to get more realistic nutritional values of wheat DDGS, samples with luminance below 50 should then be considered as abnormal and affected specific values.

In the present trial, ADF and CP in ADF appeared to be the best variables to predict the variability in nitrogen or AA SDD among samples. In addition, the present study indicated that samples with LYS content below 1.9 g/100g CP present low LYS SDD whereas constant LYS SDD (0.63) could be applied for samples with LYS content above 1.9 g/100g CP. Therefore, wheat DDGS with LYS content above 1.9 g/100g CP should be preferred for poultry. A similar approach on maize DDGS in pigs indicates that only products with LYS content above 3.2 g/100g CP should be considered as highly valuable for pigs, whereas products with LYS below 2.3 g/100g in CP should be considered as badly heat-damaged products (Fontaine *et al*., 2007). Wheat DDGS, when fed to pigs, had high and rather constant LYS SDD values for more than 1.9 g/100g CP lys (Cozannet *et al*., 2010). This 1.9 g/100g CP limit also corresponds with the previous limit defined between light and dark samples. Therefore, DDGS colour may be a good indicator of AA SDD and particularly LYS SDD.

The prediction equations for LYS SDD obtained in the present trial were established from measurements on 10 samples selected from a set of 17 samples. These 10 samples were thoroughly analysed and measured for their AA standardised ileal digestibility (SID) in pigs (Cozannet et al., 2010) and poultry (present trial). Wheat DDGS LYS SID in pigs averaged 0.56 (0.09 to 0.83), with a high correlation between SID of LYS in pigs and LYS SDD in cockerels (r=0.93) and a very comparable ranking of LYS SID and LYS SDD values (Figure 1). Values for pigs could then be estimated from values in poultry (and *vice versa*). Chung and Baker (1992) reported greater SID in pig compared with poultry, however, these authors noted that their ranking of SID was not in agreement with that of Opapeju *et al*. (2006). The 7 'discarded' samples and two additional wheat DDGS samples were also measured in adult cockerels under the conditions of the present trial, but with less complete chemical analyses.

The results have then been used for validating the prediction equations established on the 10 samples using quadratic model with LYS content in g/100g CP as predictor (Figure 2). LYS SDD of validation samples averaged 0.59 with an average predicted value of 0.56. In addition, the correlation between the measured and predicted values was quite high (r = 0.98; RSD=3.5). This indicates that the prediction equation based on LYS percentage in CP represents an acceptable predictor of LYS SDD in wheat DDGS. Figures 1 and 2 near here

Conclusions

As reported for maize DDGS, the present results indicate that wheat DDGS have variable nutrient composition and SDD of AA, with LYS content and digestibility exhibiting more variability than other AA. In addition, the total CP content in wheat DDGS is relatively high and DDGS can then be considered as a potential source of supplemental protein and AA for poultry. Nevertheless, the protein quality of DDGS is rather low due to a deficiency in several indispensable AA, especially LYS. Finally, the present study suggests a subdivision of wheat DDGS into three categories: wheat DDGS with high starch content, wheat DDGS with low starch content under normal drying conditions and overheated, and dark wheat DDGS with low starch content. The darkest DDGS have the lowest digestible LYS content and so should only be fed to poultry with caution.

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Table 1. *Composition of wheat distillers' dried grains with solubles (DDGS)*

	Wheat DDGS (n=10)			Colour[1]		Starch[2]	
	Mean	Min	Max	Dark (n =3)	Light (n = 7)	< 7% DM (n = 5) [3]	> 7% DM (n = 2)
Dry matter	926	893	944	925	927	922	939
Nutrient content, g/kg DM							
Ash	52	43	67	57	49	50	48
Protein (N*6.25)	361	326	389	358	362	366	353
Crude fat	46	36	56	51	43	45	40
Crude fibre	83	62	109	95	78	83	64
NDF[4]	292	251	338	293	292	304	262
ADF[4]	120	77	179	157	104	113	83
ADL[4]	48	21	105	84	33	36	24
Starch Ewers	41	25	95	29	47	30	87
Colour score							
L (luminance)	54	43	63	46	57	55	63
a (red index)	6.2	4.4	7.3	5.3	6.7	6.4	7.2
b (yellow index)	13.4	5.3	19.0	8.0	15.8	14.5	19.0
Protein solubility							
in water	0.18	0.09	0.30	0.22	0.16	0.19	0.09
in KOH	0.34	0.27	0.43	0.36	0.33	0.34	0.32
CP in ADFn (g/kg DM)	28	4	82	68	11	13	4
Maillard reactions							
Furosine (g/kg DM)	0.71	0.36	1.50	0.63	0.74	0.50	1.40
In vitro N digestibility[5]	0.74	0.55	0.86	0.62	0.79	0.79	0.78
Amino acid contents, g/100g CP							
Indispensable amino acids							
Arginine	3.76	2.25	4.62	2.47	4.30	4.23	4.49
Histidine	1.95	1.66	2.18	1.71	2.06	2.04	2.11
Isoleucine	3.42	3.28	3.54	3.31	3.47	3.47	3.48
Leucine	6.41	5.83	6.77	6.10	6.54	6.50	6.62
Lysine							
Total[6]	1.91	0.83	3.01	1.01	2.29	2.35	2.12
Free[7]	1.53	0.50	2.59	0.57	1.95	2.00	1.82
Bound[8]	0.26	0.13	0.54	0.24	0.28	0.18	0.50
Residual[9]	0.11	-0.22	0.42	0.20	0.07	0.17	-0.20

Methionine	1.43	1.31	1.53	1.36	1.45	1.45	1.48
Phenylalanine	4.44	4.22	4.65	4.31	4.50	4.51	4.47
Threonine	2.95	2.68	3.14	2.77	3.02	3.00	3.08
Tryptophan	1.05	0.85	1.21	0.91	1.11	1.08	1.20
Valine	4.22	3.97	4.39	4.09	4.28	4.28	4.28
Dispensable amino acids							
Alanine	3.65	3.36	4.09	3.49	3.72	3.72	3.72
Aspartic acid	4.83	4.03	5.64	4.39	5.01	4.99	5.08
Cysteine	1.76	1.10	1.95	1.52	1.86	1.84	1.90
Glutamic acid	25.71	22.63	26.79	25.75	25.69	25.52	26.12
Glycine	3.94	3.74	4.13	3.80	4.00	3.95	4.11
Proline	8.44	8.00	9.05	8.37	8.47	8.57	8.22
Serine	4.34	3.90	4.65	4.04	4.46	4.42	4.57
Tyrosine	3.02	2.80	3.12	2.90	3.07	3.06	3.09

[1]Luminance ranged from 43 to 50 for the dark group and 52 to 64 for the light group.

[2]Starch content ranged from 2.5 to 4.5% DM for starch content < 7% and 8.6 to 10.1% DM for starch content > 7% according to the classification of Sauvant *et al*. (2004).

[3]Only wheat DDGS with luminance above 52 are considered for the low starch category.

[4]Cell wall fractions determined according to the methods of Van Soest with prior amylolytic (thermamyl 120L) and proteolytic (protease extracted from *Streptomyces Griseus*) or sodium sulfite treatments (van Soest and Wine, 1967). Values provided were the average between both treatments.

[5]In-vitro N digestibility determined according to the method of Boisen and Fernandez (1995)

[6]Total lysine measured by cation exchange chromatography

[7]Free lysine measured according to Carpenter (1960)

[8]Bound lysine content calculated from furosine measurement with: Unavailable lysine = $\left(\frac{Furo\sin e \times 40}{32}\right) \times \frac{100}{CP}$

[9]Residual lysine calculated calculated from unavailable lysine, available lysine and total lysine: Residual lysine = total lysine – unavailable lysine – available lysine

Table 2. *Correlation coefficients[1] between wheat DDGS chemical characteristics and standardised digestibility (SDD) of nitrogen and lysine (n = 10[2]*

	Ash	Protein	ADF	CP in ADF	L	Furosine	IDP[3]	LYS[4]	BLYS[5]	FLYS[6]	DNv[7]	SDD N[8]
Protein	0.03											
ADF	0.34	0.05										
CP in ADF	0.49	0.12	0.84*									
L	-0.40	0.04	-0.94*	-0.86*								
Furosine	0.04	-0.11	-0.54	-0.26	0.54							
IDP[3]	0.13	-0.52	0.57	0.30	-0.69*	-0.56						
LYS[4]	-0.53	-0.04	-0.66	-0.91*	0.67	-0.06	0.07					
BLYS[5]	0.57	-0.07	-0.06	0.46	-0.12	0.64*	-0.26	-0.69*				
FLYS[6]	-0.35	0.01	-0.89*	-0.97*	0.87*	0.21	-0.37	0.87*	-0.44			
DNv[7]	-0.50	-0.18	-0.73*	-0.91*	0.70*	-0.02	-0.15	0.89*	-0.66*	0.84*		
SID N[8]	-0.50	0.00	-0.87*	-0.96*	0.87*	0.13	-0.38	0.84*	-0.51	0.96*	0.90*	
SID LYS[9]	-0.58*	0.02	-0.58	-0.87*	0.64*	-0.14	-0.10	0.97*	-0.82*	0.85*	0.88*	0.87*

[1] Correlation whose absolute value is above 0.64 is significant ($P < 0.05$).
[2] Ash, Protein, ADF, CP in ADF, furosine and LYS expressed in g/100g CP. BLYS and FLYS in g/100g LYS. DNv, SDD N and SDD LYS (%).
[3] Protein Dispersibility Index.
[4] Total LYS content.
[5] Free LYS content.
[6] Bound LYS content.
[7] *In vitro* Digestibility Index.
[8] N standardised ileal digestibility
[9] Total LYS standardised ileal digestibility

Table 3. *Basal endogenous losses (g/kg DM intake)*[1, 2]

	Mean	SD
Nitrogen[3]	1.99	0.19
Indispensable amino acids		
Arginine	0.92	0.1
Histidine	0.35	0.03
Isoleucine	0.57	0.06
Leucine	0.91	0.1
Lysine	0.70	0.07
Methionine	0.16	0.02
Phenylalanine	0.51	0.06
Threonine	1.21	0.13
Tryptophane	0.24	0.02
Valine	1.28	0.17
Dispensable amino acids		
Alanine	0.84	0.07
Aspartic acid	1.32	0.13
Cystine	0.56	0.06
Glutamic acid	1.92	0.19
Glycine	1.27	0.14
Proline	1.14	0.12
Serine	1.08	0.12
Tyrosine	0.53	0.07

[1]Animals fed with 50g fresh N free diet
[2]Endogenous amino acids losses measured on 15 experimental units
[3]Nitrogen in digesta evaluated according to methodology described by Terpstra and De Hart (1974)

Table 4. *Standardised ileal digestibilities of crude protein and amino acids in wheat DDGS*

	Wheat DDGS (n=10)			Colour[1]		Starch[2]		Statistics[3]	
	Mean	Min	Max	Dark (n=3)	Light (n=7)	< 7% (n=5)[4]	> 7% (n=2)	DDGS effect	RSD
Nitrogen	0.74	0.55	0.85	0.58	0.81	0.81	0.81	< 0.001	0.02
Indispensable amino acids									
Arginine	0.68	0.42	0.81	0.46	0.78	0.78	0.77	< 0.001	0.03
Histidine	0.70	0.48	0.83	0.50	0.78	0.78	0.78	< 0.001	0.04
Isoleucine	0.71	0.47	0.84	0.53	0.79	0.79	0.78	< 0.001	0.03
Leucine	0.76	0.55	0.86	0.59	0.83	0.83	0.82	< 0.001	0.03
Lysine	0.46	-0.04	0.71	0.12	0.61	0.64	0.52	< 0.001	0.03
Methionine	0.75	0.57	0.84	0.60	0.81	0.81	0.81	< 0.001	0.02
Phenylalanine	0.82	0.64	0.90	0.67	0.88	0.88	0.88	< 0.001	0.02
Threonine	0.65	0.42	0.76	0.46	0.73	0.73	0.73	< 0.001	0.03
Tryptophan	0.67	0.43	0.78	0.49	0.75	0.75	0.76	< 0.001	0.03
Valine	0.72	0.47	0.85	0.52	0.81	0.81	0.80	< 0.001	0.03
Dispensable amino acids									
Alanine	0.65	0.42	0.79	0.46	0.74	0.74	0.73	< 0.001	0.04
Aspartic acid	0.54	0.27	0.68	0.31	0.65	0.64	0.66	< 0.001	0.03
Cystine	0.60	0.30	0.77	0.37	0.71	0.70	0.71	< 0.001	0.03
Glutamic acid	0.82	0.65	0.92	0.69	0.87	0.87	0.88	< 0.001	0.01
Proline	0.81	0.64	0.91	0.67	0.87	0.87	0.88	< 0.001	0.01
Serine	0.70	0.51	0.81	0.53	0.78	0.78	0.78	< 0.001	0.02
Tyrosine	0.68	0.51	0.77	0.55	0.74	0.74	0.73	< 0.001	0.02

[1]Luminance ranged from 43 to 50 for the dark group and 52 to 64 for the light group.
[2]Starch content ranged from 2.5 to 4.5% DM for starch content < 7% and 8.6 to 10.1% DM for starch content > 7% according to the classification of Sauvant *et al*. (2004).
[3]From the analysis of variance on 60 results with wheat DDGS sample (n=10; 6 measurements per diet) as a fixed effect.
[4]Only wheat DDGS with luminance above 52 are considered for the low starch category.

Table 5. *Prediction equations of nitrogen (N) and lysine (LYS) standardised digestibility of wheat DDGS in cockerels*[1]

Equation number	Regression equations	R^2	RSD
Nitrogen standardised digestibility (n = 10)			
1	SDD N = - 4.9 + 1.5×L	0.75	5.9
2	SDD N = 85.4 -0.4×CP in ADF	0.92	3.4
Lysine standardised digestibility (n =10)			
3	SDD LYS = - 90.3 × 2.5×L	0.41	21.2
4	SDD LYS = 67.5 - 0.8×CP in ADF	0.75	13.7
5	SDD LYS = -16.8 + 33.0 LYS	0.88	9.6
6	SDD LYS = -56.6 + 82.7 LYS - 13.4 LYS^2	0.94	7.3
7	SDD LYS (if LYS > 1.9) = 63.0	0.90	9.3
8	SDD LYS (if LYS < 1.9) = -42.4 + 55.5 LYS		

[1]SID N and SID LYS (%), ADF and CP in ADF (g/kg of DM), LYS (g/100g of CP).

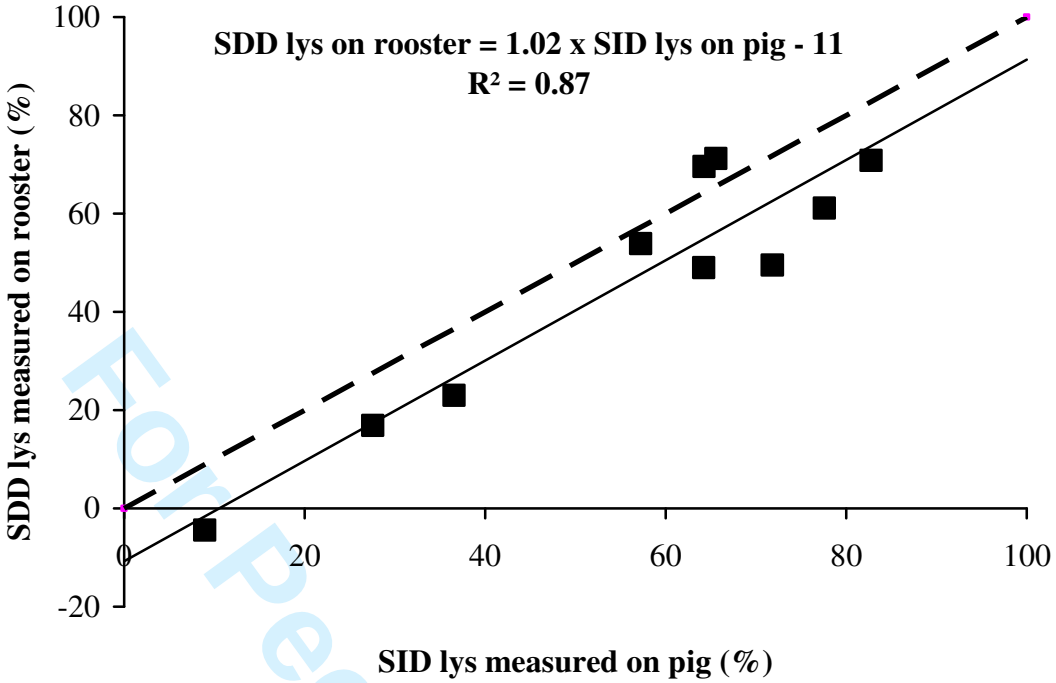

Figure 1. *Correspondence between wheat DDGS lysine standardised digestibility (LYS SDD) evaluated on cockerel and lysine standardised ileal digestibility (LYS SID) on pig (Cozannet et al., 2010).*

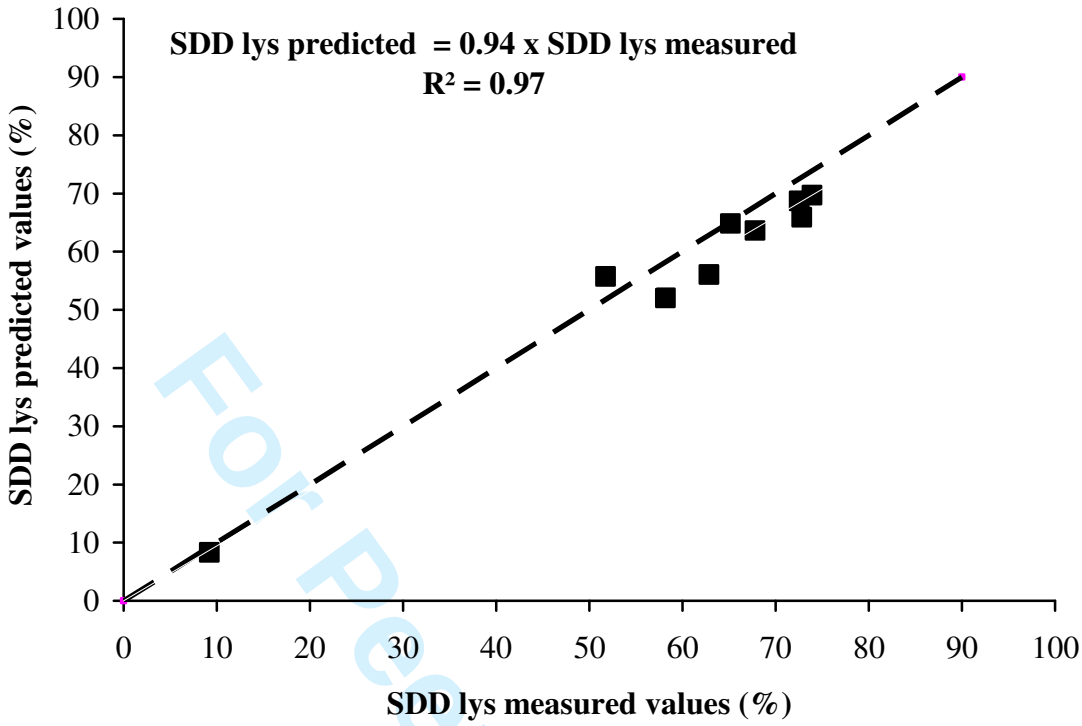

Figure 2. *Validation of the prediction model (equation 6, Table 5) of lysine standardised digestibility (LYS SDD) from an independent data set (n=9).*