Animal Feed Science and Technology 159 (2010) 68–71

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Short communication

A preliminary study on the length of incubation needed to maximize guanidination of lysine in distillers dried grains with solubles (DDGS) and in pig ileal digesta

A.A. Pahm[a], C. Pedersen[b], D. Simon[c], H.H. Stein[a,*]

[a] Department of Animal Sciences, University of Illinois, Urbana, IL 61801, United States
[b] Schothorst Feed Research, Almere Stad, The Netherlands
[c] Department of Animal and Range Sciences, South Dakota State University, Brookings, SD 57007, United States

ARTICLE INFO

Article history:
Received 2 June 2008
Received in revised form 12 April 2010
Accepted 20 April 2010

Keywords:
Distillers dried grains with solubles
Guanidination
Homoarginine
Pigs
Reactive lysine

ABSTRACT

In heat-processed feed ingredients, the concentration of bioavailable lysine (Lys) may be estimated by measuring the amount of ileal digestible Lys that has a free ε–NH_2 (*i.e.*, not bound to reducing sugars). This Lys, called ileal digestible reactive Lys, is determined by measuring the reactive Lys in the feed ingredient and in ileal digesta of pigs fed the feed ingredient. A procedure used to measure reactive Lys in feed ingredients and in ileal digesta samples is the homoarginine procedure, which consists of a guanidination to convert the Lys that has a free ε–NH_2 group into homoarginine. However, the incubation time needed to maximize the guanidination can vary among types of protein and there is no information on the time needed to maximize guanidination in ileal digesta from pigs. Thus, an experiment was conducted to determine the optimum time of incubation needed to guanidinate Lys in maize distillers dried grains with solubles (DDGS) and in ileal digesta from a pig fed a diet containing maize DDGS as the sole source of Lys and other amino acids. A DDGS sample was guanidinated in 0.6 M O-methylisourea reagent at pH 11.4 for 1, 3, 6, or 9 days. Ileal digesta from a pig fed a diet containing DDGS was also incubated in 0.6 M O-methylisourea for 1, 3 or 6 days. The incubation time that resulted in maximal amount of Lys that had been converted to homoarginine was then determined. Results showed that in DDGS, the optimum incubation time in DDGS was 3.2 days ($P<0.05$). For ileal digesta, breakpoint analysis showed that the optimum incubation time was 3.7 days ($P<0.05$). In conclusion, the optimum conversion of Lys to homoarginine in DDGS and in ileal digesta requires an incubation time of 3.2 and 3.7 days, respectively, if 0.6 M O-methylisourea is used for incubation at a pH of 11.4.

1. Introduction

During drying of distillers dried grains with solubles (DDGS), reducing sugars may bind to the ε–NH_2 group of lysine (Lys) via the Maillard reaction, which makes the Lys unavailable to animals. This bound Lys is called unreactive Lys, and varying concentrations of unreactive Lys in DDGS result in variation in the ileal digestibility of Lys in DDGS fed to pigs (Stein et al., 2006). Increased concentrations of unreactive Lys in DDGS will result in reduced Lys absorption in the small intestine of pigs

Abbreviations: DDGS, distillers dried grains with solubles; Lys, lysine.

* Corresponding author. Tel.: +1 217 333 0013; fax: +1 217 333 7088.
E-mail addresses: hstein@illinois.edu, hstein@uiuc.edu (H.H. Stein).

0377-8401/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.anifeedsci.2010.04.013

fed DDGS and increased concentrations of unreactive Lys in the ileal digesta at the end of the small intestine. Lys that has not been involved in the Maillard reaction (*i.e.*, not bound to a reducing sugar) is called reactive Lys (Hurrell and Carpenter, 1981), which is the form of Lys that is bioavailable to pigs. The concentration of reactive Lys in a feed ingredient can be measured using the homoarginine procedure. This procedure involves a guanidination reaction between O-methylisourea and Lys in proteins where the unbound Lys, but not the sugar-bound Lys, is modified by substitution of the free ε–NH_2 of unbound Lys with a guanidino group from O-methylisourea, forming 2-amino-6-guanidohexanoic acid or homoarginine (Kimmel, 1967). The concentration of homoarginine can be converted to Lys on a molar basis, which represents the amount of Lys that is considered reactive. By guanidination of Lys in a diet containing DDGS and in the ileal digesta of pigs fed a diet containing DDGS, the concentration of digestible reactive Lys can be calculated (Rutherfurd et al., 1997).

Values for reactive digestible Lys provide a more accurate estimate of the bioavailable Lys in feed ingredients than values for standardized ileal digestible Lys (Pahm et al., 2009). By measuring the reactive Lys in diets containing DDGS and in ileal digesta of pigs fed diets containing DDGS, the ileal digestible reactive Lys in DDGS can be measured (Pahm et al., 2009). However, to accomplish this, the incubation time needed to optimize guanidination in diets containing DDGS as well as in pig ileal digesta needs to be known.

The extent of the conversion of Lys to homoarginine during guanidination is affected by the type of protein that is used (Maga, 1981), and the guanidination conditions for conversion of Lys to homoarginine (Ravindran et al., 1996). By varying the incubation time of O-methylisourea guanidination, conditions for guanidination may be optimized for a particular feedstuff (Maga, 1981; Rutherfurd and Moughan, 1990). Therefore, it is necessary to determine the optimum guanidination time for each protein source.

The objective of this experiment was to determine the incubation time during guanidination with O-methylisourea that will maximize the conversion of Lys to homoarginine in DDGS and in ileal digesta from a growing pig fed a diet based on DDGS. Results of the experiment are needed to measure the concentration of ileal digestible reactive Lys in DDGS fed to pigs.

2. Materials and methods

2.1. Preparation of O-methylisourea reagent

Preparation of the O-methylisourea reagent was based on the procedure by Rutherfurd and Moughan (1990). For every 100 mL of the reagent, 20.6 g (grams) of barium hydroxide (Fisher Scientific International, Inc., Pittsburgh, PA) was dissolved in 69.0 mL of degassed water and the solution was slowly heated to 95 °C. The solution was then mixed with 10.4 g of O-methylisourea hydrogen sulfate (Sigma–Aldrich Inc., St. Louis, MO) and cooled to 25 °C by gently stirring the solution. The solution was centrifuged twice in a Jouan CR 412 centrifuge (Winchester, VA) at 4200 × *g* for 15 min. The supernatant was retained, whereas the solid phase was discarded. The supernatant, which had an initial pH between 12.0 and 12.5, was adjusted to pH 11.4 by the addition of 1.0 M hydrochloric acid. The supernatant was then adjusted to a final volume of 100 mL by adding degassed water.

2.2. Guanidination of DDGS and ileal digesta at varying incubation times

A sample of DDGS was obtained from a modern dry-grind ethanol plant (Albert Lea, MN, USA). A 0.5 g sample of DDGS was placed in each of eight 25-mL test tubes and 6 mL of 0.6 M O-methylisourea reagent with a pH of 11.4 was added to each tube. Samples of DDGS were incubated for 1, 3, 6, or 9 days. Six additional test tubes with 0.5 g each of lyophilized ileal digesta were prepared. The ileal digesta was a representative sample obtained from a 2-day collection of ileal digesta from a growing pig that had been surgically prepared with a T-cannula at the distal ileum. The pig had been fed the same diet based on maize starch (270.7 g/kg) and DDGS (667 g/kg) for 5 days before ileal digesta collection was initiated. The DDGS provided all the Lys in the diet. The digesta samples were mixed with 6 mL of 0.6 M O-methylisourea reagent and the pH was adjusted to 11.4. Two samples of ileal digesta were incubated for 1, 3, or 6 days. During incubation, all DDGS and ileal digesta samples were continuously stirred using an automatic test tube tumbler. Samples were air-dried after incubation and 0.2 g sub-samples were collected for homoarginine analysis.

2.3. Homoarginine analysis

The homoarginine analysis of DDGS and ileal digesta was performed by initially hydrolyzing the samples in 30 mL of 6.0N hydrochloric acid followed by refluxing for 24 h at 110 °C (procedure 4.1.11; AOAC International, 2000). The samples were then derivatized and analysed for homoarginine using cation exchange chromatography. Norleucine (Sigma–Aldrich Inc., St. Louis, MO) was used as an internal standard. Ninhydrin (Trione, Pickerings Laboratories, Mt. View, CA) was used to react with the primary and secondary amines including homoarginine at 130 °C. The color change was detected using a Spectrasystem UV3000 spectrophotometer (Thermo Separation Products, Riviera Beach, FL) at 570 nm. Homoarginine was eluted after arginine and quantified using a standard of homoarginine hydrochloride (Fisher Scientific International Inc., Pittsburgh, PA).

Table 1
Effect of incubation time on lysine to homoarginine conversion (g/kg) in distillers dried grains with solubles (DDGS) and in pig ileal digesta[a].

	Incubation time (days)				S.E.M.	P-value
	1	3	6	9		
DDGS						
Unreactive lysine (mmol) (kg)[b]	8.3	7.0	6.6	6.2	0.39	0.085
Homoarginine (mmol) (kg)[c]	32.2	33.6	33.2	32.2	1.49	0.873
Lysine conversion rate (g) (kg)[d,e]	783[x]	825[y]	830[y]	829[y]	6.9	0.043
Ileal digesta						
Unreactive lysine (mmol) (kg)[b]	9.1	8.4	8.1	–	0.24	0.117
Homoarginine (mmol) (kg)[c]	24.6	26.6	24.9	–	0.89	0.328
Lysine conversion rate (g) (kg)[d,e]	728	754	767	–	6.3	0.113

[a] Dry matter basis; mean of 2 replicates. The unguanidinated DDGS and ileal digesta contained 36.3 and 63.1 mmol lysine/kg DM, respectively.
[b] Lysine that was not transformed to homoarginine after guanidination.
[c] Homoarginine that was produced from guanidination of lysine with O-methylisourea.
[d] Conversion (g/kg) from Lys to homoarginine, which represents reactive lysine.
[e] The breakpoint for incubation time was at 3.2 days for DDGS (P<0.05) and 3.7 days for ileal digesta (P<0.05).
[x,y] Means within a row lacking a common superscript letter differ (P<0.05).

Calculation of the conversion rate of Lys to homoarginine was based on the amount of untransformed Lys (*i.e.*, Lys that was not converted to homoarginine after guanidination) and homoarginine (Rutherfurd and Moughan, 1990):

$$\text{Lys conversion rate}\,(\%) = 100 \times \left(\frac{\text{millimoles homoarginine}}{\text{millimoles homoarginine} + \text{millimoles Lys}}\right)$$

2.4. Statistical analyses

Data were analyzed as a completely randomized design using the GLM procedure of SAS (version 9.1, SAS Inst. Inc., Cary, NC, USA), with 2 replicates per sample. The experimental unit was the DDGS sample or the ileal digesta sample. Data for Lys conversion rate (g/kg) were further analysed for the number of days that are needed to maximize guanidination. This was accomplished using the NLREG procedure to estimate the start of a plateau or breakpoint of conversion of Lys to homoarginine at increasing incubation time. A probability of P<0.05 was considered significant.

3. Results

The effect of incubation time on the conversion of Lys to homoarginine is shown in Table 1. After 1 day of incubation, 783 g/kg of Lys in DDGS was converted to homoarginine, and after 3 days of incubation, Lys conversion was increased to 825 g/kg (P<0.05). The Lys conversion rate was lower at incubation time of 1 day than at 3, 6, or 9 days (P<0.05). Breakpoint analysis showed that the optimum incubation time was 3.2 days (P<0.05). The Lys conversion rates in ileal digesta samples that were incubated for 1, 3, and 6 days were at 728, 754 and 767 g/kg, respectively. The breakpoint analysis showed that the optimum guanidination time for ileal digesta was 3.7 days (P<0.05).

4. Discussion

In the guanidination step for reactive Lys analysis, optimizing the rate of Lys conversion is important because it increases the accuracy of the determination of reactive Lys. Some of the reactive Lys may not be guanidinated if the conditions are not optimal, leading to an underestimation of the reactive Lys concentration in the sample because any Lys that is not guanidinated is assumed to be unreactive. The rapid increase in Lys conversion during the initial 3 days of incubation that was observed in this experiment concurs with data for casein (Maga, 1981) and cottonseed protein (Ravindran et al., 1996). It has been reported that 2.5 days of guanidination results in optimum Lys conversion in DDGS (Fontaine et al., 2007), but in the present experiment, an optimum incubation time of 3.2 days for DDGS was determined. The slightly longer optimum for guanidination time that we observed compared with the data reported by Fontaine et al. (2007) may be due to differences among sources of DDGS, but to our knowledge, there is no information about the effects of source of DDGS on optimum conversion times.

The maximum Lys conversion rate for DDGS (830 g/kg) obtained in the present experiment is similar to the Lys conversion in guanidinated canola meal and barley (880 and 846 g/kg, respectively) reported by Nyachoti et al. (2002). The conversion rate obtained in this experiment is also within the range of conversion rates (630–850 g/kg) that has previously been reported for DDGS (Pahm et al., 2008). However, a conversion rate of only 361 g/kg was reported for cottonseed protein (Ravindran et al., 1996).

The greatest conversion rate for the pig ileal digesta observed in this study was 767 g/kg. The lower conversion rate of Lys in ileal digesta compared with Lys in DDGS may be a consequence of the greater concentration of unreactive Lys in the

digesta as most of the reactive Lys may have been absorbed prior to the distal ileum. The optimum time of incubation for ileal digesta that we observed was slightly longer than for DDGS, which may also be a consequence of the greater concentration of unreactive Lys in the digesta compared with the diet. To our knowledge, there is no published information on the optimum guanidination conditions for ileal digesta of pigs fed DDGS containing diets and we are not aware of other data on conversion rates or optimum time of guanidination for ileal digesta.

In conclusion, the optimum incubation time during guanidination of DDGS and pig ileal digesta using 0.6 M O-methylisourea (pH 11.4) is 3.2 and 3.7 days, respectively. Incubation at these time periods results in conversion rates of Lys to homoarginine of 830 and 767 g/kg, respectively.

References

AOAC International, 2000. Official Methods of Analysis, 17th ed. Association of Official Analytical Chemists, Gaithersburg, MD.

Fontaine, J., Zimmer, U., Moughan, P.J., Rutherfurd, S.M., 2007. Effect of heat damage in an autoclave on the reactive lysine contents of soy products and corn distillers dried grains with solubles. Use of the results to check on lysine damage in common qualities of these ingredients. J. Agric. Food Chem. 55, 10737–10743.

Hurrell, R.F., Carpenter, K.J., 1981. The estimation of available lysine in foodstuffs after Maillard reactions. Prog. Food Nutr. Sci. 5, 159–176.

Kimmel, J.R., 1967. Guanidination of proteins. Methods Enzymol. 11, 584–589.

Maga, J., 1981. Measurement of available lysine using guanidination reaction. J. Food Sci. 46, 132–134.

Nyachoti, C.M., McNeilage-Van de Wiele, E.M., de Lange, C.F.M., Gabert, V.M., 2002. Evaluation of the homoarginine technique for measuring true ileal amino acid digestibilities in pigs fed a barley–canola meal based-diet. J. Anim. Sci. 80, 440–448.

Pahm, A.A., Pedersen, C., Stein, H.H., 2008. Application of the reactive lysine procedure to estimate lysine digestibility in distillers dried grains with solubles fed to growing pigs. J. Agric. Food Chem. 56, 9441–9446.

Pahm, A.A., Pedersen, C., Stein, H.H., 2009. Standardized ileal digestibility of reactive lysine in distillers dried grains with solubles fed to growing pigs. J. Agric. Food Chem. 57, 535–539.

Ravindran, V., Imbeah, M., Angkanaporn, K., Bryden, W.L., 1996. Guanidination of lysine in cottonseed protein. J. Agric. Food Chem. 44, 1812–1815.

Rutherfurd, S.M., Moughan, P.J., 1990. Guanidination of lysine in selected dietary proteins. J. Agric. Food Chem. 38, 209–211.

Rutherfurd, S.M., Moughan, P.J., van Osch, L., 1997. Digestible reactive lysine in processed feedstuffs: application of a new bioassay. J. Agric. Food Chem. 45, 1189–1994.

Stein, H.H., Gibson, M.L., Pedersen, C., Boersma, M.G., 2006. Amino acid and energy digestibility in ten samples of distillers dried grain with solubles fed to growing pigs. J. Anim. Sci. 84, 853–860.