Absorption of Lysine and Deoxyketosyllysine in an Early-Maillard Browned Casein by the Growing Pig

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The aim was to determine the small intestinal uptake in the pig of unaltered (unreacted) lysine in a heat-damaged glucose/casein mixture, containing high concentrations of the early-Maillard compound, deoxyfructosyllysine. Diets containing a heated glucose/casein mixture, where approximately one-third of the original lysine was in the form of deoxyfructosyllysine, and an unheated glucose/casein mixture were each fed to four 10 kg live weight pigs. Chromic oxide was used as an indigestible marker, and digesta were sampled at slaughter, from the terminal ileum. The apparent ileal digestibility of unaltered lysine in the heated glucose/casein was less than unity (59 ± 2.3%, mean ± SE). Gel chromatography of digesta (heated glucose/casein) showed a 430 Da, deoxyfructosyllysine-containing peak, suggesting the presence of deoxyfructosyllysine-containing limit peptides. The FDNB reactive lysine method does not allow accurate assessment of lysine availability in heated proteins, due to incomplete digestion and absorption. Nor does the conventional ileal lysine digestibility assay provide accurate estimates due to the partial reversion of early-Maillard compounds to lysine during acid hydrolysis.

Keywords: *Maillard; lysine*

INTRODUCTION

Lysine is often the first limiting amino acid in diets for simple-stomached farm animals and for humans. Consequently, it is important to have accurate information on the lysine contents of foods and feedstuffs and on the digestibility of dietary lysine. For food material which has not undergone Maillard damage, conventional techniques of amino acid analysis and ileal digestibility determination are appropriate. However, for foods in which the Maillard reaction has occurred during storage or processing, application of the conventional techniques may lead to inaccuracies, namely an overestimation of the amount of available lysine. With early-Maillard damage, whereby early-Maillard compounds predominate, the deoxyketosyl derivative of lysine may revert to lysine during the acid hydrolysis step used in amino acid analysis. Thus, part of the determined lysine for the diet and ileal digesta will represent deoxyketosyllysine, which although being partly absorbed from the gut is of no nutritional value to the animal (Hurrell and Carpenter, 1981).

Consequently, the lysine contents of food and ileal digesta, determined by conventional amino acid analysis, are likely to be misleading. An alternative procedure with Maillard-damaged foods is to quantitate reactive lysine using a specific assay such as FDNB lysine (Carpenter, 1960). However, there is evidence that for foods which have undergone Maillard damage, the reactive lysine is incompletely absorbed (Boctor and Harper, 1967; Valle-Riestra and Barnes, 1969; Schmitz, 1988; Desrosiers *et al.*, 1989).

The aim of the present study was to determine the uptake of unaltered (reactive) lysine, in a defined heat-damaged glucose/casein mixture containing a high proportion of early-Maillard compounds, from the small intestine of the pig. The amounts of regenerated lysine (lysine regenerated from Maillard compounds during acid hydrolysis) and ϵ-*N*-deoxyfructosyllysine (altered or blocked lysine) in the diet and ileal digesta were derived from the determined concentration of furosine, and unaltered (reactive) lysine as the difference between lysine determined after acid hydrolysis and regenerated lysine.

MATERIALS AND METHODS

Preparation of the Glucose/Casein Mixtures. Unheated glucose/casein, heated glucose/casein, and heated [^{14}C]-glucose/casein mixtures were prepared for inclusion in semi-synthetic experimental diets. The unheated glucose/casein mixture was prepared by dry-blending 22 kg of spray-dried sodium caseinate (New Zealand Dairy Research Institute, Palmerston North, New Zealand) with 3 kg of anhydrous glucose, using a spiral-paddle mixer. The heated [^{14}C]glucose/casein mixture was prepared by adding 880 g of sodium caseinate to 4.4 L of water. The solution was heated to 70 °C and adjusted to pH 7.0 by the addition of 10 M NaOH. Anhydrous glucose (120 g) as a slurry and 1 mCi of D-[U-^{14}C]-glucose (230 mCi/mM, Amersham) were added, and the mixture, maintained at 70 °C, was stirred for 15 min. The mixture was subsequently freeze-dried and ground (1 mm screen). The resultant powder was subjected to heating in an autoclave at 121 °C for 1 min. The material was heated further (70 °C for 1 day) in a forced-air oven in which a moist atmosphere was maintained. The mixture was resuspended in 5 L of water at room temperature and adjusted to pH 7.0 by addition of 10 M NaOH. Unreacted glucose was removed by precipitating the protein (pH 4.5) and filtering. The precipitate was resuspended in water and reprecipitated and washed a further four times. The final precipitate was resuspended at 70 °C and the mixture adjusted to pH 7.0 by addition of 10 M NaOH. The suspension was freeze-dried, ground to pass through a 1 mm sieve, and mixed in a spiral-paddle mixer. The heated glucose/casein mixture was prepared in a similar manner to the [^{14}C]glucose/casein mixture except that no radioactive glucose was added, the casein/glucose solution was spray-dried instead of being freeze-dried, and unreacted glucose was not removed after heat treatment. The mixtures were stored in sealed plastic bags at 5 °C. The glucose/casein mixtures were subjected to duplicate analysis

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for FDNB-reactive lysine, and amino acids (including furosine), and ^{14}C radioactivity was also determined.

Chemical Analysis. FDNB-reactive lysine was determined following the procedure of Carpenter (1960) as modified by Booth (1971). The FDNB-reactive lysine values were multiplied by a factor of 1.09 to correct for dinitrophenyllysine loss during hydrolysis. Amino acids including furosine were determined in duplicate using a Beckman 119BL amino acid analyzer, utilizing postcolumn ninhydrin derivatization and detection at 570 nm, following hydrolysis in 6 M glass-distilled HCl containing 0.1% phenol for 24 h at 110 ± 2 °C in an evacuated sealed tube. Cysteine and tryptophan were not determined. Since no furosine standard was available, furosine was quantified using the peak response factor for the nearest eluting peak, arginine. Total nitrogen was determined using an automated Kjeldahl method (AOAC, 1975) and chromium by the method of Fenton and Fenton (1979). Radioactivity was measured in a toluene/Triton X-100 scintillant using a liquid scintillation counter (Beckman LS 8000). Insoluble samples were first solubilized with NCS (Nuclear Chicago Solubiliser) tissue solubilizer. [1-^{14}C]Hexadecane was included as an internal standard.

Determination of ϵ-Deoxyfructosyllysine, Regenerated Lysine, and Early- and Advanced-Maillard Compounds. Fructosyllysine (ϵ-DFL) is formed, during the heating of a food, from the reaction of glucose with lysine. Under defined acid hydrolysis conditions ϵ-DFL gives rise to pyridosine, lysine, and furosine in proportions specific to the hydrolysis conditions used (Finot and Mauron, 1972). If the latter proportions are known, and based on the estimated amounts of ϵ-DFL and reactive lysine, the relative proportions of early and advanced Maillard products relative to the lysine lost can be calculated (Hurrell and Carpenter, 1981).

In the present work, the Amadori compound, (ϵ-deoxyfructosyllysine; ϵ-DFL) was determined from the quantitation of furosine. The respective conversion factors, based on the yield of furosine from purified synthetic ϵ-DFL with the acid hydrolysis conditions employed in our laboratory, were determined in a preliminary study. The acid hydrolysis of ϵ-DFL produced three peaks which corresponded to pyridosine (retention time = 64 min), lysine (retention time = 87 min), and furosine (retention time = 152 min). Lysine generated from protein-bound ϵ-DFL during acid hydrolysis was determined by multiplying the determined furosine concentration by a conversion factor (1.28). The latter conversion factor was obtained by expressing moles of lysine (derived from the acid hydrolysis of purified free ϵ-DFL in the preliminary work) as a proportion of the moles of furosine, adjusted by a factor of 0.5 to account for the ϵ-DFL being protein bound rather than free (Finot *et al*., 1981). Free ϵ-DFL was estimated from the peak area of determined furosine on the basis of the yield of furosine from the hydrolysis of free synthetic ϵ-DFL. When the ϵ-DFL was assumed to be bound, the estimate of ϵ-DFL was increased by a factor of 60% based on the findings of Finot *et al*. (1981). Each of the glucose/casein mixtures underwent amino acid analysis, furosine was quantified using the response factor for arginine, and ϵ-DFL and regenerated lysine were calculated using the appropriate response factors.

Experimental Procedure. The three casein/glucose mixtures were each included as sole sources of protein in cornstarch-based semisynthetic diets which were formulated to meet the nutrient requirements of the young growing pig (Agricultural Research Council, 1981) (Table 1). Chromic oxide was added as an indigestible marker compound. Lysine monohydrochloride and anhydrous glucose were added as supplements to the heated glucose/casein diet.

Four littermate pairs of Landrace x Large White entire-male pigs (10 kg live weight) were individually housed in steel metabolism cages at an ambient temperature of 23 ± 1 °C. After a 3-day preliminary period, four pigs (one from each pair) were allocated to the unheated glucose/casein diet and the remaining four littermates to the heated glucose/casein diet. The animals received the diets at 0.08 metabolic body weight ($kg^{0.75}$) for a 26-day period. The food was given mixed with water in two equal meals (0830 and 1630 h) daily. The pigs were weighed at the beginning of the study and then re-

Table 1. Ingredient Compositions (g/kg Air-Dry Weight) of the Experimental Diets Given to the Pigs

ingredient	unheated glucose/casein	heated glucose/casein	heated [^{14}C]glucose/casein
maize starch	521.6	503	521.6
sucrose	50.1	48.2	50.1
dicalcium phosphate	40.0	38.6	40.0
maize oil	40.0	38.6	40.0
purified cellulose	35.1	33.8	35.1
vitamins, trace minerals[a]	5.0	4.8	5.0
potassium chloride	4.0	3.9	4.0
chromic oxide	3.0	2.9	3.0
magnesium sulfate (anhydrous)	1.2	1.2	1.2
unheated glucose/casein	300.0		
heated glucose/casein		289.3	
heated [^{14}C]glucose/casein			300.0
glucose (anhydrous)		24.3	
lysine (monohydrochloride)		11.4	

[a] Tasmix, pig creep vitamin mineral premix (Tasman Vaccines Ltd, Auckland, New Zealand).

weighed, and the meal intakes were adjusted on the basis of each pig's weight gain, on days 7 and 13. On day 26, each pig was given its daily allowance in seven equal portions as seven meals fed each hour commencing at 0400 h. The hourly feeding regime was adopted in an attempt to ensure an even flow of representative digesta at the terminal ileum. Those pigs which had previously received the heated glucose/casein diet were fed the heated [^{14}C]glucose/casein diet. One hour after the seventh meal, each pig was anaesthetized with halothane and killed by an intracardial injection of sodium pentobarbitone. The abdominal cavity was opened, and samples of jugular and portal blood were collected and allowed to clot at room temperature for 45 min. The clot was separated by centrifuging at 4500 rpm for 10 min. The serum was frozen (−20 °C) prior to analysis for furosine and radioactivity. The terminal 20 cm of ileum were removed, and the contents were gently flushed out with isotonic saline. The ileal digesta samples were freeze-dried, ground, and stored at −20 °C while awaiting analysis for chromium, total nitrogen, and amino acids (including furosine). A portion of freeze-dried digesta for each animal was pooled across animals within each treatment group (300 mg total) and subjected to gel filtration. The digesta samples were prepared for gel filtration by suspending in 0.2 M acetic acid, with the pH of the mixture being adjusted to 2.6 by addition of glacial acetic acid. The suspension was allowed to stand overnight at room temperature and was occasionally stirred to maximize solubilization. The suspension was then centrifuged at 1000*g* for 10 min to remove any particulate matter, and 3 mL of the supernatant was loaded onto a Sephadex G-15 superfine column (620 cm × 1.25 cm) in 0.2 M acetic acid, and fractions eluted with 0.2 M acetic acid at a flow rate of 20 mL/h. Absorbance at 280 nm as well as radioactivity and ninhydrin-reactive compounds were determined in all fractions. ϵ-DFL and total lysine were determined in pooled fractions corresponding to selected molecular weight ranges. For the diet samples, ϵ-DFL was assumed to be protein-bound, and a correction factor of 1.6 was applied to calculated ϵ-DFL levels, while in the small intestine ϵ-DFL was assumed to be half protein-bound and half free so a correction factor of 1.3 was applied. In the blood ϵ-DFL was assumed to be free so no correction factor was required.

Apparent digestibility coefficients for several dietary components were determined by reference to the indigestible dietary marker compound chromic oxide, using the following equation:

$$\text{digestibility of compound (\%)} = \frac{\dfrac{\text{mg compound (diet)}}{\text{mg chromium (diet)}} - \dfrac{\text{mg compound (digesta)}}{\text{mg chromium (digesta)}}}{\dfrac{\text{mg compound (diet)}}{\text{mg chromium (diet)}}} \times 100 \quad (1)$$

Table 2. Total Lysine, FDNB-Reactive Lysine, Furosine, Regenerated Lysine, and Early- and Advanced-Maillard Products (% Lysine Lost during Heating) in the Glucose/Casein Mixtures

	unheated glucose/casein	heated glucose/casein	heated [^{14}C]glucose/casein
total lysine[a] (mmol/100 g)	42.8	31.6	27.4
reactive lysine[b] (mmol/100 g)	43.6	22.7	19.3
lysine lost[c] (mmol/100 g)		20.9	24.3
furosine[d] (mmol/100 g)	0	5.15	4.86
regenerated lysine[e] (mmol/100 g)	0	6.62	6.25
ϵ-DFL[f] (mmol/100 g)	0	13.3	12.6
early-Maillard compounds (ϵ-DFL)[g]		63	52
advanced-Maillard compound[h]		37	48

[a] Determined using conventional amino acid analysis procedures. [b] Determined using the FDNB method. [c] Calculated as the difference between the total lysine and the FDNB-reactive lysine. [d] Determined, based on the response factor for arginine. [e] Calculated from furosine using a correction factor of 1.28 (refer to Materials and Methods). [f] Calculated from the furosine peak area (refer to Materials and Methods). [g] The ϵ-DFL given as a percentage of the lysine lost. [h] Calculated as the difference between lysine lost and ϵ-DFL and assumed to be advanced-Maillard compounds.

Data were subjected to analysis of variance and levels of significance were determined by reduction in sums of squares (Snedecor and Cochran, 1980). The estimated variances were tested for homogeneity by using Bartlett's test (Snedecor and Cochran, 1980).

RESULTS

Preparation of the Glucose/Casein Mixtures. The two heated glucose/casein preparations contained similar amounts of total lysine and reactive lysine respectively (Table 2). In both preparations, about 50% of the original lysine was structurally altered and 70% of the glucose was either destroyed or had become bound to the casein. No furosine was detected in the unheated glucose/casein mixture, but a considerable quantity was found in the heated material. Regenerated lysine plus the determined reactive lysine approximately equalled the determined total lysine. For the structurally altered lysine in the heated glucose/casein preparations, approximately 63% and 52% were in the form of early Maillard products in the heated glucose/casein and heated [^{14}C]glucose/casein, respectively. Furthermore, the remainder of the altered lysine (approximately 37% and 48%) was assumed to be in the form of advanced-Maillard products in both the heated glucose/casein and heated [^{14}C]glucose/casein.

Digestion and Absorption of Amino Acids and Maillard Products As Determined at the Terminal Ileum. The animals consumed their respective diets readily, and there were no food refusals. The concentrations of unaltered lysine (determined as the difference between total lysine and regenerated lysine which was estimated from determined furosine) were similar for the heated glucose/casein and the [^{14}C]glucose/casein (25.0 and 21.2 mmol/100 g, respectively) and were also similar to the estimates of reactive lysine (Table 2). The ileal digestibility of unaltered lysine was considerably lower and more variable for the heated glucose/casein, compared to the unheated diet (Table 3). However, since regenerated lysine is quantified using furosine levels and the conversion factor from furosine to regenerated lysine differs depending upon whether ϵ-DFL is free or peptide bound, the digestibilities of unaltered lysine in pigs fed the heated diet may have ranged from 53% to 65%, depending on the proportion of bound or free ϵ-DFL in the digesta. In either case, the digestibility of unaltered lysine in the heated diet was much lower than that found for the unheated diet. The mean apparent ileal digestibility for most amino acids in the unheated glucose/casein were high (Table 4). Glycine was the exception, with a digestibility of 50%, although the data for glycine were highly variable. The digestibility of total nitrogen was 90%. For several of the amino acids, the digestibility was around 4% lower for the heated glucose/casein compared to that of the unheated glucose/casein.

Table 3. Mean ± SE (n = 4) Ileal Lysine Digestibility Coefficients (%) for Pigs Fed an Unheated or Heated Glucose/Casein Diet

	unheated glucose/casein diet	heated glucose/casein diet
ileal lysine digestibility (%)	96.3 ± 0.3[a]	59.0 ± 2.3[b]

[a] There was no furosine detected in the unheated glucose/casein diet or digesta of pigs fed this diet; lysine digestibility in the unheated glucose/casein mixture was estimated by determining the lysine in diets and ileal digesta based on conventional amino acid analysis including acid hydrolysis. [b] Lysine (unaltered lysine) in diets and ileal digesta were calculated as the difference between the total lysine determined from conventional amino acid analysis and the regenerated lysine determined from furosine levels. This value is the average digestibility from a range of digestibilities (53−65%) determined using conversion factors of 1.6 and 3.2, which correspond to the amounts of unaltered lysine per unit furosine that results from hydrolysis of bound and free ϵ-DFL, respectively.

Table 4. Mean (n = 4) Apparent Ileal Digestibility of Amino Acids and Nitrogen for Pigs Fed an Unheated Glucose/Casein Diet or a Heated Glucose/Casein Diet

	unheated glucose/casein	heated glucose/casein	overall standard error	significance[a]
aspartic acid	89.05	89.33	0.69	NS
threonine	84.95	80.83	0.96	*
serine	89.68	86.88	0.41	**
glutamic acid	95.05[b]	93.30	0.21	**
proline	96.55	94.28	0.22	***
glycine	49.65	54.30	8.00	NS
alanine	89.45	87.65	1.01	NS
valine	94.20	91.10	0.41	**
methionine	98.35	95.80	0.25	**
isoleucine	95.38	93.03	0.45	*
leucine	96.58	96.10	0.37	NS
tyrosine	97.05	96.18	0.28	NS
phenylalanine	95.83	95.63	0.36	NS
histidine	96.10	93.20	0.31	***
arginine	94.78	88.00	0.81	**
nitrogen	89.83	84.25	0.73	**

[a] NS, not significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. [b] $n = 3$.

Around half (46 ± 5.9%; mean ± SE; range 34% for free to 58% for bound) of the ingested ϵ-DFL disappeared from the small intestine of the pigs fed the heated glucose/casein diet and 70 ± 1.2% (mean ± SE) of the total radioactivity disappeared during transit through the small intestine.

Molecular Weight Distribution of Compounds (Amino Acids, Peptides, and Maillard Products) in the Ileal Digesta. The molecular weight profile for ileal digesta was determined using a Sephadex G-15 column calibrated by using urea, leucine, glutathione, and bovine serum albumin. The molecular weight profile for compounds that absorb at 280 nm (Figure 1A), which include protein and peptides containing tryptophan or possibly other aromatic amino acids, for the digesta of pigs fed the unheated casein showed a large peak in the void volume (MW > 1500) and few

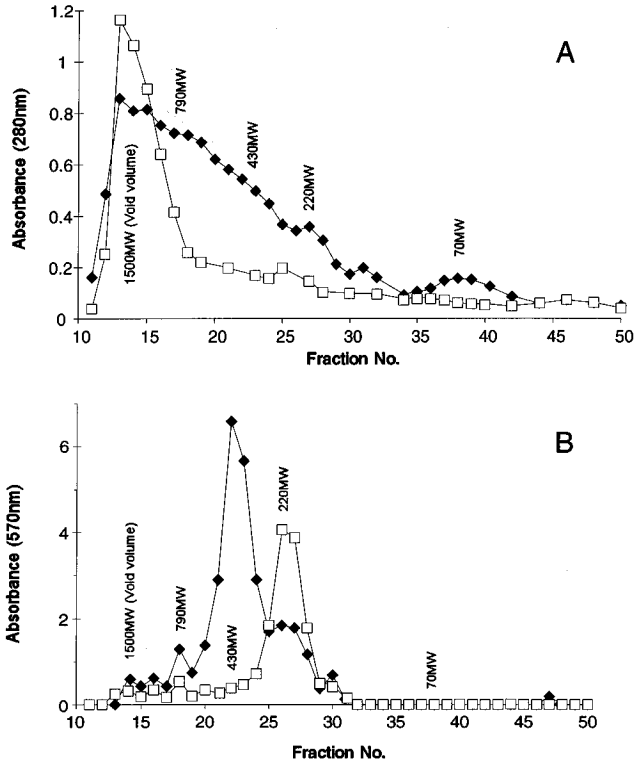

Figure 1. Elution of compounds that absorb at 280 nm (profile A) and ninhydrin-positive compounds (profile B) present in digesta collected from the terminal ileum of pigs fed an unheated glucose/casein mixture (□) and a heated [^{14}C]glucose/casein mixture (◆) from a Sephadex G-15 gel filtration column.

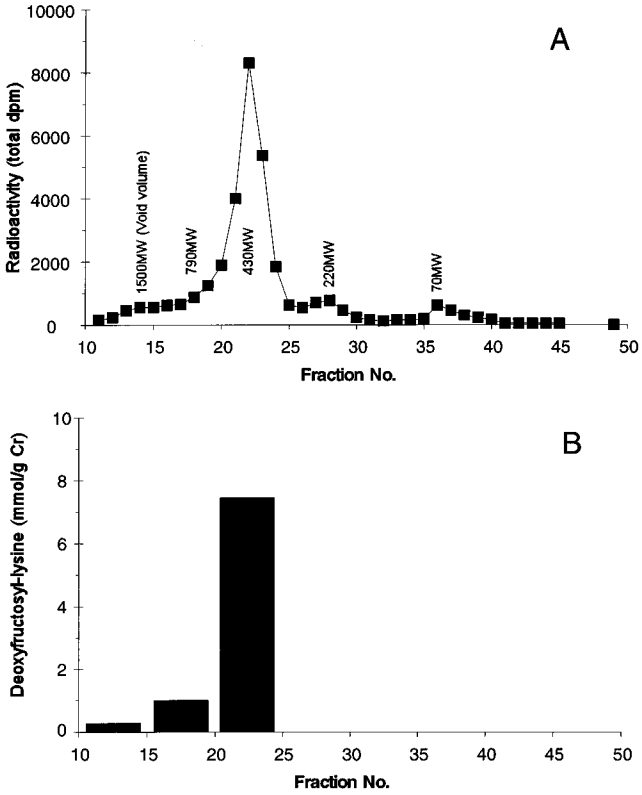

Figure 2. Elution of ^{14}C-containing compounds (profile A) present in digesta collected from the terminal ileum of pigs fed a heated [^{14}C]glucose/casein mixture from a Sephadex G-15 gel filtration column, and ϵ-DFL (determined from the furosine content) (profile B) present in digesta collected from the terminal ileum of pigs fed a heated [^{14}C]glucose/casein mixture.

peaks corresponding to fractions containing compounds of 800 Da or less. For the heated [^{14}C]glucose/casein mixture, there was also a large peak in the void volume; however, there were also considerably more compounds that absorbed at 280 nm, presumably peptides, present in the region of 100−1500 Da.

The approximate molecular weights of ninhydrin-positive compounds, including proteins, peptides, and amino acids, present in the digesta of pigs fed an unheated glucose/casein diet and a heated [^{14}C]glucose/casein diet (Figure1B), as well as radioactive compounds and ϵ-DFL (determined from the furosine content), present in the digesta of pigs fed the heated [^{14}C]glucose/casein diet (Figure 2) are shown. The void volume from profiles for both the unheated and heated glucose/casein contained only small amounts of ninhydrin-positive compounds corresponding to the large void volume peak at 280 nm. The molecular weight for most of the ninhydrin-positive compounds present in the digesta of pigs fed an unheated glucose/casein diet was about 220 Da. This peak was also present for the digesta of pigs fed the heated glucose/casein diet, although the predominant peak was one containing compounds, presumably peptides, of molecular weight about 430 Da. The 430 Da peak also contained in excess of 10 times more lysine (determined after acid hydrolysis) than any other peak in the digesta from pigs fed either the heated or unheated diets. The "430 Da" peak also contained most of the radioactivity and ϵ-DFL present in the digesta, although a small amount of radioactivity was present in peaks containing compounds of MW 220 and 70 Da and between 600 and 1500 Da, and a small amount of ϵ-DFL was present in the fraction containing compounds of higher molecular weight.

Maillard Product Concentrations in the Portal and Jugular Serum. The concentrations of ϵ-DFL and radioactivity in the portal and jugular serum obtained from pigs fed the heated [^{14}C]glucose/casein diet are reported in Table 5. The ϵ-DFL concentrations were calculated on the basis of the furosine contents of acid-hydrolyzed serum supernatants obtained by precipitating protein with 10% TCA. Furosine and radioactivity were detected in both the portal and jugular serum. The ϵ-DFL concentration in the jugular serum was approximately half that in the portal serum. A similar distribution was observed for the radioactivity. No furosine was found in the serum of pigs fed the unheated glucose/casein.

Table 5. Mean ± SE (n = 4) Concentrations of ϵ-DFL[a] and Total Radioactivity in the Portal and Jugular Serum of Pigs Fed a Heated Glucose/Casein Mixture

ϵ-DFL (nmol/mL of serum)		total radioactivity (dpm/mL of serum)	
portal	jugular	portal	jugular
23.9 ± 1.3	11.4 ± 0.4	191 ± 10	108 ± 2

[a] Calculated based on the determined furosine content of the serum.

DISCUSSION

In the presently described study, glucose and casein were heated, under defined reaction conditions, to produce early- (ϵ-DFL) and advanced-Maillard compounds. The aim was to produce a model compound in which there were high amounts of early-Maillard products. This would then allow characterization of the uptake of undamaged or "unaltered" lysine and that of the early-Maillard compounds during digestion. This objective was achieved. Approximately half of the

original total lysine (unheated mixture) was lost during processing, indicating that a considerable degree of damage had occurred. Of the lysine lost, over half could be accounted for as the early-Maillard compound, ϵ-DFL, with the remainder being converted to advanced-Maillard compounds. Therefore, the heated material which was used as the sole source of protein in the experimental diet, contained significant quantities of ϵ-DFL and was thus suitable as a model compound to examine digestion of a protein which had undergone early-Maillard damage.

In the present study "total" lysine determined using conventional amino acid analysis overestimated the structurally unaltered lysine in the heated diet but not in the unheated diet. This is consistent with the findings of other workers who have also reported that total lysine is usually considerably higher than reactive lysine (Erbersdobler and Anderson, 1983), as lysine is regenerated on acid hydrolysis of ϵ-DFL. In these situations FDNB-reactive lysine gives a better estimate of the nutritionally available lysine than the acid-hydrolyzed lysine estimates. However, in more severely heat-damaged proteins, where there is actual destruction of lysine, there is good agreement between FDNB-reactive lysine and the total lysine obtained after acid hydrolysis (Erbersdobler and Anderson, 1983).

With the exception of glycine, the amino acids in the unheated casein were highly absorbed. Furthermore, the heat treatment of the glucose/casein mixture caused significant decreases in ileal digestibility for several amino acids. Most notable, however, was the marked decrease in the digestibility of lysine from 96% in the unheated glucose/casein to 59% in the heated glucose/casein.

The true ileal amino acid digestibility assay provides an accurate assessment of lysine availability in unheated proteins, while in heated protein sources it overestimates lysine availability (Batterham, 1992). The results from the present study reflect this finding, as shown in the heated glucose/casein where the digestible total lysine (17.1 mmol/100 g) calculated from the total lysine content of the diet (31.6 mmol/100 g) and total lysine digestibility (54%) was 16% higher than the digestible unaltered lysine (14.7 mmol/100 g) calculated from the unaltered lysine content of the diet (25.0 mmol/100 g) and the unaltered lysine digestibility coefficient (59%). Since digestible unaltered lysine better reflects lysine availability than digestible total lysine, an ileal digestibility assay where unaltered lysine (i.e., chemically reactive lysine) was determined in the diets and digesta samples would be a major advance in the assessment of lysine availability in heated protein sources. The FDNB method, used in this study, accurately determined the reactive lysine in both diets, but would prove inadequate for determining reactive lysine in digesta. This is because there is a much higher proportion of free lysine and N-terminally bound peptide lysine in digesta and since FDNB will bind to both the N-terminal and side chain amino groups of lysine, so that using this method would lead to an overestimate of unaltered lysine digestibility in digesta. The reaction of *O*-methylisourea with the ϵ-amino group of lysine (guanidination) may be a suitable alternative.

The molecular weight profile at 280 nm for the digesta of pigs fed the unheated glucose/casein revealed a large peak in the void volume, which probably contained endogenous protein, but few lower molecular weight peptide-containing peaks, although at 280 nm only protein and peptides containing tryptophan and possibly other aromatic residues would be detected. Using ninhydrin, endogenous protein was not well detected, since there are relatively few amino groups present per mole of protein. However, with ninhydrin detection, there again appeared to be few peptides present in the digesta of pigs fed the unheated glucose/casein, with the exception of a peak at 220 Da, which is what would be expected for a highly digestible protein such as unheated casein. In contrast, there were considerably more peptides (MW's 220−1500) present in the digesta of pigs fed the heated [^{14}C]glucose/casein when detected at 280 nm or using ninhydrin. In addition, there was a large ninhydrin-positive, radioactivity- and ϵ-DFL-containing peak of molecular weight 430 Da for the digesta of pigs fed the heated glucose/casein. This peak most likely contains ϵ-DFL bound in limit peptides, possibly di- or tripeptides, resulting from the incomplete digestion of the heat-damaged casein. It is also possible that the reduced digestibility observed in this study may be due firstly to an inability of some digestive proteases to cleave around the sites of lysine modification and secondly to competition with other amino acids for transport carriers or blockage of these carriers by ϵ-DFL or ϵ-DFL-containing peptides. This would be consistent with the earlier findings of Sherr *et al.* (1989), who reported that free ϵ-DFL competed with lysine for the same absorption carrier, and the findings of Erbersdobler (1977), who found that ϵ-DFL had a high affinity for gut wall components and that its presence led to a decrease in the transport of neutral short-chain amino acids. Thirdly, it is possible that an increased endogenous amino acid loss may have accounted for the decrease in apparent digestibility from the unheated to heated glucose/casein diets.

Uptake of Maillard Compounds following the Ingestion of an Early-Maillard Browned Casein. Approximately half of the ϵ-DFL and 70% of total radioactivity ingested disappeared from the small intestine. The data were highly variable, indicating large interanimal differences in the degree of uptake of the Maillard compound. Furthermore, the analysis of portal and jugular serum found ϵ-DFL and radioactive compounds to be present. It is not known whether ϵ-DFL is present in the blood in a free or peptide-bound form, or both, and since total blood volumes were not determined, it is not possible to relate the ϵ-DFL level to dietary intake. However, this study provides clear evidence that Maillard compounds such as ϵ-DFL and possibly other more advanced Maillard products are absorbed from the small intestine. The proportion of ingested ϵ-DFL absorbed from the small intestine (46%) was somewhat lower than the proportion of lysine absorbed (59%). Further, it should be noted that the absorption of ϵ-DFL is a true estimate while the lysine digestibility is an apparent estimate. Consequently, the difference between ϵ-DFL absorption and lysine digestibility is even greater than presented above. Since the proportions of ϵ-DFL and lysine that are absorbed from the small intestine are different, this casts doubt as to the accuracy of the conventional ileal digestibility assay when determining lysine digestibility in heated proteins, as the proportion of ϵ-DFL and lysine absorbed from the small intestine must be the same and the proportion of ϵ-DFL reverting to lysine in both diets and digesta must also be the same if ileal digestible lysine estimates are to be accurate.

In summary, heat treatment slightly reduced the digestibility of most amino acids in a glucose/casein mixture and may have led to the formation during digestion of limit peptides which appear to be associated with Maillard compounds, particularly ϵ-DFL. Some ϵ-DFL was absorbed from the small intestine and was detected in the blood. The digestibility of unaltered lysine was considerably less than 100% for the heated glucose/casein mixture. It is apparent, therefore, that some form of correction for digestibility of reactive lysine must be made when assessing protein quality. The FDNB method, while providing an accurate assessment of the reactive lysine content of protein sources, does not allow an accurate assessment of lysine availability in heated proteins, as incomplete digestibility is not taken into account. The conventional ileal digestibility assay does not give an accurate estimation of lysine digestibility in heat-processed feedstuffs owing to the partial reversion of early-Maillard compounds to lysine during acid hydrolysis.

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