Assessment of the True Ileal Digestibility of Reactive Lysine as a Predictor of Lysine Uptake from the Small Intestine of the Growing Pig

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The aim of this study was to evaluate the accuracy of a newly developed assay for determining digestible reactive lysine in processed protein sources. Pigs were fed either a heated skim milk powder based diet or one of two control diets in which the sole source of nitrogen was a mixture of enzymatically hydrolyzed casein (EHC) and synthetic amino acids. One control diet (EHC diet A) was formulated to contain the same amount of digestible lysine (determined using a conventional true ileal amino acid digestibility assay) while the other (EHC diet B) was formulated to contain the same amount of digestible reactive lysine (determined using the new assay) present in the test diet. Lysine was the first limiting amino acid in all three diets. The whole body lysine deposition and empty body weight gain of pigs fed the heated skim milk powder diet were not significantly different from those of pigs fed EHC diet B but were significantly higher than those of animals fed EHC diet A. This experiment demonstrates that the new assay for determining digestible reactive lysine could be more accurate when applied to this heated protein source than the conventional true ileal amino acid digestibility assay.

Keywords: *Digestible reactive lysine; available lysine; lysine deposition; amino acid; digestibility; availability*

INTRODUCTION

During the processing of feedstuffs lysine can react with other compounds to produce nutritionally unavailable products. However, some of these products can revert to lysine during the acid hydrolysis step of amino acid analysis, leading to inaccuracies in compositional data and lysine digestibility estimates for processed feedstuffs (Hurrell and Carpenter, 1981). Recently, a new method that overcomes this problem has been developed with application to processed feedstuffs (Moughan and Rutherfurd, 1996). This method, which yields estimates of digestible reactive lysine in feedstuffs, utilizes the guanidination reaction and is based on a determination of the reactive lysine contents of diets and ileal digesta. The method has been applied to a number of processed feedstuffs to provide realistic estimates of digestible reactive lysine (Rutherfurd *et al*., 1997). Furthermore, digestible reactive lysine values for heated field peas determined using the digestible reactive lysine assay compared closely with available lysine estimates determined using growth studies such as the slope ratio assay and regression methods (Rutherfurd and Moughan, 1997). Despite this strong suggestive evidence that the digestible reactive lysine assay does indeed accurately predict available lysine in heat-processed feedstuffs, further direct evidence is required to fully validate the new assay.

This study aimed to evaluate the accuracy of the digestible reactive lysine assay (pig as the test animal) when applied to a heated skim milk powder. Body lysine deposition, which constituted a test for the accuracy of prediction of lysine absorption from the digestive tract, was determined in growing pigs fed one of three experimental diets. The first diet contained heated skim milk powder as the sole source of protein. The second diet contained enzymatically hydrolyzed casein (EHC) and free amino acids as the sole nitrogen source and was formulated such that the lysine content was based on the total lysine digestibility of the heated skim milk powder diet determined using a conventional ileal digestibility technique (EHC diet A). The third diet also contained EHC and free amino acids as the sole nitrogen source, but the lysine content was based on the reactive lysine digestibility of the heated skim milk powder diet determined using the new digestible reactive lysine assay (EHC diet B). It was assumed that the EHC and free amino acids were completely digested and absorbed and that all diets were first limiting in lysine. If body lysine deposition was the same for pigs fed the heated skim milk powder diet and the EHC diet B and was significantly higher than that for pigs on the EHC diet A, then this would confirm the accuracy of the new assay.

MATERIALS AND METHODS

Materials. *O*-Methylisourea was obtained from Sigma Chemical Co., St. Louis, MO, and barium hydroxide octahydrate was obtained from BDH Laboratory Supplies, Poole, England. Skim milk powder was sourced from Tui Nutriproducts, Palmerston North, New Zealand. Enzymatically hydrolyzed casein used in the growth study was obtained from New Zealand Pharmaceuticals Ltd., Palmerston North, New Zealand, and contained peptides no larger than 2000 Da, while the enzymatically hydrolyzed casein used in the digestibility study was obtained from Sigma. Centriprep 10 disposable ultrafiltration devices were obtained from Amicon, Inc., Beverly, MA. Pigs [Large White/Landrace (LW/LR) crossbred] were obtained from the Pig Research Unit, Massey University, Palmerston North, New Zealand.

Preparation of 0.6 M *O*-Methylisourea Solution. A 0.6 M *O*-methylisourea solution was prepared as described by Moughan and Rutherfurd (1996), based on the procedures of Chervenka and Wilcox (1956), Shields *et al*. (1959), Mauron and Bujard (1964), and Kassell and Chow (1966).

Preparation of Protein Sources. Skim milk powder (120 kg) was autoclaved for 5 min at 121 °C in 5 kg batches, then

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Table 1. Ingredient Compositions (Grams per Kilogram of Air-Dry Weight) of the Experimental Diets Used for the Determination of True Ileal Reactive and Total Lysine Digestibilities in a Heated SMP

	basal SMP	heated SMP	EHC
corn flour	488.2	492.4	551.2
maize oil	50.0	50.0	50.0
cellulose	50.0	50.0	50.0
sucrose	100.0	100.0	100.0
vitamin/mineral mix[a]	50.0	50.0	50.0
lactose			70.0
SMP	256.8		
heated SMP		252.6	
EHC			123.8
chromic oxide	5.0	5.0	5.0

[a] Vitamin/mineral mix was formulated to meet the pigs' requirements for vitamins and minerals in the final diets as described by the Agricultural Research Council (1981).

ground through a 1 mm mesh, and mixed thoroughly. Samples taken for chemical analysis were further ground through a 0.5 mm mesh.

Digestibility Study. A digestibility study was conducted to determine the true ileal digestibility of the heated skim milk powder (SMP). Ethics approval for the animal trial was obtained from the Animal Ethics Committee, Massey University, Palmerston North, New Zealand. Entire male pigs, of approximately 25 kg body weight, were housed individually in metabolism crates in a room maintained at 22 ± 2 °C. A semisynthetic test diet containing the heated SMP was formulated as shown in Table 1, to contain 100 g/kg crude protein. An EHC-based diet was also formulated (Table 1), to allow determination of endogenous ileal amino acid flows (Moughan *et al.*, 1990; Butts *et al.*, 1991). Chromic oxide (0.5%) was included in each diet as an indigestible marker. The diets were randomly allocated to the pigs such that there were eight pigs on each diet. The pigs were fed a basal SMP based diet for the first 7 days of the trial, after which time they were fed their respective test diet for a further 7 days. On each day each pig received its respective diet as nine meals given hourly (8:30 a.m. to 4:30 p.m.). Water was available at all times. On the 14th and final day of the study, from 6 to 8 h after the start of feeding, the pigs were euthanised by an intracardial injection of sodium pentobarbitone following deep anesthesia with halothane. The 20 cm of ileum immediately anterior to the ileocecal junction was dissected out. The dissected ileum was washed with distilled deionized water to remove any blood and hair and carefully dried on an absorbent paper towel. The digesta were then gently flushed from the ileum section with distilled deionized water from a syringe. The digesta from the pigs given the SMP diet were freeze-dried ready for chemical analysis. The digesta from pigs fed the EHC diet were processed ready for chemical analysis as described by Moughan and Rutherfurd (1996).

Chemical Analysis. Amino acids were determined in duplicate 5 mg feedstuff and digesta samples and quadruplicate 5 mg diet samples using a Waters ion-exchange HPLC system, utilizing postcolumn ninhydrin derivatization and detection using absorbance at 570 nm (440 nm for proline), following hydrolysis in 6 M glass-distilled HCl containing 0.1% phenol for 24 h at 110 ± 2 °C in evacuated sealed tubes. Cysteine, methionine, and tryptophan were not determined as they are partially destroyed during acid hydrolysis. The weight of each amino acid was calculated using free amino acid molecular weights.

Reactive lysine contents were determined in duplicate 5 mg feedstuff and digesta samples and quadruplicate 5 mg diet samples by incubation for 1, 7, and 7 days, respectively, in 0.6 M *O*-methylisourea, pH 10.6 (pH 11.0 for the digesta samples), in a shaking water bath at 21 ± 2 °C, with the reagent to lysine ratio being >1000. After incubation, the samples were taken to dryness under reduced pressure using a Speedvac concentrator (Savant Instruments, Inc., Farmingdale, NY) and analyzed for amino acid content as described above.

The chromium contents of the diet and ileal digesta samples were determined in duplicate on a GBC 902 AA absorption/emmission spectrophotometer (GBC Scientific NZ Ltd., Auckland, New Zealand) following the method of Costigan and Ellis (1987).

Data Analysis. Amino acid flows at the terminal ileum were calculated using the following equation [units are micrograms per gram of dry matter intake (DMI)]:

$$\text{ileal amino acid flow} = \text{amino acid concentration in ileal digesta} \times (\text{diet chromium/ileal chromium})$$

Endogenous amino acid flows were determined using the above equation as applied to processed ileal digesta from pigs given the EHC-based diet.

True ileal amino acid digestibility was calculated using the following equation (units are micrograms per gram of DMI):

$$\text{true digestibility} = \{[\text{dietary amino acid intake} - (\text{ileal amino acid flow} - \text{endogenous amino acid flow})]/(\text{dietary amino acid intake})\} \times 100$$

True ileal reactive lysine digestibility was calculated using the following equation (units are micrograms per gram DMI):

$$\text{true reactive lysine digestibility} = \{[\text{dietary reactive lysine intake} - (\text{ileal reactive lysine flow} - \text{endogenous lysine flow})]/(\text{dietary reactive lysine intake})\} \times 100$$

Preliminary Nitrogen Balance Study. A nitrogen balance study was conducted preliminary to the main growth study to demonstrate that lysine was the first-limiting amino acid in a heated SMP-based diet. The heated SMP-based diet (SMP was the sole source of protein) was formulated to contain similar net energy and fiber levels and to have similar amino acid balances to those of EHC diets A and B (Table 2).

Six 25 kg live weight entire male pigs were housed singly in metabolism crates designed for the complete and separate collection of urine. The pigs underwent a 6 day acclimatization period, during which time the pigs were fed the basal SMP-based diet. After this period, three pigs received the heated SMP diet for a further 6 days, while the remaining three animals received the heated SMP diet supplemented with 0.25% synthetic lysine. The total daily urine volume for each pig was determined by collecting the urine output daily for each pig for each of the first 3 days of the 6 day test period. Total urine was collected for each day of the last 3 days of this 6 day period into a bottle containing 25 mL of 1.8 M H_2SO_4/L of urine. The walls of the metabolism crates were also washed down with distilled water. After this 6 day period, the diets for the two groups of pigs were swapped and again total urine was collected for each day of the last 3 days of the 6 day period. Each urine sample for each pig and for each collection day was analyzed for creatinine content using a Roche reagents kit for creatinine followed by analysis on a COBAS FARA autoanalyzer. Urine samples for each pig were pooled over days, and the pooled samples were then analyzed for total nitrogen content. Nitrogen was determined according to the Kjeldahl method on a Kjeltec Auto 1030 analyzer. The urinary nitrogen output for the pigs fed the heated SMP diet was compared with that for the pigs receiving the heated SMP diet supplemented with lysine.

Main Study. Three experimental diets (Table 2) were formulated for the main growth study and are described below:

Heated SMP. Heated SMP and synthetic amino acids were the sole source of nitrogen.

EHC diet A contained EHC plus free amino acids formulated to contain an amount of lysine equal to the digestible lysine content of the heated SMP diet determined using the conventional ileal digestibility assay [reactive lysine in heated SMP diet × true digestibility of total lysine (determined using conventional methods) for the heated SMP diet].

Table 2. Ingredient Compositions (Grams per Kilogram of Air-Dry Weight) of the Experimental Diets for the Preliminary Nitrogen Balance Study and the Growth Study

	basal SMP	heated SMP	EHC diet A	EHC diet B
corn flour	365.0	345.0	401.4	372.1
maize oil	50.0	50.0	50.0	50.0
cellulose	50.0	50.0	50.0	50.0
sucrose	100.0	100.0	100.0	100.0
vitamin/mineral mix[a]	50.0	50.0	50.0	50.0
citric acid			6.4	6.4
sodium citrate			0.8	0.8
dicalcium phosphate			2.0	2.0
magnesium oxide			0.007	0.007
potassium chloride			1.2	1.2
lactose			191.7	191.7
SMP	385.0			
heated SMP		385.0		
EHC			90.5	108.6
asparagine			1.7	2.0
aspartic acid			1.7	2.0
threonine			2.0	2.4
serine			1.8	2.1
glutamic acid			8.1	9.7
glycine		11.0	9.7	11.6
alanine		9.0	8.3	10.0
valine			1.9	2.3
cysteine			0.6	0.7
methionine			0.6	0.7
isoleucine			1.3	1.5
leucine			4.5	5.4
tyrosine			3.2	3.9
phenylalanine			3.2	3.9
tryptophan			2.2	2.6
histidine			1.4	1.7
arginine			1.0	1.2
proline			2.9	3.5

[a] Vitamin/mineral mix was formulated to exceed the pigs' requirements for vitamins and minerals in the final diets as described by the Agricultural Research Council (1981).

EHC diet B contained EHC plus free amino acids formulated to contain an amount of lysine equal to the digestible lysine content of the heated SMP diet determined using the new ileal reactive lysine digestibility assay [reactive lysine in heated SMP diet × true digestibility of reactive lysine [determined using the new method (Moughan and Rutherfurd, 1996)] for the heated SMP diet]. Citric acid, sodium citrate, dicalcium phosphate, magnesium oxide, potassium chloride, and lactose were added to the EHC-based diets since the SMP contained more citrate, calcium, phosphorus, magnesium, potassium, and lactose than that present in the EHC (R. Lloyd, personal communication).

The macronutrient (including amino acids) and vitamin and mineral compositions of each diet are shown in Tables 3 and 4, respectively. Furthermore, the ratio of the amount of each amino acid expressed relative to the reactive lysine content for each diet is shown in Table 5.

Thirty-two entire male pigs (29.1 kg live weight, SEM 0.79, CV 15.3%) were penned in pairs at the Pig Research Unit, Massey University, in a temperature-controlled room maintained at 22 ± 2 °C. Twenty-four of the pigs were randomly allocated to the three experimental diets such that there were eight pigs on each diet. The remaining eight pigs were designated as baseline animals and were used to determine the initial whole body lysine content.

The pigs underwent a 7 day acclimatization period during which they received the basal SMP-based diet (Table 2). The pigs were fed at 0.10 metabolic body weight ($LW^{0.75}$) and were given their respective daily allowance as a slurry (approximately 50% w/w dry matter) in three equal meals. Any refusals were collected, dried, and weighed. At the end of the acclimatization period, the pigs were weighed and feed intakes were readjusted. At this time the eight baseline pigs were slaughtered and the digestive tract, gall bladder, and bladder contents were removed. Care was taken to recover any blood lost from the body. The pig bodies were sealed in plastic bags and stored at −20 °C. The remainder of the pigs were assigned

Table 3. Macronutrient Compositions (Calculated) for the Three Experimental Diets

	heated SMP	EHC diet A	EHC diet B
gross energy (MJ/kg of air-dry wt)	15.1	15.7	15.6
macronutrients (g/kg of air-dry wt)			
starch	219.0	319.2	269.6
fat	50.0	50.0	50.0
cellulose	50.0	50.0	50.0
sucrose	150.0	150.0	150.0
lactose	215.6	215.6	215.6
citric acid	8.0	8.0	8.0
protein	190.9	125.7	166.4
digestible amino acids (g/kg of air-dry wt)			
reactive lysine	7.46[a]	5.68	7.46
aspartic acid/asparagine	11.05	8.41	11.05
threonine	5.40	4.11	5.40
serine	6.33	4.82	6.33
glutamic acid/glutamine	49.02	37.31	49.02
glycine	22.32	16.98	22.32
alanine	12.84	9.77	12.84
valine	8.46	6.44	8.46
cystine/methionine	11.57	8.81	11.57
isoleucine	6.58	5.01	6.58
leucine	14.11	10.73	14.11
tyrosine/phenylalanine	13.81	10.51	13.81
tryptophan	4.78	3.64	4.78
histidine	3.73	2.84	3.73
arginine	4.57	3.48	4.57
proline	11.12	8.46	11.12

[a] The actual reactive lysine content of the final mixed diet was subsequently found by chemical determination to be 18% higher in the heated skim milk powder diet than that which had been originally formulated, based on the determined reactive lysine in the original heated skim milk powder.

Table 4. Final Vitamin and Mineral Compositions for the Three Experimental Diets

	heated SMP	EHC diet A	EHC diet B	requirement[a]
thiamin	1.53	1.53	1.53	1.50
riboflavin	2.54	2.54	2.54	2.50
niacin	14.23	14.23	14.23	14.00
pantothenate	10.17	10.17	10.17	10.00
vitamin A	0.61	0.61	0.61	0.60
vitamin D	0.0036	0.0036	0.0036	0.0035
vitamin E	5.08	5.08	5.08	5.00
vitamin K	0.20	0.20	0.20	0.20
vitamin B_6	2.54	2.54	2.54	2.50
vitamin B_{12}	0.010	0.010	0.010	0.01
Ca	9142.4	9142.4	9142.4	9000.0
P	7148.8	7148.8	7148.8	7000.0
Zn	50.84	50.84	50.84	50.00
I	0.163	0.163	0.163	0.16
Se	0.163	0.163	0.163	0.16
Mn	16.27	16.27	16.27	16.00
Fe	61.01	61.01	61.01	60.00
Mg	406.70	406.70	406.70	400.00
K	2541.89	2541.89	2541.89	2500.0
Cl	1528.44	1528.44	1528.44	1500.0
Na	1319.11	1319.11	1319.11	1300.0
Cu	4.07	4.07	4.07	4.00
choline	303.73	303.73	303.73	<1000
Co	4.71	4.71	4.71	ne[b]
F	3.62	3.62	3.62	ne
vitamin C	30.50	30.50	30.50	ne
folic acid	0.813	0.813	0.813	ne
biotin	0.049	0.049	0.049	ne
p-amino-benzoic acid	19.369	19.369	19.369	ne
inositol	189.12	189.12	189.12	ne
S	71.42	71.42	71.42	ne

[a] Agricultural Research Council (1981). [b] None established.

Table 5. Dietary Amino Acid Balances (Relative to Reactive Lysine) for the Three Experimental Diets

amino acid	heated SMP: originally calcd[a]	heated SMP: actual[b]	EHC diet A	EHC diet B	recommended balances[c]
reactive lysine	100	100	100	100	100
threonine	72	61	72	72	59
valine	113	96	113	113	66
cysteine/ methionine	155	131	155	155	54
isoleucine	88	75	88	88	46
leucine	189	160	189	189	89
tyrosine/ phenylalanine	185	157	185	185	101
tryptophan	64	54	64	64	13
histidine	50	42	50	50	34
arginine	61	52	61	61	
nonessential	1511	1281	1511	1511	693

[a] These balances are those for the heated SMP-based diet as it was originally formulated. [b] The recalculated balances of the heated SMP-based diet after it was discovered that the reactive lysine content was higher (18%) than was formulated. [c] The nutrient requirements are according to the Agricultural Research Council (1981).

to their respective test diet and fed for a further 18 days in a similar manner as described for the acclimatization period. After every 7 days, the pigs were reweighed and the feed levels were adjusted accordingly. At the end of the 18 day test period, the pigs were slaughtered and the bodies processed in a similar manner as described for the baseline pigs. The frozen pig bodies were cut into 50 cm cubes using an electrical bandsaw before being ground twice through a 10 mm aperture plate (Hobart mincer, Hobart, London) with thorough mixing of the material after each mincing. Approximately 1 kg of minced tissue was randomly sampled and further ground through a 6 mm aperture plate, after which approximately 200 g of tissue was randomly sampled and stored frozen. The minced sample was freeze-dried, then defatted using the Soxhlet fat extraction technique (Firth *et al*., 1985), and ground again through a 0.5 mm mesh in preparation for amino acid analysis, which was carried out in duplicate. Daily deposition rates for whole body lysine and protein were calculated, using the baseline animal body compositional data to correct for initial body lysine and protein contents.

Calculations. Daily body lysine and protein depositions were calculated as

daily lysine or protein deposition (g/day) =
{[whole body component at the end of the trial (g) − initial whole body component (g)]/[trial period (days)]}

Daily body weight gain was calculated as follows:

daily body wt gain (g/day) = [final empty body wt (g) − initial empty body wt (g)]/[trial period (days)]

Statistical Analysis. Daily lysine and protein depositions and the body weight gain data were subjected to an analysis of covariance (SAS, 1985) with treatment as the variable and initial live weight as the covariate. Where a significant effect of treatment on lysine and protein deposition or weight gain was obtained, the *a priori* tests were two further comparisons using Student's *t* test: EHC diet A *vs* heated SMP and EHC diet B *vs* heated SMP.

RESULTS

Digestibility Study. The mean (± SE) ($n = 8$) true ileal reactive lysine digestibility for the heated SMP was 88.2% (± 1.59). This was significantly different ($P < 0.001$) from the corresponding determined mean (± SE) true ileal total lysine digestibility 67.1% (± 2.54).

Table 6. Mean ($n = 6$) Urinary Total Nitrogen Excretion (Grams per Day) for Pigs Fed a Heated SMP-Based Diet before and after Supplementation with Synthetic Lysine

	N excretion: unsupplemented	N excretion: supplemented	overall SE	significance[a]
total N	9.1	6.6	0.50	*
total N/creatinine[b]	19.1	13.4	1.59	*

[a] *, $P < 0.05$. [b] (Grams of total nitrogen day^{-1})/(grams of creatinine day^{-1}).

Nitrogen Balance Study. The mean daily urinary excretion of total nitrogen for the six pigs fed the heated SMP diet was significantly higher than when the same pigs were fed the same diet but supplemented with synthetic lysine (Table 6). Furthermore, when total nitrogen excretion was expressed per weight of creatinine, the excretion of the pigs fed the unsupplemented diet was again significantly higher than when the pigs were fed the supplemented diet. This result showed that the heated SMP diet was first limiting in lysine and therefore was suitable for a growth study whereby body lysine deposition was the criterion of response.

Main Growth Trial. The pigs appeared to be healthy during the acclimatization period and during the first 2 days of trial. However, the majority of the pigs fed the EHC control diets then developed scours, which lasted for about 2 days, after which time the pigs appeared to recover completely. Consequently, and to avoid any influence from the diarrhea that occurred in the early part of the trial, only the data collected from the last 12 days of the growth trial were used for the statistical analysis. The pigs generally consumed their diets readily, and there were negligible feed refusals over the final 12 days of the trial.

After completion of the trial, it was found that the heated SMP diet actually contained more reactive lysine (18%) than was formulated. As such, lysine deposition data have been presented in two ways, first, uncorrected, and, second, corrected for the unplanned oversupply of reactive lysine in the heated SMP diet. The correction factor used was calculated as the actual lysine content of the heated SMP diet divided by the expected lysine content (based on the diet formulation). The lysine deposition was corrected for each pig fed the heated SMP individually. Protein deposition and weight gain data were corrected in the same manner and are presented as corrected values only.

The mean initial live weight of the pigs fed the heated SMP diet was 33.0 kg, which was significantly higher ($P < 0.013$) than that for the pigs fed EHC diet A (28.8 kg) but not significantly different from that for the pigs fed EHC diet B (31.3 kg). There was no significant difference in initial live weights between the pigs fed either of the EHC diets. Initial live weight was used as a covariate for the analysis of variance of daily lysine and protein depositions and daily empty body weight gain.

There was an overall significant difference in uncorrected daily body lysine depositions for the pigs on the three diets (Table 7). Furthermore, the uncorrected lysine deposition for the pigs fed the heated SMP was significantly ($P < 0.001$) higher than that for the pigs fed EHC diet A, which was formulated to have the same digestible lysine content determined using the conventional true ileal digestibility assay, but it was not significantly different ($P > 0.05$) from that of the pigs fed EHC diet B, which was formulated to have the same

Table 7. Least-Square Means (± SE) for Lysine Deposition (Grams per Day), Protein Deposition (Grams per Day), and Empty Body Live Weight Gain (Grams per Day) for Pigs Fed a Heated SMP-Based Diet and Two EHC Control Diets

				overall significance[a]		significance of comparison of heated SMP diet with control diets	
	heated SMP	EHC diet A[b]	EHC diet B[c]	initial live wt[d]	treatment	EHC diet A (based on traditional method)	EHC diet B (based on new method)
uncorrected lysine deposition	10.7 (0.67)	5.4 (0.68)	9.1 (0.62)	**	***	***	NS
corrected lysine deposition[e]	9.1 (0.62)	5.4 (0.63)	9.1 (0.58)	*	***	**	NS
corrected protein deposition	132.9 (6.15)	86.8 (6.26)	115.0 (5.73)	***	***	***	*
corrected wt gain	659.7 (19.0)	569.0 (19.4)	676.6 (17.7)	***	***	**	NS

[a] NS, nonsignificant, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. [b] EHC diet A was formulated to contain a lysine level equal to the digestible lysine content of the heated SMP determined using the conventional ileal digestibility assay [reactive lysine in heated SMP × true digestibility of total lysine (determined using conventional methods) for the heated SMP]. [c] EHC diet B was formulated to contain a lysine level equal to the digestible lysine content of the heated SMP determined using the new ileal reactive lysine digestibility assay [reactive lysine in heated SMP × true digestibility of reactive lysine (determined using the new method) for the heated SMP]. [d] Initial live weight was used as a covariate. [e] Corrected for the oversupply of lysine in the heated SMP-based diet.

digestible lysine content determined using the new true ileal reactive lysine digestibility assay. The corrected daily lysine deposition for the pigs fed the heated SMP was also significantly ($P < 0.01$) higher than that for the pigs fed EHC diet A (formulated on the basis of the traditional assay) but was not significantly different ($P > 0.05$) from that for the pigs fed EHC diet B (formulated on the basis of the new assay).

There was an overall difference ($P < 0.001$) between corrected daily protein deposition for pigs fed the three different diets. Pigs on the heated SMP diet had significantly higher protein depositions than both the pigs fed EHC diet A and the pigs on EHC diet B. Also, there was an overall significant ($P < 0.001$) difference in daily empty body live weight gain for pigs fed the heated SMP diet, EHC diet B, and EHC diet A. Daily live weight gain was statistically significantly higher for pigs fed the heated SMP diet compared to pigs on EHC diet A ($P < 0.01$) but was not significantly different ($P > 0.05$) from that for pigs fed EHC diet B.

DISCUSSION

It is now recognized that conventional true ileal amino acid digestibility values are inaccurate for some amino acids in processed protein sources (Batterham *et al.*, 1990; Moughan *et al.*, 1996). Lysine is one such amino acid that falls into this category, since it can undergo Maillard type reactions which can then revert to lysine during the acid hydrolysis step of amino acid analysis. Methods for measuring reactive lysine chemically (e.g. FDNB) may also not be accurate since they assume complete digestion and absorption of the reactive amino acids. Recently, a new method for determining the true ileal digestibility of reactive lysine in processed protein sources has been developed (Moughan and Rutherfurd, 1996), and while reactive lysine digestibility coefficients determined using the new method appear to be sensible (Rutherfurd and Moughan, 1997), the accuracy of the method for predicting lysine deposition has not yet been established. This study aimed to carry out such an evaluation using a heated SMP fed to growing pigs. The heated SMP was accepted as representing a moderately heated protein source containing reducing compounds.

The true ileal total lysine digestibility for the heated SMP studied here was considerably lower (21%) than the true ileal reactive lysine digestibility. This is consistent with findings for other heated SMP that have been analyzed in our laboratory. On a theoretical basis, the conventional digestibility assay is expected to considerably underestimate reactive lysine digestibility, thus leading to inaccurate measures of dietary digestible reactive lysine (available lysine).

To use whole body lysine deposition to evaluate the accuracy of the digestibility coefficients generated for the heated SMP, and using the presently described experimental approach, several criteria must be met:

(1) Lysine must be the first-limiting amino acid in the diet.

(2) Balanced protein must be fed at a level such that whole body protein deposition is lower than the maximal rate of whole body protein deposition (Pd_{max}).

(3) All other nutrients, essential fatty acids, minerals, and vitamins must be supplied in excess.

(4) The net energy content of the diets must be similar and the digestible energy (DE)/lysine ratio must be high.

(5) Amino acids in the EHC control diets must be completely digested and absorbed.

The first assumption was tested experimentally here, whereby urinary nitrogen excretion was determined for pigs fed the SMP-based diet alone or supplemented with synthetic lysine. The urinary nitrogen excretion was significantly higher in pigs fed the unsupplemented diet compared to the lysine-supplemented diet, demonstrating that the heated SMP-based diet was first limiting in lysine. The predicted whole body protein deposition for pigs fed the heated SMP diet was calculated (Moughan *et al.*, 1987) before commencement of the study to be well below the Pd_{max} for the type of pig studied. Retrospectively, the daily protein depositions determined in the study (87−132 g/day) are clearly below the Pd_{max} (177 g/day) expected for the genotype used (Morel *et al.*, 1993). Enzymatically hydrolyzed casein and free amino acids were used to supply amino acids in the EHC control diets, and it was assumed that digestion and absorption of the amino acids in these diets were virtually complete. This assumption is reasonable given that intact casein is almost completely digested and absorbed (true ileal amino acid digestibility of 97%) (S.M.R. and P.J.M., unpublished results). The gross energy contents of the diets were similar (within 4%), and the vitamins and minerals were present in excess of the pig's requirements (Agricultural Research Council, 1981). Given that the ingredient compositions of the three diets were similar, it was assumed that the net energy delivered to the pigs was also similar. The DE/lysine ratio was calculated to be 1.8 MJ DE/g (Boltshauser *et al.*, 1991), which was considered high enough to avoid a preferential catabolism of lysine for energy supply. The whole lipid to protein ratios were 0.83, 1.06, and 0.98 for pigs on the heated SMP-based diet, EHC diet A, and EHC diet B, respectively.

Assuming that the above experimental criteria were met, and since lysine deposition is directly dependent on absorbed lysine intake, a comparison of body lysine deposition of pigs fed the SMP-based diet against that for the pigs fed the control diets would constitute a suitable test for the accuracy of the true ileal total lysine and reactive lysine digestibility coefficients in SMP. Unfortunately, the reactive lysine content of the heated SMP diet was found, post trial, to be 18% higher than expected on the basis of the formulation of the diet using the determined reactive lysine content of the heated SMP. Consequently, the lysine and protein depositions and live weight gains for these animals were corrected on the basis of the ratio of formulated lysine to actual lysine in the heated SMP diet. The uncorrected and corrected daily whole body lysine depositions for the pigs fed the heated SMP were significantly higher (approximately double) than those for the pigs fed EHC diet A, which was formulated to contain a similar digestible lysine content as determined using conventional methods. Consequently, it would appear that the conventional true ileal total lysine digestibility assay considerably underestimated lysine digestibility in the heated SMP. In contrast, there was no significant difference in uncorrected and corrected lysine depositions for the pigs fed the heated SMP diet and those given EHC diet B, which was formulated to contain a similar digestible lysine level as determined using the new true ileal reactive lysine digestibility assay. This is evidence that the new assay accurately predicted digestible lysine (available lysine) in the heated SMP. It should also be noted that when daily whole body lysine deposition was determined over the whole trial period, the absolute values were slightly lower than those determined over the last 12 days of the study; however, the relative performances of the pigs fed the three diets were similar for both trial periods.

Broadly, the live weight gain and whole body protein deposition data obtained in the present study supported the conclusions based on lysine deposition.

From the results of the present study, it appears that the new assay predicts lysine availability accurately and therefore offers considerable promise as a bioassay for determining available lysine in heat-processed protein sources. The assay has application to monogastric species generally, including humans.

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