Application of a New Method for Determining Digestible Reactive Lysine to Variably Heated Protein Sources

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The study evaluated a recently developed bioassay for determining digestible reactive lysine when applied to skim milk powder and field peas, which had been exposed to varying degrees of heat treatment. Semi-synthetic corn starch-based diets containing the respective material as the sole source of protein were formulated and fed to growing rats. Chromic oxide was included as an indigestible marker. Digesta were sampled from the terminal ileum post-mortem, and diet and digesta were analyzed for amino acid content (including reactive lysine, which was determined following guanidination). True ileal reactive lysine and total lysine digestibilities were calculated. For the heated skim milk powder, both total and reactive lysine digestibility decreased from 97% and 100% (unheated) to 44% and 85%, respectively (121 °C for 10 min). A similar trend was observed for the heated peas where total and reactive lysine digestibility decreased from 83% and 88% (unheated) to 43% and 67%, respectively, in the maximally heated peas. In all cases, total lysine digestibility significantly underestimated reactive lysine digestibility. In contrast, digestible total lysine (conventional method) overestimated digestible reactive lysine for all the skim milk powder samples and some of the pea samples. This overestimation was almost double for the maximally heated skim milk powder where digestible total lysine was 11.2 g kg^{-1} and digestible reactive lysine was 5.7 g kg^{-1}.

Keywords: *Lysine; digestibility; availability; processing; heat*

INTRODUCTION

In feedstuffs that have undergone processing or prolonged storage, the ϵ-amino group of lysine can react with other compounds, particularly reducing sugars, rendering the lysine nutritionally unavailable (Hurrell and Carpenter, 1981). Some of these Maillard compounds can in part revert back to lysine during the acid hydrolysis step of conventional amino acid analysis, leading to inaccuracy in the determination of the lysine content and the digestible lysine content of processed foods (Finot and Mauron, 1972). Several chemical assays allow accurate measurement of the reactive lysine contents of foods, but since they do not account for the incomplete digestion and absorption of lysine from the small intestine (Moughan *et al*., 1996), they do not enable an accurate determination of digestible reactive lysine (available lysine). A method (International Patent Application No. PCT/NZ96/0066) has recently been developed that affords the determination of digestible reactive lysine (available lysine) in feedstuffs (Moughan *et al*., 1996). In this method, the guanidination reaction is used to determine reactive lysine in a test diet and in the digesta of animals fed that diet, thus allowing the calculation of true ileal reactive lysine digestibility and consequently true ileal digestible reactive lysine. In the present study, the new assay was applied to a heated skim milk powder and heated field peas with the aim of investigating the effectiveness of the new assay for detecting differences in digestible reactive lysine content in variably heated protein sources.

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MATERIALS AND METHODS

Materials. *O*-Methylisourea was obtained from Sigma Chemicals (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). Heated pea samples were supplied by Dr R. J. van Barneveld (South Australian Research and Development Institute, Adelaide). Enzymatically hydrolyzed casein was obtained from New Zealand Pharmaceuticals LTD (Palmerston North, New Zealand) and contained peptides no larger than 2000 Da. Centriprep 10 disposable ultrafiltration devices were obtained from Amicon, Inc. (Beverly, MA). Laboratory rats were obtained from the Small Animal Production 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. Four 1-kg batches of skim milk powder were autoclaved for 1, 3, 5, and 10 min at 121 °C, respectively. Each batch of skim milk powder was then ground through a 0.5-mm mesh. The four pea samples had been heated for 15 min in a forced air dehydrator at 110, 135, 150, and 165 °C. These samples were also ground through 0.5-mm mesh before use. All samples and diets were stored at −20 °C when not in use.

Digestibility Study. Ethics approval for the animal trial was obtained from the Animal Ethics Committee, Massey University (Palmerston North, New Zealand). Male Sprague−Dawley rats, of approximately 150 g body weight, were housed individually in stainless steel wire-bottomed cages in a room maintained at 22 ± 2 °C, with a 12 h light/dark cycle. Ten semi-synthetic test diets were formulated as shown in Table 1 to contain 100 g/kg crude protein. An enzymatically hydrolyzed casein (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 was included (0.5%) in each diet as an indigestible marker. The diets were randomly allocated to the rats such that there were

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Table 1. Ingredient Compositions[a] (g kg^{-1} Air Dry Weight) of Experimental Diets

	EHC[b]	unheated or heated SMP[c] diets	unheated or heated pea diets
cornstarch	631.9	505.7	305.7
soybean oil	50.0	50.0	50.0
cellulose	50.0	50.0	50.0
sucrose	100.0	100.0	100.0
Vit/Min mix[d]	39.3	39.3	39.3
EHC	123.8		
unheated or heated SMP[e]		250.0	
unheated or heated peas[f]			130.0
chromic oxide	5.0	5.0	5.0

[a] All diets were formulated to contain equal crude protein contents. [b] Enzymatically hydrolyzed casein diet used for determining endogenous amino acid losses at the terminal ileum (Moughan *et al.*, 1990; Butts *et al.*, 1991). [c] Skim milk powder. [d] Vitamin/mineral mix was formulated to meet the laboratory rat's requirements for vitamins and minerals in the final diet as described by the National Research Council (National Academy of Sciences, 1972). [e] Unheated or heated SMP diets were the five diets consisting of either unheated SMP or SMP heated at 121 °C for either 1, 3, 5, or 10 min as the sole protein source. [f] Unheated or heated pea diets were the five diets consisting of unheated peas or peas heated for 15 min at 110, 135, 150, or 165 °C as the sole protein source.

eight animals on each diet. The rats were fed hourly as described by Moughan and Rutherfurd (1996). After 14 days, the rats were killed, and digesta from the terminal ileum (20 cm) were sampled. Endogenous amino acids in the ileal digesta were determined after separating the larger endogenous proteins from undigested dietary peptides by centrifugation at 1400*g* for 30 min and ultrafiltration using a Centriprep 10 ultrafiltration device (exclusion limit 10000 Da) as described by Moughan and Rutherfurd (1996).

Chemical Analysis. Amino acid contents were determined in duplicate 5-mg feedstuff and digesta samples and quadruplicate 5-mg diet samples using a Waters ion-exchange HPLC system, utilizing post-column ninhydrin derivatization and detection using absorbance at 570 and 440 nm, 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 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 greater than 1000. After incubation, the samples were dried down 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 an Instrumentation Laboratory atomic absorption spectrophotometer following the method of Costigan and Ellis (1987).

Data Analysis. Ileal and endogenous (for EHC fed rats) ileal amino acid flows at the terminal ileum were calculated using the following equation (units are μg g^{-1} dry matter intake (DMI)):

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

True ileal amino acid digestibility was calculated using the following equation (units are μg g^{-1} DMI unless stated otherwise):

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

Table 2. Mean[a] (n = 8) True Ileal Amino Acid Digestibility (%) for Variably Heated Skim Milk Powder[b]

	heating time (min)					overall	overall
amino acid	0	1	3	5	10	SE	significance[c]
aspartic acid	94^{a}	94^{ab}	93^{ab}	91^{bc}	87^{c}	1.2	***
threonine	93^{a}	94^{ab}	91^{b}	90^{abc}	87^{c}	1.2	**
serine	83^{a}	85^{a}	82^{a}	79^{ab}	74^{b}	1.9	**
glutamic acid	92^{a}	92^{ab}	91^{ab}	89^{bc}	85^{c}	1.0	***
proline	98^{a}	97^{ab}	96^{b}	94^{c}	91^{c}	0.9	***
glycine	72	72	75	78	66	3.8	NS
alanine	98^{a}	98^{a}	97^{a}	97^{a}	94	0.9	**
valine	94^{a}	94^{a}	94^{a}	92^{ab}	90^{b}	0.9	**
isoleucine	91^{a}	91^{ab}	89^{bc}	86^{bc}	82^{c}	1.3	***
leucine	98^{a}	98^{ab}	98^{a}	98^{ab}	97^{b}	0.4	*
tyrosine	99	99	99	99	98	0.5	NS
phenylalanine	99	100	100	99	99	0.6	NS
histidine	96^{a}	95^{a}	90	83^{b}	79^{b}	1.5	***
arginine	99^{a}	99^{a}	96^{b}	95^{b}	94^{b}	0.9	***

[a] Means in each row with different superscripts are significantly different ($P < 0.05$). [b] Skim milk powder was autoclaved at 121 °C for 1, 3, 5, and 10 min. [c] NS, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

True ileal reactive lysine digestibility was calculated using the following equation (units are μg g^{-1} DMI unless stated otherwise):

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

$$\text{digestible total lysine (g kg}^{-1}) = \text{true ileal total lysine digestibility (\%)} \times \text{total lysine content of the protein source (g kg}^{-1})$$

$$\text{digestible reactive lysine (g kg}^{-1}) = \text{true ileal reactive lysine digestibility (\%)} \times \text{reactive lysine content of the protein source (g kg}^{-1})$$

The amino acid digestibility data were subjected to a one-way analysis of variance for each diet and for each amino acid singly (GLM Procedure, SAS Institute Inc., Cary, NC).

RESULTS

Comparison of True Ileal Digestibility of Amino Acids Other Than Lysine in Variably Heated Skim Milk Powder and Peas. For the skim milk powder, the true ileal digestibility of all amino acids, with the exception of glycine tyrosine and phenylalanine, significantly decreased after heating at 121 °C for 10 min (Table 2). This decrease ranged from 1% unit for leucine to 17% units for histidine with the average decrease being approximately 7% units. For some amino acids, significant decreases were also observed after shorter heating periods. For the heated pea samples there was a significant increase in digestibility as the heating temperature was increased to 135 °C for all amino acids examined except glycine (Table 3). This increase ranged from 5% units for arginine to 15% units for threonine and isoleucine, with the average increase being 12% units. This was followed by a decrease in digestibility for all amino acids as the temperature was further increased to 165 °C. Overall, when the maximally heated peas were compared to the unheated peas, digestibility had decreased after heat treatment for all amino acids except proline and leucine. This decrease ranged from 2% units for phenylalanine to 32% units for aspartic acid and glycine, with the average decrease being 21% units.

Table 3. Mean[a] ($n = 5$) True Ileal Amino Acid Digestibility (%) for Variably Heated Field Pea Sample[b]

amino acid	heating temperature (°C) unheated	110	135	150	165	overall SE	overall significance[c]
aspartic acid	74[a]	79[a]	83[b]	70[a]	42[c]	2.8	***
threonine	67[a]	76[a]	82[b]	70[ab]	45[c]	3.4	***
serine	73[a]	80[b]	85[c]	76[abc]	51[d]	2.9	***
glutamic acid	80[a]	85[ab]	89[b]	79[a]	53[c]	2.2	***
proline	61[a]	75[b]	74[b]	72[ab]	51[a]	3.3	***
glycine	64[a]	74[a]	75[a]	63[a]	33[b]	4.5	***
alanine	76[a]	82[b]	88[c]	79[abc]	55[d]	3.2	***
valine	72[a]	80[a]	86[b]	79[ab]	58[c]	3.2	***
isoleucine	74[a]	81[a]	88[b]	80[ab]	60[c]	3.2	***
leucine	75[ac]	81[a]	89[b]	81[ab]	64[c]	2.9	***
tyrosine	69[a]	74[a]	84[b]	72[ab]	51[c]	4.2	***
phenylalanine	73[a]	79[b]	86[c]	80[abc]	61[d]	2.9	***
histidine	69[a]	77[a]	83[b]	70[a]	41[c]	3.2	***
arginine	84[a]	87[a]	92[b]	86[ab]	71[c]	2.2	***

[a] Means in each row with different superscripts are significantly different ($P < 0.05$). [b] Peas were heated for 15 min at 110, 135, 150, and 165 °C. [c] ***, $P < 0.001$.

Table 4. Reactive Lysine Contents (±SE) (mg g^{-1} Air Dry Weight) in Heated Skim Milk Powder[a] and in Heated Field Peas[b] Determined Using Guanidination Method and Total Lysine Contents (±SE) (mg g^{-1} Air Dry Weight) Determined Using Conventional Amino Acid Analysis

heating time[a] (min)	skim milk powder total lysine	skim milk powder reactive lysine	heating temp (°C)	peas total lysine	peas reactive lysine
unheated	38.1 (0.65)	38.1 (1.40)	unheated	15.1 (0.23)	14.9 (0.58)
1	35.7 (0.13)	28.1 (2.03)	110	15.3 (0.13)	14.8 (0.07)
3	28.6 (2.19)	17.7 (0.11)	135	14.0 (0.06)	13.0 (0.15)
5	26.5 (1.03)	11.9 (1.26)	150	12.1 (0.42)	10.6 (0.13)
10	25.6 (0.49)	6.7 (0.30)	165	8.7 (0.15)	5.3 (0.12)

[a] Skim milk powder was autoclaved at 121 °C for 1, 3, 5, and 10 min. [b] Peas were heated for 15 min at 110, 135, 150, and165 °C.

Comparison of Total Lysine and Reactive Lysine Contents of the Variably Heated Skim Milk Powder and Peas. Reactive lysine and total lysine contents were similar for the unheated skim milk powder (38.1 mg g^{-1}, Table 4). As the heating time increased, both the total lysine and reactive lysine contents decreased accordingly. However, the 82% decrease in reactive lysine content resulting from heating (38.1 mg g^{-1} with no heating to 6.7 mg g^{-1} after 10 min heating) was considerably greater than the 33% decrease (38.1 mg g^{-1} with no heating to 25.6 mg g^{-1} after 10 min heating) observed for the total lysine content.

Reactive lysine and total lysine contents were similar for the unheated peas (15.0 mg g^{-1}, Table 4). However, with increased temperatures the reactive lysine content decreased by up to 64% (to 5.3 mg g^{-1}) for the peas heated at 165 °C, while total lysine decreased by only 42% (to 8.7 mg g^{-1}).

Comparison of True Ileal Total Lysine Digestibility and True Ileal Reactive Lysine Digestibility for Variably Heated Skim Milk Powder and Peas. For the skim milk powder, both true ileal total lysine digestibility and true ileal reactive lysine digestibility decreased with increased heating, with decreases in total and reactive lysine digestibilities after 10 min heating of 55% and 15%, respectively (Table 5). True ileal total lysine digestibility was significantly lower than true ileal reactive lysine digestibility for all heat treatments. This difference was least in the unheated skim milk powder (3.4% difference between total and reactive lysine digestibility) and became progressively greater as time of heating increased.

For the field peas, both true ileal total lysine digestibility and true ileal reactive lysine digestibility initially increased with increased heating temperatures up to 135 °C, after which there was a decrease in total and reactive lysine digestibility of 53% and 28%, respectively, observed after heating peas at 165 °C for 15 min (Table 5). There were highly statistically significant differences between true ileal total lysine digestibility and true ileal reactive lysine digestibility for all the heat treatments with the digestibility based on total lysine being lower. The greatest difference was observed for the most severely heated peas.

Table 5. Comparison of Mean Total Lysine Digestibility (%) Determined Using Conventional Methods and Reactive Lysine Digestibility (%) Determined Using Reactive Lysine Digestibility Assay for Heated Skim Milk Powder ($n = 8$) and Heated Peas ($n = 5$)

heat treatment	lysine digestibility total[a]	lysine digestibility reactive[b]	overall SE	significance
Skim Milk Powder				
unheated	96.6	100.0	0.24	***
121 °C for 1 min	88.5	99.4	1.50	***
121 °C for 3 min	69.1	94.0	0.94	***
121 °C for 5 min	51.6	92.3	5.14	***
121 °C for 10 min	43.7	84.7	3.06	***
Peas				
unheated	82.6	87.9	1.75	***
110 °C for 15 min	85.8	90.2	1.63	**
135 °C for 15 min	89.9	93.1	3.58	***
150 °C for 15 min	87.0	83.6	0.80	***
165 °C for 15 min	42.7	67.3	4.86	***

[a] Total lysine digestibility was determined using a true ileal amino acid digestibility assay (rat) with conventional amino acid analysis being used to quantitate lysine in the diets and digesta. [b] Reactive lysine digestibility was determined using a true ileal amino acid digestibility assay (rat) with the guanidination reaction being used to quantitate reactive lysine.

Table 6. Digestible Total Lysine and Digestible Reactive Lysine Contents (g kg^{-1} Air Dry Weight) of Variably Heated Skim Milk Powder and Heated Peas

heat treatment	digestible lysine total[a]	digestible lysine reactive[b]	overall SE	significance
Skim Milk Powder				
unheated	36.8	38.1	0.09	***
121 °C for 1 min	31.6	28.0	0.53	***
121 °C for 3 min	19.8	16.6	0.25	***
121 °C for 5 min	13.7	11.0	0.62	*
121 °C for 10 min	11.2	5.7	0.73	***
Peas				
unheated	12.5	13.1	0.25	***
110 °C for 15 min	13.2	13.3	0.28	NS
135 °C for 15 min	12.6	12.6	0.54	NS
150 °C for 15 min	9.5	8.8	0.10	***
165 °C for 15 min	3.7	3.6	0.40	NS

[a] Digestible total lysine was calculated from total lysine digestibility determined using a true ileal amino acid digestibility assay (rat) where conventional amino acid analysis was used to quantitate lysine and from the total lysine content in the protein source determined using conventional amino acid analysis. [b] Digestible reactive lysine was calculated from the reactive lysine digestibility determined using a true ileal amino acid digestibility assay (rat) where the guanidination reaction was used to detect reactive lysine and the reactive lysine content in the protein source was determined using guanidination.

Comparison of True Ileal Digestible Total Lysine and True Ileal Digestible Reactive Lysine in Variably Heated Skim Milk Powder and Peas. The true ileal digestible total lysine content was generally significantly higher than the true ileal digestible reactive lysine content (available lysine) for the heated skim milk powders (Table 6). Digestible total lysine overestimated the digestible reactive lysine content by 13, 19, 25, and 96% after 1, 3, 5, and 10 min heating, respectively. For the heated field peas, true ileal digestible total lysine content was not significantly different from the true ileal digestible reactive lysine content (available lysine) for samples heated at 110, 135, and 165 °C but was signif-

icantly higher for the peas heated at 150 °C, although the actual difference was small (8%) (Table 6).

DISCUSSION

Heat treatment of the skim milk powder resulted in decreases in true ileal amino acid digestibility for almost all of the amino acids examined. In contrast, for the heated pea samples, the true ileal amino acid digestibility for almost all amino acids initially increased after exposure to the milder heat treatments (110 and 135 °C), but then decreased dramatically upon exposure to the more severe heat treatments. Overall, the digestibility of most amino acids had declined when the most severely heated pea samples were compared with the unheated peas. The increase in amino acid digestibility for the peas heated at mild temperatures may have resulted from an inactivation or destruction of antinutritional factors as well as from denaturing of the proteins, rendering them more susceptible to proteolysis. Similar effects have been noted in soybean meal (Wright, 1981). The overall decreases in amino acid digestibility were greater in the heated peas as compared to the heated skim milk powder for all amino acids examined. Clearly exposure of protein sources to heat treatment can result in significant reductions in amino acid digestibility and hence reduced protein quality.

The reactive lysine content (reactive lysine being the lysine units whose ϵ-amino group is still free to react with the test reagent) of the skim milk powder and field peas was reduced considerably by heating. Furthermore, as heating times increased, reactive lysine content continued to decrease. This result probably reflects the formation of Maillard type compounds (probably lactulosyl-lysine for the skim milk powder) in the two protein sources. Estimates of total lysine content may include not only the lysine present in a protein but also the reverted lysine from the Maillard compounds. Consequently, for such protein sources total, lysine estimates will often overestimate the lysine content. In this study, total lysine overestimated the lysine content by 27%, 62%, 123%, and 282% for the skim milk powder heated for 1, 3, 5, and 10 min, respectively, and by 4%, 8%, 14%, and 64% for the field peas heated for 15 min at 110, 135, 150, and 165 °C, respectively.

The reactive lysine content of the skim milk powder was reduced by 26%, 54%, 69%, and 82% after heating for 1, 3, 5, and 10 min, respectively, while in the field peas it was reduced by 1%, 12%, 29%, and 64% after heating for 15 min at 110, 135, 150, and 165 °C, respectively. It would appear that with the increasing heat treatment more lysine underwent Maillard type reactions. This is supported by the proportionally much smaller decrease in total lysine observed after heating, the difference most likely being Maillard compounds that have reverted back to lysine during the acid hydrolysis step of amino acid analysis. Clearly, in heated protein sources lysine should be determined as reactive rather than total lysine.

True ileal reactive lysine digestibility decreased up to 15% (after 10 min heating at 121 °C) in the skim milk powder and up to 24% (after 15 min at 165 °C) in the field peas. This decrease was most likely due to a reduced ability of protease enzymes to cleave peptide bonds in the vicinity of the modified amino acid residues. In contrast, true ileal total lysine digestibility decreased 49% after 10 min heating of the skim milk powder and 48% after 15 min at 165 °C for the field peas. In both protein sources, the decrease in true ileal total lysine digestibility after heat treatment was considerably more than that observed for the reactive lysine digestibility. In all treatments total lysine digestibility underestimated reactive lysine digestibility, by as much as 44−48% after 5−10 min heating in the skim milk powder and 37% in field peas heated at 165 °C. It is interesting to note that in the unheated skim milk powder the difference between the true ileal total and reactive lysine digestibility values was considerably smaller than for the heated skim milk powders. This was not the case for the heated peas, for which the difference between true ileal total and reactive lysine digestibility was similar for the unheated peas and those heated up to 150 °C. It should be noted that the unheated peas had been dried prior to any experimental treatment and as such had probably been subjected to some form of heating. This may explain why there was a quantitatively significant difference (6%) between the reactive lysine and total lysine digestibilities for the "unheated" peas. It is also notable that both reactive lysine and total lysine digestibility, as well as the digestibility of most of the other amino acids examined, increased in the field peas as they were heated up to 135 °C. This may reflect the inactivation of antinutritional factors and denaturing of dietary protein resulting from these heat treatments. While total lysine digestibility may accurately reflect reactive lysine digestibility in unheated proteins, the true ileal total lysine digestibility assay should not be used to determine reactive lysine digestibility (lysine availability) in heated protein sources.

Heat treatment dramatically reduced the digestible reactive lysine content (available lysine) of the skim milk powder. After only 1 min heating at 121 °C, one-fourth of the original lysine in the unheated skim milk powder was no longer nutritionally available, and this increased to one-half after only 2 min heating. After 10 min heating, over 85% of the original lysine was rendered unavailable. The heat treatment used in this study was not particularly severe especially when compared to many processes used by commercial protein suppliers and highlights the large extent to which protein quality can be reduced with heat processing. For the skim milk powder the true ileal digestible total lysine estimates, in all cases, overestimated available lysine content. This overestimation ranged from 13 to 25% after 1−2 min heating to 96% after 10 min heating.

In contrast, for the heated field peas, digestible total lysine was similar to the digestible reactive lysine for most of the heat treatments. It should be noted that true ileal total lysine digestibility was significantly different from the true ileal reactive lysine digestibility for those samples. Also, total lysine content and reactive lysine content were quite different for some of the heated pea samples. It would appear that in these cases, the underestimation in total lysine digestibility was compensated for by the overestimation of the total lysine content and as such the errors cancel when digestible total lysine was calculated. This phenomenon does not always occur, as was seen for the heated skim milk powder where digestible total lysine was very different from digestible reactive lysine, and consequently, the accuracy of the digestible total lysine estimates cannot always be relied upon when determined in heated protein sources.

The new digestible reactive lysine assay was sensitive for detecting differences in digestible reactive lysine between unheated skim milk powder and skim milk powders heated for only 1 min at 121 °C. Furthermore, for the skim milk powder at least, relatively mild heat treatment can result in a significant reduction in available (digestible reactive) lysine. In contrast, for the

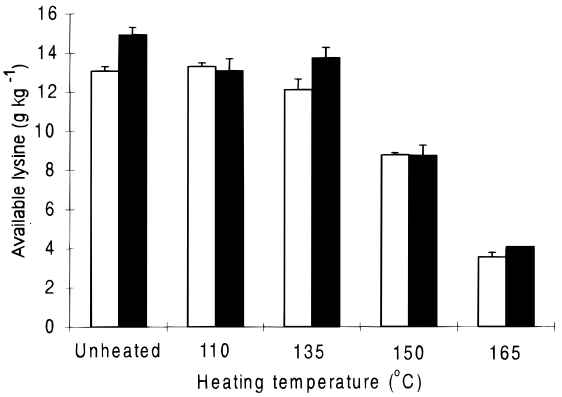

Figure 1. Comparison of available lysine (g kg^{-1}) in field peas determined by the digestible reactive lysine assay (□) or by growth assay (van Barneveld *et al*., 1994) (■). Available lysine was determined in our laboratory as digestible reactive lysine using the digestible reactive lysine assay (□). Available lysine was also determined by van Barneveld *et al*. (1994) (■) and is presented in this figure for the raw peas and peas heated at 110, 135, and 150 °C as mean lysine availability determined using both the slope ratio assay (using daily empty-body weight gain, empty body weight gain:food intake, protein deposition, and protein deposited:food intake) as indicators of lysine utilization, and regression methods (using daily empty-body weight gain versus lysine intake and estimated lysine retained versus lysine intake) multiplied by the total lysine content of each pea sample. For the peas heated at 165 °C available lysine is presented as lysine availability determined using only the regression of daily empty-body weight gain versus lysine intake multiplied by the total lysine content of each pea sample, since this lysine availability estimate was the only valid one (Barneveld *et al*., 1994). The latter data were calculated from van Barneveld *et al*. (1994) with the author's permission.

peas, more severe heat treatment was required before quantitatively significant reductions in available lysine were observed. The new assay was successful in describing the progressive loss of available lysine with increasing heat treatment.

Prior to this study, lysine availability had been determined on the same heated field pea samples used in this study, using both the slope ratio assay and regression methods (van Barneveld *et al*., 1994). In this case, we are able to compare the digestible reactive lysine estimates determined using the new digestible reactive lysine assay and available lysine estimates obtained using conventional growth assays. In the study of van Barneveld *et al*. (1994), lysine availability coefficients were determined in several ways. First, they used the slope ratio assay based on either daily empty-body weight gain, empty body weight gain:food intake, protein deposition, or protein deposited:food intake. Second, daily empty-body weight gain had been regressed against lysine intake, and estimated lysine retained had been regressed against lysine intake. There appeared to be considerable variation among the lysine availability estimates determined using the above six measures. Consequently, for the present purpose of comparing the digestible reactive lysine assay with growth assays for determining available lysine, the lysine availability estimates considered to be valid by van Barneveld *et al*. (1994) were averaged for each pea sample and multiplied by the total lysine content of each pea sample, respectively, to give an overall available lysine estimate. These were then compared with available lysine estimates determined using the digestible reactive lysine assay (Figure 1). There was quite good agreement between available lysine determined using the new bioassay and that determined using conventional growth assays, especially for the peas heated at 110, 150, and 165 °C. Although it must be remembered that the two studies were carried out independently by two different laboratories at different times, it would appear that the new digestible reactive lysine assay is sensitive for monitoring the effects of heat treatment and appears to accurately predict available lysine content. This highlights the potential of the new assay for assessing available lysine in heated protein sources.

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