Digestible Reactive Lysine in Processed Feedstuffs: Application of a New Bioassay

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A new bioassay for determining digestible reactive lysine was applied to a range of processed feedstuffs. Semisynthetic diets containing various processed feedstuffs as the sole sources of protein and including chromic oxide as a marker were fed to growing rats. Digesta from the terminal ileum were collected and, with samples of the diets, analyzed for reactive lysine following reaction with *O*-methylisourea. True reactive lysine digestibility was determined by correcting for endogenous lysine loss at the terminal ileum of rats fed enzyme-hydrolyzed casein. True ileal digestibility of reactive lysine was similar to that of total lysine for blood meal, wheat meal, meat and bone meal, and soybean meal but significantly higher for dried maize (84.3% and 80.5%, respectively), an alfalfa-based mix (86.3% and 74.2%, respectively), heated skim milk powder (94.0% and 69.1%, respectively), and cottonseed meal (71.9% and 62.1%, respectively). When compared to digestible total lysine, digestible reactive lysine contents were lower for wheat (2.9 and 3.2 g kg^{-1}), maize (1.9 and 2.6 g kg^{-1}), heated skim milk powder (16.6 and 19.8 g kg^{-1}), cottonseed meal (10.3 and 12.9 g kg^{-1}), and the alfalfa-based mix (10.8 and 14.4 g kg^{-1}). The new assay leads to different estimates of available lysine in processed feedstuffs compared to assays based on conventional analysis.

Keywords: *Lysine; digestibility; availability; protein; ileal*

INTRODUCTION

Lysine is a dietary essential amino acid that is often first limiting for pigs and poultry. In feedstuffs that have undergone processing or prolonged storage, the ϵ-amino group of lysine can react with other compounds present in feedstuffs to render the amino acid nutritionally unavailable (Hurrell and Carpenter, 1981). Some of these reacted lysine derivatives are acid labile and can revert back to lysine during the acid hydrolysis step used in conventional amino acid analysis, leading to an overestimation of the lysine content and the digestible lysine content of processed feedstuffs. While chemical assays that determine the reactive lysine contents of feeds partly overcome this inaccuracy, they do not account for the incomplete digestion and absorption of lysine from the small intestine (Moughan *et al*., 1996). A method is needed, therefore, to measure the digestibility of reactive lysine in feedstuffs. Moughan and Rutherfurd (1996) (New Zealand Patent Application 272486) have described a new procedure, whereby a true ileal amino acid digestibility assay is used in conjunction with the guanidination reaction (the reaction of *O*-methylisourea and the ϵ-amino group of lysine to produce homoarginine) to determine the true ileal digestibility of reactive lysine and reactive lysine concentrations are determined in the test diet and in the digesta of animals fed that diet. Coefficients derived using the latter assay and the reactive lysine contents of processed feedstuffs can be used to estimate digestible reactive lysine (available lysine) contents. The present study compares true ileal lysine digestibility determined using conventional methodology (based on total lysine) with true ileal reactive lysine digestibility determined using the newly developed assay (Moughan and Rutherfurd, 1996), for a range of processed feedstuffs.

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MATERIALS AND METHODS

Materials. 1-Fluoro-1,4-dinitrobenzene (FDNB), (dinitrophenyl)lysine (DNP-lysine), and *O*-methylisourea were obtained from Sigma Chemicals, St. Louis, MO. Barium hydroxide octahydrate was obtained from BDH Laboratory Supplies, Poole, England. Skim milk powder was obtained from Tui Nutriproducts, Palmerston North, New Zealand, while wheat meal, blood meal, meat and bone meal, soybean meal, dried maize, and an alfalfa-based mix were obtained from the Feed Processing Unit, Massey University, Palmerston North, New Zealand, and cottonseed meal was from Cargill Oilseed Ltd., Brisbane, Australia. Enzymatically hydrolyzed casein was obtained from New Zealand Pharmaceuticals Ltd., Palmerston North, New Zealand, and contained free amino acids and 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.

FDNB Method. FDNB-reactive lysine was determined on samples containing approximately 10 mg of reactive lysine (estimated previously using amino acid analysis), according to the method of Carpenter (1960), and using the modifications described by Booth (1971). Correction factors were used to adjust for the loss of (dinitrophenyl)lysine during acid hydrolysis. These were determined from the amount of lysine present (determined using amino acid analysis) after hydrolysis of the FDNB-reacted feedstuff.

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. To ensure that at least one of the protein sources was sufficiently heat damaged to allow a sizable difference between the assays, approximately 1 kg of skim milk powder was autoclaved for 3 min at 121 °C before use. The autoclaved skim milk powder along with a

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

	EHC[b]	blood meal	wheat meal	meat and bone meal	soybean meal	heated skim milk powder	dried maize	alfalfa-based mix	cottonseed meal
wheat starch	625.7	646.7		572.7	542.7	495.7		355.7	504.7
soybean oil	50	50	50	50	50	50		50	50
purified cellulose	50	50		50	50	50		50	50
sucrose	100	100	20.7	100	100	100		100	100
vitamin/mineral mix[d]	39.3	39.3	39.3	39.3	39.3	39.3	39.3	39.3	39.3
EHC	130								
blood meal		109							
wheat meal			885						
meat/bone meal				183					
soybean meal					213				
heated skim milk powder						260			
dried maize							955.7		
alfalfa-based mix[c]								400	
cottonseed meal									251
chromic oxide	5	5	5	5	5	5	5	5	5

[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, the EHC contained free amino acids and small peptides (<2000 Da). [c] The alfalfa-based mix consisted of 55% alfalfa, 10% meat and bone meal, and 5% each of blood, wheat, barley, maize, sorghum, soybean, and broll meals and was initially in a pelleted form. [d] Vitamin/mineral mix was formulated to meet the requirements for vitamins and minerals in the final diets as described by the National Research Council (National Academy of Sciences, 1972).

selection of readily available feedstuffs, including wheat meal, blood meal, soybean meal, meat and bone meal, dried maize, cottonseed meal, and a pelleted alfalfa-based mix containing 55% alfalfa, 10% meat and bone meal, and 5% each of blood, wheat, barley, maize, sorghum, soybean, broll meals, were each ground through a 0.5 mm mesh. The blood meal, soybean meal, and wheat meal represented processed feedstuffs which were expected to be of high quality, whereas the other materials, being subjected to a higher degree of processing during manufacturing, were expected to have a lower protein quality.

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, approximately 150 g of 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. Eight semisynthetic test diets were formulated (Table 1) to each contain 100 g/kg crude protein. An enzymatically hydrolyzed casein (EHC)-based diet was also formulated (Table 1) to allow determination of endogenous ileal lysine flows (eq 1) (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, and the animals were fed the diets for a 14 day experimental period. On each day each rat received its respective diet as nine meals given hourly (0830−1630 h). At each meal time, the diet was freely available for a 10 min period, and the feed containers were weighed after each meal. Water was available at all times. On the final day of the study, from 5.5 to 7 h after the start of feeding, the rats were asphyxiated in carbon dioxide gas and then decapitated. 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 gently flushed from the ileum section with distilled deionized water from a syringe. The digesta from the rats fed the test diets were freeze-dried ready for chemical analysis. The digesta of rats fed the EHC diet were adjusted to approximately pH 3 with 6 M HCl, to minimize protease activity. The EHC digesta were then centrifuged at 1400*g* for 30 min at 3 ± 1 °C, the precipitate was washed and recentrifuged, and the washings were pooled with the supernatant. The supernatant was ultrafiltered using a Centriprep 10 disposable ultrafiltration device, after which the filtrate was discarded and the retentate washed and ultrafiltered for a second time. The resulting retentate was added to the precipitate from the centrifugation step and freeze-dried ready for chemical analysis.

Chemical Analysis. Amino acid contents were determined in duplicate 5 mg diet and digesta samples and quadruplicate 5 mg semisynthetic diet samples using a Waters ion-exchange HPLC system, utilizing postcolumn 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), at 21 °C in a shaking water bath, with the reagent to lysine ratio being greater than 1000 according to the procedure of Moughan and Rutherfurd (1996). After incubation, the samples were dried 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 GBC 902 AA absorption/emmission spectrophotometer (GBC Scientific NZ Ltd., Auckland, New Zealand) following the method of Costigan and Ellis (1987).

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

$$\text{ileal amino acid flow} = \text{amino acid concentration in ileal digesta} \times \frac{\text{diet chromium}}{\text{ileal chromium}} \quad (1)$$

True ileal amino acid digestibility was calculated as follows (units are μg g^{-1} of DMI):

$$\text{true digestibility (\%)} = [\text{dietary amino acid intake} - (\text{ileal amino acid flow} - \text{endogenous amino acid flow})]/\text{dietary amino acid intake} \times \frac{100}{1} \quad (2)$$

True ileal reactive lysine digestibility was calculated as follows (units are μg g^{-1} of DMI):

$$\text{true ileal reactive lysine digestibility (\%)} = [\text{dietary reactive lysine intake} - (\text{ileal reactive lysine flow} - \text{endogenous lysine flow})]/\text{dietary reactive lysine intake} \times \frac{100}{1} \quad (3)$$

Reactive lysine was determined using the guanidination method. The amino acid digestibility data were subjected to

Table 2. Reactive Lysine Contents (mg g^{-1} of Sample) of Several Protein Sources Determined Using the FDNB or Guanidination Methods in Comparison with Total Lysine Contents (mg g^{-1} of Sample) Determined Using Conventional Amino Acid Analysis[a]

		reactive lysine	
	total lysine	FDNB	guanidination
blood meal	89.1	84.4	88.0
wheat meal	3.5	3.1	3.1
meat and bone meal	36.5	30.4	34.6
soybean meal	32.3	27.1	32.3
cottonseed meal	20.6	14.7	14.4

[a] The correction factors used for the FDNB method were 1.06 for blood meal, 1.03 for wheat meal, 1.08 for meat and bone meal, 1.04 for soybean meal, and 1.05 for cottonseed meal and were determined as described in Materials and Methods.

a one-way analysis of variance for each amino acid singly (GLM Procedure, SAS Institute Inc.).

RESULTS

Comparison of Reactive Lysine Contents Determined Using the Guanidination or FDNB Method. The reactive lysine contents, determined using the guanidination method (where the homoarginine content is equated to reactive lysine) and the FDNB-reactive lysine method, were compared to the total lysine content, determined using conventional amino acid analysis for five of the protein sources (Table 2). Reactive lysine determined using guanidination was generally similar or higher than the FDNB-reactive lysine content for all five protein sources.

Comparison of True Ileal Lysine Digestibility (Conventional Assay) with True Ileal Reactive Lysine Digestibility. The rats appeared healthy throughout the 14 day digestibility study. Meal intakes were relatively constant over the first six meals on the last day of the study, and therefore a relatively constant flow of digesta through the gut should have been achieved. Mean meal intakes (g) ± SE for the first six meals on the last day were 1.7 ± 0.08 g for the wheat meal diet, 1.8 ± 0.05 g for the cottonseed diet, 1.9 ± 0.06 g for the meat and bone diet, 2.0 ± 0.06 g for the soybean diet, 0.8 ± 0.04 g for the blood meal diet, 1.7 ± 0.12 g for the heated skim milk powder diet, 0.9 ± 0.15 g for the dried maize diet, 1.9 ± 0.28 g for the alfalfa-based mix diet, and 1.7 ± 0.07 g for the EHC-based diet.

True ileal digestibility values (eq 2) based on "total" lysine as determined using conventional amino acid analysis were compared with true ileal digestibility values for reactive lysine (eq 3), determined following the guanidination reaction, for eight different protein sources (Table 3). For blood meal, wheat meal, and meat and bone meal, the digestibilities of total lysine and reactive lysine were high (generally greater than 90%) and there was no significant difference between total lysine digestibility and reactive lysine digestibility. For soybean meal, the digestibility of total lysine, which was also high, was significantly lower than the reactive lysine digestibility, although the actual difference was less than 3% units. For the dried maize, alfalfa-based mixed diet, cottonseed meal, and heated skim milk powder, digestibility of total lysine was significantly lower ($p < 0.05$) than that of reactive lysine. Differences between the digestibility values for the two approaches were 4%, 12%, 10%, and 25% units, respectively.

Digestible lysine (based on total lysine determined by conventional analysis) and digestible reactive lysine contents are shown in Table 4. For blood meal and meat and bone meal, there were no significant differences between the two values. In contrast, the two values were significantly different for the six remaining protein sources. However, for soybean meal there was less than a 2% difference between digestible total lysine and digestible reactive lysine. For the alfalfa-based mix, the dried maize, cottonseed meal, and heated skim milk powder, all of which had undergone more severe heat processing, the differences (34%, 37%, 25%, and 19%, respectively) between digestible total lysine and digestible reactive lysine were quantitatively significant.

Table 3. Comparison of the Mean[a] True Ileal Lysine Digestibility (%) Determined Using Conventional Amino Acid Analysis (Total) and True Ileal Lysine Digestibility (%) Based on Determined Reactive Lysine (Reactive)

	lysine digestibility		overall	
	total[b]	reactive[c]	SE	
blood meal	96.3	96.7	0.41	NS
wheat meal	92.6	92.1	0.45	NS
meat and bone meal	88.9	91.5	0.76	NS
soybean meal	94.5	96.5	0.41	*
dried maize	80.5	84.3	1.54	*
heated skim milk powder	69.1	94.0	1.11	***
cottonseed meal	62.1	71.9	1.75	**
alfalfa-based mix	74.2	86.3	0.63	***

[a] For blood meal, wheat meal, soybean meal, meat and bone meal, heated skim milk powder, and cottonseed meal, $n = 8$; for the dried maize and alfalfa-based mix, $n = 5$. [b] Lysine digestibility was determined using a true ileal amino acid digestibility assay (rat), and conventional amino acid analysis was used to quantitate total lysine in the diets and digesta. [c] Lysine digestibility was determined using a true ileal amino acid digestibility assay (rat), and the guanidination reaction was used to quantitate reactive lysine in the diets and digesta.

Table 4. Mean[a] Digestible Total Lysine and Mean Digestible Reactive Lysine Contents (g kg^{-1} of Sample) in Several Protein Sources

	digestible lysine		overall	
	total[b]	reactive[c]	SE	
blood meal	85.9	85.1	0.34	NS
wheat meal	3.2	2.9	0.02	***
meat and bone meal	32.5	31.6	0.24	NS
soybean meal	30.6	31.2	0.12	*
dried maize	2.6	1.9	0.04	***
heated skim milk powder	19.8	16.6	0.30	***
cottonseed meal	12.9	10.3	0.29	***
alfalfa-based mix	14.4	10.8	0.10	***

[a] For blood meal, wheat meal, soybean meal, meat and bone meal, heated skim milk powder, and cottonseed meal, $n = 8$; for the dried maize and alfalfa-based mix, $n = 5$. [b] Digestible total lysine was calculated from true ileal lysine digestibility (rat), with lysine determined by conventional amino acid analysis, and the total lysine content in the protein source, also determined using conventional amino acid analysis. [c] Digestible reactive lysine was calculated from true ileal reactive lysine digestibility (rat, guanidination analysis) and the reactive lysine content of the protein source, also determined using guanidination.

DISCUSSION

Lysine can undergo chemical reactions (*e.g.*, the Maillard reaction) with other compounds present in a complex feed rendering the lysine structurally altered and nutritionally unavailable. Such reactions may be particularly marked during processing of the feedstuffs or during prolonged storage. Furthermore, some of the lysine derivatives are acid labile and can revert back to lysine during the acid hydrolysis step used in conventional amino acid analysis, leading to an overestimate of lysine content. This conversion does not occur in the animal's digestive tract. Consequently, ileal digest-

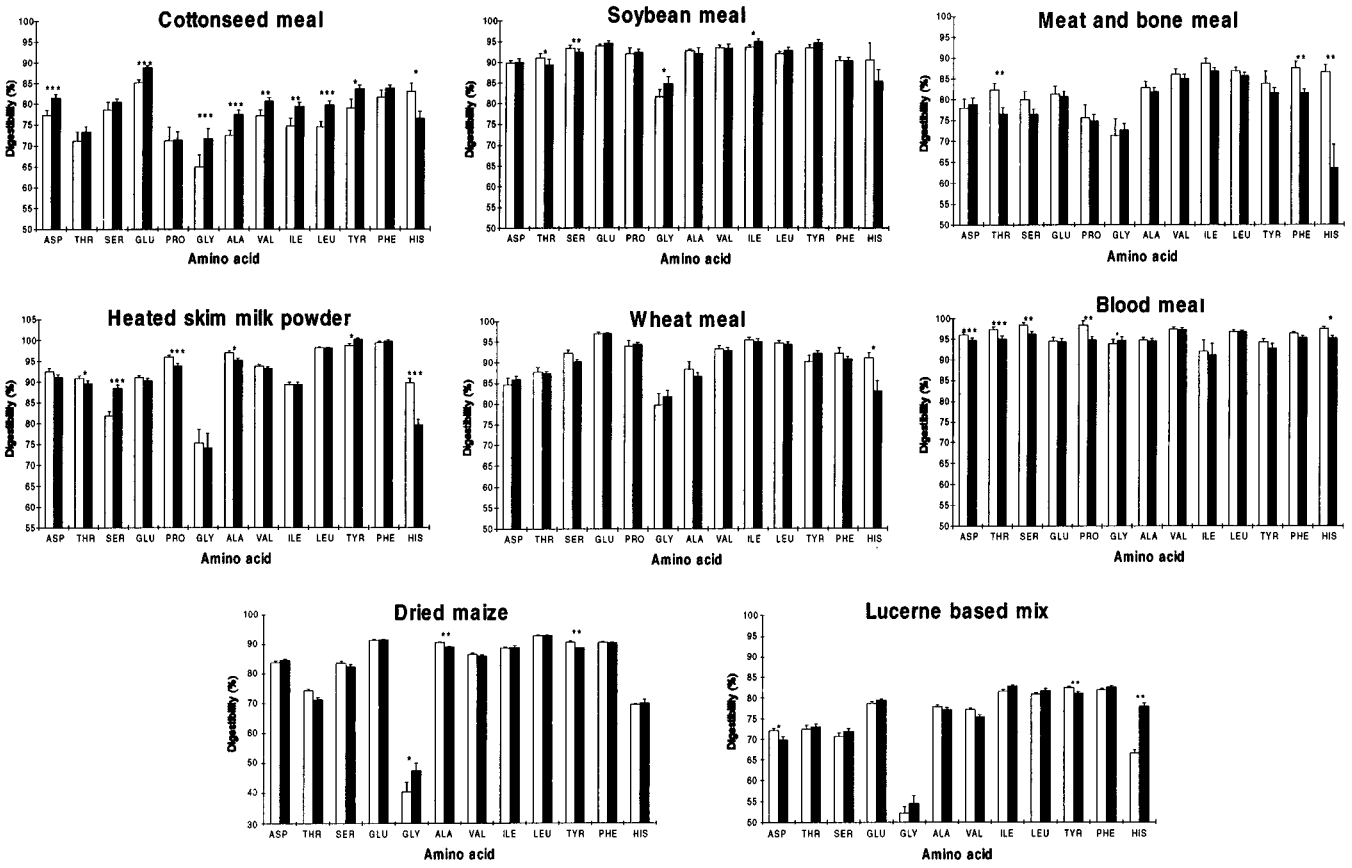

Figure 1. Comparison of the true ileal digestibility of amino acids (other than lysine) as determined using conventional amino acid analysis (□) or following the guanidination reaction (■).

ibility assays which use conventional amino acid analysis to determine lysine contents of diets and digesta are likely to lead to inaccuracies. Methods, such as the FDNB method, have been developed to determine chemically reactive lysine in feedstuffs. These methods, however, do not account for the incomplete digestion and absorption of structurally unaltered lysine. It appears that not all of the structurally unaltered lysine present in a processed feedstuff may be absorbed (Moughan *et al.*, 1996). A better approach would be to determine the reactive lysine concentrations in diets and ileal digesta to determine the digestibility of "reactive" lysine rather than the "total" lysine and thus to describe the digestible reactive lysine contents of feedstuffs. This present study aimed to apply a true ileal reactive lysine digestibility assay (Moughan and Rutherfurd, 1996) to a range of commercially available protein sources, some of which had undergone processing.

Reactive Lysine in Different Protein Sources. Generally good agreement was found between the reactive lysine contents of the feedstuffs determined using the FDNB and guanidination methods, especially for blood meal, wheat meal, and cottonseed meal. For soybean meal and meat and bone meal, the reactive lysine contents determined using the FDNB method were lower than those determined using guanidination. Since theoretically the guanidination method cannot overestimate reactive lysine, it would appear that these differences are most likely an artifact of the FDNB method in which correction factors must be used.

In an unprocessed protein source the reactive lysine content should be equivalent to the "total" lysine content, where total lysine is the lysine determined by conventional amino acid analysis. In contrast, in a protein source which has sustained early heat damage, the total lysine content may be higher than the reactive lysine content due to reversion of lysine during acid hydrolysis. In some processed protein sources where more severe processing damage has occurred, structurally altered lysine derivatives may be acid-stable. In this case reactive and total lysine values would be expected to be similar. For the blood meal, meat and bone meal, and soybean meal in the present study, the reactive lysine content determined using the guanidination method was similar to the total lysine content, suggesting that either these protein sources did not contain structurally altered lysine derivatives or, if they were present, they were in a form that is stable to acid conditions. For wheat meal the reactive lysine content was lower than the total lysine content suggesting that some reversible modification of lysine may have occurred. For dried maize, the alfalfa-based mix, cottonseed meal, and heated skim milk powder, the reactive lysine content was considerably lower than the total lysine, reflecting protein sources in which lysine had undergone early Maillard type reactions during processing. Cottonseed meal undergoes considerable heat processing, in order to reduce the toxicity of the antinutritional factors known to be present (Berardi and Goldblatt, 1980), while the skim milk powder in the present study was subjected to controlled heating in our laboratory.

Comparison of the True Ileal Digestibility of Acid-Stable Amino Acids in Protein Sources Determined Using Conventional Amino Acid Analysis or following Guanidination of the Diet and Digesta Prior to Amino Acid Analysis. While the recently developed true ileal reactive lysine digestibility assay was designed to determine reactive lysine digestibility, it would be desirable if digestibility data for the

remaining acid-stable amino acids could also be obtained. The present study provided important information on this. The true ileal digestibilities of amino acids, other than lysine, were determined using the true ileal digestibility assay applied to unguanidinated diet and digesta samples or to samples which had undergone guanidination, and the results are given in Figure 1. For most of the protein sources tested, including wheat meal, soybean meal, blood meal, dried maize, meat and bone meal, skim milk powder, and the alfalfa-based mix, there was no statistically significant or practical difference (less than 3% units) for most (89%) of the amino acids between digestibility determined using conventional amino acid analysis with or without prior guanidination. In contrast for cottonseed meal there were significant and practical differences for nine of the amino acids examined. There was no one amino acid for which digestibility differed between the two approaches for all protein sources. Depending on the level of accuracy required, it may be possible to obtain digestibility coefficients for amino acids other than lysine following guanidination of the diet and digesta samples.

Comparison of the True Ileal Digestibility of Reactive Lysine and Total Lysine in Protein Sources Determined Using Conventional Amino Acid Analysis or following Guanidination of the Diet and Digesta Prior to Amino Acid Analysis. True ileal "total" lysine digestibility was compared with the true ileal digestibility of reactive lysine for the eight different protein sources. For blood meal, wheat meal, and meat and bone meal, the digestibilities of total lysine and reactive lysine were similar and both were high. For soybean meal, total lysine digestibility, which was also high, was significantly ($p < 0.05$) different from reactive lysine digestibility, although the difference was small. These results again reflect protein sources containing minimal amounts of acid-labile "damaged" lysine derivatives, and as such both the conventional true ileal amino acid digestibility assay and the new true ileal reactive lysine digestibility assay may be suitable methods for determining lysine digestibility. For the alfalfa-based mix, which had been pelleted, and for the dried maize, cottonseed meal, and heated skim milk powder, which had all undergone heat processing, there were large differences between total lysine digestibility and reactive lysine digestibility. Further, total lysine digestibility underestimated the actual digestibility of reactive lysine. The conventional true ileal amino acid digestibility assay appears to be unsuitable for assessing lysine availability in heat-processed feedstuffs as it underestimates the digestibility of structurally unaltered lysine. The new true ileal reactive lysine digestibility assay may provide a more accurate assessment of digestibility of structurally unaltered (available) lysine in processed protein sources.

The digestible total lysine and digestible reactive lysine contents were similar for both blood meal and meat and bone meal. For soybean meal, there was a statistically significant difference between digestible total lysine and digestible reactive lysine, although the actual difference was small. For these protein sources, both digestible total lysine and digestible reactive lysine provide accurate measures of available lysine. For wheat meal, there was a significant difference between the digestible total lysine content and the digestible reactive lysine content. This result was unexpected, as wheat meal undergoes only minimal processing. It is possible, however, that Maillard type reactions may have occurred during the storage of the wheat meal prior to submission to our laboratory. For dried maize, the alfalfa-based mix, cottonseed meal, and heated skim milk powder, the digestible reactive lysine content was considerably lower than the digestible total lysine content. The conventional true ileal lysine digestibility assay is known to overestimate lysine availability in processed feeds (Batterham, 1990). It is likely that digestible reactive lysine more accurately reflects available lysine in these protein sources.

There is need for an assay to determine digestible reactive lysine (available lysine) contents in processed feedstuffs, and the true ileal reactive lysine digestibility assay described here may be suitable for this purpose. The most significant shortcoming of the new assay is that the guanidination time required for the digesta is long (3−7 days). It may be possible to significantly reduce this reaction time by increasing the incubation temperature. The second shortcoming is that the assay most likely cannot distinguish between L-lysine and D-lysine; therefore, the assay is limited to feedstuffs in which significant racemization of lysine has not occurred. However, since lysine will only racemise in strong alkali (Liardon and Hurrell, 1983) and since it is one of the more difficult amino acids to convert to its D-enantiomer, then for most protein sources this should not significantly reduce the effectiveness of the assay. There appears to be considerable potential with the new assay for determining lysine availability in processed feedstuffs.

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