Nutritional Quality and Variation of Meat and Bone Meal

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ABSTRACT : Meat and bone meal is a valuable protein and mineral source in diets of production animals and contributes to the protein, energy and mineral component of diets. The aim of the present study was to more accurately characterise the apparent ileal amino acid digestibility of meat and bone meals produced in New Zealand and evaluate routine *in vitro* assays used in practise to measure meat and bone meal quality. A total of 94 commercial meat and bone meals from 25 New Zealand rendering plants over a two and a half year period were analysed for proximates, gross energy, gross amino acid content (incl. hydroxyproline, hydroxylysine and lanthionine), apparent ileal amino acid digestibility, pepsin nitrogen digestibility, protein solubility and bone content. The mean crude protein content of the 94 meat and bone meal samples was 56.8% with a range of >35% units and a coefficient of variation of 9.8%. The mean crude fat and ash content were 10.0 and 28.4% respectively. These latter components showed a large range (16 and 43%, respectively) with coefficients of variation above 22%. Amino acid digestibility between samples was highly variable with lysine and sulphur amino acids digestibility ranging between 45.8-89.0 and 38.2-85.5%, respectively. Pearson correlation coefficients are presented between crude protein content and individual gross amino acids, crude protein content and individual digestible amino acid content, and pepsin N digestibility and individual digestible amino acid content. There was a significant relationship between the digestible amino acid nitrogen content and the crude protein content while pepsin nitrogen digestibility was not correlated to ileal amino acid nitrogen digestibility (r=-0.06). Meat meals with a high protein content had relatively low hydroxyproline and hydroxylysine levels something that was attributed to the levels of collagen from bone. The data indicated that lanthionine (formed upon heat treatment of cysteine with a hydroprotein) is not a good indicator of the heat treatment employed to meat and bone meals. Step-wise multiple regression equations to predict the apparent digestible content of amino acids from rapid *in vitro* assays are presented. The most selected variables included ash and crude fat content. In general the equations derived for the essential amino acids had a higher degrees of fit (R^2) compared to the non-essential amino acids. The R^2 for the essential amino acids ranged from 0.43 for histidine and 0.68 for leucine. These equations provide a means of more rapidly estimating the apparent ileal digestible amino acid content (protein quality) of meat and bone meal using standard analyses. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 10 : 1507-1516)***

Key Words : Meat and Bone Meal, Nutritional Quality, Ileal Digestibility, Gross Composition, Amino Acids

INTRODUCTION

Meat and bone meal is included in diets for pigs and poultry as a protein source and can contribute up to 30% of the dietary protein supply. Besides being a valuable protein source, meat and bone meal also contributes to the energy component of the diet and is a good source of calcium, phosphorus and trace minerals. The nature of the raw materials as well as the processing methods used to produce meat and bone meal, however, can result in a highly variable product in terms of chemical composition and protein quality. Ashley (1983) reported wide variability in the crude protein, fat and ash contents of meat and bone meals produced in the United Kingdom and Europe. Crude protein levels ranged from 31 to 66% (as is) while ash levels ranged from 12 to 40% (as is) in meat and bone meals produced in the United Kingdom. Skilton et al. (1991) determined the apparent ileal nitrogen digestibility of New Zealand meat and bone meals using a rat bioassay and reported a range of 54 to 75%. Donkoh et al. (1994a) found a similar range in apparent ileal nitrogen digestibility for New Zealand meat and bone meals (53 to 79%). Parsons et al. (1997) determined the lysine bioavailability of North American meat and bone meals for chickens using a slope-ratio assay and reported a range from 43 to 89%. The variability in gross composition and protein quality is a major concern to the feed industry and emphasises the inappropriateness of the use of tabulated analytical values thereby limiting the inclusion of meat and bone meal in diets for pigs and poultry.

Limited data are available on the protein quality of meat and bone meals and the variability in protein quality between meals (Parsons et al., 1997). Earlier work on meat and bone meals produced in New Zealand (Skilton et al., 1991; Donkoh et al., 1994a) involving a total of 20 samples, have found that the quality of New Zealand meat and bone meals is variable. It was concluded in these studies that there was a need for a routine relatively inexpensive assay for the measurement of the protein quality of meat and bone meal.

The aim of the present study was to characterise the nutritional value of meat and bone meals produced in New Zealand in terms of its apparent ileal amino acid digestibility

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using a larger number of samples. A total of 94 meat and bone meals were analysed from 25 New Zealand rendering plants over a two and a half year period. In addition, the current study also aimed to evaluate the value of routine *in vitro* assays currently used to measure meat and bone meal quality to predict the apparent ileal digestibility of protein and amino acids.

MATERIALS AND METHODS

Meat and bone meals

A total of 94 meat and bone meal samples were obtained over a two and a half year period from 17 rendering companies throughout New Zealand. Eighty-nine samples were directly obtained from a total of 25 plants. The samples (5 kg each) were collected from newly made batches of meat and bone meal, sealed in plastic bags and transported to the laboratory within two days of manufacture. Five samples were obtained from feed compounders. A subsample (approximately 1.5 kg) of each 5 kg meat and bone meal sample was passed through a 0.8 mm sieve and the fraction retained finely ground (1 mm mesh). The two fractions were thoroughly mixed and the homogenized sample was used for all chemical and biological analyses. All samples were stored at -20°C or -85°C until required for analysis.

Chemical analysis

All chemical analyses were determined in duplicate unless otherwise stated. Dry matter was determined by oven drying for 16 h at 105°C while ash was determined by heating samples to 550°C for 16 h. Nitrogen and sulphur contents were determined by the Dumas method using a LECO CNS-2000 Carbon, Nitrogen and Sulphur Analyzer. Lipid content was determined using the method of Folch (1957).

Amino acids were determined in 5 mg samples by hydrolyzing with 1 ml of 6 M glass-distilled HCl (containing 0.1 g phenol/l) for 24 h at 110°C in glass tubes, sealed under vacuum. The tubes were opened and norleucine was added to each tube as an internal standard, and the tubes were then dried under vacuum (Savant Speedvac Concentrator AS 290, Savant Instruments Inc., Farmingdale, NY). Amino acids were dissolved in 2 ml sodium citrate buffer (pH 2.2) and loaded onto a Waters ion-exchange HPLC system (Millipore, Milford, MA) employing postcolumn derivatization with ninhydrin and detection at 570 nm. Proline was detected at 440 nm. The chromatograms were integrated using dedicated software (Millenium, Version 3.05.01, Waters, Milford, MA) with amino acids (including 4-hydroxyproline, hydroxylysine and lanthionine) identified by retention time against a standard amino acid mixture (Sigma, St. Louis, MO). Cysteine and methionine were determined following performic acid oxidation of the samples prior to hydrolysis. Samples (±5 mg) were accurately weighed into 10 ml pyrolyzed glass hydrolysis tubes and 2 ml of freshly prepared performic acid (1 part 30% H_2O_2 to 8 parts of 88% formic acid) was added. The tubes were kept at 0°C for 16 h after which time the reaction was terminated using 0.3 ml of 48% HBr. The hydrolysis tubes were dried under vacuum and the oxidised samples were hydrolyzed and quantitated using the procedure and equipment described previously. Cysteine and methionine were detected as cysteic acid and methionine-sulphone, respectively. Tryptophan was not determined. Amino acid concentrations were corrected for recoveries of norleucine and converted to a weight basis using molecular weights of free amino acids.

Chromium contents of diet and ileal digesta samples were determined on a GBC 902 AA absorption/emission spectrophotometer (GBC Scientific NZ Ltd, Auckland, New Zealand) following the method of Costigan and Ellis (1987). Gross energy was determined by bomb calorimetry. The pepsin nitrogen digestibility was essentially that of AOAC (1984) but with the following modifications. The samples were defatted prior to pepsin digestion using the method of Folch (1957) and the final results were expressed as % digestible crude protein where the crude protein was measured by the N content (%), using the Dumas rather than the Kjeldahl method, multiplied by the factor 6.25. Protein solubility was determined by the procedure described by Parsons et al. (1991) except that inadequate separation of the soluble and insoluble fractions obtained with centrifugation was overcome by using fluted paper filters (Whatman No. 1) suspended in glass funnels. An estimate of bone content of each meat and bone meal was carried out using a chloroform flotation procedure (Dale, 1997). Each sample (20 g) was placed in a 100 ml graduated measuring cylinder with 65 ml chloroform. After agitation, more chloroform was added to bring the total volume to 90 ml. The stoppered measuring cylinders were left undisturbed for 16 h overnight allowing the bone fraction to sink below the floating protein and lipid layers. A reading of the bone volume was then taken.

Apparent ileal amino acid digestibility

The apparent ileal amino acid digestibility of the meat and bone meal samples was determined in six batches, 14-18 samples being tested in each batch. Within each trial 6 rats were randomly allocated to one of the meat and bone meal diets. Ethics approval for the ileal digestibility procedure was given by the Palmerston North Crown Research Institutes' Animal Ethics Committee.

Weaned male Sprague Dawley rats (21 days of age; 45-55 g body weight) were obtained from the Food Evaluation Unit, Crop and Food Research (Palmerston North, New Zealand). The rats were housed in family weaning groups in

shoebox cages in a temperature (22±1°C) and humidity-controlled (60±5%) room with a 12 h light/dark cycle (06:00-18:00 h). They were provided commercial rat pellets (Sharpes Grain and Seed, Carterton, New Zealand) *ad libitum* for 14 days. At an approximate body weight of 140 g, the rats were transferred to individual raised stainless steel cages with mesh floors and fed a 120 g kg^{-1} lactic casein-based semi-synthetic diet *ad libitum* for 14 days. At approximately 200 g bodyweight, the rats were accustomed to a single daily 3 h meal period (09:00-12:00 h) over a 7 day period using the lactic casein diet. After the training period, the rats were fed the meat and bone meal diets as a single daily meal for a further eight days. Water was freely available to the rats at all times.

The lactic casein-based diet comprised (g kg^{-1}): lactic casein, 120; maize oil, 80; mineral salt mix, 50; vitamin mix, 50; cellulose, 10 and wheaten corn flour, 690. The meat and bone meal diets were formulated to contain 100 g crude protein (N×6.25) and 65 g fat kg^{-1} diet. Meat and bone meal was the sole source of protein for the diets while the 65 g kg^{-1} fat was supplied by fat from the meat and bone meal sample and maize oil. Chromic oxide (3 g kg^{-1}) was included in each meat and bone meal diet as an indigestible marker. The remainder of the diet comprised 50 g kg^{-1} mineral salt mix, 50 g kg^{-1} vitamin mix, 50 g kg^{-1} cellulose and sufficient corn flour to make 1,000 g kg^{-1}. The diets were formulated to meet the nutrient requirements of the growing rat (NRC, 1995).

On the last day of the assay, four hours after the start of feeding, the rats were asphyxiated with carbon dioxide gas and decapitated (immediately ceasing all neural stimulation of the gut). The abdomen was opened by an incision along the mid ventral line and the skin and musculature were folded back to expose the viscera. The stomach contents were inspected for signs of faecal contamination which would result from coprophagy. The final 20 cm of the ileum was dissected out from the body and the digesta were slowly flushed out with 10 ml deionised water from a plastic syringe. The digesta were immediately frozen (-20°C) and later stored at -85°C. The ileal digesta was then freeze-dried, finely ground and weighed. Equal amounts (by weight) of the freeze-dried ileal digesta for each of the six rats fed the same meat and bone meal diet were pooled. The pooled digesta sample was mixed well and subjected to amino acid and chromium analyses.

The flow of amino acids at the terminal ileum (mg g^{-1} DM) was calculated for each meat and bone meal diet using the following equation:

$$\text{Ileal digesta amino acid content (mg g}^{-1}\text{ DM)} \times \frac{\text{Diet Cr (mg g}^{-1}\text{ DM)}}{\text{Ileal digesta Cr (mg g}^{-1}\text{ DM)}}$$

Apparent ileal amino acid digestibility (%) was calculated for each meat and bone meal diet using the following equation:

$$= \frac{\text{Diet amino acid content (mg g}^{-1}\text{ DM)} - \text{Ileal amino acid flow (mg g}^{-1}\text{ DM)} \times 100}{\text{Diet amino acid content (mg g}^{-1}\text{ DM)}}$$

The data on crude protein and the digestible amino acid nitrogen contents as well as the pepsin nitrogen digestibility and the digestible amino acid nitrogen content data were subjected to linear regression. Pearson correlation coefficients were determined between crude protein content and gross amino acid contents, crude protein content and digestible amino acid contents, and pepsin N digestibility and individual digestible amino acid content. Pearson correlation coefficients were also determined between selected variables. A stepwise regression procedure was used to obtain other equations for the prediction of the apparent ileal digestible amino acid, amino acid nitrogen and sulphur amino acid content from proximate analyses. Variables in the stepwise regression included dry matter, crude protein, crude fat, ash, gross energy, sulphur, crude protein to fat ratio, ash to crude protein ratio and ash to fat ratio. All statistical analyses were performed using SAS (1999) and probabilities were considered significant at the 5% level.

RESULTS

The variation in nutrient composition and *in vitro* digestibility data are presented in Table 1. There was a high variability, with the exception of dry matter, for all the components measured. The mean crude protein content was 56.8% with a range of >35% units and a coefficient of variation of 9.8%. The mean crude fat and ash content of the 94 meat and bone meal samples were 10.0 and 28.4%, respectively. These components also showed a wide range (16 and 43%, respectively) with coefficients of variation above 22%. The lowest and highest levels measured in the 94 meat and bone meals for both components differed by a factor of 7 and 4, respectively. The variation in the content of nutrients was also reflected by the gross energy content of the samples, which had a range of 12 kJ/g and a coefficient of variation above 10%. Pepsin nitrogen digestibility was generally high (mean 89.9%) with a coefficient of variation of approximately 4%. Total sulphur content, protein solubility and the bone content measurements (Table 1) showed the largest range in values of all components measured and high coefficients of variation.

The variation in gross amino acid contents of the 94 samples is shown in Table 2. On average, meat and bone meal samples contained higher levels of the non-essential amino acids glutamic acid, aspartic acid, alanine and proline

Table 1. Variation in the nutrient composition and *in vitro* digestibility data of 94 New Zealand meat and bone meal samples

Component	Range	Mean	SD[1]	CV[2]
Dry matter (%)	91.2-98.6	95.4	1.6	1.7
Crude Protein (N×6.25) (%)	38.5-73.6	56.8	5.6	9.8
Fat (%)	2.5-18.5	10.0	2.7	26.6
Ash (%)	13.0-56.5	28.4	6.5	22.9
Gross energy (kJ/g)	9.4-22.3	17.1	1.9	11.3
Pepsin N digestibility (%)	79.7-94.4	89.9	3.3	3.7
Protein solubility (%)	6.7-62.0	25.8	16.0	61.9
Bone content[3] (ml)	20.1-93.5	57.6	14.9	25.9
Sulphur (%)	0.1-1.0	0.4	0.1	36.2

[1] Standard deviation.
[2] Coefficient of variation.
[3] Chloroform flotation method.

(4.2 to 7.4%) as well as high levels of the essential amino acids arginine, leucine and lysine (3.1 to 4.2%). The average contents of hydroxyproline and hydrolysine were 2.74 and 0.35%, respectively. Of the essential amino acids methionine had the lowest SD (0.2) while leucine had the highest (0.57). The CV of the essential amino acid content of the meat and bone meal samples ranged from 10.2 to 21.2%. On average, the non-essential amino acids (with the exception of lanthionine) were found to have lower CVs (7.4 to 15.6). The semi-essential amino acids, cysteine and tyrosine, had SDs of 0.12 and 0.25, respectively. The amino acid N content of the samples was about 8 g per 100 g dry matter or about 85-90% of the total N content. The average CV for amino acid N (Table 2) was similar to the CV of total N (crude protein, Table 1).

The results of the *in vivo* apparent ileal digestibility measurements as determined in rats are provided in Table 3. In general the apparent digestibilities of the essential amino acids were higher than those of the non-essential amino acids. There was a large range in the apparent digestibility of most essential and non-essential amino acids. The SD of the apparent digestibility of the amino acids ranged from 8.8 to 15.1% and the SD for amino acid N was 10.0%. Histidine and leucine had the largest CVs of the essential amino acids while of the non-essential amino acids aspartic acid, serine, hydroxylysine and hydroxyproline had the largest CVs. Low apparent digestibility values were found for cysteine while negative digestibility values were obtained for three meat and bone meal samples for the two amino acids aspartic acid and hydroxylysine. The variation in the apparent digestibility of amino acid nitrogen and sulphur amino acids was similar.

There was a significant relationship between the crude protein content and the digestible amino acid N content in meat and bone meals (Figure 1). The intercept was found to be not significantly different from zero and after fitting a no intercept model to the data, the following equation was obtained: dcAAN=0.0861×CP where dcAAN is the apparent ileal digestible amino acid nitrogen content (%) and CP is the crude protein content (%). The slope of the linear regression was found to be significant at a probability level of 0.01%. The degree of fit (R^2) of the linear regression equation was 0.24. Figure 2 shows the *in vitro* digestibility of protein by pepsin against the *in vivo* digestibility of amino acid N. The slope of the linear regression line was found to be not significantly different from zero.

Table 4 shows the Pearson correlation coefficients between crude protein content and gross content of individual amino acids, and protein content and the digestible content of individual amino acids of the 94 meat and bone meal samples. The correlation coefficients between crude protein and individual gross amino acids were generally between 0.60 and 0.80 with the exception of hydroxylysine, hydroxyproline and proline. The correlation coefficients were weaker between crude protein content and the apparent digestible content of individual amino acids. There were no

Table 2. The variation in gross amino acid, amino acid nitrogen and sulphur amino acid content of 94 New Zealand meat and bone meal samples

Component	Range	Mean	SD	CV
Essential amino acids				
Arginine	3.17-5.15	4.15	0.42	10.2
Histidine	0.48-1.85	1.05	0.21	20.0
Isoleucine	0.84-2.56	1.60	0.26	16.5
Leucine	1.82-5.21	3.53	0.57	16.1
Lysine	1.73-4.28	3.04	0.44	14.5
Methionine	0.44-1.54	0.90	0.19	21.2
Phenylalanine	1.07-3.22	1.88	0.30	16.0
Threonine	1.23-2.70	1.95	0.32	16.6
Valine	1.31-3.62	2.44	0.39	15.8
Semi-essential amino cids				
Cysteine	0.14-0.78	0.42	0.12	29.5
Tyrosine	0.60-2.07	1.34	0.25	18.9
Non-essential amino acids				
Alanine	3.49-5.23	4.21	0.32	7.6
Aspartic acid	3.27-6.06	4.33	0.58	13.3
Glutamic acid	5.07-8.45	6.82	0.78	11.4
Glycine	5.40-9.42	7.36	0.64	8.8
Proline	3.84-5.75	4.66	0.34	7.4
Serine	1.63-2.84	2.23	0.30	13.2
Hydroxylysine	0.23-0.46	0.35	0.05	13.1
Hydroxyproline	1.72-4.04	2.74	0.43	15.6
Lanthionine	0.01-0.28	0.07	0.06	88.8
Amino acid nitrogen	6.37 - 9.58	8.01	0.69	8.6
Sulphur amino acids	0.58 - 2.13	1.32	0.29	22.0

Values are expressed as % in the dry matter.

Table 3. Variation in apparent ileal digestibility (%) of amino acids, amino acid nitrogen and total sulphur amino acid of 94 New Zealand meat and bone meal samples

Component	Range	Mean	SD	CV
Essential amino acids				
Arginine	36.9-90.7	73.2	10.3	14.1
Histidine	15.5-81.3	56.1	13.5	24.0
Isoleucine	46.6-87.7	70.8	8.8	12.5
Leucine	45.0-88.8	71.9	9.2	12.9
Lysine	45.8-89.0	72.7	9.5	13.1
Methionine	47.1-89.6	74.8	9.5	12.7
Phenylalanine	47.3-89.6	74.2	8.9	12.0
Threonine	31.6-82.4	57.6	11.3	19.6
Valine	43.9-87.0	68.8	9.4	13.7
Semi-essential amino acids				
Cysteine	10.0-79.2	54.0	15.1	27.9
Tyrosine	45.1-88.9	69.8	9.9	14.2
Non-essential amino acids				
Alanine	38.8-84.8	67.2	10.1	15.0
Aspartic acid	-4.8-75.2	43.1	16.0	37.1
Glutamic acid	37.5-84.7	64.4	10.1	15.6
Glycine	28.2-80.3	58.0	11.7	20.2
Proline	27.3-79.5	59.2	11.5	19.4
Serine	23.5-78.5	53.0	12.1	22.9
Hydroxylysine	-1.1-76.8	38.9	14.8	38.2
Hydroxyproline	20.9-84.8	56.4	14.0	24.8
Amino acid nitrogen	37.6-83.6	63.7	10.0	15.7

significant correlations between pepsin nitrogen digestibility and any of the apparent ileal digestible amino acids in the meat and bone meal samples.

Pearson correlation coefficients for selected correlations between assays are shown in Table 5. Total sulphur content was moderately but significantly correlated to gross cysteine, methionine and sulphur amino acid contents as well as apparent digestible cysteine and methionine contents. Protein solubility was weakly but significantly correlated to apparent amino acid nitrogen digestibility. Pepsin nitrogen digestibility was not correlated to the apparent digestible amino acid nitrogen content in the meat and bone meal samples. The highest significant correlation was observed between bone content and ash content (r=0.80). Lanthionine content was not correlated to apparent ileal amino acid nitrogen digestibility.

Table 6 shows the step-wise multiple regression equations and the degree of fit of the equations using selected variables to predict the apparent ileal digestible amino acid, amino acid nitrogen and sulphur amino acid content. The equations for the essential amino acids in general had a higher degree of fit (R^2) compared to the non-essential amino acids (0.43-0.68 compared to 0.22-0.49, respectively). Variables selected by the statistical programme included crude protein content, ash content, crude fat content, sulphur content, gross energy content,

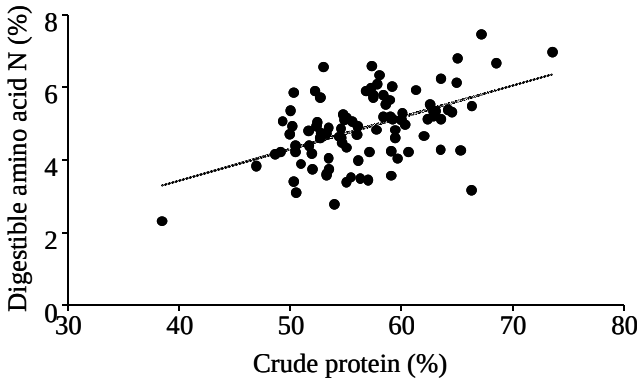

Figure 1. Relationship (Y=0.0861×X) between crude protein content and the digestible amino acid nitrogen content of 94 New Zealand meat and bone meals.

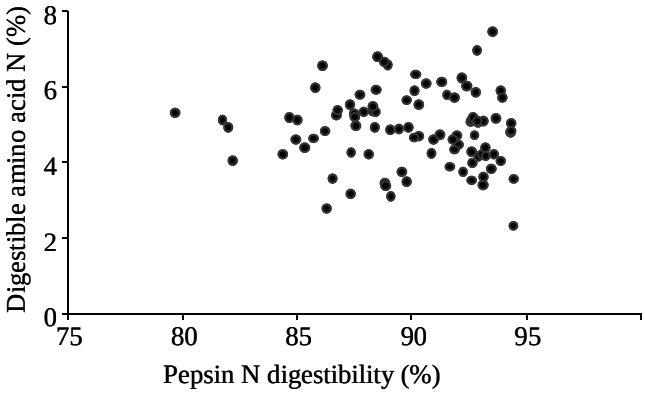

Figure 2. Relationship between pepsin nitrogen digestibility and the digestible amino acid nitrogen content of 94 New Zealand meat and bone meals.

(crude fat content)^2, (sulphur content)^2, crude protein to crude fat ratio and ash to crude protein ratio. No variables were selected at the 5% probability level to predict the apparent ileal digestible glycine content.

DISCUSSION

The data in the present study show that meat and bone meals produced in New Zealand are highly variable in nutritional quality. In particular, the contents of crude protein, crude fat and ash were highly variable. The ash content in meat and bone meal does not contribute to the energy component of meals. As a result, the large variability in ash content together with the variability in protein and fat content resulted in the observed large range in gross energy content (9.4 to 22.3 MJ/kg). The ranges and variation found in the present study for crude protein, fat and ash were larger compared to previous data on New Zealand samples (Skilton et al., 1991; Donkoh et al., 1994a) but comparable to those reported by Ashley (1983) for 299 meat and

Table 4. Pearson correlation coefficients between crude protein content and individual gross amino acids, and crude protein content and individual digestible amino acids

Component	Protein vs gross AA	Protein vs digestible AA
Essential amino acids		
Arginine	0.74***	0.57***
Histidine	0.62***	0.49***
Isoleucine	0.75***	0.60***
Leucine	0.80***	0.64***
Lysine	0.74***	0.61***
Methionine	0.71***	0.62***
Phenylalanine	0.73***	0.60***
Threonine	0.75***	0.49***
Valine	0.74***	0.57***
Semi-essential amino acids		
Cysteine	0.64***	0.44***
Tyrosine	0.80***	0.62***
Non-essential amino acids		
Alanine	0.61***	0.44***
Aspartic acid	0.66***	0.24*
Glutamic acid	0.75***	0.53***
Glycine	-0.08NS	0.14NS
Proline	0.26*	0.28**
Serine	0.76***	0.41***
Hydroxylysine	-0.23*	0.02 NS
Hydroxyproline	-0.45***	-0.13NS
Amino acid N	0.77***	0.49***
Sulphur amino acids	0.74***	0.60***

NS=Not significant (p>0.05), * p<0.05, ** p<0.01, *** p<0.001.

Table 5. Selected Pearson correlation coefficients between assay parameters

Parameter 1	Parameter 2	r
Total sulphur	vs. gross cysteine	0.57***
	vs. gross methionine	0.52***
	vs. gross sulphur amino acids	0.58***
	vs. app. digestible cysteine	0.44***
	vs. app. digestible methionine	0.44***
Protein solubility	vs. app. amino acid N digestibility	0.29***
Pepsin N digestibility	vs. app. digestible amino acid N	-0.06^{NS}
Protein	vs. app. digestible amino acid N	0.60***
Bone content	vs. ash	0.80***
Lanthionine	vs. app. amino acid N digestibility	0.20^{NS}

NS=Not significant (p>0.05), *** p<0.001.

bone meals produced in the United Kingdom and Europe. The variation in meat and bone meal composition is mainly due to the composition of the raw material source used for production (Donkoh et al., 1994a; Johnson and Parsons, 1997).

Kirby et al. (1993) examined the assumption of a normal distribution for protein content in feedstuffs by collecting data on the nutrient content of meat and bone meals from broiler feed mills. Statistical analyses of 264 meals showed that the protein content of meat and bone meal was non-normally distributed. Statistical analyses of the results in the present study showed that the protein content of New Zealand meat and bone meals was normally distributed (Kolmogorov-Smirnov normality test, p>0.15; skewness 0.14; kurtosis 0.75). The reason for this may be because the samples obtained by Kirby et al. (1993) were from only four broiler feed mills. These samples may not have been representative of the population of meat and bone meals produced as renderers may only sell meals that fall within a specified range to feed mills. In addition Kirby et al. (1993) obtained samples from March to November while the samples obtained in the present study were obtained over a two and a half year period.

In typical New Zealand diets for finishing pigs, meat and bone meal can make up to 10% of the total diet representing approximately 30% of the dietary protein supply. Diets for production animals such as pigs and poultry are formulated based on the digestible nutrient content of ingredients and as such the digestibility of amino acids is an important characteristic of feed ingredients. Information on the relative ability of feed ingredients to supply digestible rather than total amino acids is necessary for accurate diet formulation (Furuya and Kaji, 1989). In addition feed ingredients have to be “predictable” in terms of nutrient delivery from batch to batch. Various studies (Moughan et al., 1984, 1987; Picard et al., 1984; Donkoh et al., 1994b; Pearson et al., 1999) showed that the laboratory rat is a suitable model for the growing pig for the determination of the ileal protein and amino acid digestibility, particularly for meat and bone meal (Donkoh et al., 1994b). The present study shows that the digestible amino acid content of New Zealand meat and bone meals is highly variable. The digestibility of amino acid nitrogen, which represents the digestibility of the ‘true’ protein in meat and bone meal, ranges from 37.6 to 83.6%. The digestible amino acid nitrogen content ranged from 6.37 to 9.58% in the present study. Similar to results obtained in other studies (Parsons et al., 1997; Shirley and Parsons, 2000), the digestibility of cysteine was found to be low and highly variable in the present study. The reason for the high variability is likely to be caused by variability in raw materials and processing conditions of the meals. Cysteine has been shown to be the amino acid most affected by processing conditions (Wang and Parsons, 1998; Shirley and Parsons, 2000). The large variability in the digestibility and digestible content of amino acids in meat and bone meal and the dependence on meat and bone meal as a source of amino acids for monogastric production animals has a large impact on animal performance. Over-formulation of diets in terms of digestible amino acid content, results in inefficient production while under-formulation results in the deposition of fat by the animal due to a sub optimal protein to energy ratio.

Table 6. Multiple regression equations, degree of fit (R^2) and standard error of the estimation (SEE) for the prediction of the apparent ileal digestible content of selected components in meat and bone meal

Digestible (% as is)	Regression equation (% as is)	R^2	SEE
Essential amino acids			
Arginine	$5.025-0.083\times Ash-1.206\times S^2+0.075\times R_1$	0.55	0.149
Histidine	$1.323-0.023\times Ash-0.001\times CF^2$	0.43	0.022
Isoleucine	$2.545-0.039\times Ash-0.035\times CF$	0.60	0.030
Leucine	$5.494-0.084\times Ash-0.067\times CF$	0.68	0.103
Lysine	$4.925-0.072\times Ash-0.075\times CF$	0.65	0.080
Methionine	$1.610-0.025\times Ash-0.025\times CF$	0.53	0.016
Phenylalanine	$2.905-0.043\times Ash-0.034\times CF$	0.62	0.035
Threonine	$3.056-0.080\times Ash-0.045\times CF+1.471\times R_2$	0.51	0.052
Valine	$3.554-0.053\times Ash-0.042\times CF$	0.58	0.065
Semi-essential amino acids			
Cysteine	$-0.1301+0.005\times CP+0.186\times S$	0.26	0.007
Tyrosine	$2.447-0.057\times Ash-0.034\times CF+0.773\times R_2$	0.67	0.020
Non-essential amino acids			
Alanine	$4.442-0.054\times Ash-0.002\times CF^2$	0.38	0.164
Aspartic acid	$14.18-0.163\times Ash-0.451\times GE$	0.22	0.474
Glutamic acid	$8.064-0.116\times Ash-0.005\times CF^2$	0.49	0.466
Glycine	No variable selected at $p<0.05$		
Proline	$4.141-0.046\times Ash-1.127\times S^2$	0.22	0.255
Serine	$5.067-0.063\times Ash-0.126\times GE$	0.36	0.068
Amino acid nitrogen	$8.665-0.115\times Ash-0.005\times CF^2$	0.44	0.553
Sulphur amino acids	$2.264-0.035\times Ash-0.041\times CF$	0.50	0.033

CP=Crude protein, CF=Crude fat, GE=Gross energy, S=Sulphur, R_1=CP/CF, R_2=Ash/CP.

Dry matter, crude protein, crude fat, ash, pepsin nitrogen digestibility and data on hair content and residues (sieve test) are normally provided by suppliers of meat and bone meal. This information is of limited value in diet formulation where accurate data on the digestible content of protein and amino acids is required. Using a conversion factor of 6.25, the present study showed that New Zealand meat and bone meals, on average, contain 56.8% crude protein which is higher than the protein content of skim milk powder (~38%) and soyabean meal (~44%) but the digestibility of the protein is much less (~67% for meat and bone meal vs. ~95 and ~82% for skim milk powder and soybean meal, respectively) (Sauer et al., 1982; Ravindran et al., 1998; Morel et al., 1999). The digestible protein content however is similar between all three feed ingredients (38, 36 and 36%, respectively, for meat and bone meal, skim milk powder and soyabean meal) but the variability in protein quality of meat and bone meal is significantly greater compared to the other two protein sources (Ravindran et al., 1998; Rutherfurd and Moughan, 1998). The large variability observed in the contents of gross and digestible protein and amino acids between meat and bone meal samples in our study indicates that a large safety margin is required in diet formulation and highlights the need for a rapid and accurate assay to determine the protein quality of each batch before use in diets for pigs and poultry.

All AA's were positively correlated with crude protein content, except hydroxyproline and hydroxylysine which were negatively correlated while glycine was not correlated to crude protein content (Table 4). These results indicate that meat meals with a high protein content have relatively lower hydroxyproline and hydroxylysine levels. Raw bone and connective tissue contains collagen and elastin, two types of protein extraordinarily rich in hydroxyproline and hydroxylysine as well as glycine and proline, compared to protein in other tissues (Asghar and Henrickson, 1982; Jobling and Jobling, 1983). A significant positive correlation was observed between ash content and hydroxyproline ($r=0.67$, $p<0.001$), hydroxylysine ($r=0.52$, $p<0.001$) and glycine ($r=0.39$, $p<0.001$) with all other amino acids showing a significant ($p<0.001$) negative correlation except for proline which was not correlated to ash content ($r=0.01$, $p=0.96$). These results indicate that meals with a low protein content have higher levels of collagen from bone. Lower correlations were observed between crude protein content and digestible essential amino acids compared to gross amino acids while pepsin nitrogen digestibility was not correlated to any significant degree to the digestibility of any essential or non-essential amino acid (Table 4). Knabe et al. (1989) also found that the pepsin nitrogen digestibility assay (AOAC. 1984) poorly correlates to the differences in ileal and faecal nitrogen digestibility. Correlation coefficients between crude protein and individual digestible essential amino acids were on average 0.15 units lower compared to those observed between crude protein and gross essential amino acids. These results indicate that pepsin nitrogen digestibility estimates cannot be used to accurately predict the apparent ileal

digestible content of amino acids. Interestingly, crude protein can moderately predict the apparent ileal digestible content of amino acids.

A number of assays have been used to determine the protein quality of meat and bone meals including protein efficiency ratio, net protein ratio, pepsin nitrogen digestibility, relative nutrient value using *Tetrahymena furgasoni*, dye-binding scoring, ileal amino acid and nitrogen digestibility and chemical scoring (Johnson and Coon, 1979; Skilton et al., 1991; Donkoh et al., 1994a; Parsons et al., 1997). The present study shows that individual assays for crude protein content, total sulphur content, ash content, pepsin N digestibility or protein solubility cannot accurately predict the digestible protein content (apparent digestible amino acid N content) or digestibility of the protein (apparent digestibility of amino acid nitrogen) of meat and bone meal (Table 5). There is limited information in the literature on the suitability of a combination of routinely conducted assays to determine the digestible content of protein and amino acids in meat and bone meal. Combining several routine assays in a multiple regression approach to predict the digestible content of nutrients could potentially provide the information required on the quality of individual meals and be used to provide information required for least-cost formulation of diets from pigs and poultry. Table 6 shows the step-wise multiple regression equations to predict the digestible protein content and the digestible content of individual amino acids from proximate analyses parameters. Ash was selected as a variable in all but one equation (cysteine) to predict the digestible content of individual amino acids. This is unexpected as it is the protein content in feedstuffs which is composed of amino acids and can be expected to be used as a predictor of the digestible content of individual amino acids. In contrast, the ash component does not contribute to the digestible content of amino acids in feedstuffs. However, high levels of ash can be expected to be negatively correlated to high levels of protein, amino acids and digestible amino acids. This is apparent also from the equations in Table 6 as ash was always negatively associated with the digestible content of individual amino acids. In general the equations derived for the essential amino acids have higher degrees of fit compared to the non-essential amino acids. Knabe et al. (1989) and Skilton et al. (1991) presented linear regression equations for the prediction of apparent ileal amino acid digestibility from the apparent ileal nitrogen digestibility in pigs and rats, respectively. The regression equations determined in the present study for the calculation of apparent ileal digestibility of amino acids, amino acid nitrogen and sulphur amino acids have degrees of fit similar to those provided by Skilton et al. (1991) and slightly lower to values presented by Knabe et al. (1989). Although the equations reported by Knabe et al. (1989) and Skilton et al. (1991) are useful, the apparent ileal digestibility of nitrogen needs to be determined to obtain estimates for the apparent ileal digestibility of individual amino acids, which is time consuming and costly. The current study presents equations from which the apparent ileal digestibility of amino acids can be calculated using values which, with the exception of gross energy and sulphur contents, are routinely provided by meat and bone meal producers. Gross energy as well as sulphur content can be obtained relatively rapidly and inexpensively and together with the equations provided here allows the rapid estimation of the protein quality of meat and bone meal samples.

Raw material composition and the heat treatment employed to produce meat and bone meal contributes to the variability in gross composition and the digestibility of amino acids (Skurray and Herbert, 1974; Knabe et al., 1989; Donkoh et al., 1994a; Wang and Parsons, 1998; Shirley and Parsons, 2001). The present study showed a large variation in the digestibility of amino acids between meals. The apparent digestibility of lysine, the first limiting amino acid in diets for pigs fed cereals, ranged between 45.8-89.0% while the apparent digestibility of the first limiting sulphur-containing amino acids for poultry, cysteine and methionine ranged from 38.2 to 85.5%. Similarly, larger ranges in apparent digestibility values were observed for other amino acids. In three cases a negative apparent digestibility value was obtained for amino acids which indicates that the endogenous losses of these amino acids were higher than the absorption of the corresponding dietary amino acids. Lanthionine is an amino acid formed in foods and feed ingredients upon heat treatment by reaction of cysteine with a hydroprotein (Freidman, 1999). Besides the formation of new amino acids, the digestibility of amino acids is generally lowered by increasing heat treatment of meat and bone meal (Shirley and Parsons, 2000). Lanthionine therefore, may be a useful indicator for the extent to which meat and bone meals have been heat-treated. The results from the present study, however showed that there was no significant correlation between lanthionine content and the digestibility of amino acid N (Table 5).

It can be concluded from the present study, that the composition of New Zealand meat and bone meals is highly variable and large coefficients of variation for most gross compositional analyses are observed. The digestibility of amino acids in meat and bone meal is also highly variable and cannot be predicted with great accuracy by the crude protein content or pepsin nitrogen digestibility. By using data from a number of routine assays in a stepwise multiple regression approach the digestible content of individual amino acid meat and bone meals can be predicted.

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