Amino Acid Digestibility in Meat and Bone Meal for the Growing Pig: The Development of a Digestibility Assay Based on the Laboratory Rat

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CORRIGENDA

e 9, line 16 from the top should read "--- amino acids ---". On page 14, last line read "--- reference ---". On page 29, line 21 from the top should read "--- ginine ---". On page 33, line 34 from the top, "identical" should be "similar". On page 11 from the top should read "--- sampling time may vary with ---". On page 51, line the top should read "--- offered freely ---". On page 81 line 30 from the top should appears ---".

ABSTRACT

The study involved developing and applying a routine ileal amino acid digestibility assay, based on the sampling of digesta from the euthanased laboratory rat, with specific application to the digestion of meat and bone meal (MBM) protein by the growing pig. The work was conducted in six separate experiments.

1. The first experiment aimed to evaluate the slaughter method as a technique for sampling ileal digesta in the pig under defined sampling conditions. In a preliminary investigation, the influence of time after feeding (3, 4, 5, 7, 9 and 11 hours) on the amount of digesta obtained from the terminal 20 cm of ileum and apparent ileal nitrogen (N) digestibility was determined. Thirty-six 30 kg liveweight entire male pigs were given a semi-synthetic diet containing meat and bone meal (MBM) as the sole protein source and chromic oxide as an indigestible marker. The animals were given the MBM diet for 14 days and were killed by intracardial injection of a barbiturate on the 14th day. Digesta were flushed from the terminal ileum using deionised water. Secondly, the effect of site in the ileum for sampling digesta at 9 hours post-feeding was determined using 12 entire male pigs. The animals were equally and randomly allocated to two sampling sites (the terminal 0-20 cm or 0-40 cm of ileum). Further samples of ileal digesta were taken at regular 20 cm intervals up the final 140 cm of ileum of each pig. Sampling at 9 hours after the start of feeding resulted in the greatest and least variable sample size as well as the highest N digestibility. Sampling site within the terminal ileum had no significant ($P > 0.05$) influence on the apparent digestibility of nitrogen.

In the same study, comparison of apparent ileal amino acid digestibility in MBM, under the defined sampling conditions, was made between 8 pigs whose digesta were sampled from the terminal 20 cm of ileum at death 9 hours after the commencement of feeding, and 8 pigs with simple T-cannulas and with hourly collection of digesta over 10 hours on the final 2 days of the 14-day study. The simple T-cannulated animals were accepted as the control. There was no significant ($P > 0.05$) effect of digesta collection procedure on the apparent ileal N and amino acid digestibility coefficients. Faecal N and amino acid digestibility coefficients in intact pigs were identical to those in pigs fitted with a simple T-piece cannula in the distal ileum. Faecal digestibility values in both intact and cannulated pigs were, however, considerably ($P < 0.05$) higher than the corresponding ileal values. It was concluded that the slaughter technique is a viable alternative to simple T-cannulation for the determination of N and amino acid digestibility in the pig given a semi-synthetic MBM diet.

2. The second experiment determined the optimal digesta sampling conditions with the slaughter method applied to the laboratory rat. The effect of time after feeding (1, 2, 3, 4, 5 and 6 hours) on the amount of digesta obtained from the terminal 20 cm of ileum and apparent ileal N digestibility was investigated. Thirty-six 190 g male rats received a semi-synthetic diet whose sole protein source was meat and bone meal. Chromic oxide was

added to the diet an indigestible marker. The animals were given the MBM diet for 14 days and were killed by CO_2 asphyxiation on the 14th day. Digesta were sampled from the terminal ileum. Furthermore, the effect of site within the terminal ileum (0-5, 0-10, 0-15 and 0-20 cm) for sampling digesta 4 hours post-feeding was determined with 72 male rats. The optimal time for sampling digesta was 4 hours after the start of feeding, while 20 cm of ileum was the maximum length of ileum sampled without affecting apparent N digestibility. Significantly ($P < 0.05$) higher quantities of digesta were collected from the terminal 20 cm of ileum compared to the shorter ileum lengths.

3. In experiment three, the use of the rat as a model animal for allowing the determination of apparent ileal amino acid digestibility in the growing pig, using the slaughter method was evaluated. Sixteen male rats and 16 male pigs were fed diets containing chromic oxide and as the sole protein source, meat and bone meals which were expected to differ in their quality. Ileal contents from the terminal 20 cm of ileum were collected after slaughter of the rats and pigs, 4 and 9 hours after the start of feeding, respectively. Inter-species comparison made under the defined conditions, indicated close agreement between the rat and pig for the ileal digestibility of N and most of the amino acids in each of the two different meat and bone meals evaluated. It was concluded that the growing rat is a satisfactory model for the growing pig, for determining ileal amino acid digestibility in meat and bone meal. The measurement of digestibility at the terminal ileum indicated differences in amino acid digestibility between two meat and bone meals, however, the faecal approach which generated significantly higher digestibility coefficients than the ileal digestibility method, did not allow the differences in amino acid digestibility to be detected.

4. Experiment four was undertaken to compare a recently-developed peptide alimentation method and the protein-free and regression methods for determining endogenous ileal N and amino acid excretion in the rat, and was aimed at the development of a true ileal digestibility assay with application to meat and bone meal. Preliminary investigations determined the effect of the time of sampling of digesta from rats given a protein-free (PF) or an enzymically hydrolysed casein (EHC) based diet, on ileal digesta and endogenous N excretion. There was a significant ($P < 0.05$) effect of the time of sampling on the amount of digesta collected and the endogenous N excretion for both the EHC- and PF-fed rats. The amount of digesta collected from the terminal 20 cm of ileum and the endogenous N excretion for both the EHC- and PF-fed rats were least variable at 3 hours post-feeding.

In the main study, endogenous ileal amino acid excretions were determined in the growing rat fed an EHC-based diet and with subsequent treatment of the digesta using ultrafiltration ($n = 6$) or in 6 rats given a PF diet or by extrapolation from data for 30 animals given 5 diets which contained graded levels of MBM as the protein source. For the EHC treatment, the ileal digesta precipitate plus retentate was used to determine the endogenous flows. Such processing excludes unabsorbed dietary amino acids from the measure of endogenous loss. Chromic oxide was the reference marker in all the diets. The endogenous

flows determined by the protein-free and the regression method were similar but both significantly ($P < 0.01$) lower than those for rats fed the EHC-based diet. The mean (±SE) endogenous ileal N flows determined by the peptide alimentation method, the protein-free and regression approaches were 1866 (±30.8), 1103 (±22.6) and 1019 (±3.6) $\mu g\ g^{-1}$ FDMI, respectively. It was concluded that endogenous amino acid flows at the terminal ileum were underestimated when determined using the traditional protein-free or regression methods.

5. The effect of dietary protein content (25, 60, 95, 130, 165 and 200 g CP kg^{-1} diet) on the apparent and true ileal digestibility of N and amino acids was investigated. Semi-synthetic diets in which the protein content was varied by the inclusion of graded amounts of MBM (50 to 400 g kg^{-1} diet) at the expense of maize starch were fed to 36 growing rats for 14 days. On the 14th day, the rats were fed and euthanased 4 hours after the start of feeding and digesta were collected from the terminal 20 cm of ileum. Endogenous amino acid excretion was determined for 18 rats given an EHC-based diet. The EHC-fed rats were euthanased 3 hours after the start of feeding and digesta were collected from the terminal 20 cm of ileum. The true ileal digestibility values determined with reference to chromium as a marker, were higher than the corresponding apparent estimates. Apparent digestibility of N and amino acids significantly ($P < 0.001$) increased with increasing dietary protein level, however, dietary protein content had no significant ($P > 0.05$) effect on the true ileal digestibility of N and amino acids. The mean apparent ileal digestibility of N in MBM ranged from 65.6 to 75.3%. The corresponding range of true ileal digestibility of N was 76.9 to 78.2%

6. The final experiment generated data on the nutrient compositions of meat and bone meals collected from eight processing plants throughout New Zealand. The true ileal digestibility of the amino acids in the meat and bone meals were also determined using the rat assay developed in the previous studies. Endogenous amino acid excretion, used for the correction of apparent digestibility values to true estimates, was determined for rats given a protein-free or an enzymically hydrolysed casein diet. The meat and bone meals were variable in their nutrient composition and in the true ileal digestibility of protein and amino acids. The true nitrogen and amino acid digestibility coefficients based on endogenous flows determined by the EHC method were markedly higher than with the protein-free diet. The true ileal N and lysine digestibility coefficients based on the respective endogenous flows for rats fed the EHC diet ranged from 62.7 to 88.9% and 66.4 to 92.3%, respectively. Values determined with endogenous flows for rats fed the protein free diet ranged from 59.0 to 85.2% for N, and 63.2 to 88.9 for lysine. The variable ileal digestible N and amino acid contents of meat and bone meals emphasise the limitation of tabulated analytical values and the need for a routine relatively inexpensive digestibility assay.

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