ENDOGENOUS PROTEIN FLOW IN THE GUT OF THE SIMPLE-STOMACHED MAMMAL

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ABSTRACT

The set of studies was undertaken to examine different aspects of the measurement of endogenous ileal nitrogen and amino acid loss in simple-stomached mammals and specifically to investigate the effect of the concentration of protein and peptides in the diet on endogenous ileal amino acid flows. Seven separate studies were conducted using rats and pigs.

1. The aim of the first study was to determine whether endogenous nitrogen (N) and amino acid flows at the terminal ileum change over time in the growing pig fed a protein-free diet. Male pigs (n=7, mean bodyweight 82 kg) with surgically implanted post-valve T-caecum (PVTC) cannulas received a casein-based diet for 8 days after which food was withheld from the pigs for 24 hours. The pigs then received a protein-free diet for a further 8 days during which time ileal digesta were collected continuously via the cannulas from 1300h to 1800h on each day. Endogenous ileal N and amino acid flows were determined on the digesta. There were no significant ($P>0.05$) effects of the duration of feeding of the protein-free diet on endogenous ileal total N or amino acid flows except for the amino acids glycine and cysteine, the mean flows of which significantly decreased over the 8-day experimental period ($P<0.01$ and $P<0.05$ for glycine and cysteine, respectively), from 1639 to 892 μg/g dry matter intake (DMI) for glycine, and 173 to 127 μg/g DMI for cysteine.

2. The enzyme hydrolysed protein, isotope dilution and guanidination methods can be used to determine endogenous ileal protein flows. The aim of the second study was to determine whether the isotope dilution and guanidination methods give similar estimates of endogenous ileal N and lysine flows, respectively, as the enzyme hydrolysed protein method. A test diet was prepared that contained guanidinated and enzymatically hydrolysed (MW <5,000 Da) casein labelled with ^{15}N. Male rats (n=30, mean bodyweight 178 g) and male pigs (n=6, mean bodyweight 19.2 kg) received a preliminary EHC-based diet for 7 days. The test diet was given to the rats and pigs on the following day and digesta were sampled at the terminal ileum of the animals following euthanasia. Endogenous ileal lysine flows were determined using the enzyme hydrolysed protein and guanidination methods. Endogenous ileal N flows were determined using the enzyme hydrolysed protein and isotope dilution methods. The guanidination method led to significantly ($P<0.05$ and $P<0.01$ for the rat and pig, respectively) lower mean endogenous lysine flows compared with the enzyme hydrolysed protein method (298 vs 382 and 214 vs 287 μg/g DMI in the rat and pig, respectively). The isotope dilution method led to significantly ($P<0.001$ for the rat and $P<0.05$ for the pig) lower mean endogenous ileal N flows compared with those determined using the enzyme hydrolysed protein

method (means of 1034 vs 1942 and 1011 vs 1543 μg/g DMI for the rat and pig, respectively). Given that the enzyme hydrolysed protein method is known to somewhat underestimate actual endogenous N and amino acid flows, it appears that the guanidination and isotope dilution methods notably underestimate endogenous flows at the terminal ileum.

3. The third experiment involved an *in vitro* study to examine the effectiveness of Centriprep-10 Concentrator devices for the ultrafiltration of digesta with the enzyme hydrolysed protein method for the determination of endogenous ileal N and amino acid flows. Different amounts of enzyme hydrolysed casein (EHC) were added to test tubes containing digesta collected from pigs that had received a protein-free diet for 5 to 8 days. The samples were centrifuged and then ultrafiltered using Centriprep-10 concentrators. The amount of N and amino acids that was deemed to have originated from the EHC and remained in the precipitate plus retentate fraction of digesta after processing, expressed as a percentage of the total amount of N or amino acid added to the tubes as EHC, ranged from 1.0 to 5.0% for N and averaged 2.4 to 5.8% for the amino acids. With Centriprep-10 concentrators there is a less than complete separation of N and amino acids originating from EHC from endogenous material in the digesta, which could potentially lead to a small overestimation (up to 2%) of endogenous ileal N and amino acid flows.

4. In experiment 4, endogenous ileal N and amino acid flows were determined using the enzyme hydrolysed protein method using a molecular weight (MW) cut-off for ultrafiltration of 10,000 Da, and were compared with flows determined using a MW cut-off of 3,000 Da. Digesta were sampled from the terminal ileum of male rats (n=24, mean bodyweight 179 g) that had received a diet containing EHC for 8 days. The digesta were pooled to give 6 pooled samples, each containing the digesta from 4 rats. Endogenous ileal N and amino acid flows were determined using the enzyme hydrolysed protein method with ultrafiltration using MW cut-offs of 10,000 Da and 3,000 Da. The endogenous ileal N and amino acid flows determined using a MW cut-off of 3,000 Da were greater than those determined following ultrafiltration at 10,000 Da, by 17% for N and on average 12% for the amino acids, with a range from 1.7% for arginine and phenylalanine to 26% for serine.

5. In the fifth experiment, the diurnal pattern of endogenous N flow at the terminal ileum of the pig was examined using the enzyme hydrolysed protein method. Male pigs (n=7, mean bodyweight 33 kg) had PVTC cannulas surgically implanted. The pigs received an EHC-based diet for 8 days. Digesta were continuously collected for 24 hours (0800h - 0800h) on each of the fifth and eighth days. During each hour of digesta collection, 10% (by weight) of the digesta collected for that hour for each

pig was sampled. Flows of dry matter and chromium were determined in the digesta, and endogenous N was determined after centrifugation and ultrafiltration (10,000 Da MW cut-off) of the digesta. The concentration of chromium in the digesta expressed on a digesta dry matter basis was relatively constant over the 24-hour periods, with no statistically significant ($P>0.05$) differences from 1200h - 0800h. The ratio of endogenous N to chromium at the terminal ileum was also relatively constant with no statistically significant ($P>0.05$) differences from 1300h - 0800h. The net outcome of endogenous protein secretion and reabsorption in the small intestine appears to be relatively constant over time in the meal-fed animal.

6. The aim of experiment 6 was to determine whether dietary peptide concentration affects endogenous ileal N and amino acid flows in the growing pig. Entire male pigs (n=8, mean bodyweight 33 kg) had PVTC cannulas surgically implanted. The pigs received the diets (0, 5, 10 and 20% EHC) for 8-day periods in a Latin Square design with a basal casein-based diet given to the pigs for 6-day periods in between the experimental diets. Digesta were collected continuously for 24 hours on each of the fifth and eighth days. The endogenous ileal N and amino acid flows were determined directly for pigs receiving the protein-free diet or after centrifugation and ultrafiltration (10,000 Da MW cut-off) for pigs on the EHC-based diets. Mean endogenous ileal N flows were 1753, 1948, 2851 and 5743 μg/g DMI when the pigs received diets containing 0, 5, 10 and 20% EHC, respectively. There was a significant ($P<0.05$) effect of dietary peptide concentration on the endogenous ileal flow of N and for all of the amino acids, with an increase in endogenous ileal N and amino acid flow with increasing dietary EHC concentration.

7. The final experiment was conducted to corroborate the results described in the sixth study on the effect of dietary peptide/protein concentration on endogenous ileal lysine flow. Male rats (n=108, mean bodyweight 170 g) received diets containing 5, 10, 15, 20, 25 or 30% zein over a period of 8 days. Zein is nearly devoid of lysine and tryptophan, so the diets were supplemented with lysine and tryptophan for the first 6 days and lysine and tryptophan were given to the rats via intraperitoneal injections over the final 2 days of this period. Digesta were sampled (after euthanasia) from the terminal ileum of the rats on the eighth day and pooled to give 6 samples (3 rats per pooled sample) per diet. All lysine present in the digesta was assumed to be of endogenous origin. Increasing the amount of zein in the diet led to a significant ($P<0.0001$) increase in endogenous lysine flow through the terminal ileum.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 9