

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Endogenous Ileal Amino Acid Excretion in Monogastric Animals

A thesis presented in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy in Animal Science
at Massey University

Christine Ann Butts
1991

ABSTRACT

A new method for the determination of endogenous ileal amino acid excretion under conditions of peptide alimentation was refined and evaluated with studies involving the laboratory rat. The refined method was used to investigate aspects of endogenous ileal amino acid flow in the pig. Five studies were conducted, three with rats and two involving the growing pig.

1. Preliminary investigations evaluated the filtration efficiency of ultrafiltration devices, and examined three pre-filtration treatments for rat ileal digesta: trichloroacetic acid (TCA) and perchloric acid (PCA) precipitation, and centrifugation (SPIN). The recovery of nitrogen following ultrafiltration (molecular weight exclusion limit 10,000 Daltons) of fifteen purified protein, peptide and amino acid solutions indicated an effective filtration (>90%) on nominal molecular weight by the ultrafiltration devices. Determination of nitrogen and amino acids in the resulting fractions following TCA and PCA precipitation and centrifugation of rat ileal digesta indicated that PCA was the most effective precipitant. Endogenous ileal amino acid excretions in the growing rat fed an enzymically hydrolysed casein (EHC) based diet with subsequent treatment of the digesta using the ultrafiltration technology were then determined. Twelve 100 g male rats were fed either an EHC-based diet or a protein-free diet and samples of digesta were collected after slaughter. The digesta from the 6 EHC-fed rats were ultrafiltered after centrifugation and the high molecular weight fraction added to the precipitate. The protein-free fed rats had significantly ($P<0.05$) lower amino acid flows than those rats fed the EHC-based diet with subsequent treatment of the digesta.

2. The proportions of endogenous protein-, peptide- and free amino acid nitrogen (N) in digesta N from the distal ileum of the rat immediately after collection or following storage frozen (-20°C and -196°C) were compared. Eighteen growing rats were given a protein-free diet for 6 days, euthanased and samples of digesta were collected from the terminal 20 cm of ileum. The storage of digesta did not significantly affect the proportions of N-containing substances in the precipitate plus retentate or ultrafiltrate fractions. On average, 67% of the total digesta N was in the precipitate plus retentate fraction and 33% of total digesta N was in the ultrafiltrate fraction. Free amino acid N and peptide N were 10.4 and 10.6% of total digesta N, respectively.

3. The effect of using different flushing media for the collection of ileal digesta on the composition of endogenous N was examined. Twelve growing rats were given a protein-free diet and samples of ileal digesta were collected from the

ethanased animal using either distilled water or physiological saline as the flushing medium. There was no significant effect of collection method on the levels of N-containing substances in rat endogenous ileal digesta.

4. The effects of state of body nitrogen balance and the presence of dietary peptides and protein in the digestive tract on the excretion of endogenous amino acids from the ileum of the pig were investigated. Endogenous lysine excretion was determined for pigs given a protein-free diet, an EHC-, a zein- or a synthetic amino acid-based diet. Endogenous flows for amino acids other than lysine were determined for pigs on the protein-free and EHC-based diets. Six male pigs (15 kg liveweight) were allocated to each of the four diets and received the diet for 10 days. The mean endogenous ileal lysine flows for the zein and EHC fed pigs were not significantly different but were higher ($P < 0.05$) than those for the protein-free and synthetic amino acid fed pigs whose mean flows were not significantly different from each other. The mean endogenous ileal flows for amino acids other than lysine were higher ($P < 0.05$) for the EHC fed pigs compared to the animals on the protein-free diet, except for proline, glycine and arginine.

5. The effect of food dry matter intake on endogenous ileal amino acid excretion of the pig under peptide alimentation was determined. Sixteen male pigs (50 kg liveweight) each fitted with a T-cannula in the terminal ileum were fed at 8 levels of food dry matter intake for periods of 8 days. The experiment involved two trials of 8 pigs each, comprising a cross over design. Each trial involved 4 pairs of pigs with each pair receiving one of 4 sequences of treatment. Each sequence comprised 4 levels of food dry matter intake arranged in a Latin square. The food dry matter intakes were 0.06, 0.08, 0.10 and 0.12, and 0.05, 0.07, 0.09 and 0.11 metabolic liveweight ($W^{0.75}$) day^{-1} for the first and second trials, respectively. There was an increase in ileal excretion of amino acids, nitrogen and dry matter with increasing food dry matter. There were significant ($P < 0.05$) linear relationships between endogenous ileal amino acid and nitrogen excretion and food dry matter intake except for lysine, glutamic acid and phenylalanine which increased in a curvilinear manner. These relationships, determined under physiologically more normal conditions than under protein-free alimentation, provide preliminary data on the magnitude of small intestinal amino acid losses in the pig.

ACKNOWLEDGEMENTS

I sincerely thank my supervisors, Dr Paul Moughan and Associate Professor Bill Smith, for their guidance and encouragement throughout this study.

I am also grateful for the willing support and expertise of Dr Gordon Reynolds, Dr David Carr and Dr Cam Reid for the surgery and post-operative care of the pigs. Thanks are also due to: Ms Felicity Jackson, Miss Rosemary Watson, Mrs Geraldine Wood, Mr Julian Reid, and Mrs Carol Flyger for their assistance with chemical analyses; Mrs Irene Hall for assistance during surgery; Mr Barry Parlane and Mr Graham Pearson for technical assistance; Dr Dorian Garrick and Mr Carlos Sosa for advice on statistical analyses.

I wish to acknowledge The New Zealand Pork Industry Board for funding the research.

I was the grateful recipient of a Massey University Graduate Assistantship and a Massey University Vice Chancellors Study Grant.

The guidance of the Massey University Animal Ethics Committee in approving the experimental protocols involving the use of animals is acknowledged.

The companionship and advice of Alison Darragh and other post-graduate students, and staff members of the Department of Animal Science is gratefully acknowledged. Finally, I wish to express my fondest appreciation to my husband Murray for his loving support, encouragement and assistance throughout this study.

CONTENTS

	PAGE
Abstract	ii
Acknowledgements	iv
List of Tables	ix
Introduction	1
Chapter 1	
Review of Literature	3
1.1 Introduction	3
1.2 Protein Digestion and Absorption	3
1.2.1 Gastric Digestion	4
1.2.2 Digestion in the Small Intestine	4
1.2.3 Mucosal Digestion	5
1.2.4 Absorption of the Products of Protein Digestion	5
1.2.5 Digestion in the Large Intestine	7
1.3 Gastrointestinal Protein Secretions	8
1.3.1 Salivary Protein Secretion	9
1.3.2 Gastric Protein Secretion	10
1.3.2.1 Physiological Control of Gastric Secretion	11
1.3.2.2 Effect of Age and Diet on Gastric Protein Secretion	12
1.3.3 Pancreatic Protein Secretion	13
1.3.3.1 Physiological Control of Pancreatic Secretion	14
1.3.3.2 Effect of Age and Diet on Pancreatic Protein Secretion	15
1.3.3.2.a Dietary protein	15
1.3.3.2.b Dietary fat and starch	16
1.3.3.2.c Dietary fibre	17
1.3.3.2.d Rate of adaptation of pancreatic secretion to diet	18
1.3.3.2.e Summary of pancreatic adaptation to diet	18
1.3.4 Bile Protein Secretion	19
1.3.4.1 Physiological Control of Bile Secretion	21
1.3.4.2 Effect of Diet on Bile Secretion	21

	PAGE
1.3.5 Intestinal Secretion	22
1.3.5.1 Brush Border Enzymes	23
1.3.6 Plasma Protein Secretion	24
1.3.7 Epithelial Cell Loss	24
1.3.8 Microbial Protein	25
1.3.9 Mucus Secretion	26
1.4 Total Quantities of Endogenous Nitrogen and Amino Acids Secreted Into the Gastrointestinal Tract	29
1.5 Digestion of Endogenous Protein Secretions	32
1.6 Determination of Endogenous Nitrogen and Amino Acid Excretion in Ileal Digesta - Methodology	34
1.6.1 Protein-Free Method	35
1.6.2 Synthetic Amino Acid Diet	38
1.6.3 Regression Method	38
1.6.4 Homoarginine Method	39
1.6.5 Tracer Method	42
1.6.6 Peptide Alimentation	43
1.7 Conclusion	44
1.8 References	45

Chapter 2

Endogenous Amino Acid Flow at the Terminal Ileum of the Rat Determined Under Conditions of Peptide Alimentation

	69
2.1 Abstract	70
2.2 Introduction	70
2.3 Experimental	72
2.3.1 Validation of Ultrafiltration Technology	72
2.3.2 Evaluation of Pre-Filtration Treatments	72
2.3.2.1 Collection of Ileal Digesta	72
2.3.2.2 Treatment of Digesta	73
2.3.2.3 Fractionation of the Supernatants	73
2.3.3 Determination of Endogenous Amino Acid Flows	74
2.3.4 Chemical Analysis	74
2.3.5 Data Analysis	75

	PAGE
2.4 Results	75
2.4.1 Validation of Ultrafiltration Method	75
2.4.2 Pre-filtration Treatments	75
2.4.3 Endogenous Amino Acid Flows	77
2.5 Discussion	79
2.5.1 Validation of Ultrafiltration Technology	79
2.5.2 Pre-Filtration Treatment of Digesta	80
2.5.3 Endogenous Amino Acid Flow	81
2.6 References	83
Chapter 3	
Protein-, Peptide- and Free Amino Acid-Nitrogen in Endogenous Digesta Nitrogen at the Terminal Ileum of the Rat	85
3.1 Abstract	86
3.2 Introduction	86
3.3 Experimental	88
3.4 Results	91
3.5 Discussion	95
3.6 References	99
Chapter 4	
Composition of Endogenous Ileal Digesta Nitrogen For the Rat - The Use of Distilled Water for Digesta Collection	102
4.1 Abstract	103
4.2 Introduction	103
4.3 Experimental	103
4.4 Results	104
4.5 Discussion	105
4.6 References	107
Chapter 5	
Endogenous Lysine and Amino Acid Flows at the Terminal Ileum of the Growing Pig (15-25 kg liveweight) Determined	

	PAGE
Under Protein-Free, Synthetic Amino Acid, Peptide and Protein Alimentation	108
5.1 Abstract	109
5.2 Introduction	110
5.3 Experimental	111
5.3.1 Animals	111
5.3.2 Diets	112
5.3.3 Experimental Procedure	112
5.3.4 Chemical Analysis	114
5.3.5 Data Analysis	115
5.4 Results	116
5.5 Discussion	119
5.6 References	127
Chapter 6	
The Effect of Food Dry Matter Intake on Endogenous Ileal Amino Acid Excretion Determined Under Conditions of Peptide Alimentation in the 50 kg Liveweight Pig	
6.1 Abstract	132
6.2 Introduction	132
6.3 Experimental	134
6.3.1 Animals and Housing	134
6.3.2 Surgical Implantation of Simple T-cannulas in the Terminal Ileum	134
6.3.3 Diets and Feeding	134
6.3.4 Experimental Procedure	135
6.3.5 Chemical Analysis	136
6.3.6 Data Analysis	137
6.4 Results	137
6.5 Discussion	138
6.6 References	142
Summary and Conclusions	147

LIST OF TABLES

TABLE		PAGE
1.1	Amounts of nitrogen (g day^{-1}) secreted into the gastrointestinal tract of the 50 kg liveweight pig	30
1.2	Endogenous amino acid secretions (g day^{-1}) into the gastrointestinal tract of the pig	31
1.3	Endogenous amino acid secretions (g day^{-1}) remaining undigested and unabsorbed at the end of the ileum of the pig	33
1.4	Summary of literature values for endogenous ileal amino acid excretion (g kg^{-1} dry matter intake) in the pig determined under protein-free alimentation	36
1.5	Summary of the literature values for endogenous ileal amino acid excretion (g kg^{-1} dry matter intake) in the pig determined by the regression method	40
2.1	Ingredient composition (g kg^{-1} air dry weight) of the enzymically hydrolysed casein (EHC) and protein-free diets	73
2.2	The recovery of nitrogen (N) from purified protein, peptide and amino acid solutions in the retentate and filtrate after ultrafiltration	76
2.3	Mean (\pm SE) endogenous amino acid flows at the terminal ileum of rats ($n=6$) fed an enzymically hydrolysed casein-based diet determined on the total untreated digesta or digesta subjected to centrifugation plus ultrafiltration	78
2.4	Mean (\pm SE) endogenous amino acid flows at the terminal ileum of rats fed an enzymically hydrolysed casein (EHC) based diet determined on digesta samples subjected to centrifugation plus ultrafiltration and flows for rats fed a protein-free diet	79
3.1	Ingredient composition (g kg^{-1} air dry weight) of a protein-free diet fed to growing rats	89
3.2	Mean proportions of nitrogen-containing compounds in the precipitate and ultrafiltrate of ileal digesta for rats	

	given a protein-free diet and after different treatments of the digesta	92
3.3	Mean proportions of total amino acid nitrogen in the precipitate plus retentate (MW>10,000 Da) and the ultrafiltrate (MW<10,000 Da) of ileal digesta for rats given a protein-free diet	93
3.4	Mean proportions of free amino acid nitrogen and peptide nitrogen in the ultrafiltrate (MW<10,000 Da) of ileal digesta for rats given a protein-free diet	94
3.5	Mean amino acid nitrogen in the free amino acid and peptide fractions of the ultrafiltrate (MW<10,000 Da) expressed as percentages of the total amount of nitrogen for the respective amino acid in whole ileal digesta of rats given a protein-free diet	96
3.6	Overall mean endogenous amino acid flows in the whole ileal digesta for rats given a protein-free diet	97
4.1	The effect of flushing medium for the collection of digesta on the mean protein nitrogen (N), peptide N, free amino acid N, and non-amino acid-N in endogenous ileal digesta for the growing rat	105
4.2	Mean (n=6) endogenous amino acid flows in the whole ileal digesta for growing rats given a protein-free diet and after collection of the digesta with distilled water or physiological saline	106
5.1	Ingredient composition (g kg ⁻¹ air dry weight) of the experimental diets	113
5.2	Excretion rates for urinary total nitrogen (N), urea N, urea N:creatinine N, and total N:creatinine N for growing pigs given a synthetic amino acid or zein diet with oral or parenteral supplementation of lysine and tryptophan	117
5.3	Endogenous amino acid flows at the terminal ileum of the growing pig given an enzymically hydrolysed casein-based diet and determined on the total unprocessed digesta or digesta subjected to centrifugation plus ultrafiltration	118
5.4	Endogenous lysine flows at the terminal ileum of the growing pig given protein-free, synthetic amino acid-, enzymically hydrolysed casein- or zein-based diets	119

5.5	Endogenous amino acid flows at the terminal ileum of the growing pig under protein-free or peptide alimentation	120
5.6	Mean apparent and true ileal amino acid digestibility for growing pigs given a zein-based diet	121
5.7	Mean apparent and true ileal amino acid digestibility for growing pigs given a diet containing synthetic amino acids as the sole nitrogen source	124
5.8	Mean endogenous amino acid flows at the terminal ileum for the growing pig given either a protein-free or a synthetic amino acid-based diet	125
6.1	Ingredient composition (g kg^{-1} air dry weight) of the experimental diets	136
6.2	Least squares means for daily ileal amino acid, nitrogen and dry matter excretions for the 50 kg liveweight pig at different levels of dietary intake	139
6.3	Regression relationships between daily ileal excretion of endogenous amino acids and nitrogen and food dry matter intake for the 50 kg liveweight pig	140
6.4	Regression relationships between daily ileal excretion of lysine, glutamic acid and phenylalanine and food dry matter intake for the 50 kg liveweight pig	140