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**THE EFFECT OF ENZYMATIC HYDROLYSIS OF A
DIETARY PROTEIN ON THE EXCRETION OF
URINARY NITROGEN METABOLITES**

**A thesis submitted in partial fulfilment of the requirements for the
degree of Master of Science in Nutritional Science at Massey
University, Palmerston North, New Zealand.**

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**TO TERESA CINTORA
AND
ANTONIO QUINTINO,

MY PARENTS**

ABSTRACT

Hydrolysed milk proteins are used for many purposes in human nutrition. Although it is assumed that the nutritive value of a protein hydrolysate is the same, or even superior to the corresponding intact protein, there is limited research available to support this assumption.

The aim of this study was to compare amino acid utilisation and the pattern of excretion in the urine of the nitrogenous metabolites (urea, ammonia and creatinine) as an immediate response to the ingestion of a meal containing an intact protein or its enzymatic hydrolysate. This involved a novel technique, 'acute urine collection' (AUC), in which urine was drained from the bladder at short time periods (30 min to 2 hr) through a catheter.

The performance and nitrogen balance results indicated that the two sources of amino acid were equally effective in supporting nitrogen retention and growth of the pigs. Nevertheless, the pattern of excretion of the metabolites of nitrogen digestion suggested important differences in the metabolism of the pigs on the two diets.

Both groups of pig excreted creatinine nitrogen, at constant and comparable rates over the sampling period indicating similar rates of catabolism in the muscle. The total excretion of nitrogen by AUC by the two groups was similar but the pattern of excretion over the day differed which indicated a difference in the metabolism of the amino acids in the diets. This may have been in part due to a more rapid absorption of amino acids from the hydrolysed diet and in part due to a higher rate of glutamine and asparagine breakdown in the gut of pigs fed the hydrolysate.

Excretion of nitrogen as urea and ammonia was similar for the two groups but there were differences between the groups in the pattern of excretion of these metabolites. In addition, the excretion of ammonia was significantly lower ($P < 0.0001$) in the pigs fed the hydrolysate. This was due to a higher content of fixed cations in the diet containing the hydrolysate that led to a compensatory reduction in ammonia excretion. There was a proportional increase in the excretion of urea in the pigs on the hydrolysed diet as a result of the reduction in ammonia excretion but the differences were small relative to the total urea excretion and not significant.

AUC not only gives comparable information to the nitrogen balance if it is carried out over a 24 hr period but it also provides detailed information about the protein utilisation during the immediate postprandial period. In particular, AUC can indicate differences and/or similarities in protein absorption by allowing the observation of the pattern of production of urea directly related to the catabolism of dietary amino acids. In addition, it may be possible to use this technique to estimate the optimum time between meals.

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LIST OF ABBREVIATIONS

ADG	Average Daily Gain
AT/TN	Amino acid nitrogen in the hydrolysate relative to the total amount of nitrogen in the substrate
AOAC	Association of Official Analytical Chemistry
APU	Animal Physiology Unit
AUC	Acute Urine Collection
D	Daltons
DEB	Dietary Electrolyte Balance
DH	Degree of Hydrolysis
GLM	the general linear method procedure
HP	Diet containing hydrolysed protein
IFNHH	Institute of Food Nutrition and Human Health
IP	Diet containing intact protein
MBW	Metabolic Body Weight
MW	Molecular Weight
N	Nitrogen
NAD	Nicotinamide Adenine Dinucleotide
NADH	Nicotinamide Adenine Dinucleotide (reduced)
NB	Nitrogen Balance
PDR	Protein Deposition Ratio
UTI	Urinary Tract Infections

INTRODUCTION

Proteins are vital molecules for life. Their importance has been recognised since the 19th century, when they were called the 'primary material of life'. Even though protein structure is built using only 20 different amino acids, the different arrangement and repetition in the chain make it possible to have thousands of different proteins performing unique and specific functions.

The human being requires a constant supply of protein in the diet for maintaining protein homeostasis in the body. The body of a normal 70 kg man contains about 11 kg of protein (Forbes, 1987). This protein mass contains thousands of different proteins each of different weight and chemical composition and each one with a definite function. Some provide the structure for cells and organs; others control the speed of biochemical reactions, and others control intracellular and intercellular communication. Although the total mass of protein in the body is relatively stable in the adult, proteins are continually degraded into their constituent amino acids. In order to keep the protein mass constant, new proteins must be synthesised to replace the degraded ones. About 0.3 kg of protein are degraded and replaced each day in a normal 70 kg man (Morais *et al.*, 1997). Synchronised degradation and synthesis of protein, referred to as 'protein turnover', consumes approximately 20% of the energy consumption of a person at rest after an overnight fast (Welle and Nair, 1990). Most of this energy is used for protein synthesis rather than degradation (Welle, 1999). The rapid alteration of the concentration of certain enzymes, such as occurs after ingestion of a meal, alcohol or stress and the replacement of altered proteins from oxidation,

glycation, racemisation and isomerisation consumes much more energy than cell replication and growth (Berneis, 1997; Boirie *et al.*, 1997; Welle, 1999).

Under normal conditions, an individual can maintain the turnover of his/her body protein by eating a diet containing an adequate quantity and balance of proteins. The normal physiological processes of digestion and absorption will ensure the dietary amino acids reach the sites of protein metabolism. Under some circumstances, however, dietary proteins are unable to meet the amino acid requirements of the individual or whole dietary proteins are not tolerated. For example, an insufficient gut absorptive surface, inefficient function of some gastrointestinal organs, degenerated digestive and absorptive function, may all lead to insufficient absorption of amino acids from the gastrointestinal tract and therefore malnutrition. Alternatively, activation of allergic reactions to dietary proteins can induce life threatening anaphylactic reactions. Many of these problems may be avoided by replacing the protein in the diet with partially digested proteins that are readily digested and have low allergenicity.

Given the nutritional dependence that consumers, such as allergenic infants, and patients with pancreatic and Crohn's disease, have on pre-digested proteins, it is crucial to assess their nutritional value in comparison with the intact protein. Even though several studies have compared the nutritional value of intact proteins and their hydrolysates, there is still much controversy arising from the methods used in the comparisons. The objective of the present study was to compare both the immediate and long-term responses of pigs to a milk protein that was fed intact or hydrolysed. The response variables measured included the weight gain of the pigs, nitrogen balance and the pattern of excretion of nitrogenous compounds in the urine.
