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**The Role of the  $\gamma$ -Glutamyl Cycle in Milk Protein Synthesis  
in the Ruminant**

A thesis presented in partial fulfilment of the requirements for  
the degree of

**Doctor of Philosophy**  
in  
**Biochemistry**

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**Massey University,**  
Palmerston North, New Zealand.

**Sarah Louise Johnston**

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# MASSEY UNIVERSITY

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Name of Candidate: Sarah Louise Johnston I.D. Number: 93004712

Degree: PhD in Biochemistry Dept / Institute / School: Food, Nutrition and Human Health

Thesis Title: The Role of the Gamma-Glutamyl Cycle in Milk Protein Synthesis  
in the Ruminant

Name of Chief Supervisor: Associate Professor Kathy Kitson Telephone Ext: 7703

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## Abstract

Dairy products are New Zealand's primary export commodity. The manufacturing efficiency for dairy products would be maximised if the Dairy Industry had the ability to control milk protein production to suit the manufacture of specific products. Understanding the role of amino acid transport in regulating milk protein synthesis may allow manipulation of proteins in milk.  $\gamma$ -Glutamyl transpeptidase ( $\gamma$ -GT), an enzyme thought to play a key role in mediating amino acid transport, has been demonstrated in mammary tissue, but the role of this enzyme and its associated biochemical pathway, the  $\gamma$ -glutamyl cycle, has not been fully elucidated. The  $\gamma$ -glutamyl cycle consists of synthetic and degradative enzymes for the cysteine-containing tripeptide glutathione.  $\gamma$ -GT transfers the  $\gamma$ -glutamyl moiety from glutathione to amino acids, and has a high affinity for cyst(e)ine. The vascular supply of cysteine is thought to be insufficient to maintain milk protein synthesis. In this study, the role of the  $\gamma$ -glutamyl cycle in amino acid transport for milk protein synthesis was investigated using two systems, firstly, in acini isolated from the udder of lactating sheep, and secondly in lactating goats. Milk protein secretion from isolated acini significantly decreased (70%) as a result of  $\gamma$ -GT inhibition with acivicin, and significantly increased (250%) when supplied with cysteine as N-acetylcysteine (NAC). In lactating goats, acivicin did not affect milk yield or total protein concentration or yield, but significantly increased  $\alpha_2$ - and  $\kappa$ -casein concentration in milk. This may have resulted from increased uptake of some amino acids by the mammary gland and suggests that  $\gamma$ -GT negatively regulates uptake of some amino acids for milk protein synthesis. NAC significantly increased milk yield, protein concentration and protein yield as a result of increased uptake of some amino acids, which may have been due to increased mammary blood flow. This increase was prevented by acivicin, however, suggesting that  $\gamma$ -GT plays an important role in amino acid supply. Inhibition of  $\gamma$ -GT may up-regulate sub-saturated transport systems leading to increased uptake of amino acids required for milk protein synthesis. Further testing of NAC and a greater understanding of the function and regulation of  $\gamma$ -GT may allow increased, and targeted, milk protein production as required by the Dairy Industry.

To my parents Graham and Jacqueline Cridland

‘I have nothing but a book.  
Nothing but that to prove your blood and mine.’

W B Yeats

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## List of Abbreviations

$^3\text{H-AIB}$	$\alpha$ -amino-isobutyric acid [Methyl- $^3\text{H}$ ]
A	arterial
AA	amino acid
ATP	adenosine triphosphate
A-V	arterial venous difference
BBB	blood brain barrier
BBM	brush border membrane
Bq	becquerel ( $\text{s}^{-1}$ )
BSO	buthionine sulphoximine
CA	cellulose acetate
Ci	Curie ( $37 \times 10^9$ Bq)
Da	Daltons
D-MEM	Dulbecco's modified eagle medium
DTT	dithiothreitol
EAA	essential amino acids
EDTA	ethylenediaminetetra-acetic acid di-sodium salt
FCS	foetal calf serum
FIA	flow injection analysis
g	grams
h	hours
HPLC	high performance liquid chromatography
kg	kilograms
LHS	left hand side
MBW	metabolic body weight ( $\text{kg}^{0.75}$ )
min	minutes
MQ H <sub>2</sub> O	Millipore deionised water
NAC	N-acetylcysteine
NADP <sup>+</sup>	nicotinamide adenine dinucleotide phosphate
NEAA	nonessential amino acids
NIT	near infrared transmittance spectrophotometry
OTCA	(-)-2-oxo-4 thiazolidine-carboxylic acid
PBS	phosphate buffered saline

PCA	perchloric acid
PITC	phenylisothiocyanate
RHS	right hand side
rpm	revolutions per minute
SBD-F	4-fluro-7-sulfobenzo-furazan ammonium salt
SDS	sodium dodecyl sulphate
SRA	specific radioactivity
TCA	trichloroacetic acid
TEA	triethylamine
U	units
V	venous