The Role of the γ-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

at

Massey University,

Palmerston North, New Zealand.

Sarah Louise Johnston

2002

MASSEY UNIVERSITY

APPLICATION FOR APPROVAL OF REQUEST TO EMBARGO A THESIS
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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

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The research presented in this thesis indicates milk protein production in the ruminant can be increased by supplying N-acetylcysteine to the mammary gland and this information would considerably benefit the dairy industry. AgResearch Limited has funded this research and it is requested that this thesis be embargoed to allow time to achieve a competitive advantage.

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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. γ-Glutamyl transpeptidase (γ-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 γ-glutamyl cycle, has not been fully elucidated. The γ-glutamyl cycle consists of synthetic and degradative enzymes for the cysteine-containing tripeptide glutathione. γ-GT transfers the γ-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 γ-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 γ-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 α_{s2}- and κ-casein concentration in milk. This may have resulted from increased uptake of some amino acids by the mammary gland and suggests that γ-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 γ-GT plays an important role in amino acid supply. Inhibition of γ-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 γ-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

Acknowledgements

I wish to express my appreciation to my supervisors Julian Lee, Kathy Kitson, Steve Davis, and John Tweedie for support and encouragement during my studies.

This research was funded by the Foundation for Research, Science and Technology. Thank you to AgResearch Limited for continued financial and technical support.

Ethics approval was obtained for this study from the Crown Research Institutes' Animal Ethics Committee under proposals numbered 26/97, 19/98, 4/99 and 28/99.

I am grateful for financial support from the Royal Society of New Zealand, the Kathleen Spragg Trust, the Massey University Institute of Molecular BioSciences, the New Zealand Society of Biochemistry and Molecular Biology (NZSBMB), and AgResearch, that allowed my participation in my first international conference, the 15th biennial Gordon Research Conference on Mammary Gland Biology held in New Hampshire, USA, June 1999. I thank NZSBMB and AgResearch for support in attending the Microbes and Molecules conference in Masterton 1998. I also thank the Massey University Institute of Food Nutrition and Human Health and AgResearch for support in attending the New Zealand Society of Animal Production (NZSAP) 61st conference in Lincoln, and I thank NZSAP for their young scientist paper prize.

As with all animal trials there are a number of people to thank. To Nicole Roy and Warren McNabb thank you for your help in designing and running the experiment, for your supervision and your friendship. The trial would have been very difficult without you. My thanks to surgeon Gordon Reynolds. Thank you for involving me in the surgery and for the check-ups on the goats that you made in your own time. I am also grateful to Dave Shelton, Scott Waghorn, Luke Waghorn, Jessie Chan, Penny Back and Emma Bermingham, for taking on goat milking, feeding and cleaning duties, and acknowledge the work of Andrew Graham. Thanks to Jason Peters for help in the preliminary sheep trial, to Clare Reynolds for assistance with sample preparation, and to Dean Corson for assistance with NIR. I am indebted to Don Otter of the New Zealand Dairy Research Institute for HPLC analysis of the goat milk proteins. The animal experiment was designed with help from AgResearch statistician David Baird

and input for data analysis was obtained from AgResearch statistician Fred Potter. My thanks to David Pacheco-Rios for help in statistically analysing the goat data.

Thank you to the members of the Nutrition and Behaviour Group analytical lab, Willy Martin, Renate Simenauer and especially Bryan Treloar for assistance with HPLC which was used in a large part of my work. Thank you to Abdul Molan for assistance with PAGE. Thank you to Penny Back and Emma Bermingham for assistance in cysteine analysis using Gaitonde's method. Thanks also to members of the Rumen micro lab especially Kerri Reilly for autoclaving and various borrowing. Thank you to our dishwashers Bev Breslin, Malcolm Alley, and Patrice Evitt who have kept us running. Thanks to Ben Frecklington for assisting with sheep for the *in vitro* part of this thesis. I would also like to thank Steve Davis and co-workers of the AgResearch Dairy Science Group in Ruakura for collecting ewe mammary tissue and analysing lactose in the acini medium samples.

To Bruce Sinclair, single most helpful person, walking encyclopaedia, mentor, and friend, a big thank you.

From the Nutrition and Behaviour Group, I wish to thank the senior scientists for guidance and scientists, technicians and students for help and friendly banter. I wish to thank Catherine Cameron for making life easier for us all.

I would like to thank my 6^{th} and 7^{th} form chemistry teacher Dr Jan Giffney who was an excellent teacher and friend. Dr Giffney has a PhD in biochemistry and has been my role model since secondary school.

Lastly, I wish to thank the advice and support of family and friends. To Penny Back and Kerri Reilly thank you for support and sanity. To my Mum and Dad, thank you for your understanding, guidance, and love. Finally, to my husband Jason, I would not have entered into this PhD or completed it without your support and encouragement. Thank you for your motivation and for showing me the brighter side when things got rough.

Mi amore.

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List of Abbreviations

^{3}H-AIB	α-amino-isobutyric acid [Methyl-^{3}H]
A	arterial
AA	amino acid
ATP	adenosine triphosphate
A-V	arterial venous difference
BBB	blood brain barrier
BBM	brush border membrane
Bq	becquerel (s^{-1})
BSO	buthionine sulphoximine
CA	cellulose acetate
Ci	Curie (37×10^9 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 ($kg^{0.75}$)
min	minutes
MQ H_2O	Millipore deionised water
NAC	N-acetylcysteine
$NADP^+$	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