AMINO ACID UTILISATION BY THE MAMMARY GLAND OF DAIRY COWS FED FRESH FORAGE

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DECLARATION

The studies presented in this thesis were completed by the author whilst a postgraduate student in the Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand. I hereby affirm that the content of this thesis is original research conducted by the author. All views and conclusions are the sole responsibility of the author. All references to previous work are included in the References section of each chapter. Any assistance received during the preparation of this thesis has been acknowledged.

I certify that the content of this thesis has not already been submitted for any degree and is not being currently submitted for any other degree. I certify that to the best of my knowledge any help received in preparing this thesis, and all sources of materials used, have been acknowledged in this thesis

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ABSTRACT

A series of experiments was conducted to assess the utilisation of amino acids by the mammary gland of lactating dairy cows fed fresh forages. Experiments were conducted at Palmerston North (AgResearch and Massey University) and Hamilton (Dairying Research Corporation), New Zealand with the purpose of identifying the amino acids which may limit milk protein synthesis.

The first two experiments (Chapter 3) were conducted to assess the effect of methionine supplementation on the productive performance of Friesian cows fed fresh cut pasture (ryegrass (*Lolium perenne)* / white clover (*Trifolium repens*) at two different stages of lactation (mid and late lactation). Methionine has been nominated as one of the first limiting amino acids in concentrate-fed cows, and theoretical calculations showed that its supply could also be limiting milk protein synthesis in pasture-fed cows. Supplementation with methionine (15 g d^{-1}) did not have significant effects upon the concentrations and yields of milk protein, fat or lactose in either experiment. In late lactation, methionine-supplemented cows produced less β-casein ($P < 0.05$). In mid lactation, intravenous infusion of methionine increased ($P < 0.05$) the efficiency of conversion of pasture nitrogen to milk protein.

Results from the methionine supplementation trials highlighted the lack of reliable information available to explain the responses to extra supply of amino acids in pasture-fed dairy cows. Therefore, a third experiment was conducted to provide information about the utilisation of amino acids by the mammary gland of Friesian cows fed two levels of dry matter intake (*ad libitum* and 75% of *ad libitum*). This experiment was designed to provide information on amino acid metabolism from two different approaches. The first approach consisted in the measurement of amino acid utilisation by the mammary gland using an arterio-venous preparation (Chapter 5). The second approach consisted of the use of isotopic markers to measure the total flux of amino acids in the whole body (Chapter 6). Additionally, an evaluation of two methods for measuring mammary blood flow was conducted as part of this experiment (Chapter 4).

The comparison of methods for blood flow measurement (Chapter 4) showed that the arterio-venous difference and output in milk protein of methionine and phenylalanine+tyrosine yielded similar estimates of mammary blood flow (8.1 and 8.8 litres per minute, respectively) when used as markers with the Fick principle. On the other hand, the use of tritiated water as a marker gave a significantly lower ($P < 0.05$) estimate of mammary blood flow (5.3 litres per minute) than the method using methionine or phenylalanine+tyrosine. Therefore, it was concluded that methionine and/or phenylalanine can be used as an indirect approach for measuring mammary blood flow when direct methods such as flow meters are not available.

Results from the arterio-venous preparation of the mammary gland identified plasma as the main source of free amino acids for milk protein synthesis. Contribution from erythrocytes was limited to isoleucine, leucine, phenylalanine and tyrosine and accounted for 5-14% of the total uptake of these amino acids by the mammary gland. However, uptake of free amino acids from plasma was in some cases not enough to account for their appearance in milk protein. Histidine was the amino acid with the greatest deficit between output and uptake. Sources other than free histidine appear to provide up to 4.2 g d^{-1} of histidine to the mammary gland for milk protein synthesis. It is speculated that the high contribution of non-free histidine (most likely small blood peptides) is the result of a limitation in the transfer of free histidine from the blood to the mammary gland.

The whole body fluxes of amino acids were measured by isotopic dilution using continuous infusion of a mixture of universally labelled ^{13}C- AA. The whole body flux of essential amino acids was reduced by 20% as a result of restricted feed intake, with exception of glutamic acid, for which the whole body flux was up to 8% higher in restricted animals. On average, the mammary utilisation accounted for one third of the whole body flux of essential amino acids. The branched-chain amino acids, plus lysine, are the amino acids with the greatest partitioning towards the mammary gland. It is concluded that the high mammary demand for this group of amino acids may create potentially limiting conditions in terms of their supply for milk protein synthesis.

From this series of studies on amino acid utilisation by lactating dairy cows fed fresh forage, it can be concluded that a) it is unlikely that extra supply of only one amino acid may elicit positive responses in milk protein production; b) potentially limiting amino acids have to be identified using several criteria for different metabolic conditions. Summarising several methods of assessment of limiting amino acids, it can be concluded that histidine, lysine, phenylalanine, threonine and leucine are the main candidates for limiting amino acids in pasture-fed dairy cows.

In the General Discussion, methods for confirming or rejecting that the supply of these amino acids restrict milk protein production in forage-fed dairy cows are proposed, and practical methods for increasing the supply of limiting amino acids in grazing dairy cows are discussed.

THIS THESIS IS DEDICATED TO MY PARENTS

AARON AND MARGARITA PACHECO

I walk the maze of moments
but everywhere I turn to
begins a new beginning
but never finds a finish

I walk to the horizon
and there I find another
it all seems so surprising
and then I find that I know

Enya. *Anywhere is*

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TABLE OF CONTENTS

LIST OF TABLES

Chapter 2

LIST OF FIGURES

LIST OF ABBREVIATIONS

AA	amino acid(s)
AgR	AgResearch
Ala	alanine
Arg	arginine
Asn	asparagine
Asp	aspartic acid
A-V	arterio-venous difference
BCAA	branched-chain amino acid(s)
BF	blood flow
CN	casein
CP	crude protein
Cys	cysteine
DCRU	Dairy Cattle Research Unit, Massey University
DIM	days in milk
DM	dry matter
DMI	dry matter intake
dpm	disintegrations per minute
EAA	essential amino acid(s)
EI	electron impact
FAA	free amino acid(s)
GC-MS	gas chromatography-mass spectrometry
GIT	gastrointestinal tract
Gln	glutamine
Glu	glutamic acid
Gly	Glycine
His	histidine
IE	isotopic enrichment
Ig	immunoglobulins
Ile	isoleucine
ILR	irreversible loss rate

Leu	leucine
Lys	lysine
MBF	mammary blood flow
ME	metabolisable energy
Met	methionine
MPE	mole percent excess
MPS	milk protein score
N	nitrogen
NCN	non-casein nitrogen
NEAA	non-essential amino acid(s)
NPN	non-protein nitrogen
P+T	phenylalanine plus tyrosine
PBAA	peptide-bound amino acid(s)
PCV	packed cell value (haematocrit)
PF	equivalent protein flux
Phe	phenylalanine
Pro	Proline
RBC	red blood cell
RPAA	ruminally-protected amino acid(s)
RPM	ruminally-protected methionine
SA	serum albumin
Ser	Serine
TBDMS-	N, O- *tert.* butyldimethylsylil derivative
Thr	threonine
TN	total nitrogen
TOH	tritiated water
Trp	tryptophan
$U^{13}C$-	universally-labelled with ^{13}C
Val	valine
α-LA	alpha-lactalbumin
β-Lg	beta-lactoglobulin