The metabolic cost of an intestinal parasite infection on amino acid kinetics in sheep fed fresh forages.

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

Doctor of Philosophy

in
Animal Science
at Massey University
Palmerston North
New Zealand

Emma Natasha Bermingham
2004

This thesis is dedicated to my father
George H. Bermingham

Massey University
COLLEGE OF SCIENCES

Institute of Food, Nutrition and Human Health
Private Bag 11 222,
Palmerston North,
New Zealand
Telephone: 64 6 350 4336
Facsimile: 64 6 350 5657

23 February 2004

CANDIDATE'S DECLARATION

This is to certify that the research carried out for my Doctoral thesis entitled:

"The metabolic cost of intestinal parasites in lambs fed fresh forages"

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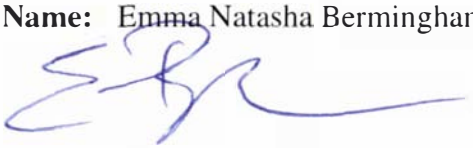
Signature: 

Date: 23 February 2004

Massey University
COLLEGE OF SCIENCES

Institute of Food, Nutrition and Human Health
Private Bag 11 222,
Palmerston North,
New Zealand
Telephone: 64 6 350 4336
Facsimile: 64 6 350 5657

23 February 2004

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This is to certify that the research carried out for the Doctoral thesis entitled:

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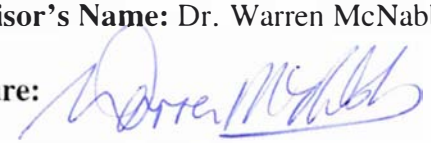
Supervisor's Name: Dr. Warren McNabb

Signature: 

Date: 23 February 2004

Massey University
COLLEGE OF SCIENCES

Institute of Food, Nutrition and Human Health
Private Bag 11 222,
Palmerston North,
New Zealand
Telephone: 64 6 350 4336
Facsimile: 64 6 350 5657

23 February 2004

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(a) is the original work of the candidate, except as indicated by appropriate attribution in the text and/or in the acknowledgements;

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Date: 23/02 2004

Supervisor's Name:

Dr Warren McNabb

Signature:

Date: 23/2/2004

Everyone will forget soon,
The fourth man on the moon,
But I've got it in my mind.

"Alan Bean" Hefner, 2001

I. *Abstract*

There is mounting evidence that parasitic infections change nutrient utilisation within the tissues, and that this is responsible for the reduction in animal performance that has been observed. Feeding forages that contain condensed tannins (CT) are thought to alleviate the impact of parasite infection on amino acid (AA) and protein metabolism by improving protein supply post-ruminally. However, there has been no quantification of how nutrients are partitioned in the lamb fed fresh forages during a parasitic infection. Therefore, the objective of this study was to quantify the partitioning of AA between the gastrointestinal tract (GIT), liver and the hind limb tissues (muscle, skin, fat) in lambs during an established parasite infection. It was hypothesised that the feeding of CT would alter the partitioning of AA between the GIT, liver and hind limbs in lambs with an established parasite burden due to the increased availability of dietary AA to the small intestine. This hypothesis was tested in two separate experiments, which had a similar experimental design. In the first experiment (Experiment One; 1999) lambs were fed fresh Lucerne (*Medicago sativa*; contains no CT). In Experiment Two, which was conducted in 2000, the lambs were fed fresh Sulla (*Hedysarum coronarium*; 2.2% CT; Experiment Two).

One week prior to infection, permanent indwelling catheters were placed in the mesenteric artery, and the mesenteric, portal and hepatic veins and vena cava for blood sampling. Additional permanent catheters were placed in the mesenteric vein (upstream from the sampling catheter) and abdominal aorta for infusion of para-aminohippuric acid (PAH) and indocyanin green (ICG) respectively, to measure plasma flow across the splanchnic tissues (PAH) and the hind limbs (ICG). A permanent Teflon cannula was fitted in the abomasum for the infusion of [1-^{13}C]-valine and [^{35}S]-cysteine (Chapters Five, Six and Seven only) on day 48 post infection to measure valine and cysteine kinetics across the mesenteric-drained viscera (MDV), portal-drained viscera (PDV), liver, total splanchnic tissues (TSP; PDV + liver) and hind limbs. A temporary catheter was inserted into the jugular vein two days before the start of blood sampling for the infusion of deuterium oxide (D_20), and [^{13}C]-sodium bicarbonate and [^{35}S]-sulfate (Chapters Five, Six and Seven only) on day 45 post infection, and [3, 4-^{3}H]-valine on day 48 post infection.

Lambs were dosed with 6 000 L3 *T. colubriformis* larvae for 6 d (n=5) or kept as parasite free controls (n=6). Faecal egg production was monitored every second day from day 22 to day 48 post infection and total intestinal worm burdens were determined at slaughter.

Blood was continuously collected from the mesenteric, portal and hepatic veins, the mesenteric artery and the vena cava in 2-hour aliquots. Plasma was harvested and AA and metabolite concentrations measured and the specific radioactivity (SRA) and isotopic enrichment (IE) of valine and cysteine were determined. After the completion of blood sampling, but while the [3, 4-^{3}H]-valine infusate was still being administered, the sheep were euthanased by an intravenous overdose of sodium pentobarbitone. Tissue samples were rapidly collected from the sheep in the following order: skin, muscle (*biceps femoris*), liver, duodenum, ileum, spleen, mesenteric lymph nodes and thymus. Digesta was also sampled from the abomasum and ileum after slaughter in order for the apparent absorption of AA to be determined.

The results from Experiment One (Lucerne-fed lambs) suggest that there is no re-partitioning of AA from the posterior hind limbs to the GIT and liver during an established infection. The changes that occurred within the PDV suggests that an established parasitic infection may trigger a localised alteration in AA metabolism and/or protein turnover without significantly changing the metabolism of AA and proteins in tissues peripheral to the TSP tissues and impacting negatively on the growth of the parasitised lambs.

In Experiment Two (Sulla-fed lambs) a reduction in feed intake was likely to be the reason for the alterations in the first pass metabolism of AA in the TSP tissues due to the decreased apparent AA absorption by the MDV observed in the parasitised lambs. However, the results from this experiment are in agreement with those from Experiment One confirming that there is no increase in partitioning of AA from the hind limbs to the GIT or liver during an established parasite infection.

Although a statistical comparison cannot be made between the data in Experiment One (Lucerne-fed) and Experiment Two (Sulla-fed), it appears that the beneficial

effects of feeding CT during a parasitic infection is due to the reduction in larval establishment in the GIT of the lamb, rather than increased AA availability.

In conclusion, an established infection imposes no measurable metabolic cost on the lamb, when feed intake is not reduced. When feed intake is reduced, there is no detectable mobilisation of protein from the hind limb. Therefore, localised or other sources of AA and/or energy substrates may be utilised.

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VI. *List of Abbreviations*

AA	Amino acid
ADG	Average daily gain
APE	Atoms percent enrichment
APP	Acute phase proteins
ASR	Absolute protein synthesis rate
ATP	Adenosine triphosphate
AV	Arterio-venous
BCAA	Branched-chain amino acid
c.	Approximately
CCK	Cholecystokinen
CO_2	Carbon dioxide
CT	Condensed tannin
cv	Cultivar
d	Day
D_2O	Deuterium oxide
DI	Deionised
DM	Dry matter
DTT	Dithiothreitol
EAA	Essential amino acid
EGF	Eggs per gram of wet faeces
Eqn	Equation
FEC	Faecal egg counts
FSR	Fractional protein synthesis rate
g	Gram
GC	Gas chromatography
GIT	Gastrointestinal tract
h	Hour
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
ICG	Indocyanin green
IE	Isotopic enrichment
ILR	Irreversible loss rate
kg	Kilogram
L	Litre
LSmeans	Least squares means
M	Molar
m	Meter
MDV	Mesenteric-drained viscera
mg	Milligram
min	Minute
mL	Millilitre
mm	Millimetre
mmol	Millimole
μm	micrometer
MS	Mass spectrometry
N	Nitrogen
NaCl	Sodium chloride

NAN	Non-ammonia nitrogen
NEAA	Non-essential amino acid
nm	Nanometer
NZ	New Zealand
O_2	Oxygen gas
P	Probability
PAH	Para-ammino hippuric acid
PDV	Portal-drained viscera
PEG	Poly ethylene glycol
PF	Plasma flow
PITC	Phenylisothiocynate
QT	Quebracho tannin
SAA	Sulphur amino acid
SD	Standard deviation
SDS	Sodium dodecyl suplhate
Spp.	Species
SRA	Specific radioactivity
TCA	Trichloroacetic acid
TDMAC	Tridecylmethammonium chloride heparin
TEA	Triethylamine
TSP	Total splanchnic tissues
t-RNA	Transfer ribonucleic acid