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

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Emma Natasha Bermingham
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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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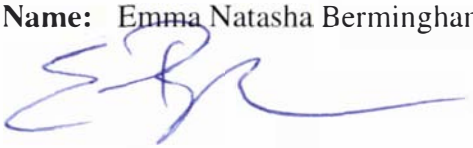
Signature: 

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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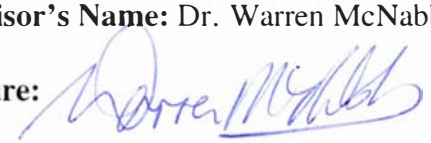
was done by **Emma N Bermingham** in the Institute of Food, Nutrition and Human Health, Massey University, Turitea Campus, New Zealand. The thesis material has not been used in part or in whole for any other qualification, and I confirm that the candidate has pursued the course of study in accordance with the requirements of the Massey University regulations.

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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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.

II. *Acknowledgements*

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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

1 LITERATURE REVIEW

1.1. Introduction

Parasites impose a severe financial cost on farmers in New Zealand (NZ) and around the world. Firstly, there is the direct cost of combating the parasitic infection – approximately NZ$ 59 million is spent annually in NZ on anthelmintic drenches (Bisset, 1994; Vlassoff & McKenna, 1994). The increased incidence of drench resistance means that the chemical control of parasites is becoming more difficult and there is the threat of trade barriers against agricultural products due to chemical residues in both meat and wool. Secondly, there is the indirect cost of decreased production estimated to be around NZ$ 200 million annually in NZ alone (Vlassoff *et al.*, 2001). These factors imply an urgent need to develop alternative methods of parasite control.

Despite this, there is very little information on tissue-specific effects of parasite infection. Protein is the major constituent of muscle and wool. It is also a large component of the gastrointestinal tract (GIT) where the parasite comes into contact with the host. The liver, due to its role in the regulation of the distribution of nutrients to the peripheral tissues and its function in the immune response may also be indirectly influenced by parasitic infection. All these tissues have high rates of protein turnover and any alterations in the protein turnover can have follow-on effects on production. Therefore, quantification of amino acid (AA) utilisation and partitioning in the GIT, liver and muscle is required.

Condensed tannins (CT) have been identified as an alternative method in the control of parasitic infection (Niezen *et al.*, 1993). While the effects of CT on improving AA supply to the uninfected animal have been documented (Waghorn *et al.*, 1987; 1994b), there is little information available on the effect of CT on nutrient utilisation within the parasite-infected animal.

This review will outline the effects of parasites on animal production and the current methods of controlling parasitic infection. Amino acid and protein metabolism in the GIT, liver and muscle in the uninfected animal, and the effects of parasitic infection on

these processes will be outlined. Finally, the effects of CT in uninfected and infected animals will also be discussed.

1.2. Intestinal parasitic infections

Sheep in grazing systems invariably come into contact with a range of nematode species including *Ostertagi spp.* (now known as *Teladorsagia spp.*) and *Haemonchus contortus*, which inhabit the abomasum of ruminants and the *Trichostrongylus* species which are found in the small intestine (Table 1.1). In NZ the two major parasites of economic significance are *Ostertagi spp.* and *Trichostrongylus colubriformis* as these are the predominant species on pasture (Vlassoff & McKenna, 1994).

Abomasal parasites are generally thought to exert their effects by decreasing food intake (Coop & Sykes, 2002) due to the alterations in abomasal morphology, mucosal secretions and the secretion of gastrointestinal hormones (e.g., Fox *et al.*, 1989a; 1989b; Lawton *et al.*, 1996; Simpson *et al.*, 1997; Scott *et al.*, 1998; 2000). The intestinal species impact on the animals' ability to utilise absorbed nutrients rather than by decreasing nutrient absorption *per se* (Coop & Sykes, 2002).

Table 1.1 Internal parasites common on New Zealand farms from Bisset (1994) and Vlassoff & McKenna (1994).

Location	Parasite	Common Name
Lungs	*Dictyocaulus filaria*	Lungworm
	D. viviparus	
Liver	*Fasciola hepatica*	Liver fluke
Abomasum	*Haemonchus contortus*	Barbers' pole worm
	Ostertagia[1] *circumcincta*	Small Brown stomach worm
	Ostertagia ostertagi	
	Trichostrongylus axei	Stomach hair worm
Small intestine	*Cooperia spp.*	
	Nematodirus spp.	
	Trichostrongylus colubriformis	Black scours worm
	Trichostrongylus vitrinus	

1. Now known as *Teladorsagia*

Trichostrongylus colubriformis is a major sheep parasite in NZ (Vlassoff & McKenna, 1994; Pomroy, 1997). Its life cycle is typical of many nematodes (Figure 1.1) with stages both inside and outside of the host animal (Familton & McAnulty, 1997). Sheep ingest the parasites as L3 larvae from pasture, which then ex-sheath in the abomasum. The larvae then move to the small intestine where they lodge and grow into adult worms after a series of molts. These adult parasites then burrow into the epithelial lining of the small intestine, mate and produce eggs which are voided out of the host via the faeces. The eggs hatch and develop into L3 larvae in the faeces and migrate to the pasture, where they are subsequently eaten by the host animal to continue the cycle. Larvae present on pasture are particularly sensitive to environmental conditions, with cool, dry weather resulting in prolonged larval survival and exposure to particularly hot or cold temperatures lethal to many of the parasites present on pastures (Barger, 1999). New Zealand's mild climate means high levels of parasitic larvae present on pastures all year round (Barger, 1999) and therefore, the need to manage parasitic infections is vital in order to minimise their effects on animal production.

The numbers and species of the parasite burden within the animal is influenced by the age and the immune status of the host with season also having an affect. For example a rise in nematode burdens during spring was observed in both breeding ewes (Brunsdon, 1970b) and young lambs (Brunsdon, 1970a) with peak numbers occurring in late summer/early autumn, followed by a rapid decline in winter.

Parasite infections 'on farm' are most likely to be mixed infections rather than with one species. Studies done in the early 1970's showed that *Ostertagia* and *H. contortus* are both major contributors to parasite burdens in sheep (Brunsdon, 1970b). Farm location will play a role in the exact composition of the parasite population present in the animal (Brunsdon, 1970a; Vlassoff *et al.*, 2001). For example, *T. colubriformis* was present in the highest numbers in all regions in NZ reported by Vlassoff & McKenna (1994). However, the contribution of *Ostertagia* and *Haemonchus* species varied from 1-11 and 15-25% respectively (Vlassoff & McKenna, 1994). Infection with multiple parasite species will result in damage to several tissues at the same time (Sykes *et al.*, 1988), and

this may have a compounding impact on the animal. For example, experiments conducted by Steel *et al.* (1982) and Sykes *et al.* (1988) found that when *Ostertagia* was mixed with *T. colubriformis* the impacts on animal production were greater than the effects of single infections alone (Table 1.2).

1.2.1. Experimental approaches to parasitic infection

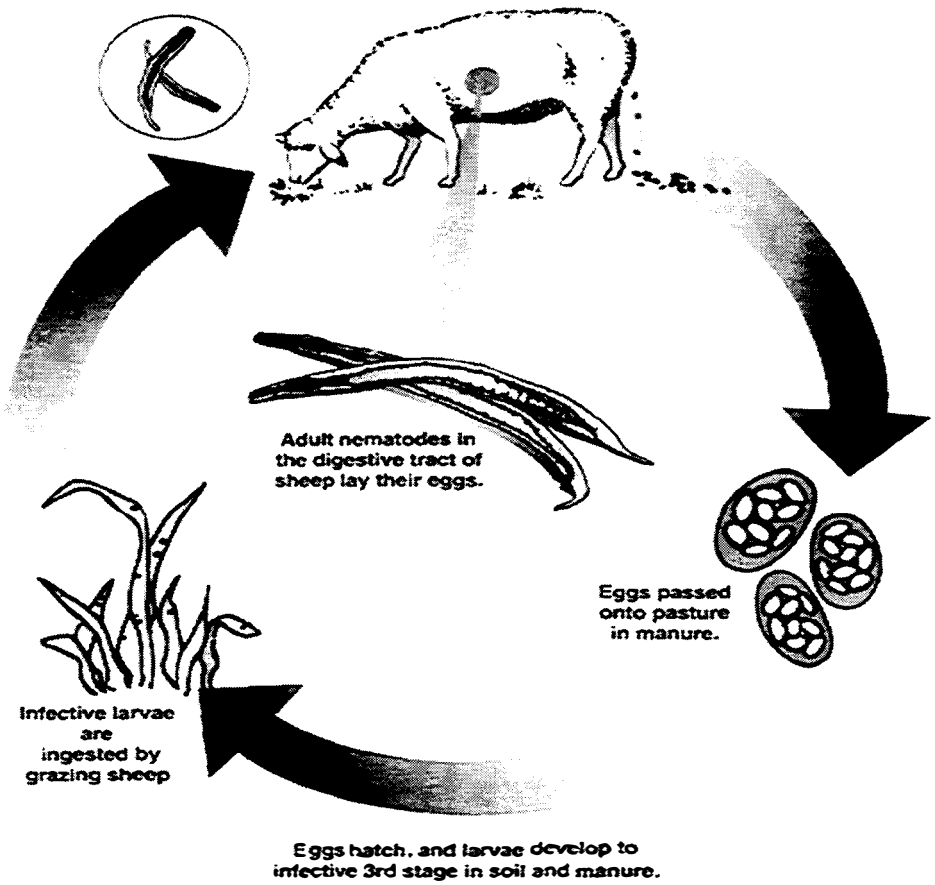
Trickle infections (i.e., a constant exposure to infective larvae), rather than a single large dose, are more representative of what occurs during farming practices (MacRae, 1993) and the majority of scientific studies have been based around the trickle infection (Appendix Table A.1). However, the interpretation of the effects of trickle infection may be difficult. This is because trickle infected animals may have continual stimulation of the immune system which may impact on host metabolism. The host will also have larvae at different stages of development, as well as the presence of adult worms both of which will have different impacts on host metabolism. The nutritional demands of the different stages of parasite growth (e.g., larvae *vs.* adult) have not been quantified both in terms of parasite requirements and their impacts on the host, and these are important areas for future research.

Table 1.2 The effects of a single or mixed infection on dry matter intake and body weight (Sykes *et al.*, 1988) and wool growth (Steel *et al.*, 1982) in lambs.

	T. colubriformis[1]	*Ostertagia*	Mixed
Intake†	-10%	-8%	-30%
Body weight (g d^{-1})	-43	-13	-92
Wool growth†	NS	-25%	-66%

1. ***Trichostrongylus colubriformis***
†: as% difference from control lambs
NS: Not significantly different from uninfected animals

Figure 1.1 The generalised lifecycle of intestinal parasites (Whittier *et al.*, 1997).



1.2.2. Effects of intestinal parasites on animal production

Gastrointestinal parasites impair production, and can even result in the death of the host animal (Parkins & Holmes, 1989). Clinical infections are an obvious source of economic loss, while subclinical infection results in poor animal production, poor breeding performance and premature culling (Parkins & Holmes, 1989). For example, decreases in liveweight gain (43-66%; Sykes & Coop, 1976; van Houtert *et al.*, 1995), wool production (20-77%; Steel *et al.*, 1980; 1982; Leyva *et al.*, 1982) and milk production in ewes (17-23%; Leyva *et al.*, 1982; Thomas & Ali, 1983) have been observed following gastrointestinal infection.

The presence of parasites in the host animal can result in decreased feed intake and nutrient utilisation and increased loss of nitrogen (N) from the body either as endogenous proteins (e.g., mucus, plasma leakage or sloughed cells) or excretion as N in urine and faeces (e.g., Sykes & Coop, 1976). The hosts' response to the infection includes altered metabolism of protein, carbohydrates and minerals (Nielsen, 1982), resulting in changes in liveweight and body composition (Parkins & Holmes, 1989).

1.2.3. Effects of intestinal parasites on nitrogen metabolism

During different physiological states (e.g., lactation, pregnancy, growth or infection) the animal has different metabolic requirements, which are met by short- and long-term changes in metabolism (Stehle & Furst, 1983). During parasitic infection these changes allow the partitioning of nutrients to the repair of damaged tissues and the immune response against the parasite (Coop & Kyriazakis, 1999). The tissues that have the most vital roles in these changes are the GIT, liver and muscle, due to their roles in the supply, metabolism and storage of nutrients.

Parasitic infections are thought to disrupt the supply of AA to muscle, and other peripheral tissues, and therefore have a major impact on animal production (Kimambo *et al.*, 1988). The impact on animal production may be due to an increase in the loss of AA-N into the GIT (compared to pair-fed controls; Kimambo *et al.*, 1988) resulting in a lower N retention in infected animals (compared to pair-fed controls; Sykes & Coop,

1976). Even at subclinical levels of infection, it has been reported that parasites decrease whole body protein gain in sheep by 43-66% (Sykes & Coop, 1976; van Houtert *et al.*, 1995). This decrease in net protein gain may occur due to the greater AA requirement by parasitised animals resulting in the diversion of AA from growth to the repair of the damaged GIT tissue (Butter *et al.*, 2000) or to support the subsequent immune response elicited by the presence of parasites (Parkins & Holmes, 1989). The changes in protein utilisation during parasitic infection are indicated by increased protein synthesis in the small intestine and liver (Symons & Jones, 1975) and decreased protein synthesis in the muscle and skin (Symons & Jones, 1975; Jones & Symons, 1982).

1.2.4. Approaches to studying amino acid and protein metabolism

Protein metabolism in the ruminant and the methodologies commonly used to estimate protein metabolism have been the focus of many reviews (MacRae & Lobley, 1991; Lobley, 1992; Reynolds, 1992; Lobley, 1994; Davis *et al.*, 1999). The most common procedures include isotopic-incorporation into bound proteins and the constant infusion procedure combined with an arterio-venous (AV) technique.

Incorporation studies, using either a flooding (large) dose or continuous infusion (Section 1.2.4.1) involve measuring the incorporation of labelled AA into tissue proteins, and have been used extensively in the literature (Garlick *et al.*, 1980; Lobley *et al.*, 1980; Davis *et al.*, 1981; Attaix *et al.*, 1988; Baracos *et al.*, 1991; Breuillé *et al.*, 1994; Connell *et al.*, 1997). Protein synthesis (as fractional protein synthesis rate; FSR) is calculated from the ratio of AA incorporation into bound-protein and intracellular (free) AA (Davis *et al.*, 1999).

The constant infusion of labelled AA combined with an arterio-venous (AV) technique is the most widely used technique for measuring AA and protein metabolism (Harris *et al.*, 1992; Roy *et al.*, 1998; Lapierre *et al.*, 1999; Bush *et al.*, 2002). This technique requires the estimation of blood flow across the tissue bed, determination of metabolite(s) concentration in blood supplied to the tissue and venous drainage, together with isotopic activity of the metabolite in these pools (Lobley, 1994). However, this method is limited

as the choice of the AA precursor pool can affect the estimation of protein metabolism, and it also requires accurate estimations of metabolite concentration and enrichment and blood flow (Lobley, 1994).

1.2.4.1. Infusion protocols

Determination of AA and protein metabolism in ruminants using stable or radioactive isotopes has been the focus of much research over the last three decades (Garlick *et al.*, 1975; Beckett *et al.*, 1992; Harris *et al.*, 1992; Lapierre *et al.*, 1996; Lobley *et al.*, 1996; Roy *et al.*, 1998; Milano *et al.*, 2000). The rationale behind the use of radioactive and stable isotopes has been the subject of recent reviews (Davis *et al.*, 1999; Rennie, 1999). The use of a labelled AA as a tracer assumes a two or three-compartmental model, that the tracer is chemically indistinguishable from the unlabelled substrate and is not metabolised over the measurement period, and that the substrate under investigation is in steady state (Taylor & Low, 1999).

The use of different techniques to measure the turnover of body protein have been reviewed by Schaefer & Scott (1993) and Davis *et al.* (1999). Briefly, the infusion of a labelled AA at a constant rate (constant infusion procedure) provides the best estimation of tissues with slow rates of turnover (e.g., muscle) while the flooding dose method (a large, rapid injection of the tracee and tracer AA) is more suited to tissues with faster rates of turnover and/or that secrete large amounts of the protein that they synthesise (Davis *et al.*, 1999). The choice of the labelled AA to use has been discussed previously (Davis *et al.*, 1981; Schaefer & Scott, 1993), however it must meet four important criteria. These are that the AA is essential, its metabolism is uncomplicated and not reversible (or if so, the intermediate isotopic activity is measurable), byproducts of its metabolism should not influence protein turnover and finally the AA should be easily analysed and have a small pool size. The cost of labelled AA differ widely, and even though an essential AA might meet all these considerations its cost might prohibit its use.

Aminoacyl-tRNA is the immediate precursor to protein synthesis and therefore this pool is ideal for measuring the isotopic activity in the tracee pool (Davis *et al.*, 1999). However, this pool is not easily measured (Lobley, 1994; Ljungqvist *et al.*, 1997; Davis *et al.*, 1999) therefore a more easily sampled pool is often used. This generally involves the sampling of the free AA in arterial or venous blood, or in the intracellular pool. This can result in overestimation (arterial pool) or underestimation (venous or intracellular pool) of protein synthesis (Lobley, 1994).

1.2.4.2. Estimation of blood flow

Estimates of blood flow across tissue beds can be achieved by various methods including dye dilution (Fick principle), ultrasonic probe and the use of tritiated water (Tepperman & Jacobson, 1982). The dye dilution technique involves the infusion of a dye into the blood vessel supplying the tissue bed and measuring its concentration in the blood inflow and outflow downstream of the infusion catheter. Compounds that are not chemically modified by the target tissue, and are extracted by the body (but not the target tissues) in order to prevent re-circulation make suitable dyes (Tepperman & Jacobson, 1982). The use of para-aminohippuric acid to measure blood flow across the splanchnic tissues is well documented (Katz & Bergman, 1969; Harris *et al.*, 1992; Lobley *et al.*, 1995), while the infusion of indocyanin green has been used to measure blood flow across the hind limbs (Wester *et al.*, 2000). Blood flow measurements can be highly variable, especially in the mesenteric drainage, due to incomplete mixing of the dye in the vessel of interest (Ortigues *et al.*, 1994; Greany, 2001). Catheter movement caused by peristaltic movements of the small intestine are also thought to cause large variations (coefficients of variation of 20% between animals) in mesenteric blood flow (MacRae *et al.*, 1997b).

Blood flow and its regulation have been the focus of many reviews (Granger *et al.*, 1980; Chou & Kvietys, 1981; Chou, 1982; Mailman, 1982; Richardson & Withrington, 1982; Shepard, 1982). Blood flow to the digestive tract increases after feeding, while it decreases in skeletal muscle (Chou & Kvietys, 1981). However, blood flow does not increase in all the organs of the digestive tract suggesting that there is localised control

of blood flow (Granger *et al.*, 1980; Chou & Kvietys, 1981). Mechanisms that control blood flow include gastrointestinal hormones and GIT motility (Granger *et al.*, 1980). Hormones that stimulate blood flow include gastrin, and cholecystokinin (CCK), whilst stomatostatin decreases blood flow (Chou, 1982; Mailman, 1982; Richardson & Withrington, 1982).

Intestinal parasite infection has been reported to increase blood flow to both the mesenteric-drained viscera (MDV) and the portal-drained viscera (PDV; Yu *et al.*, 2000) and this may be exerted by hormonal changes. For example, gastrin secretion, which has been seen to increase during *Ostertagia* infections (Fox *et al.*, 1989a; 1989b) can increase blood flow (Chou, 1982; Mailman, 1982). However, during intestinal parasitic infections, gastrin secretion does not increase (Fox, 1997), but CCK has been found to increase. Symons & Hennessy (1981) demonstrated that CCK increased by 65% during the anorexia associated with a *T. colubriformis* infection. The effects of parasitic infection on blood flow to the liver and hind limbs have not been quantified to my knowledge.

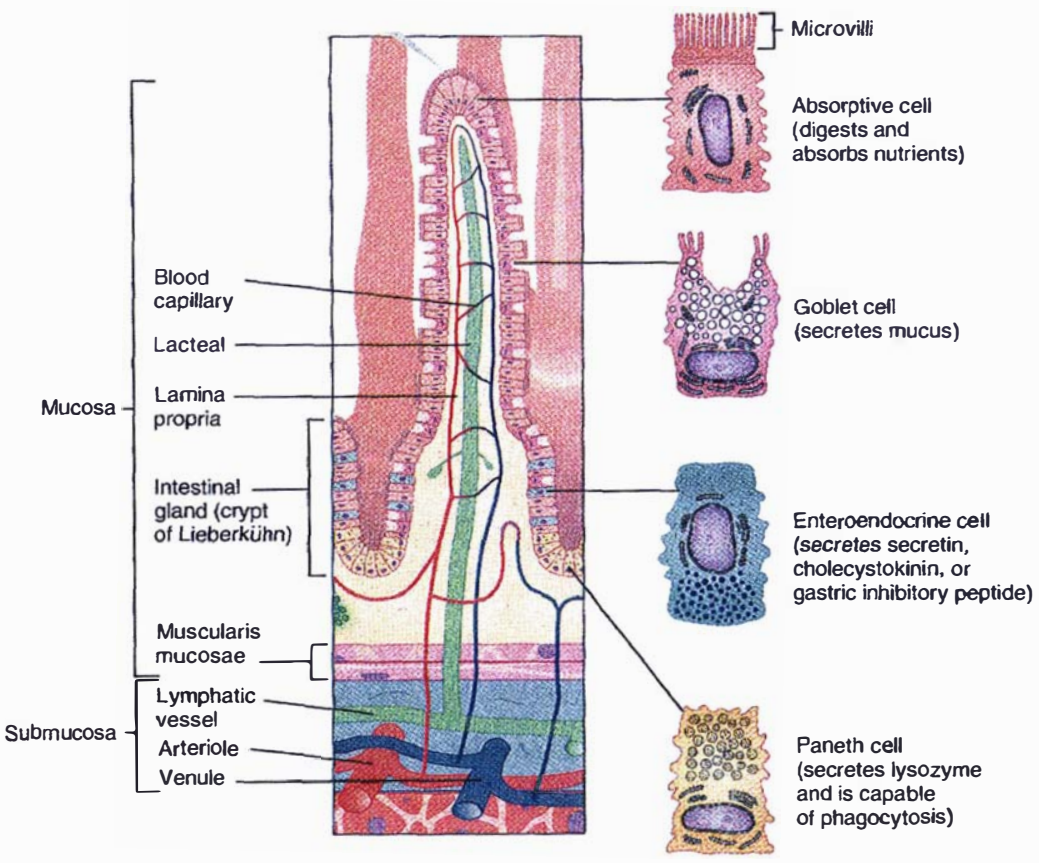
1.2.5. The gastrointestinal tract and its metabolic activity

The GIT comprises of the MDV (small intestine), and the PDV (rumen, abomasum, small and large intestine, pancreas and spleen). The GIT is a complex system with many different functions including the digestion and absorption of nutrients, protection against lumen acid and production of gut hormones (Seare & Playford, 1998). Tissues within the PDV such as the spleen have a role in maintaining the health and well being of the host, while the pancreas is involved in hormone production. These functions result in a large requirement for nutrients (e.g., energy and AA) and oxygen. The small intestine (duodenum, jejunum and ileum) accounts for over 70% of the protein synthesis that occurs in the splanchnic tissues (Table 1.3) and it is also the point of contact with intestinal parasites. Therefore, intestinal tissue will be the focus of this review.

The two major types of tissue in the intestine are the smooth muscle, which has a role in digesta flow through this tissue, and the mucosal epithelium, which is involved in

secretion and nutrient absorption. The mucosal cells have a life span of 38-46 hours. They differentiate in the crypt regions of the epithelium (Figure 1.2), migrate to the tips of the villi and are then shed into the lumen of the GIT (Moog, 1981; Parkins & Holmes, 1989). The mucosal cells and the digestive proteins that are secreted in the GIT lumen require digestion before the animal can reabsorb them, with 70–85% of the secretions reabsorbed in the terminal ileum (Bown *et al.*, 1986; Poppi *et al.*, 1986). The remaining unabsorbed proteins (15-30%) represent a major loss of protein from the animal as they are fermented to ammonia in the large intestine or excreted in the faeces (Parkins & Holmes, 1989).

Figure 1.2 Structure of the small intestine of humans (Tortora & Grabowski, 1996).



1.2.5.1. Protein and amino acid metabolism

The contributions of different tissues to protein synthesis in the splanchnic bed are given in Table 1.3. The FSR of the rumen and abomasum ranges from 20-79 % d^{-1} (Davis *et al.*, 1981; Lobley *et al.*, 1994). The small intestine ranges from 20-100 % d^{-1} (Symons & Jones, 1983; Baracos *et al.*, 1991; Lobley *et al.*, 1994). The large intestine has a lower protein turnover rate at 20-26 % d^{-1} (Lobley *et al.*, 1994). Rates of FSR in the spleen are 9-32 % d^{-1} (Garlick *et al.*, 1980; Baracos *et al.*, 1991; Breuillé *et al.*, 1998).

Fractional protein synthesis rates will be determined by the metabolic demand of the tissue. For example, the high rate of FSR in the small intestine is due to the constant renewal of the epithelium of the small intestine. This means that the epithelial layer has the most rapid rate of tissue renewal in the body (Seare & Playford, 1998). This is shown in Table 1.4, where the small intestine has a high FSR compared to muscle or skin in the rat, sheep and heifer. However, despite the high rates of protein synthesis, very little protein is actually retained by the small intestine. For example, in the growing lamb only 1 g protein per day is retained by the small intestine (Figure 1.3).

Amino acids taken up by the tissues of the small intestine may be either incorporated into protein or catabolised into CO_2 and water and are therefore unavailable to the animal (Reeds *et al.*, 1999). The small intestine has a major role in the utilisation of AA absorbed from the digestive tract, with both essential AA (EAA) and non essential AA (NEAA) undergoing substantial metabolism. For example, only 30-80% of the EAA present in the intestinal digesta appears in the portal blood (Tagari & Bergman, 1978; MacRae *et al.*, 1997b). This suggests that the intestinal epithelium has a requirement for AA, either for energy production, protein synthesis or other processes (Bergman & Heitmann, 1980).

Studies investigating the utilisation of AA by the small intestine in the monogastric have shown that there is substantial metabolism of AA as they pass through the small intestine (Stoll *et al.*, 1998b), with one-third of the dietary AA used by the small intestine. However, in the sheep, it has been suggested that the utilisation of AA from the lumen is

Table 1.3 The contribution of different tissues to protein synthesis in the splanchnic tissue (from Lobley *et al.*, 1994).

		FSR† % d^{-1}	Protein content§ g kg^{-1}	Protein synthesis§ g d^{-1}	% of total
Rumen		22	95	21	5
Abomasum		24	77	18	4
Duodenum	Mucosa	60	126	75	18
	Serosa	42	87	36	9
Jejunum	Mucosa	64	118	76	18
	Serosa	36	122	44	10
Ileum	Mucosa	47	103	49	12
	Serosa	31	90	28	7
Caecum		20	79	16	4
Colon		26	85	22	5
Liver		22	169	37	9
Total splanchnic tissues				421	100

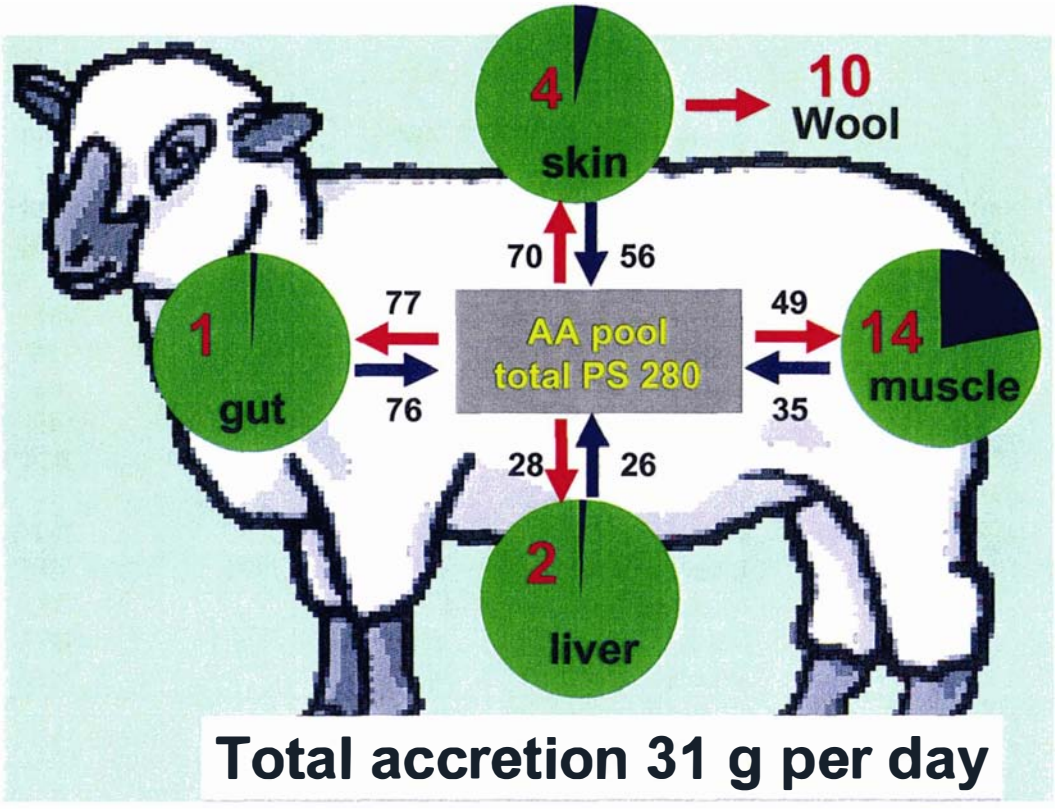
†FSR: fractional protein synthesis rates (% day^{-1}) based on intracellular amino acid pool enrichment
§ Based on wet weight of tissue

Table 1.4 Protein content, fractional protein synthesis rates (FSR) and protein synthesis per day in rats (McNurlan & Garlick, 1980), growing lambs (Davis *et al.*, 1981) and heifers (Lobley *et al.*, 1980).

Species	Liveweight (kg)	Tissue	Protein Content (g)	FSR[1] (% d^{-1})	Protein synthesis[2] (g d^{-1})
Rat†	0.04	Stomach	50	74	30
		Small intestine	460	103	480
		Large intestine	120	62	80
		Liver	70	105	740
		Whole body	9300	34	3100
Lamb	16	Gut	250	14	38
		Liver	100	15	14
		Muscle	1710	3	51
		Skin	350	12	42
		Whole body	3100	NS[3]	214
Heifer	240	Gut	1900	53	1015
		Liver	600	32	186
		Muscle	17300	2	335
		Skin	7600	6	467
		Whole body	34700	7	2498

† units for protein content and protein synthesis are in mg.
1. FSR = percentage (%) of protein pool synthesised per day.
2. Protein synthesis = FSR x protein content.
3. Not stated

Figure 1.3 Protein turnover (g d^{-1}) in the lamb (adapted from MacRae, 1993). Numbers in the pie diagram represent the amount of protein retained by the tissue (g d^{-1}).



much smaller than in the pig, with approximately 20% of the AA utilised within the tissue derived from the GIT lumen (MacRae *et al.*, 1997a; Yu *et al.*, 2000). It was initially suggested that the uptake of AA by enterocytes in the pig may indicate protein synthesis within the cell (Rerat *et al.*, 1992). However, subsequent studies suggest that, approximately 60% of the AA taken up by the small intestine of the pig are catabolised (Stoll *et al.*, 1998b).

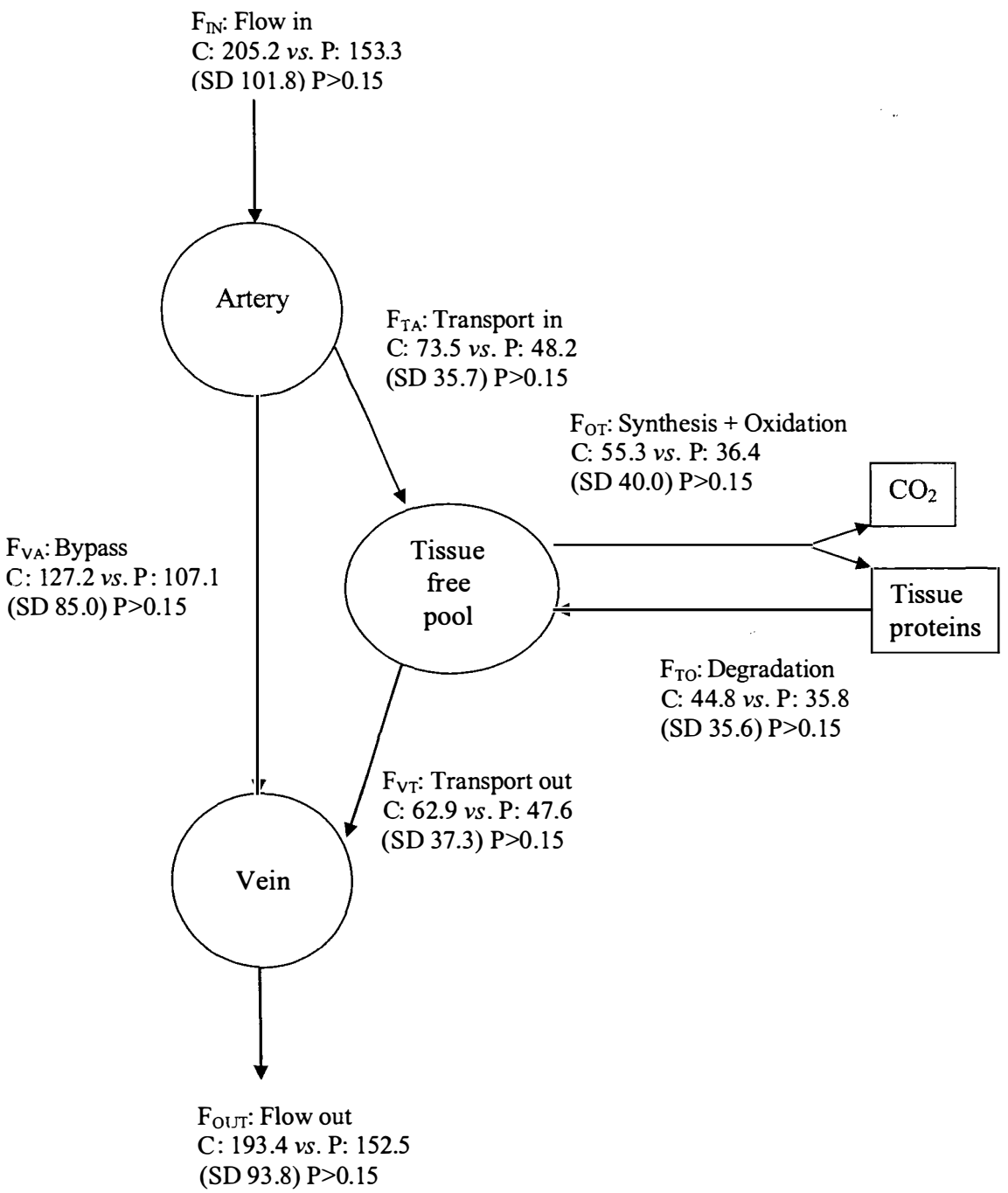
Amino acids of particular importance in the GIT include glutamine and cysteine. Glutamine has a role in the maintenance, structure and function of the digestive tract (Souba, 1991), while cysteine is present in high concentration in gut enzymes (MacRae & Lobley, 1991). Other AA that are important include threonine, serine and proline due to their high concentration in mucin produced by the GIT (Roberton *et al.*, 1991; Van Klinken *et al.*, 1998; Reeds *et al.*, 1999).

1.2.5.2. Parasitic infections and the gastrointestinal tract

Damage to intestinal tissue by *T. colubriformis* infection is caused by adult parasites as they tunnel into the intestinal crypts and the villi epithelium (Coop & Angus, 1975; Holmes, 1985). Coop & Angus (1975) noted that *T. colubriformis* infection resulted in noticeable changes in the histology in the proximal section of the small intestine. Subsequent studies showed that *T. colubriformis* did not affect the digestibility and absorption of protein (Poppi *et al.*, 1986; Bown *et al.*, 1991b). More recently Coop & Sykes (2002) have suggested that *T. colubriformis* impairs the animals' ability to utilise absorbed nutrients due to alterations in protein and energy metabolism, rather than affecting nutrient absorption.

The changes in GIT structure and function in response to parasitic infection (Table 1.5) induce changes to the metabolism of the GIT. This causes changes to whole-animal metabolism due to the large influence that protein turnover in the GIT has on the whole animal (MacRae *et al.*, 1997a; Stoll *et al.*, 1998b). An alteration in protein synthesis in the GIT due to the presence of intestinal parasites has been observed in guinea pigs infected with *T. colubriformis* with a 40% increase in the large intestine FSR, but with

Figure F.2 ^{13}C-Valine kinetics (F; mmol d^{-1}) in the muscle of lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with *Trichostrongylus colubriformis* (P; n=6) or kept as parasite free controls (C; n=6). Results are presented as LSmeans and their associated pooled standard deviation (SD).



no change in small intestine FSR (Symons & Jones, 1983). The alterations in FSR in the large intestine may be due to the changes in anatomy and increased mucus production that occur during parasitic infection (Cheema & Scofield, 1982). Additionally, daily absolute protein synthesis (ASR) increased by 24% and 70% in the small- and large intestine, respectively. These effects on FSR and ASR were not due to changes in feed intake, demonstrated by the inclusion of "pair-fed" control animals (Symons & Jones, 1983), and indicate an increase in the amount of constitutive protein synthesised.

Catabolism of AA within the small intestine is already high, with approximately 60% of the AA that are absorbed by the tissue catabolised (Stoll *et al.*, 1998b). Compared to uninfected controls, parasite infection increased the oxidation of leucine in the small intestine by a further 20-40% (Yu *et al.*, 2000). This increase in oxidation, together with the increase in leucine sequestration (leucine removed from the circulation for either oxidation or protein synthesis) by the GIT had the net effect of decreasing its availability to other tissues (Yu *et al.*, 2000). Therefore, increases in protein synthesis in the GIT will decrease the availability of AA (and energy) to other tissues (Stehle & Furst, 1983; MacRae *et al.*, 1997a; Stoll *et al.*, 1998b; Yu *et al.*, 2000) and may explain the decreases in animal production observed during parasitic infection.

The low N retention that has been observed in lambs dosed with 2500 infective *T. colubriformis* larvae per day (Sykes & Coop, 1976) may be due to the significant increase in endogenous N (e.g., mucus, plasma proteins and cell sloughing) entering the GIT lumen and increases in urinary-N (Parkins & Holmes, 1989). The increase in AA catabolism in the GIT decreases the efficiency of AA utilisation due, in part, to the increase in urinary-N loss (Parkins & Holmes, 1989). The increase in non-ammonia-N (NAN) leaving the small intestine of lambs exposed daily to *T. colubriformis* larvae (3-20 g NAN d^{-1}) could result from endogenous protein secretions such as plasma leakage, sloughed cells or additional mucus production (Poppi *et al.*, 1986). *Trichostrongylus colubriformis* increased the amount of endogenous protein secretion in lambs (Yu *et al.*, 1999). Increased endogenous secretions will increase demand for the

replacement of these products, representing an additional energy and protein cost to the animal (Poppi *et al.*, 1986). If AA absorption is low, such as the case when feed intake is reduced, there is a subsequent decrease in the availability of dietary AA for supporting the synthesis of these products (Poppi *et al.*, 1986). Therefore, the additional AA required must be sourced from stores within the animal.

Table 1.5 The effects of intestinal parasites on the histology, function and secretion by the gastrointestinal tract.

Effect		Impact	Source
Histology	Villi atrophy	Decrease nutrient absorption	1, 2, 3
	Flattened Mucosa	Decrease nutrient absorption	1, 3
	Thickened mucosa	Decrease nutrient absorption	2
	Decreased villi height	Decrease nutrient absorption	4
	Goblet cell hyperplasia	Increased mucus production	1, 4
	Crypt elongation	Decrease nutrient absorption	1
	Epithelial erosion	Decrease nutrient absorption	2
Function	Decreased rumen clearance rate	Decrease feed intake	5
	Increased volume of liquid in small intestine	Decrease feed intake	5
	Decreased digesta flow	Decrease feed intake	5
Secretion	Increased endogenous nitrogen loss into small intestine	Decreased nitrogen retention	6
	Increased NAN* leaving small intestine	Decreased nitrogen retention	6
	Decreased brush border enzymes	Decreased nitrogen retention	1
	Increased gastrin secretion	Decrease feed intake	7

1. Coop & Angus, 1975.
2. Holmes, 1985.
3. Symons & Jones, 1970.
4. Stephenson *et al.*, 1980.
5. Roseby, 1977.
6. Parkins & Holmes, 1989.
7. Nielsen, 1982.

*NAN: non -ammonia nitrogen.

1.2.6. Liver

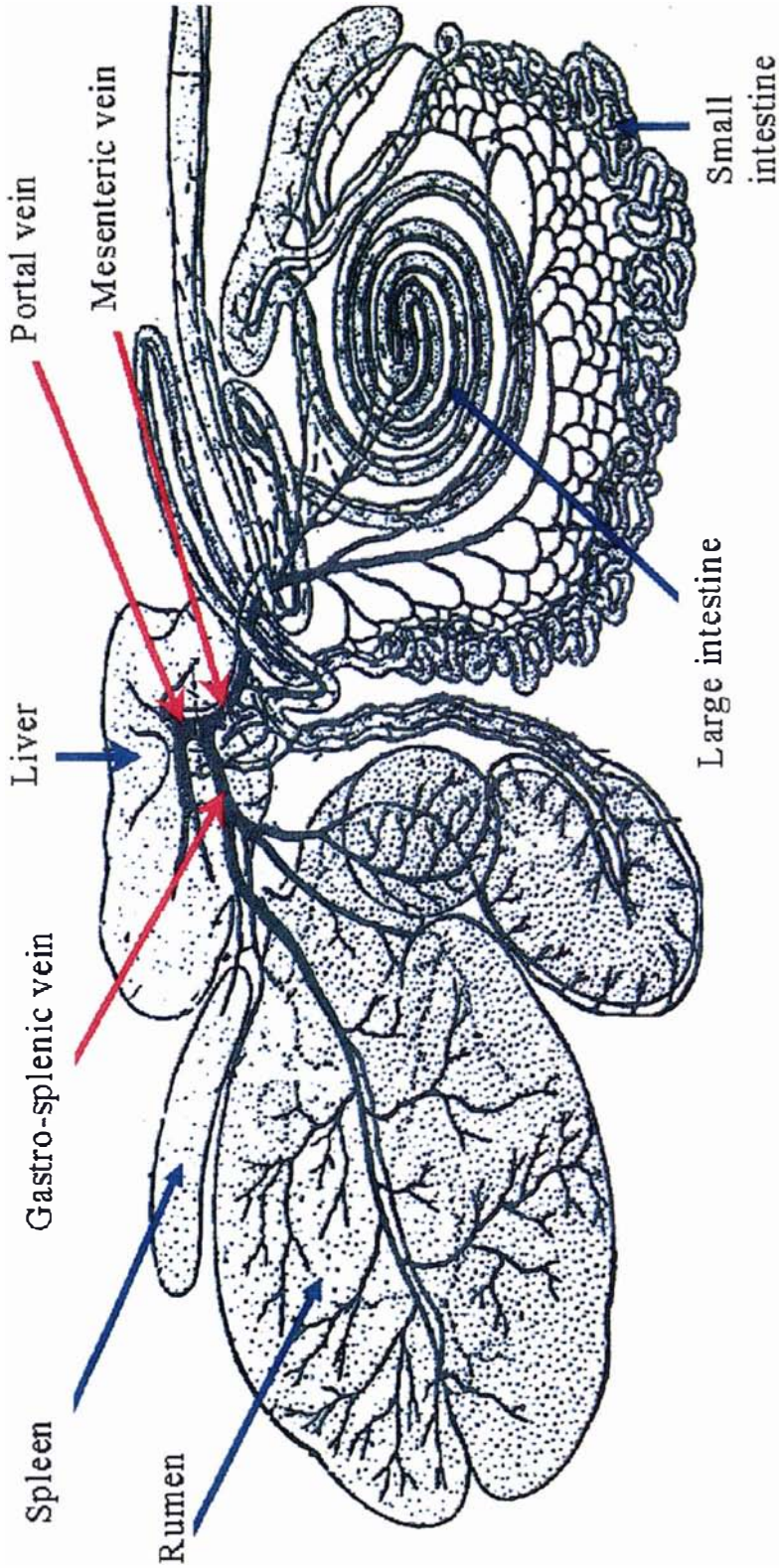
The liver has many functions, including the metabolism of carbohydrate, protein, and lipid. It is also vital for the detoxification of compounds that pose a threat to the health of the animal. The liver is responsible for the synthesis of acute phase proteins (APP) which include C reactive protein and fibrogen (Moshage, 1997; Obled, 2002) required to elicit an immune response against pathogens.

Hepatocytes are responsible for the extraction of nutrients and absorbed toxins delivered to the liver by the portal vein (Figure 1.4). Nutrients not utilised by the liver, or those that are synthesised for export, are released into the hepatic vein to be distributed to other tissue beds within the body. It was suggested that the liver has a vital role determining the amount and pattern of absorbed nutrients that are available to the rest of the body, due to its roles in gluconeogeneis, ureagenesis and AA metabolism (Stehle & Furst, 1983). However, a recent review, has suggested that the converse is true, with metabolism in the liver regulated by the immediate demands and metabolic activity of the peripheral tissues (Lobely, 2002). This is referred to as the "push or pull" theory of regulation of AA metabolism and this hypothesis is gaining worldwide acceptance.

1.2.6.1. Protein and amino acid metabolism

The liver consists mostly of "labile" protein stores – i.e. protein which undergoes degradation and re-synthesis regularly (Daniel *et al.*, 1977; Parkins & Holmes, 1989). The rates of FSR in the liver are high (Davis *et al.*, 1981), with the balance between protein synthesis, degradation and the export of protein to extracellular fluid determining the protein content in the liver (Meijer *et al.*, 1999).

Figure 1.4 Organs and blood vessels in the splanchnic tissue bed (Goshal, 1975).



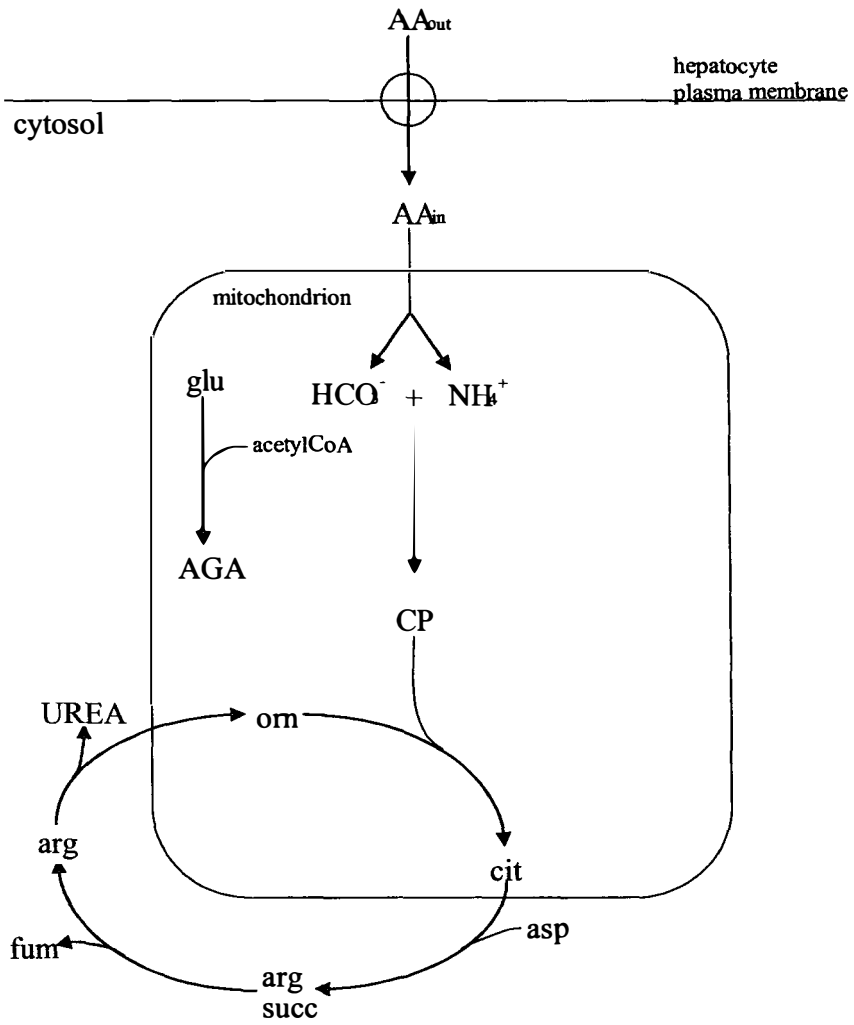
The liver removes considerable amounts of AA from the portal circulation (Wolff *et al.*, 1972; Lobley *et al.*, 1995; 1996). The AA that are removed by the liver have four fates (Wray-Cahen *et al.*, 1997). Firstly, they may be retained in free form in intra- and extra-vascular fluid. Secondly, they can be converted to N metabolites (e.g., transamination products, urea). Thirdly, they can be oxidised to carbon dioxide (CO_2), ammonia (NH_4) and water yielding adenosine triphosphate (ATP). And finally, they can be used in the synthesis of constitutive or export proteins. Results from an experiment conducted by Stoll *et al.* (1998a) suggest that hepatic protein synthesis is the final outcome for many of the AA taken up by the liver from the portal blood of pigs. For example, in the case of leucine, phenylalanine and lysine, 73-90% of these AA taken up by the liver were incorporated into hepatic proteins (both constitutive and export proteins). The synthesis of export proteins, such as albumin, account for 30-40% of total hepatic protein synthesis (Vary & Kimball, 1992; Connell *et al.*, 1997).

Oxidation of AA in the liver results in the production of ammonium and bicarbonate ions, which then combine with an additional AA-N to form urea (Figure 1.5). In effect, this "scavenging" of the ammonium and bicarbonate ions prevents toxic build up of ammonia and alterations in blood pH, respectively (Meijer *et al.*, 1999). However, the formation of urea from the oxidation of AA represents an inefficient use of AA, as the urea is eventually excreted from the body in urine, although there is a small proportion of urea recycled to the GIT.

1.2.6.2. Parasitic infections and liver protein metabolism

Trichostrongylus colubriformis infections also increased ASR and FSR in the liver of sheep (Jones & Symons, 1982) and guinea pigs (Symons & Jones, 1971; 1978). In the rat, bacterial sepsis (as a model of the inflammatory response) increased protein FSR by 22% and the contribution of the liver to whole body protein synthesis was doubled (Breuillé *et al.*, 1994). These increases may be associated with the loss of serum proteins that are synthesised by the liver into the GIT (Poppi *et al.*, 1986), increased APP production (Stehle & Furst, 1983), and increased synthesis of other export proteins (Vary & Kimball, 1992).

Figure 1.5 Urea synthesis in the liver and the end products of amino acid breakdown and the formation of the urea molecule (Meijer *et al.*, 1999).



Where:
AA, amino acids; CP, carbamoyl-phosphate; glu, glutamate; asp, aspartate; orn, ornithine; cit, citrulline; argsucc, argininosuccinate; arg, arginine; fum, fumarate; AGA, N-acetylglutamate

$$2\,NH_4^+ + HCO^{3-} \longrightarrow NH_2\text{-}CO\text{-}NH_2 + H^+ + 2H_2O$$
$$H^+ + HCO^3 \longrightarrow CO_2 + H_2O$$
$$2\,NH_4^+ + 2HCO^{3-} \longrightarrow NH_2\text{-}CO\text{-}NH_2 + CO_2^+ + 3H_2O$$

Protein exported by the liver, such as APP have high concentrations of glycine, serine, methionine and cysteine, branched-chain AA (BCAA; leucine, isoleucine and valine), histidine and phenylalanine (Stehle & Furst, 1983; Grimble, 1990). Data summarised by Reeds *et al.* (1994) suggest that the aromatic AA (tyrosine, phenylalanine and tryptophan) are also of importance in APP synthesis. In the rat, sepsis increased the production of liver export proteins by 40%, and total protein synthesis increased by 30-50% compared to control animals (Vary & Kimball, 1992). Therefore, it is likely that the hepatic requirement for AA will increase during parasitic infection, and when dietary supply of AA decreases, these AA will be sourced from the breakdown of muscle protein (Bergman & Heitmann, 1980)

The liver is an important site of the synthesis of metabolites such as glucose and urea. However, while there is information available on the concentration of these metabolites there is very few data relating to their flux across tissue beds in the infected animal. Parasitic infection did not affect the plasma concentration of glucose (Coop *et al.*, 1976). However, results in the literature relating to plasma urea concentrations in parasitised lambs are contradictory. For example, Coop *et al.* (1976) reported that a trickle infection of 2500 *T. colubriformis* larvae had no effect on the concentration of urea during 14 weeks of infection, whilst other studies have shown that a single dose of 30 000 *T. colubriformis* resulted in a 18-26% increase in urea concentration 30 days post infection (Roseby, 1973; Roseby & Leng, 1974). An increase in the production of urea by the liver may result in an increase in the loss of AA-N from the body, as urea synthesis requires an additional AA to supply a N-group (Meijer *et al.*, 1999).

1.2.7. Muscle

Muscle is the largest AA reserve in the body and has an important role in supplying AA during disease, injury or starvation (Daniel *et al.*, 1977; Rennie & Harrison, 1984). Muscle also forms the basis of payment for many livestock farmers, and is therefore an important production trait.

1.2.7.1. Protein and amino acid metabolism

In the short term, the muscle generally does not release AA to a great extent (Oddy *et al.*, 1987). However, during periods where AA from the diet are limited or non-existent (e.g., fasting Bergman & Heitmann, 1980; Heitmann & Bergman, 1980), muscle releases considerable amounts of AA. These include the BCAA, glutamine and gluconeogenic AA, such as alanine, glutamine, serine and glycine. During fasting, BCAA are used as an energy source for the brain (Felig, 1975). Alanine has roles in N transport around the body via the glucose-alanine cycle (Figure 1.6). Glutamine is converted to alanine in the kidneys (Rodwell, 2000) and is therefore also involved in the glucose-alanine cycle.

Muscle protein is a moderately labile store of protein – it undergoes considerable amounts of protein synthesis and degradation largely due to its considerable protein mass rather than a high turnover rate (Davis *et al.*, 1981; Figure 1.3). This characteristic of muscle enables muscle to act as a regulatory organ (Daniel *et al.*, 1977). The rate of protein synthesis in muscle is 16-22% of whole body protein synthesis in cattle, while in the GIT this figure is doubled (32-45%) despite being a significantly smaller percentage of total body protein mass (5% *vs.* 50%, respectively; Lobley *et al.*, 1980). However, nutrient utilisation by muscle seems more efficient than that by the GIT (MacRae & Lobley, 1991), with 50-75% of the protein synthesised in the muscle deposited, in contrast to the GIT and liver where less than 20% of the synthesised protein is retained by these tissues.

1.2.7.2. Parasitic infections and amino acid and protein metabolism in muscle

Short-term protein degradation in the muscle is beneficial to the animal during parasitic infection or any other type of challenge. Protein degradation in the skeletal muscle releases AA, partly to provide the liver with additional AA for the immune system (Hasselgren, 1999; Vary, 1999). However, in the long term the breakdown of muscle protein is undesirable for the animal for several reasons including liveweight loss. Reed *et al.* (1994) suggested that the requirement of AA for APP production would result in

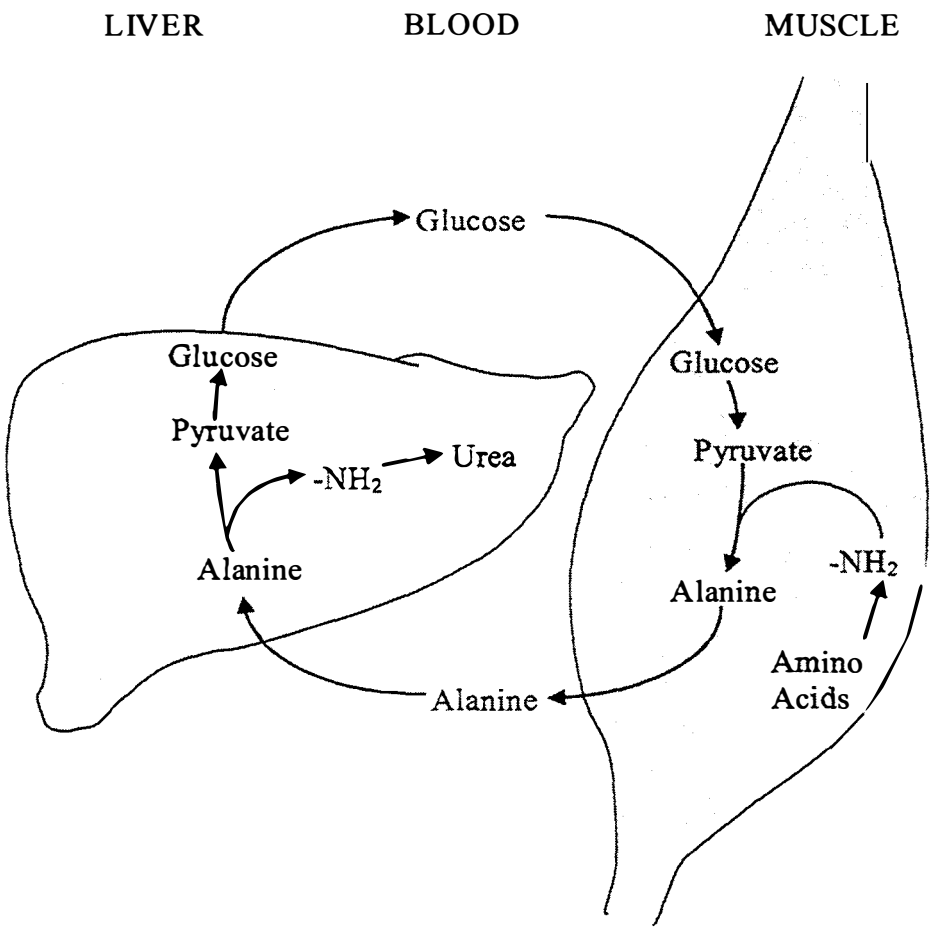
the breakdown of muscle protein. For example, Preston *et al.* (1998) estimated that 2.6 g of muscle is required to synthesis 1 g of fibrogen (an acute phase protein).

During sepsis in the rat, muscle atrophy was observed and protein FSR was decreased by 36% compared to pair-fed controls (Breuillé *et al.*, 1998). Sheep given a gelatin capsule containing 60 000 infective *T. colubriformis* larvae showed a decrease in body weight that was explained by a decrease in muscle protein synthesis and an increase in muscle protein degradation (Symons & Jones, 1975). Decreases in muscle protein synthesis have also been observed in mice, sheep and guinea pigs given *T. colubriformis* (Symons & Jones, 1971; 1972; 1978).

1.2.8. Interactions between the gastrointestinal tract, liver and muscle

The main sources of AA for the body tissues are from the dietary supply from the GIT, breakdown of storage proteins, or from synthesis of AA within the animal (NEAA only). It has been shown that the AA released from the breakdown of skeletal muscle protein are preferentially released into the bloodstream, rather than re-synthesised into protein within the muscle (Biolo *et al.*, 1995b). These AA are mainly diverted to the small intestine (Biolo *et al.*, 1995a; Reeds *et al.*, 1999) or metabolised within the liver (Wray-Cahen *et al.*, 1997). In the small intestine, the majority of AA used for protein synthesis are derived from arterial plasma rather than the GIT lumen (MacRae *et al.*, 1997a; Reeds *et al.*, 1999) supporting the conclusion by Biolo *et al.* (1995b) that there is a net transport of AA from the muscle to the small intestine during the post-absorptive state. However, in the liver much of the AA used for hepatic protein synthesis originates from dietary supply (Stoll *et al.*, 1998a).

Figure 1.6 The glucose-alanine cycle (Felig, 1975).



In the uninfected animal, nutrient interactions between the GIT, liver and skeletal muscle are carefully maintained, because different tissues have different metabolic requirements. For example, the splanchnic tissues (PDV and liver) operate at high rates of protein synthesis. This is due to their roles in digestion, absorption, and immune responses. This can lead to competition with the "storage" tissues (Stehle & Furst, 1983). The integration of AA metabolism between the GIT, liver and muscle has been reviewed previously (Felig, 1975; Bergman & Heitmann, 1980; Seal & Parker, 2000), with the overall conclusion that the fate of AA are specific to the individual AA. For example, the net portal appearance of AA ranges from 40-80% of the amount present in the digestive tract (Bergman & Heitmann, 1980). The use of individual AA within the PDV is also variable (Seal & Parker, 2000). The exact interactions between the different tissues will also depend on the animal species and physiological state.

Few experiments have simultaneously measured the rate of AA utilisation and protein synthesis and degradation rates. The quantification of these relationships is difficult, and extensive mathematical modelling is required (Gill *et al.*, 1984; 1989a; 1989b; Baldwin *et al.*, 1987). Data obtained from a model of protein metabolism in the growing lamb (Gill *et al.*, 1989b) demonstrated that the GIT (25%), skin (23%) and muscle (21-26%) had the largest contribution to total protein synthesis followed by the liver (13-14%). These estimations are comparable to the rates measured and reported in the literature (e.g., Lobley *et al.*, 1980). The GIT contributes only 5% to whole body protein mass yet has the highest rate of oxygen consumption (23%) and protein synthesis (28%), in comparison to the muscle which, despite a higher protein mass, has a lower oxygen consumption and protein synthesis (Table 1.6; Gill *et al.*, 1989b; Lobley, 1994).

Table 1.6 Percent contribution of different organs to protein mass, whole body oxygen consumption and protein synthesis in the sheep (adapted from Lobley, 1994).

Organ	Protein mass	Oxygen uptake	Protein synthesis
GIT[1]	5	23	28
Liver	2	21	9
Muscle	32	17	18
Skin	14	1	25

1. Gastrointestinal tract

1.2.8.1. The effects of parasitic infections

During times of stress, such as parasitic infection, there is a shift in the partitioning of nutrients between organs in order to supply nutrients to the organs with the highest priority. It has been proposed that in the infected animal, nutrients are partitioned firstly to the maintenance of body proteins (including repair and replacement of damaged tissues), secondly to the development of immunity towards the parasite, thirdly to protein gain, and then finally to the deposition of fat (Coop & Kyriazakis, 1999). This theory is supported by carcass composition data in the literature which indicated lambs infected with *O. circumcincta* had reductions in both fat (25-33%) and protein (10-18%) deposition (Sykes & Coop, 1977; Coop *et al.*, 1982).

The immune system and its components has been extensively reviewed in the literature (Grimble, 1990; 1998; Hasselgren, 1995; Moshage, 1997; Spurlock, 1997). During the immune response there is increased protein synthesis and changes in the metabolism and physiology within the animal (Moshage, 1997; Grimble, 1998). These changes are brought about by cytokines, which as well as having a direct role in the immune response, are thought to have roles in normal growth and development (Spurlock, 1997). The components of the immune response have a large requirement for glycine, serine, methionine, cysteine and BCAA as they occur in high concentrations in many of the compounds synthesised during the immune response (Grimble, 1990). Glutamine is also important as it is used as fuel in many tissues involved in the immune response including the lymph nodes, thymus and spleen (Wu *et al.*, 1991; Calder, 1995). The increase in

AA requirements for the immune response means that the protein turnover of these tissues will be affected. For example, in the septic rat FSR in the spleen was 2-3 times higher during sepsis (Breuillé *et al.*, 1998).

The effects of parasite infection on protein metabolism in the animal have not been fully quantified. However, the effects of sepsis on protein metabolism in man have been reviewed by Wannemacher (1977). It was suggested that, compared to the starvation-adapted patient, the amount of protein being used by the liver of the septic patient would more than double (60 *vs.* 140 g protein d^{-1}), with the synthesis of plasma proteins increasing by almost 70% in the septic patient. The amount of glucose synthesised from AA was estimated to increase four-fold (15 *vs.* 75 g protein d^{-1}). To meet this increase in AA demand by the liver, it was suggested that the contribution from skin protein would double (5 *vs.* 10 g protein d^{-1}) while muscle contributions would increase by 75% (80 *vs.* 140 g protein d^{-1}).

An increased in the contribution of AA from the productive tissues (muscle and skin) agrees with the changes observed in protein metabolism in parasitised animals. During parasitic infection muscle protein synthesis is decreased (Symons & Jones, 1971; 1972; 1978) and the rate of liver protein synthesis is increased in sheep (Symons & Jones, 1971; 1978; Jones & Symons, 1982). These alterations may be due to the diversion of AA from growth to the repair of damaged tissue (Butter *et al.*, 2000; Coop & Sykes, 2002) and the immune responses (APP synthesis, increased albumin synthesis) due to the increased requirement for AA by these tissues (Coop & Sykes, 2002). The effects of parasitic infection on protein metabolism in sheep have been hypothesised in Figure 1.7. However, with the exception of the MDV and PDV (Yu *et al.*, 2000), the quantification of AA partitioning in the lamb have not been determined. Yu *et al.* (2000) found that a *T. colubriformis* infection in sheep resulted in increased sequestration of leucine by the tissues of the MDV and PDV. An increase in the oxidation of leucine was also noted in the PDV and overall there was a decrease in the availability of leucine to the peripheral tissues.

The increased AA requirements of the tissues affected directly by the parasites (GIT and liver) may lead to an increase in the degradation of protein from the skeletal muscle to supply the specific AA required by the GIT and liver. This is because the reserves of some AA used by the liver and GIT during infection (e.g., cysteine) are in low concentrations in the skeletal muscle compared to the concentration in APP (Figure 1.8; MacRae & Lobley, 1991; MacRae *et al.*, 1993). Therefore, proportionally more muscle may need to be catabolised in order to supply these to the GIT and liver (Reeds *et al.*, 1994), with excess AA to these requirements catabolised in the liver. This will be exacerbated if feed intake is decreased during the parasite infection. However, a recent review on AA requirements during inflammatory states highlighted the lack of experimental evidence on the actual AA requirements for the synthesis of APP during infection (Obled, 2002).

1.3. Strategies for alleviating parasitic infection

Currently chemical anthelmintics (e.g., benzimidazoles, imidothiazole, and avermectin) are used as the major method of controlling parasites (Parkins & Holmes, 1989; Niezen *et al.*, 1996; Rew, 1999). Their chemical structure and hence mode of action has been reviewed by McKellar (1997). Briefly, the avermectins and imidazoles are absorbed by the parasites and interfere with the neurotransmitters in the parasite. Metabolism of the bezimidazole family by the parasite results in altered secretion of enzymes in the parasite gut and therefore reduces nutrient absorption by the parasite.

Resistance to many of the chemical anthelmintics available (e.g., benzimidazoles, imidothiazole, and avermectin) has been reported (Sangster, 1999) due, in part, to the incorrect use of the anthelmintic drenches. Incorrect drenching with chemical anthelmintics have resulted in the development of anthelmintic-resistant parasites (Niezen *et al.*, 1996) due to the inheritance of resistance genes from parasites that survive drug treatment (Sangster, 1999). Alternative approaches to alleviate the dependence on current chemical anthelmintic drenches are therefore required, not only to combat anthelmintic resistance, but also for economic reasons such as potential tariff

barriers (Neizen *et al.*, 1993; 1996). Several approaches, outlined by Niezen *et al.* (1996), to place less reliance on anthelmintics, include changing grazing practises, genetic selection of parasite resistant stock, nutritional supplementation or growing speciality forage crops (e.g., those containing CT; Robertson *et al.*, 1995).

A study completed in Denmark found that by shifting lambs after weaning to clean pasture for 3 to 4 months reduced FEC and resulted in good production even with out chemical drenching (Githigia *et al.*, 2001). However, this practise may not be suitable for the NZ grazing system as parasites are present on pastures at high levels all year round. Breeding sheep for resilience to parasitic infection has been reviewed by Bisset & Morris (1996), however negative correlations with wool growth and liveweight in NZ breeds of sheep (Howse *et al.*, 1992; McEwan *et al.*, 1992) suggests that this option may not be economically viable in the short-term. Therefore, nutritional supplementation or the use of specialty forages are the most suitable approaches, in the short-term at least.

1.3.1. Nutrient supplementation

Increasing the availability of protein post-ruminally may reduce the effects of parasitism (Neizen *et al.*, 1993) as it may enhance the animals' immune response to parasites (Kahn & Diaz-Hernandez, 1999). The level of nutrition has a role in the development of resistance to nematode infection with greater resistance to *H. contortus* in lambs fed at a higher plane of nutrition (Roberts & Adams, 1990). Recent experiments showed that the infusion of specific AA (cysteine and glutamine) increased N retention and decreased faecal egg counts (FEC) in parasitised sheep (Miller *et al.*, 2000; Hoskin *et al.*, 2002). Protein supplementation was also successful in improving resistance to both intestinal and abomasal parasites in the periparturient ewe (Donaldson *et al.*, 1997; Houdijk *et al.*, 2000). The level of energy fed to lambs affected the fecundity of female *T. circumcincta*, with lower FEC at higher energy intakes (Valderrábano *et al.*, 2002). It was suggested that this effect may be due to the increase in the immune response at higher energy intakes, as indicated by higher levels of circulating eosinophils (Valderrábano *et al.*, 2002).

Figure 1.7 The effect of parasitic infection on protein metabolism in the ruminant (Coop & Sykes, 2002).

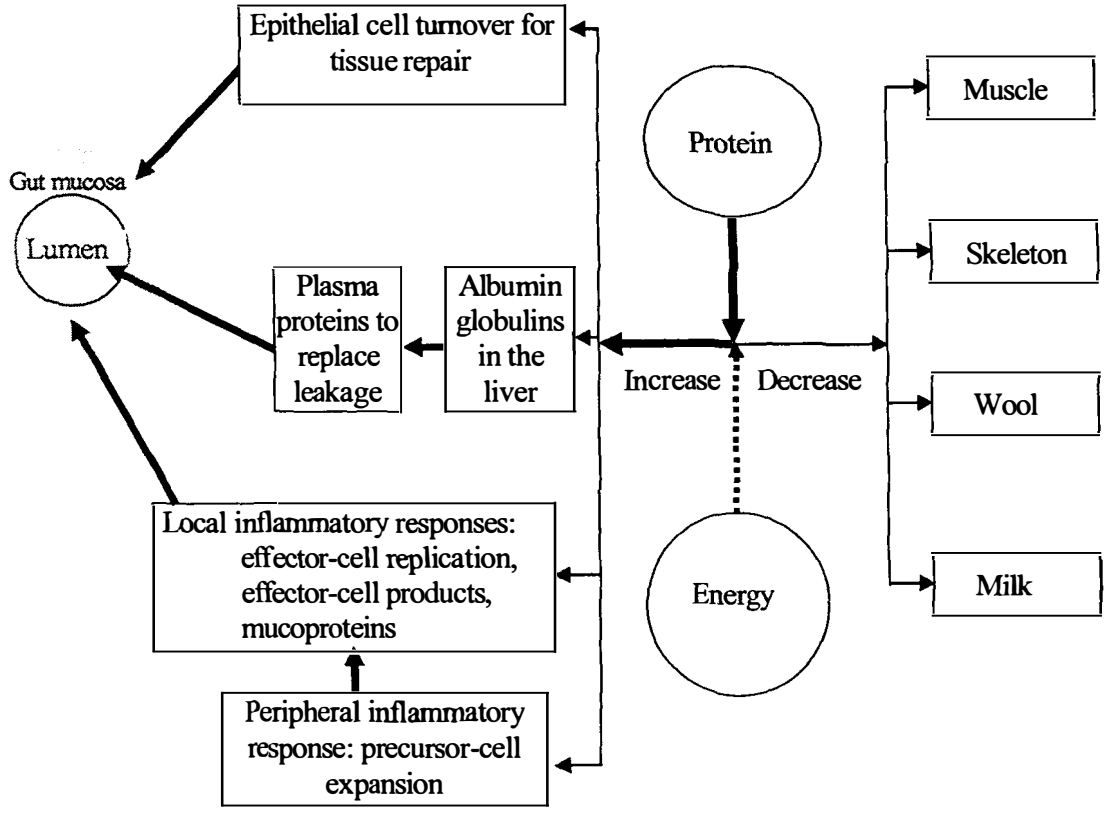
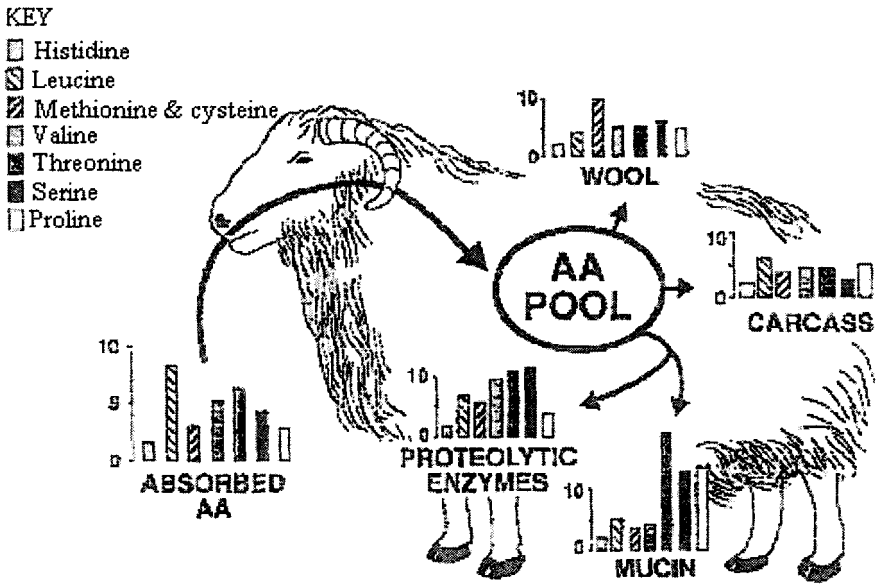


Figure 1.8 Amino acid absorption and composition of body tissues and secretions in sheep (MacRae & Lobley, 1991).



Despite both energy and protein being important for growth and production, it has been suggested in a recent review that protein is of more importance in the infected animal unless the animal is severely underfed (Coop & Kyriazakis, 2001). However, the importance of protein and energy supply to the infected lamb shows an interesting contradiction. Protein or energy was infused into the abomasum of Coopworth-cross lambs infected with *T. colubriformis*, however only the protein-supplemented animals showed a reduction in FEC and worm burdens (Bown *et al.*, 1991a). In contrast, Merino lambs infused with protein showed no enhancement of the immune response (as indicated by intestinal worm burdens) while animals with greater energy intakes had lower intestinal worm burdens (Kahn *et al.*, 2000). This may suggest a species difference with regards to how nutrients are partitioned within the body during infection, and is an important area for future research.

Therefore, an increase in the supply of protein post-ruminally may decrease the need to mobilise skeletal muscle protein, as there will be more AA available to the liver and the GIT. One known method of increasing protein supply post-ruminally is by feeding forages that contain condensed tannins (CT; Neizen *et al.*, 1993).

1.3.2. Condensed tannins

Condensed tannins are naturally occurring phenolic compounds found in plant vacuoles (Reid *et al.*, 1974), in the leaves, bark and reproductive organs of trees, shrubs, and a few legume forages including the *Lotus* species, Sainfoin (*Onobrychis viciifolia*) and Sulla (*Hedysarum coronarium*). Condensed tannins are known to shift the digestion of plant protein from the rumen to the small intestine and therefore can be used to increase the efficiency of protein utilisation (Ulyatt *et al.*, 1977; Waghorn, 1987; Lobley, 1992).

Plant protein degradation, ammonia production, and its subsequent utilisation by microorganisms for protein synthesis, are characteristic of rumen digestion. In fresh forages, such as those commonly fed in NZ, approximately 70% of the plant protein is broken down in the rumen to ammonia, with only 30% of the dietary protein reaching the small intestine for digestion (Waghorn & Barry, 1987). The rate of ammonia

production from the breakdown of plant protein is greater than the rate at which microbial protein can utilise it and therefore much of the ammonia is absorbed into the blood stream through the rumen wall (Ulyatt, 1981). The rate of microbial incorporation of ammonia into protein in the rumen is also dependant on the rate of microbial growth in the rumen (Egan, 1980), which is limited by the availability of fermentable energy.

Excessive digestion of plant protein in the rumen is undesirable for three reasons (Ulyatt *et al.*, 1977; Lobley *et al.*, 1995). Firstly, there is the production of ammonia which is toxic to the animal and requires conversion to urea. Secondly, there is an energetic cost (in terms of ATP) in the production of urea. And thirdly, there is an AA cost for the synthesis of urea from ammonia (Figure 1.5). Therefore, reducing the excessive production of ammonia from plant protein by increasing the NAN flow to the small intestine without affecting microbial N flow would represent a major improvement in the efficiency of production from grazing ruminants (Lobley, 1992; Waghorn *et al.*, 1994b).

Condensed tannins preferentially form strong bonds with proteins at pH 3.5-7.0 (Jones & Mangan, 1977). The CT-protein complex is stable over this range in pH and this complex is less degradable in the rumen (Waghorn & Barry, 1987). The CT protein complex becomes unstable at pH less than 3.0 (Jones & Mangan, 1977) thus enabling the protein to disassociate from the complex in the abomasum where pH is approximately 2.5 (Waghorn & Barry, 1987). As a result, there is an increase in the amount of plant protein entering the small intestine (pH 7) which may be available to the ruminant for digestion and subsequent absorption (Waghorn *et al.*, 1990). This essentially means that there is more protein from the feed available to the animal (McNabb *et al.*, 1996) and this can lead to increased production in ruminant animals (Waghorn *et al.*, 1987; McNabb *et al.*, 1993) without increasing food intake (Wang *et al.*, 1996c).

The effects of CT in ruminant nutrition are usually determined by daily drenching or by infusing of polyethylene glycol (PEG) into the rumen. Polyethylene glycol preferentially binds to CT minimising the binding between CT and plant protein (Kumar & Singh, 1984). Therefore, animals infused with PEG can be considered to be free of

the actions of CT. However, PEG is inappropriate to use in determining the effects of CT in parasitised lambs (Niezen *et al.*, 1998b) therefore CT-fed animals have to be compared to animals fed a forage of similar nutritive value which does not contain CT.

The effects of CT on animal production in ruminants has been reviewed previously (e.g., Waghorn, 1996; Barry & McNabb, 1999). The effects of CT are dependent on the concentration and type of CT present in the forage consumed with the chemical structure of the CT thought to be a major determinant of the effects of the CT (McNabb *et al.*, 1998; Aerts *et al.*, 1999). The net effects of the CT in *L. corniculatus* (*cv* Goldie; birdsfoot trefoil) have been beneficial to animal production, with improvements in liveweight gain reported in the literature (Douglas *et al.*, 1995; Rameriz-Restrepo *et al.*, 2002). Milk secretion was increased by 21% and its composition altered (increases of 14% and 12%, in milk protein and lactose, respectively) in sheep in mid to late lactation (Wang *et al.*, 1996a). Wool growth increased by 10% (Wang *et al.*, 1996b), clean fleece weight increased by 10% and staple length by 12% in sheep (Min *et al.*, 1998). None of these production parameters were due to increased feed intake. In contrast *L. pedunculatus* (*cv* Maku) has had a negative impact on animal production, with reductions in dry matter (DM) intake (Waghorn *et al.*, 1994a) and N digestibility (Waghorn *et al.*, 1994b), and these effects were independent of CT concentration (Waghorn & Shelton, 1995). Feeding Sulla improved lean carcass gains (28-33%) and wool production (8-30%; Terrill *et al.*, 1992; Burke *et al.*, 2002) when compared to pasture-based diets and is highly palatable at low (4-5%; Terrill *et al.*, 1992) and high (7.2%; Stienezen *et al.*, 1996) concentrations of CT.

The alterations in animal performance when CT-containing forages are fed may be due to the alterations in nutrient digestion and AA availability that have been seen in *L. corniculatus* (Waghorn *et al.*, 1987), *L. pedunculatus* (McNabb *et al.*, 1993; Waghorn *et al.*, 1994a; 1994b), Sulla and Sainfoin (Bermingham *et al.*, 2001). For example, CT increased the flow of N to the abomasum by 10-85% and the ileum by 30-75%. This is reflected by increases in AA flows to the abomasum of 10% with Sainfoin, 24% with Sulla and 50% with *L. corniculatus*. However, only *L. corniculatus* and Sulla increased

the apparent absorption of AA (59% and 16% in *L. corniculatus* and Sulla, respectively). Feeding CT to sheep has increased the amount of cysteine available for use within the body (McNabb *et al.*, 1993; Wang *et al.*, 1994), which may explain the increases in wool growth observed in other studies (Terrill *et al.*, 1992; Wang *et al.*, 1996b; Min *et al.*, 1998; Burke *et al.*, 2002).

Condensed tannins are generally considered to be anti-nutritional in monogastric animals, (Jansman, 1993; Dawson *et al.*, 1999) and some CT depending on their chemical structure, can also have negative impacts in ruminant species. For example, the feeding of Quebracho tannin (QT; a tannin extracted from the *Schinopsis* species) to lambs (50 g kg^{-1} DM) reduced DM and N digestibility and altered the morphology of the jejunum and ileum (Dawson *et al.*, 1999). Similar concentrations of *L. pedunculatus* (5% DM) have shown no negative impacts on the histology of the intestine in sheep (Walton *et al.*, 2001). This is despite the fact that this legume could be viewed as marginally antinutritional due to the lowered intakes observed in some studies (Barry & Duncan, 1984).

1.3.2.1. Parasites and condensed tannins

Feeding forages that contain CT during parasitic infection has in most cases improved average daily gain (ADG) compared to grass-fed animals. For example, when lambs were fed either *L. pedunculatus* (% CT assumed) or grass and infected with both abomasal (1500 *Ostertagia*) and intestinal larvae (1500 *T. columbriformis*) the ADG was significantly higher in *L. pedunculatus* (184 g d^{-1}) compared to the grass-fed lambs (33 g d^{-1}; Neizen *et al.*, 1993). However, there are differences between the CT-containing forages. For example, when lambs with a naturally-acquired intestinal nematode burden were fed either *L. pedunculatus*, *L. corniculatus* or Sulla, the lambs fed Sulla and *L. pedunculatus* had the highest live weight gains (226 g d^{-1} and 232 g d^{-1}, respectively) while the *L. corniculatus* had the lowest (86 g d^{-1}; Robertson *et al.*, 1995; Niezen *et al.*, 1998a).

Infected lambs fed Sulla (Niezen *et al.*, 1994; 1998a; Robertson *et al.*, 1995) and *L. pedunculatus* (Neizen *et al.*, 1993; 1998a) have shown a reduction in FEC compared to grass fed animals. Intestinal parasite burdens were decreased when Sulla was fed (Niezen *et al.*, 1994; 1998a; Robertson *et al.*, 1995), however they were not affected when fed either *L. pedunculatus* or *L. corniculatus* (Robertson *et al.*, 1995; Niezen *et al.*, 1998a). When QT was given to parasitised lambs as a drench, this resulted in a reduction in FEC and intestinal parasite burdens (Athanasiadou *et al.*, 2001).

Improvements in ADG in parasitised lambs may be due to direct action on the parasite, or indirect actions through improved protein supply (Kahn & Diaz-Hernandez, 1999; Coop & Kyriazakis, 2001). A study completed by Butter *et al.* (2001) showed that QT present in the feed was ineffective at reducing worm burdens of *T. spiralis* – a mucosal dwelling parasite, however it significantly reduced the numbers of the lumen-dwelling *N. brasiliensis*. This suggests that the tannin has a direct impact on the parasite, which is consistent with *in vitro* results that indicate that extracted CT inhibit both parasite motility (Molan *et al.*, 2000a; 2000b; Athanasiadou *et al.*, 2001), larval development, and the viability of parasite eggs (Molan *et al.*, 2002).

Resistance to parasitic infections can be defined as the ability of the host to resist the establishment, development, persistence or reproduction of parasites. Resilience on the other hand is the ability of the animal to be productive despite infection with parasites. From these definitions it seems that Sulla confers resistant-like properties through its mode of action, with decreases in parasite numbers and FEC. This effect is not complete, as there is a small detrimental effect of the parasite burden on liveweight gain (Robertson *et al.*, 1995; Niezen *et al.*, 1998a). *Lotus pedunculatus* seems to confer resilient-like properties, due to the high liveweight gains observed despite high parasite burdens. Although both Sulla and *L. pedunculatus* are interesting in terms of how their CT may act to improve liveweight during parasitic infection, Sulla is of more value to the NZ farmer due to the reduction in parasite burdens and FEC. If parasite numbers can be decreased on pasture, then in the long term, there will be a reduced requirement for chemical anthelmintics.

1.4. Conclusion

Parasites in the GIT impair production, and can even result in the death of the host animal (Parkins & Holmes, 1989). Parasitic infection has decreased liveweight gain (Sykes & Coop, 1976; van Houtert *et al.*, 1995), wool production (Steel *et al.*, 1980; 1982; Leyva *et al.*, 1982) and milk production in ewes (Leyva *et al.*, 1982; Thomas & Ali, 1983). Decreased N retention (Sykes & Coop, 1976) and altered body composition (Sykes & Coop, 1977; Coop *et al.*, 1982) have also been reported. Parasitic infection increased the loss of N from the body either as endogenous protein (e.g., mucus, plasma leakage, sloughed cells) or excretion as N in urine and faeces (e.g., Sykes & Coop, 1976).

Parasitic infection increased ASR in the small and large intestine and this may explain the increased AA requirements by the GIT. This may be due to increased mucus production (Cheema & Scofield, 1982), plasma leakage of serum proteins (Kimambo *et al.*, 1988) and sloughed cells, together with increased AA oxidation in the small intestine (Yu *et al.*, 2000).

Trichostrongylus colubriformis infections have increased FSR in the liver (Symons & Jones, 1971; Symons & Jones, 1978; Jones & Symons, 1982), and this may be due to either increased synthesis of export proteins for sustained increases in acute phase protein production (Stehle & Furst, 1983) or for replacing serum proteins lost into the GIT (Poppi *et al.*, 1986). Consequently, the AA requirement by the liver will also increase during parasitic infection.

The concentration of specific AA such as cysteine in muscle protein are low compared to the liver and GIT (MacRae *et al.*, 1993), therefore to meet the increased requirements by the liver and GIT during infection, proportionally more muscle protein needs to be mobilised to supply the increase in demand. Excess AA above these demands will be catabolised in the liver resulting in a net loss of AA from the body.

It is hypothesised that the infected animal may be required to break down its own body reserves or re-prioritise its metabolic activities in order to meet the additional demands due to infection (Coop & Kyriazakis, 1999; Butter *et al.*, 2000). Supplementation of additional protein (Bown *et al.*, 1991a) or AA (Hoskin *et al.*, 2002) has resulted in enhanced immune responses and improved N retention in infected animals, and it is known that the presence of CT in some fresh forages, such as *L. corniculatus* and Sulla improve the post-ruminal supply of AA to the animal. This may explain how the impacts of parasite infection in animals fed forages containing CT occur (Robertson *et al.*, 1995; Niezen *et al.*, 1998a). Sulla is of particular interest for future research due to its negative impacts on FEC and worm burdens (Niezen *et al.*, 1994; 1998a; Robertson *et al.*, 1995) and therefore its effects on AA utilisation during parasitic infection is of importance. Poly-ethylene glycol is inappropriate to use to elucidate the effects of CT in parasitised animals (Niezen *et al.*, 1998b) therefore to determine the effects of CT on nutrient partitioning within the body, fresh Lucerne (*Medicago sativa*) will be fed as a CT-free comparison as it is similar in chemical composition to Sulla.

1.5. Hypothesis and objectives

It is hypothesised that the presence of an established parasite infection will result in the mobilisation of AA from the muscle to the GIT and liver. Additionally, feeding forages that contain CT will improve the lambs ability to alleviate the effect of the parasite burden on the mobilisation of AA from the muscle, resulting in similar liveweight gain between control and parasitised lambs.

Gastrointestinal parasites in growing lambs limit animal production in the pastoral grazing system, and there is mounting evidence that parasitic infections change nutrient utilisation within the tissues. However, there has been no quantification of how nutrients (especially AA) are partitioned in the lamb fed fresh forages during a parasitic infection. Therefore, the objective of this study was to firstly quantify the partitioning of AA and metabolites (glucose, lactate, ammonia, urea, O_2 and CO_2) between the GIT, and liver and in the hind limb tissues (muscle, skin, fat) in lambs during a parasite infection, when

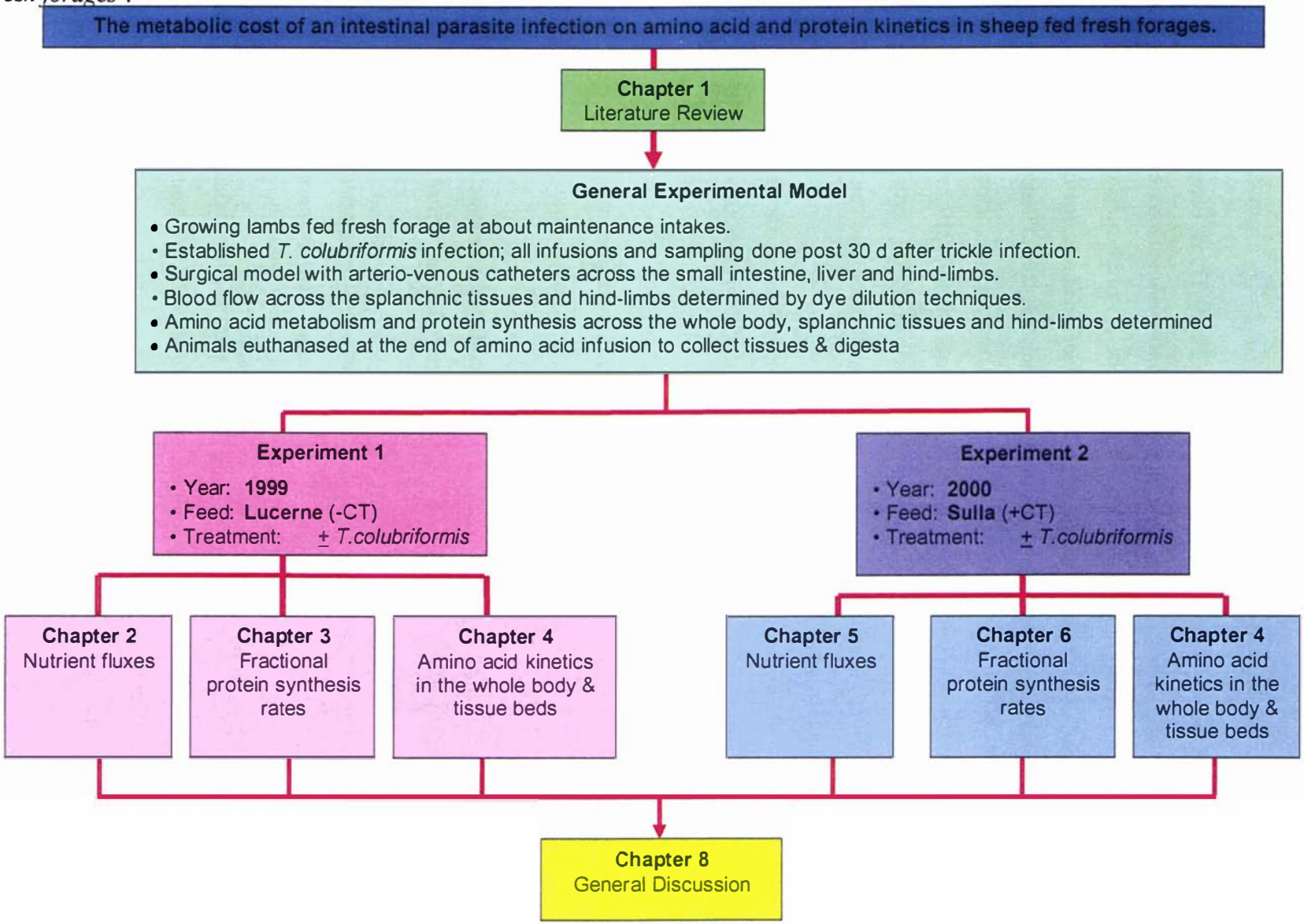
the lamb is fed fresh Lucerne (contains no CT). Secondly, in order to determine how the feeding of CT-containing forages improves animal performance, fresh Sulla will be fed. The objectives of this thesis will be investigated by conducting two similar experiments. In Experiment One, Lucerne (- CT) will be fed to investigate the effects of an established *T. colubriformis* infection on AA partitioning within the lamb. A second experiment (Experiment Two) will then be conducted in order to elucidate the effects of feeding Sulla (+ CT) during parasite infections (Figure 1.9).

Several methodological approaches will be used in this thesis. Firstly, the apparent availability of dietary AA to the small intestine and the net flux of nutrients and metabolites across the MDV, PDV, liver, TSP and hind limbs will be measured in order to asses the nutrient requirement by the different tissue beds (Chapters Two and Five).

Secondly the incorporation of labelled AA into the intracellular pool and protein bound fraction of the small intestine (duodenum and ileum), liver, lymphoid tissues (spleen, thymus, mesenteric lymph nodes) and productive tissues (muscle and skin; Chapters Three and Six) will be determined to measure FSR in these tissues.

Finally a constant infusion procedure combined with an AV-technique will utilised determine AA oxidation, and protein synthesis (Chapters Four and Seven) in the whole body, MDV, PDV, liver, TSP and hind limbs.

Figure 1.9 Experimental layout of '*The metabolic cost of an intestinal parasite infection on amino acid and protein kinetics in lambs fed fresh forages*'.



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2 NUTRIENT FLUXES IN LAMBS FED FRESH LUCERNE (*MEDICAGO SATIVA*) AND INFECTED WITH OR WITHOUT *TRICHOSTRONGYLUS COLUBRIFORMIS*

2.1 Abstract

The effect of an established *Trichostrongylus colubriformis* infection on amino acid (AA) availability in the small intestine and the partitioning of AA and nutrients across the mesenteric-drained viscera (MDV), portal-drained viscera (PDV), liver, total splanchnic tissues (TSP; PDV and liver) and hind limbs were determined in lambs 48 days post infection. The lambs were fed fresh Lucerne (*Medicago sativa*; 800 g DM d^{-1}). Lambs were dosed with 6 000 L3 *T. colubriformis* larvae for 6 d (n=5) or kept as parasite free controls (n=6) and faecal egg production was monitored every second day from day 22 to day 48. A nitrogen balance was conducted on days 35 to 43 post infection, and digesta flow measurements were made on day 44. On day 48 post infection, the lambs were continuously infused with para-aminohippuric acid (PAH; 750 mg h^{-1}) and indocyanin green (ICG; 16 mg h^{-1}) for 8 h in order to measure blood flow across the TSP and hind limbs, respectively. Blood was continuously collected from the mesenteric, portal and hepatic vein, the mesenteric artery and the vena cava, plasma harvested and PAH, ICG, AA and metabolite concentrations measured. Faecal egg production peaked on day 26 post infection ($P<0.001$), and intestinal worm burdens on day 48 post infection were significantly higher in the infected lambs ($P<0.001$). There was no effect of parasitic infection on feed intake or liveweight gain ($P>0.15$). The digestibility of dry matter (DM) and nitrogen (N), and the flow of DM and N through the digestive tract were also unaffected by parasite infection ($P>0.15$). Apparent AA absorption from the small intestine was unaffected ($P>0.15$) despite higher abomasal AA flux ($P<0.10$). Parasitic infection increased plasma flow in the PDV, TSP and hepatic artery ($P<0.15$). However, there was very little effect of infection on the net flux of non-essential, essential, branched-chain (BCAA) and total AA across the MDV or PDV. In the TSP, the BCAA were released by both infected and control lambs, however this was significantly lower in the parasitised lambs ($P<0.10$). There was no effect of parasite infection on the net flux of AA across the hind limbs ($P>0.15$). There were relatively small effects of parasitic infection on O_2, CO_2, glucose, lactate, ammonia and urea metabolism across all tissues. These results suggest that an established parasite infection had little effect on the partitioning of AA and the use of energy substrates within lambs fed fresh Lucerne in this study.

2.2 Introduction

During parasitic infection, there is a shift in the nutrient utilisation by tissues in order to meet the demands of the affected tissues, with priority placed on the maintenance of body protein, and repair of damaged tissues (Coop & Kyriazakis, 1999). The animals' metabolic requirements differ between physiological states (e.g., lactation, pregnancy, growth, health status etc.). These are met by short- and long-term changes in the animals' metabolism (Stehle & Furst, 1983). Tissues within the body are heterogeneous in their requirements, for example, the gastrointestinal tract (GIT) and liver have a high metabolic requirement for nutrients despite a small contribution to protein mass (Lobley, 1994). In contrast, the muscle and skin represent over 50% of protein mass, but have low rates of protein synthesis (Lobley, 1994). Also, the amino acid (AA) composition of the tissue protein varies (MacRae *et al.*, 1993) and this could affect the way nutrients are partitioned within the body.

The already-high metabolic requirement of the GIT and liver is likely to increase further with infection, and often these are times when feed intake is also reduced (Sykes *et al.*, 1988). *Trichostrongylus colubriformis* infection has increased protein synthesis in the small intestine and liver (Symons & Jones, 1975) and decreased protein synthesis in the muscle and skin (Symons & Jones, 1975; Jones & Symons, 1982). An increase in leucine oxidation and protein turnover in the GIT during parasitic infection decreased the availability of leucine to the other tissues (Yu *et al.*, 2000) and this could impact on animal production (Kimambo & MacRae, 1988). Parasitic infection also increased the loss of nitrogen (N) from the body either as endogenous protein in the GIT (e.g., mucus, plasma leakage or sloughed cells; Kimambo *et al.*, 1988) or excretion as N in urine and faeces (e.g., Sykes & Coop, 1976). Therefore, the impact of parasitic infection on animal production may be due to an increase in the repartitioning of AA from other tissues such as skeletal muscle, to the GIT and liver. However, there has been no quantification of how nutrients are partitioned in the lamb during a parasitic infection.

The hypothesis of this study is that the infected animal may be required to breakdown its own body reserves or re-prioritise its metabolic activities to meet the additional demands due to infection (Coop & Kyriazakis, 1999; Butter *et al.*, 2000). Therefore, the objective

of this study was to quantify the partitioning of AA and metabolites (glucose, lactate, ammonia, urea, oxygen and CO_2) between the GIT, liver and the hind limb tissues (muscle, skin, fat) in lambs during a parasite infection. This was achieved by measuring the apparent absorption of dietary AA and the net flux of nutrients and metabolites across the mesenteric-drained viscera (MDV; small intestine), portal-drained viscera (PDV; rumen, small intestine, large intestine, pancreas, spleen), liver, total splanchnic tissues (TSP; PDV and liver) and hind limbs of lambs with an established parasite infection.

2.3 Materials and Methods

2.3.1 Animals and surgery

The experimental procedures for this trial were reviewed and approved by the Crown Research Institute Animal Ethics Committee in Palmerston North, New Zealand according to the Animals Protection Act (1960) and Animals Protection Regulations (1987) and amendments.

Wether lambs (29 kg) were weaned from their dams and transported to AgResearch Limited, Grasslands Research Centre, Palmerston North. The lambs were shorn, drenched twice on consecutive days with Ivomectin (Ivomec® Merial, New Zealand Ltd.) and treated for external parasites using Wipeout (Coopers, Schering-Plough Animal Health Limited, New Zealand). The lambs were fed Lucerne pellets (*Medicago sativa*; 800 g dry matter (DM) d^{-1}) and chaffed Lucerne hay (200 g DM d^{-1}) for approximately 3 weeks. One week before surgery the lambs were brought indoors and housed in individual metabolism crates and maintained on the same diet.

Three weeks prior to the start of surgery, catheters were prepared using Tygon® tubing (Scientific supplies Ltd., Wellington, New Zealand), adapted from the method outlined by Huntington *et al.* (1989; see Appendix B.1 for full details). Catheters were treated with tridodecylmethylammonium chloride heparin (Polysciences Inc., Warrington, PA, US) to prevent blood clotting between sampling periods (Huntington *et al.*, 1989). The

catheters were sterilised in ethylene gas at 55°C for 2.5 h, with an 8 h aeration cycle. Catheters were left for a minimum of 1 week before being used for surgery to minimise residues of ethylene gas.

Twelve lambs were fasted for 24 h before surgery. Due to the invasive nature of the surgical preparation, the 12 sheep were prepared over 4 weeks with a maximum of 4 sheep in each week. Anaesthesia was initiated with Nembutal (60 mg mL^{-1}; 0.5 mL kg body $weight^{-1}$; Vetworks, Cambridge, New Zealand) and maintained with isoflurane (1.5%; Vetworks, Cambridge, New Zealand) administered through an endotracheal tube. Permanent indwelling catheters were placed in the mesenteric artery, and the mesenteric, portal and hepatic veins (Huntington *et al.*, 1989) and vena cava (Ortigues & Durand, 1995) for blood sampling (see Appendix B.1 for catheter placement). 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 TSP tissues and the hind limbs (Appendix B.1). A permanent Teflon cannula was fitted in the abomasum (see Appendix B.1 for details) for the infusion of [1-^{13}C]-valine on day 48 post infection to measure valine kinetics across the MDV, PDV, liver, TSP 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 and [^{13}C]-sodium bicarbonate on day 45 post infection, and [3, 4-^{3}H]-valine on day 48 post infection. Radioactive and stable isotopes were purchased from Amersham Life Science (Buckinghamshire, UK) and Mass Trace, Inc. (Woburn, MA, USA), respectively. Results relating to the valine kinetics are presented in Chapters Three and Four.

After surgery, the catheters were filled with sterile 250 iu mL^{-1} heparinised saline, containing 1% procaine penicillin (Bomacillin, Bomac Laboratories Ltd., Auckland, New Zealand; see Appendix B.2.1.4). Catheters were flushed every 14 d with a sterile solution of heparin (50 iu mL^{-1}) containing 1% procaine penicillin (Bomacillin, Bomac Laboratories Ltd., Auckland, New Zealand) in isotonic saline and then filled with sterile heparinised saline (250 iu mL^{-1}; containing 1% procaine penicillin) to maintain their

patency. The lambs received a daily intramuscular injection of procaine penicillin (3 mL; Bomacillin, Bomac Laboratories Ltd., Auckland, New Zealand) for 4 days post-surgery.

The lambs were fed Lucerne pellets (800 g DM d^{-1}) and chaff (200 g DM d^{-1}) for 3 d after surgery and then were offered approximately 800 g DM d^{-1} of fresh Lucerne (Table 2.1) until the conclusion of the trial. The Lucerne was harvested every 2 d with a sickle bar mower by 10.00 am and stored at 4°C. Dry matter content of the Lucerne was determined daily in order to adjust the amount of wet forage given to maintain DM offered at approximately 800 g d^{-1}. The lambs were fed at hourly intervals from overhead feeders and water was available *ad libitum*. The lambs were weighed weekly to monitor liveweight changes during the experimental period.

One week after surgery (day 1 of the experimental period) six sheep were given 6000 *T. colubriformis* L3 larvae per day orally for 6 d (parasite treatment) while the remaining six sheep were drenched once with Ivomectin (Ivomec® Merial, New Zealand Ltd) to serve as controls (control treatment). The lambs were assigned to either the control or parasite group according to a completely randomised block design. The L3 larvae were obtained from faeces of sheep infected with pure cultures of *T. colubriformis*. After a 14-d incubation period at 20°C in high humidity, the larvae were extracted over a 24 h period using the standard Baermannisation procedure (Jørgensen *et al.*, 1998).

2.3.2 Parasitology

Faecal egg counts (FEC) from individual sheep were measured every second day from day 20 to day 45 of infection using the modified McMaster method (Whitlock, 1948), where the presence of one egg represents 50 eggs per gram of wet faeces. Total intestinal worm burdens were determined after slaughter. The proximal 10 m of the small intestine was detached from the abomasum, ligated and refrigerated. The contents of the intestine were washed and a 10% subsample of the washings was passed through a 38 μm sieve to collect worms for counting (Sutherland *et al.*, 1999b).

2.3.3 Digesta flow

On day 32 of the experiment the sheep were dosed with a pre-weighed, slow release alkane capsule (Captec™ Alkane CRC; Captec New Zealand Ltd) into the rumen. The plant alkanes (odd-numbered carbons) present in the Lucerne and the synthetic alkanes present in the Captec™ capsules (even-numbered carbons) were used to estimate digesta flow. On day 44, abomasal digesta (approximately 100 g) was sampled four times during a 24 h period and bulked in order to estimate digesta flow. The bulked digesta was freeze dried and ground (0.5 mm sieve) and analysed for DM, N, alkane and AA concentrations.

2.3.4 Nitrogen balance

Faecal collection harnesses were placed on the sheep on day 32 and the total daily output of faeces and urine were measured from day 35 to day 43 for measuring DM and N digestibility and N retention. Urine was collected by gravity into a container containing 150 mL of 50% HCl and mixed several times a day to minimise ammonia losses. Faeces and urine were collected and weighed daily during the N balance period with a 10% aliquot collected and stored at -20°C for analysis. A sub-sample of feed offered and refused was taken daily and bulked to give one sample per group.

Table 2.1 Nutrient composition of Lucerne (*Medicago sativa*) fed to lambs. Values based on feed samples taken during the nitrogen balance period (day 35 to 42) and are presented on a dry matter (DM) basis.

Nutrient	Concentration (g kg DM^{-1})
Non-essential amino acids	123
Essential amino acids	91
Total amino acids	214
Nitrogen	32
Lipid*	26
Ash*	99
Acid detergent fibre*	290
Neutral detergent fibre*	352
Soluble sugars and starch*	74
Organic matter digestibility*	66
Metabolisable energy (MJ kg DM^{-1})*	9

* based on samples analysed by near infra-red reflectance spectroscopy analysis.

2.3.5 Infusions and blood sampling

On day 48, in order to measure plasma flow across the MDV, PDV, liver and TSP tissues, PAH (749.7 mg h^{-1}; 0.15 mmol L^{-1} Na form, dissolved in water and then autoclaved) was infused continuously into the mesenteric vein for 8 h. The lambs also received a continuous infusion of ICG into the abdominal aorta for 8 h (16.2 mg h^{-1}; 0.93 mmol L^{-1} ICG) to measure plasma flow across the hind limbs (Wester *et al.*, 2000). The ICG was dissolved in deionised water and 1% albumin and 0.9% NaCl were added to stabilise the ICG and maintain isotonic molarity, respectively. The resultant infusate was filter-sterilised (0.2 μm) immediately prior to infusion. To prevent blood clotting during the continuous sampling, 6000 iu ovine heparin h^{-1} was infused into the jugular vein with the [^{3}H]-valine (see Chapters Three and Four for details) over the 8 h infusion period. Sampling lines and syringes were kept in an ice-water bath in order to minimise the degradation of blood constituents (Lobley *et al.*, 1995). As part of the larger study, 30 mL of blood was withdrawn continuously every 2 h from the mesenteric artery, the mesenteric, portal, and hepatic veins, and the vena cava over the infusion period. The data presented in this chapter represent the average of samples taken from the last 2 sampling periods (time 4 to 6, and 6 to 8 h of infusion).

After each 2-h collection period, the syringes were removed from the collection lines and carefully mixed by gentle rotation. Packed cell volume (haematocrit) was determined. Immediately following the collection of blood samples approximately 0.2 mL of blood was injected into a blood gas analyser (ABL3, Radiometer Pacific Limited, Copenhagen) to determine the concentration of O_2 and CO_2 in whole blood. The remaining 25 mL of whole blood was centrifuged (4°C; 3270 g for 15 min) and the plasma harvested and either processed or stored at -85°C for further analysis as described below or in Chapters Three and Four.

2.3.6 Slaughter

On day 48 of the experiment, the sheep were euthanased by an intravenous overdose of sodium pentobarbitone (300 mg mL^{-1}; 0.5 mL kg^{-1} liveweight). The duodenum (comprising the proximal 3 m of the small intestine) was isolated in order to sample duodenal digesta, however at the time of slaughter there was no duodenal contents in any of the sheep. The ileo-caecal junction of the small intestine was located and sectioned in order to collect ileal digesta from the final 3 m of the ileum for digesta flow measurements. Ileal digesta was stored at -20°C until analysis. The alkane capsule was recovered from the rumen of each sheep, dried overnight and weighed to determine the release rate of C32 and C36 alkanes in the rumen over a 15-day period (day 32 to day 48 post infection).

2.3.7 Sample processing and chemical analysis

Dry matter of feeds and refusals were determined daily after oven drying at 85°C for 24 h. Faeces and digesta samples were dried at 85°C for 48 h at the completion of the experiment. Feed, feed refusals, faeces and digesta samples were freeze-dried and ground (0.5 mm seive) for alkane, N and AA analysis. The concentration of N in feed, feed refusals, faeces, urine and digesta samples were determined by automated analysis of ammonia following Kjeldahl digestion (Williams & Twine, 1967). In order to determine the fibre content of the diet a sub-sample (0.5 g) of feed offered was submitted for near infra-red reflectance spectroscopy analysis (NIRSystems, Foss Ltd, New Zealand), where the sample was scanned between 1100 nm and 2500 nm

wavelengths at 2 nm intervals. The calibration was based on principal component analysis using a first derivative modified partial least squares mathematical interpretation (Shenk & Westerhaus, 1991).

The alkanes present in freeze-dried feed and digesta samples were determined by gas chromatography (Hewlett Packard 5890 series II Gas Chromatograph; Avondale, CA, USA) according to the method of Mayse *et al.* (1986).

2.3.7.1 Amino acids

Amino acid hydrolysates were prepared from feed and digesta samples by hydrolysing approximately 50 mg of freeze-dried material in 6.0 M HCl at 110°C for 22 h. The hydrolysates were filtered, rotory evaporated to near dryness, washed in distilled deionised water and reconcentrated before being dissolved in 0.2 M sodium citrate buffer (pH 2.2). Amino acids in the hydrolysates were analysed by ion exchange chromatography (Shimadzu Scientific Instruments Limited, Columbia, MD 21046, USA) with a post-column reaction using ninhydrin as the derivitising agent.

To determine the concentration of AA in plasma, 0.5 mL of plasma was treated with 80 mM dithiothreitol (DTT) as an antioxidant and 3 mM norleucine (in 0.1% phenol; as an internal standard) and stored at -85°C. The samples were thawed and transferred to a Centrisart® filter (10000 molecular weight cut off), and then centrifuged at approximately 28000 g for 60 min, with the filtrate containing free AA removed and stored at -85°C for analysis. A 50 μL aliquot of the filtrate was dried under vacuum before the addition of 20 μL redry solution (2:2:1, methanol: 1M Na acetate: triethylamine (TEA; under $N_{2(g)}$) mixed by vortex and again dried down. The derivatisation reagent (20 μL containing 7:1:1:1 ratio of methanol, MilliQ water, TEA (under $N_{2(g)}$), phenylisothiocyanate (PITC; under $N_{2(g)}$)) was added to each dried sample which was then vortexed and incubated at room temperature for 10 min and then dried down under vacuum. Dried derivatised samples were resuspended in 200 μL diluent containing 5 % CH_3CN and 95 % phosphate buffer (5 mM Na_2HPO_4 adjusted to pH 7.40

using 10 % (v/v) H_3PO_4). Samples were vortexed and transferred to 1.5 mL microfuge tubes and centrifuged at approximately 14000 g for 5 min. The supernatant was then transferred to autosampler vials. A stock standard solution containing 0.5 mM AA was prepared using 200 µL Pierce A/N (Pierce C Chemicals, Lab Supply Pierce (NZ) Ltd, Auckland, New Zealand) and 200 µL Pierce B (Pierce Chemicals, Lab Supply Pierce (NZ) Ltd, Auckland, New Zealand) standard solutions together with 0.5 mM norleucine in a final volume of 1 mL 0.1 M HCl. Stock standards were diluted 10-fold using 0.1 M HCl before use. Derivitised plasma samples (50 µL) were injected onto a Picotag C_{18} reverse phase column in an oven set to 46°C, with a 90 min run time between each injection (see Appendix B.3 for chromatograpical settings).

Measurement of AA concentration in plasma was determined using a method modified from Bidlingmeyer *et al.* (1984). The samples were analysed after reverse-phase high performance liquid chromatography (HPLC) separation of phenylisoothiocyanate derivatives, using a Waters Pico-Tag® column (3.9 x 300 mm, Waters Corporation, Milford, NMA 01757, USA) and a Shimadzu LC-10/A HPLC system (Shimadzu Scientific Instruments Ltd., Columbia, MD 21046, USA).

2.3.7.2 Ammonia, urea, glucose and lactate

Plasma (1.5 mL) was mixed with 0.5 mL trichloroacetic acid (TCA; 30% w/v) and the resulting solution was centrifuged at 3270 g for 15 min at 4°C on the day of sampling. The supernatant was filtered and stored at -85°C until it was injected onto a Tecator FIAstar Flow Injection 5010 Analyser (Tecaort Ltd., Höganäs, Sweden) to determine the absorbance at 590 nm wavelength in order to calculate the concentration of ammonia in plasma.

Urea concentration in plasma was determined using a commercial assay (Catalogue number 07 3685 6; Roche Diagnostics Ltd, Basel, Switzerland) that utilised the enzymes urease and glutamate dehydrogenase and followed the production of NADH at 340 nm. This assay measures urea concentration in 0.5 mL of plasma by converting it to

ammonia using the enzyme urease. The urea concentration was corrected for plasma ammonia using a second assay that lacked urease (Catalogue number 171C; Sigma Diagnostics Ltd, St Louis, Missouri, USA), where the ammonia underwent reductive amination using L-glutamate dehydrogenase. The urea and ammonia assays were performed on a Cobas Fara II analyser (Hoffmann la Roche, Basel Switzerland) using protocols recommended by the kit manufacturers.

The concentration of oxygen (O_2) and carbon dioxide (CO_2) in blood, and glucose and lactate in plasma were determined on a blood gas analyser (ABL 615, Radiometer Pacific Limited, Copenhagen) and a metabolite analyser (EML 105, Radiometer Pacific Limited, Copenhagen). Oxygen concentration was determined by measuring the decrease in electric potential caused as O_2 diffuses across the membrane of the electrode. Carbon dioxide concentration was determined by measuring the change in pH caused by the CO_2 as it dissolves in the electrolyte. Glucose and O_2 present in the plasma sample were metabolised by glucose oxidase present in the electrode membrane to form hydrogen peroxide (H_2O_2) and gluconic acid. The breakdown of H_2O_2 creates a flow of electrons which produces a current that is proportional to the concentration of glucose in the sample. Lactate concentration was determined in a similar manner, with lactate oxidase present in the lactate electrode producing H_2O_2 and pyruvate.

2.3.7.3 Plasma flow

Para-aminohippuric acid dye dilution was used to determine the flow of plasma though the MDV, PDV, liver and TSP tissues as described by Katz & Bergman (1969) with an additional deacylation step as described by Lobley *et al.* (1995). Plasma (0.5 mL) that had been stored at -85°C was deproteinised with 12% (w/v) TCA (5 mL), mixed and centrifuged at 3270 g for 15 min. Four mL of the resulting supernatant was mixed with 0.5 mL of 1.2 N HCl, capped and heated for 60 min at 90°C in order to deacylate the PAH. After cooling to room temperature, 0.5 mL of sodium nitrite (1 mg mL^{-1}), 0.5 mL of ammonium sulphamate (5 mg mL^{-1}) and 0.5 mL of N-1-napthyl-ethylene-diamino-dihydrochloride (1 mg mL^{-1}) was added sequentially at intervals of 4-5 min, with the

weight recorded at the end. The addition of sodium nitrite enables diazotization to take place (forming the diazo compound), with the excess nitrite produced destroyed by the addition of ammonium sulfamate. The diazo compound reacts with the NEDC to form the azo dye (a reddish purple colour) which is subsequently measured at 540 nm using a spectrophotometer (GBC UV/VIS 918, GBC Scientific Equipment Pty Ltd., Victoria, Australia). The greater the concentration of the PAH in the plasma sample, the more intense the purple colour.

Standards for each lamb were prepared by gravimetrically diluting a sample of the PAH (1:200) that was infused into that lamb. Aliquots of 1:200 infusate were added at 10, 25, 50 and 100 μL to deproteinised plasma (0.5 mL plasma, 5 mL TCA) which contained no PAH. The standards were prepared in the same manner as the plasma samples and read at 540 nm in a spectrophotometer (GBC UV/VIS 918, GBC Scientific Equipment Pty Ltd., Victoria, Australia).

Plasma flow across the hind limbs was calculated using the ICG concentration measured in plasma. One mL of plasma was thawed and then centrifuged at 3270 g for 15 min at 4°C before measuring the absorbance at 790 nm in a spectrophotometer (GBC UV/VIS 918, GBC Scientific Equipment Pty Ltd., Victoria, Australia). The plasma concentration of ICG was determined from a standard curve generated from known concentrations of ICG and their corresponding absorbance (Wester *et al.*, 2000).

2.3.8 Calculations

2.3.8.1 Digesta flow

The invasive nature of the surgery that the lambs in this experiment underwent prevented the insertion of a rumen cannula. Therefore, the infusion of a liquid phase marker (e.g., CoEDTA, CrEDTA) was not possible. Dry matter flow was calculated by the dilution of natural plant alkanes and synthetic plant alkanes in the abomasum and ileum. Dry matter flow (g DM d^{-1}) through the abomasum was determined according to Equation 2.1. Ileal digesta DM flow was determined following correction for the

recovery of C36 from Captec™ according to Equation 2.2. Ileal DM flow was corrected for C36 alkane (CF) because C36 is an indigestible marker, therefore the assumption is that C36 flux at the ileum should be the same as C36 flux at the abomasum.

Equation 2.1

$$\text{Abomasal DM flow (g d}^{-1}) = \frac{\text{Alkane intake (mg d}^{-1})}{\text{[alkane] in abomasum (mg g DM}^{-1})}$$

Equation 2.2

$$\text{Ileal DM f ow (g d}^{-1}) = \left(\frac{\text{Alkane intake (mg d}^{-1})}{\text{[alkane] in ileum (mg g DM}^{-1})}\right) * \frac{1}{CF}$$

$$\text{where } CF = \frac{\text{DM flow at ileum (g DM d}^{-1}) * \text{[C36] in ileum (mg g DM}^{-1})}{\text{DM flow at abomasum (g DM d}^{-1}) * \text{[C36] in abomasum (mg g DM}^{-1})}$$

Amino acid flows at the abomasum and ileum were calculated by multiplying AA concentration in the digesta by the corresponding DM flow at the abomasum or ileum (Waghorn *et al.*, 1987). Apparent absorption of AA in the small intestine was determined using Equation 2.3. Digestibility of AA in the small intestine was calculated by dividing apparent absorption of AA in the small intestine (Equation 2.3) by AA flux at the abomasum.

Equation 2.3

$$\text{Apparent AA absorption (g d}^{-1}) = \text{AA flux at abomasum} - \text{AA flux at ileum}$$

2.3.8.2 Plasma flow

Plasma flow (Pf) across the MDV, PDV, TSP, liver and hind limbs were determined by Equation 2.4 to 2.8.

Equation 2.4

$$\text{MDV Pf } (\text{mL min}^{-1}) = \frac{[\text{PAH}]_I \times \text{infusion rate}}{[\text{PAH}]_M - [\text{PAH}]_A}$$

where : $[\text{PAH}]_I$ = concentrat ion of PAH in the infusate
$[\text{PAH}]_M$ = concentrat ion of PAH in the mesenteric vein
$[\text{PAH}]_A$ = concentrat ion of PAH in the mesenteric artery

Equation 2.5

$$\text{PDV Pf } (\text{mL min}^{-1}) = \frac{[\text{PAH}]_I \times \text{infusion rate}}{[\text{PAH}]_P - [\text{PAH}]_A}$$

where : $[\text{PAH}]_P$ = concentrat ion of PAH in the portal vein

Equation 2.6

$$\text{TSP Pf } (\text{mL min}^{-1}) = \frac{[\text{PAH}]_I \times \text{infusion rate}}{[\text{PAH}]_H - [\text{PAH}]_A}$$

where: $[\text{PAH}]_H$ = concentration of PAH in the hepatic vein

Equation 2.7

$$\text{ART Pf } (\text{mL min}^{-1}) = \text{TSP Pf} - \text{PDV Pf}$$

where : ART Pf = blood flow in the hepatic artery

Equation 2.8

$$\text{Hind limb Pf } (\text{mL min}^{-1}) = \frac{[\text{ICG}]_I \times \text{infusion rate}}{[\text{ICG}]_V - [\text{ICG}]_A}$$

where : $[\text{ICG}]_I$ = concentrat ion of ICG in the infusate
$[\text{ICG}]_V$ = concentrat ion of ICG in the vena cava
$[\text{ICG}]_A$ = concentrat ion of ICG in the mesenteric artery

2.3.8.3 Metabolite flux across the tissue beds

The arterio-venous (AV) concentration differences of metabolites (data not presented) across the tissue beds were calculated as the difference between the concentration in the

arterial supply (mesenteric artery; A) and that in the venous drainage of the tissue bed (mesenteric, portal and hepatic veins; M, P, H, respectively or the vena cava (V)). The net nutrient fluxes across the MDV, PDV, liver, TSP and hind-limb tissues were calculated using an AV approach as outlined in Equation 2.9 to 2.13. The Z refers to the venous concentration of the metabolite in question (e.g., AA, glucose etc.).

Equation 2.9

Net flux of metabolite across MDV = $([Z]_A - [Z]_M) \times$ MDV Pf

where: $[Z]_A$ = concentration of metabolite in mesenteric artery

$[Z]_M$ = concentration of metabolite in mesenteric vein

Equation 2.10

Net flux of metabolite across PDV = $([Z]_A - [Z]_P) \times$ PDV Pf

where: $[Z]_P$ = concentration of metabolite in portal vein

Equation 2.11

Net flux of metabolite across Liver =

$([Z]_A \times$ ART Pf) + $([Z]_P \times$ PDV Pf) – $([Z]_H \times$ TSPT Pf)

where: $[Z]_H$ = concentration of metabolite in hepatic vein

Equation 2.12

Net flux of metabolite across TSP = $([Z]_A - [Z]_H) \times$ TSP Pf

Equation 2.13

Net flux of metabolite across hind limbs = $([Z]_A - [Z]_V) \times$ Hind limb Pf

where: $[Z]_V$ = concentration of metabolite in vena cava

2.3.9 Statistical analysis

Statistical analysis was performed using a General Linear Model (SAS version 8, 1999), with treatment and group (the week that the lamb underwent surgery) used as sources of variation in the model. Feed intake, FEC and liveweight were analysed using Proc Mix repeated measures. The data were checked for normality and the presence of outliers by plotting residuals versus the predicted residuals. The FEC were transformed by *ln* $(x+1)$ before analysis in order to ensure symmetry in the data and to standardise variances across the treatments (Sutherland *et al.*, 1999a).

Probability values less than 0.10 were considered to indicate a significant difference and values between 0.10 and 0.15 to indicate a trend. The use of 0.15 as the limit of significance is used extensively in metabolic studies (e.g., Lapierre *et al.*, 2000) due in part to the small numbers of degrees of freedom analysed statistically due to catheter failure or erroneous values within the data set

Results are presented as least squares means (LS means) and associated pooled standard deviation (SD). Statistical difference from zero for AV concentration difference of AA and metabolites was determined using the T-statistic, with P>T values greater than 0.05 considered as non significant. Unless otherwise indicated in the results section, all AV values presented are statistically different from zero.

One sheep from the parasite treatment was omitted from all statistical analysis as the Captec^{TM} Alkane CRC capsule blocked the reticulo-rumen orifice and prevented feed intake 2 days prior to the blood sampling period.

2.4 Results

2.4.1 Feed intake and liveweight

Over the course of the experiment feed intake was not affected by the presence of parasites in the small intestine (826 *vs.* 796 (SD 18) g DM d^{-1} in the control and parasite

lambs, respectively; P>0.10) and is consistent with the feed intakes measured during the N balance (Table 2.2).

The parasitised lambs were initially lighter than the control lambs before infection (34 *vs.* 38 (SD 2.5) kg in the parasite and control lambs, respectively; P<0.01), however, the gain in liveweight over the course of the experiment was unaffected by the presence of parasite infection (240 *vs.* 190 (SD 100) g d^{-1} in the parasite and control lambs, respectively; P>0.15).

2.4.2 Parasitology

Faecal eggs counts were significantly higher in the infected lambs compared to the control lambs throughout the course of the experiment (Figure 2.1; P<0.001). Worm burdens in the small intestine were also significantly higher in the parasite lambs compared to the control lambs (Table 2.3; P<0.001).

2.4.3 Nitrogen balance

The presence of parasites in the small intestine did not have any effect on the intake and digestibility of DM and N (P>0.15; Table 2.2). Parasitic infection had no effect on the excretion of N in urine (16 *vs.* 18 (SD 3) g N d^{-1} in the control and infected lambs, respectively; P>0.15) and faeces (6 *vs.* 7 (SD 0.3) g N d^{-1} in the control and parasite lambs, respectively; P>0.15). Subsequently, N retention was also unaffected (Table 2.2; P>0.15). The fluxes of DM and N through the abomasum and ileum were similar for control and parasite-infected lambs (P>0.10; Table 2.2).

2.4.4 Amino acid balance in the gastrointestinal tract

Amino acid intakes were similar between the control and parasite-infected lambs (Table 2.4; see Appendix B.5 Tables B.1 to B.5 for individual AA data). The fluxes of total and non-essential AA (NEAA) through the abomasum of infected lambs were approximately 8% higher (P<0.05) than those of the control lambs, while those for the essential AA (EAA) and branched-chain AA (BCAA) were higher than those for the control lambs (P<0.10).

Despite higher fluxes of AA through the abomasum in the parasite-infected lambs, the fluxes of AA to the ileum was not significantly different (Table 2.4). The apparent absorption of AA and their digestibility in the small intestine were not affected by the presence of parasites (Table 2.4).

Figure 2.1 Faecal egg counts in lambs fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* (n=5) or kept as parasite-free controls (n=6) on days 0 to 6 of the experimental period. Results are presented as LSmeans, with the error bar representing the pooled standard deviation.

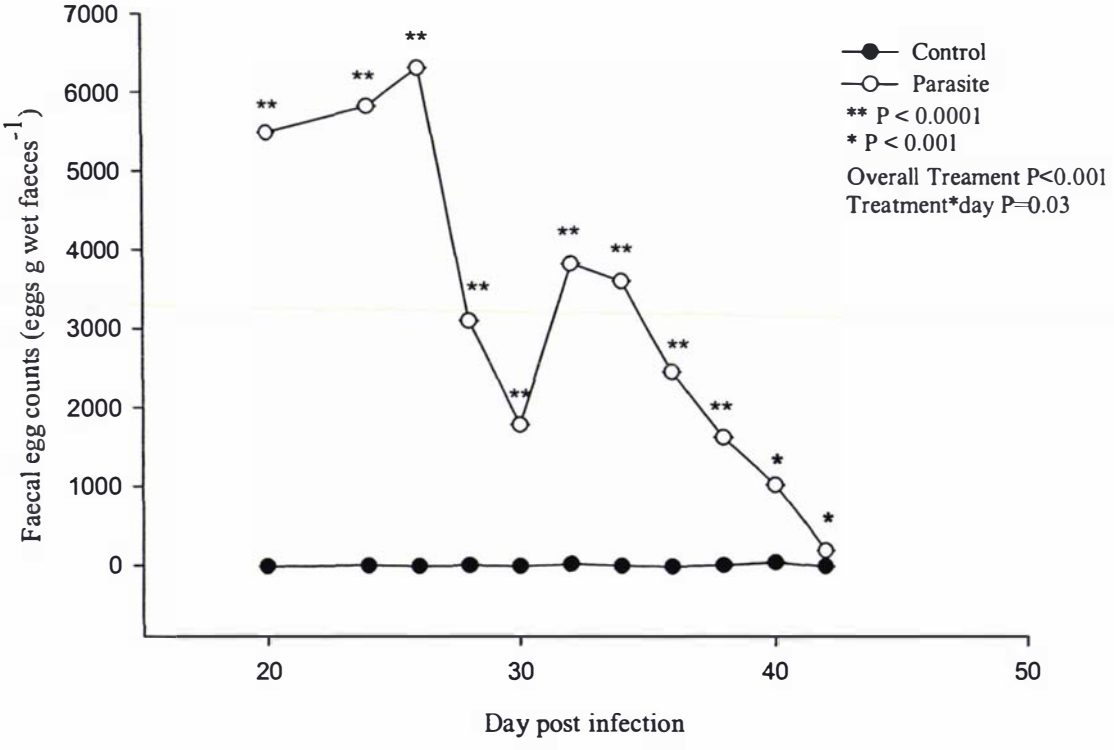

Table 2.2 Dry matter (DM) and nitrogen (N) intake, digestibility and flux in the abomasum and ileum (g d^{-1}) and N retention (g d^{-1}), in lambs fed fresh Lucerne (*Medicago sativa*) with a *Trichostrongylus colubriformis* infection or kept as parasite-free controls. Results are presented as LSmeans and the associated pooled standard deviation (SD).

		Control n=6	Parasite n=5	Pooled SD	P
Intake	DM	763	754	16	0.49
	N	26	26	0.4	0.74
Abomasum Flux	DM	468	479	31	0.62
	N	19	20	1.0	0.37
Ileum Flux	DM	310	306	46	0.90
	N	9	9	2	0.85
Digestibility	DM	0.65	0.65	0.01	0.24
	N	0.74	0.72	0.02	0.29
Retention	N	3.6	1.2	2.9	0.28

Table 2.3 Parasite burdens in the small intestine of lambs fed fresh Lucerne (*Medicago sativa*) and infected with a *Trichostrongylus colubriformis* infection or kept as parasite-free controls. Results are presented as the LSmeans and associated pooled standard deviation (SD).

	Control n=6	Parasite n=5	Pooled SD	P
Male	180	9043	543	0.001
Female	263	12699	1561	0.001
Total	443	21741	1101	0.001

Table 2.4 Non-essential (NEAA), essential (EAA), branch-chained (BCAA) and total amino acid intakes and abomasum and ileum fluxes (g d^{-1}) of lambs fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=5	Pooled SD	P
Intake[1]	NEAA	92.9	88.9	5.4	0.30
	EAA	71.0	67.7	4.1	0.30
	BCAA	28.4	27.1	1.6	0.30
	TOTAL	163.9	156.6	9.4	0.30
Abomasal flux[2]	NEAA	52.7	56.4	2.3	0.05
	EAA	46.2	49.4	2.2	0.06
	BCAA	19.1	20.2	0.8	0.09
	TOTAL	98.9	105.8	4.5	0.05
Ileal flux[3]	NEAA	20.9	21.6	4.2	0.81
	EAA	16.9	17.8	3.7	0.80
	BCAA	7.9	8.4	1.7	0.80
	TOTAL	37.3	39.4	7.8	0.80
Apparent absorption of amino acids from the SI	NEAA	31.8	34.8	4.5	0.63
	EAA	29.3	31.6	4.1	0.68
	BCAA	11.2	11.8	2.0	0.76
	TOTAL	61.1	66.4	8.5	0.65
Proportion of amino acids digested in the SI	NEAA	0.60	0.62	0.08	0.93
	EAA	0.63	0.64	0.08	0.98
	BCAA	0.59	0.58	0.10	0.93
	TOTAL	0.62	0.63	0.08	0.98

1. Based on data obtained during the nitrogen balance (days 35-43).
2. Based on digesta samples taken on day 44.
3. Based on samples taken at slaughter (day 48).

NEAA: alanine, aspartate, glutamate, glycine, proline, serine, and tyrosine.
EAA: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine.
BCAA: isoleucine, leucine and valine.
Total = NEAA + EAA.

2.4.5 Nutrient and metabolite fluxes

Catheter patency was approximately 99%, with only one catheter in the hepatic vein failing. In lambs infected with *T. colubriformis*, plasma flows across the MDV and hind limbs, were unaffected by the presence of parasites (P>0.10; Table 2.5). However, plasma flow in the PDV and TSP was higher in the infected lambs (P<0.10; Table 2.5), as was plasma flow through the hepatic artery (P<0.15; Table 2.5).

Table 2.5 Plasma flow (mL min^{-1}) across the mesenteric-drained viscera (MDV), portal-drained viscera (PDV), total splanchnic tissues (TSP), hepatic artery (ART) and hind limbs of lambs fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD).

	Control n=6	Parasite n=5	Pooled SD	P
MDV	545	772	222	0.22
PDV	1182	1353	111	0.06
TSP	1281	1676	238	0.07
ART	101	346	199	0.14
Hind limbs	489	464	141	0.81

The presence of parasites in the small intestine did not effect the concentration of AA in plasma (Table 2.6), with a few exceptions. Parasitic infection had no effect on the concentration of AA in the mesenteric vein (P>0.15; Table 2.6 and Appendix Table B.6). Alanine, serine, isoleucine and lysine concentrations were increased in the portal vein of infected lambs (P<0.15; Appendix Table B.7). Infection increased the plasma concentration of isoleucine, lysine (P<0.15; Appendix Table B.8) and the BCAA (P<0.15; Table 2.6) in the hepatic vein. In the mesenteric artery parasitic infection increased the concentration of serine, lysine, isoleucine, valine (P<0.15; Appendix Table B.9) and the BCAA (P<0.15; Table 2.6). The concentration of lysine, serine, isoleucine, leucine, valine (P<0.15; Appendix Table B.10) and the BCAA (P<0.05; Table 2.6) were higher in the vena cava.

Across the MDV, AV concentration differences of AA were significantly different from zero (P>0.05; data not presented) with the exception of the following AA: aspartate, hydroxyl proline, glutamine, ornithine, taurine and threonine. The net fluxes of NEAA,

EAA and BCAA across the MDV were not affected by the presence of parasites (P>0.15; Table 2.7), with a net release of most AA in the mesenteric drainage (Appendix Table B.11).

The AV concentration difference across the PDV of AA (except aspartate, hydroxyl proline, glutamine, taurine, threonine, and ornithine) were significantly different from zero (P>0.05; data not presented). The net fluxes of NEAA, EAA and BCAA across the PDV were not affected by the presence of parasites (P>0.15; Table 2.7), with a net release of most AA in the portal drainage (Appendix Table B.12).

Across the liver, all AV concentration differences were significantly different from zero (P>0.05; data not presented). Although parasitic infection did not affect the net fluxes of total AA in the liver (P<0.15; Table 2.7), individual AA were affected, with the presence of parasites resulting in an increase in the utilisation of aspartate and alanine by the liver (P<0.15; Appendix Table B.13).

Across the TSP, only glutamate and citrulline AV concentration differences were significantly different from zero (P>0.05; data not presented). Parasitic infection increased the release of glutamate, serine, isoleucine, leucine, lysine (P<0.15) and methionine (P<0.15; Appendix Table B.14) and the BCAA (P<0.10; Table 2.7) by the TSP. Parasitic infection increased the use of glutamate (P=0.15; Appendix Table B.14) by the TSP.

Across the hind limbs hydroxyl-proline, taurine, histidine, threonine, carnithine, proline, tyrosine, phenylalanine, ornithine and lysine were not significantly different from zero (P<0.05; data not presented). There was no effect of parasite infection on net AA fluxes across the hind limbs (P>0.15; Table 2.7). Glutamate was the only individual AA affected by parasitic infection, with an increase in utilisation by the hind limbs of infected lambs (P<0.15; Appendix Table B.15). Most AA with the exception of the BCAA and some NEAA which were utilised by the hind limbs, were released by the hind limbs of both the control and parasite lambs (Table 2.7 and Appendix Table B.15).

Parasitic infection did not have any significant effect on the concentration of CO_2 in blood taken from any vessels ($P>0.15$; Table 2.9) and consequently the net production of CO_2 by the tissue beds was unaffected ($P>0.15$; Table 2.9). Oxygen concentration was higher in the mesenteric vein in the parasite-infected lambs ($P<0.15$; Table 2.8) but not in any other blood vessels. However, net oxygen consumption by all the tissues was unaffected by the presence of parasitic infection ($P>0.15$; Table 2.9).

The AV concentration differences of glucose and lactate across the tissue beds were not significantly different from zero across the MDV, PDV, TSP or hind limbs ($P<0.05$; data not presented). However, the AV concentration differences of glucose and lactate across the liver were significantly different from zero ($P>0.05$; data not presented). Glucose concentration in all vessels (Table 2.8) and net flux across tissue beds was similar between treatment groups (Table 2.9). Glucose was used by the MDV and hind limbs, whilst it was released by the PDV and liver in both control and parasitised lambs. Overall, the TSP released glucose into the peripheral circulation (Table 2.9). Parasitic infection increased the concentration of lactate in the mesenteric vein ($P<0.15$; Table 2.8). Consequently, there was a net appearance of lactate across the MDV in parasitised lambs compared to a net uptake in control lambs ($P=0.10$; Table 2.9). The PDV and liver released lactate, however there was no effect of parasite infection ($P>0.15$; Table 2.9). In the TSP, parasitised lambs had a lower lactate flux than the control lambs ($P<0.15$) despite no significant effect of parasitic infection on lactate concentration in the mesenteric artery and hepatic vein (Table 2.8; $P>0.10$).

Table 2.6 Non-essential (NEAA), essential (EAA), branch-chained (BCAA) and total amino acid plasma concentrations (μmol L^{-1}) in the mesenteric, portal and hepatic veins, mesenteric artery and vena cava of lambs infected with *Trichostrongylus colubriformis* or kept as parasite-free controls and fed fresh Lucerne (*Medicago sativa*). Results are presented as LSmeans and associated standard deviation (SD).

		Control n=6	Parasite n=5	Pooled SD	P
Mesenteric vein	NEAA	930	849	283	0.63
	EAA	1048	917	294	0.55
	BCAA	502	427	118	0.39
	Total AA	1978	1766	575	0.58
Portal vein	NEAA	812	904	143	0.37
	EAA	865	983	131	0.26
	BCAA	410	447	38	0.21
	Total AA	1677	1887	270	0.31
Hepatic vein*	NEAA	726	844	173	0.36
	EAA	798	940	148	0.24
	BCAA	394	451	48	0.15
	Total AA	1525	1785	317	0.29
Mesenteric artery	NEAA	769	865	117	0.24
	EAA	764	870	119	0.28
	BCAA	380	432	37	0.08
	Total AA	1533	1735	229	0.23
Vena cava	NEAA	804	893	140	0.37
	EAA	776	893	102	0.18
	BCAA	363	410	27	0.04
	Total AA	1580	1787	238	0.27

*Hepatic vein: n=4 for the parasite treatment
NEAA: alanine, asparagine, aspartate, glutamate, glutamine, glycine, hydroxy proline, proline and serine.
EAA: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine and valine.
BCAA: isoleucine, leucine and valine.
Total = EAA + NEAA.

Table 2.7 Net flux (μmol min^{-1}) of non-essential (NEAA), essential (EAA), branched-chain (BCAA) and total amino acids in the mesenteric-drained viscera (MDV), portal-drained viscera (PDV), liver, total splanchnic tissues (TSP) and hind limbs in lambs infected with *Trichostrongylus colubriformis* or kept as parasite-free controls and fed fresh Lucerne (*Medicago sativa*). Positive values represent a net utilisation by the tissue, while a negative value represents a net release. Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=5	Pooled SD	P
MDV*	NEAA	-74.0	-10.2	83.9	0.35
	EAA	-136.5	-62.5	111.9	0.42
	BCAA	-58.1	-13.1	46.1	0.24
	Total AA	-210.6	-72.7	195.2	0.39
PDV	NEAA	-57.4	-63.7	70.5	0.90
	EAA	-118.5	-142.1	109.6	0.87
	BCAA	-36.1	-28.1	29.9	0.71
	Total AA	-175.9	-205.8	167.0	0.87
Liver*	NEAA	109.4	122.1	76.4	0.85
	EAA	63.9	63.6	59.2	0.48
	BCAA	14.5	4.0	17.4	0.50
	Total AA	173.3	185.7	148.2	0.77
TSP*	NEAA	29.4	-18.7	53.5	0.82
	EAA	-39.2	-162.5	91.5	0.87
	BCAA	-15.2	-43.4	8.8	0.08
	Total AA	-9.8	-181.2	78.6	0.73
Hind limbs	NEAA	-15.1	-4.4	10.8	0.21
	EAA	-9.3	-11.7	30.7	0.73
	BCAA	9.2	12.2	9.0	0.64
	Total AA	-24.3	-16.1	31.2	0.91

* n=4 for the parasite treatment.
NEAA: alanine, asparagine, aspartate, glutamate, glutamine, glycine, hydroxyl-proline, proline and serine.
EAA: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine and valine.
BCAA: isoleucine, leucine and valine.
Total = EAA + NEAA.

Table 2.8 The concentration of oxygen (O_2), carbon dioxide (CO_2) in blood (mmol L^{-1}), and glucose, lactate, urea (mmol L^{-1}) and ammonia (μmol L^{-1}) in plasma of the mesenteric, portal, and hepatic veins, mesenteric artery and vena cava in lambs fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Results are presented as LSmeans and the pooled standard deviation (SD).

		Control n=6	Parasite n=5	Pooled SD	P
Mesenteric vein	O_2	10.38	11.24	0.75	0.14
	CO_2	26.84	26.91	2.51	0.97
	Glucose	2.94	2.81	0.14	0.23
	Lactate	0.65	0.81	0.14	0.14
	Ammonia	0.59	0.95	0.35	0.19
	Urea	7.85	8.34	0.91	0.45
Portal vein	O_2	11.11	11.10	0.24	0.96
	CO_2	27.14	26.70	2.44	0.80
	Glucose	2.99	3.00	0.15	0.92
	Lactate	0.70	0.70	0.13	0.99
	Ammonia	0.92	0.90	0.38	0.92
	Urea	7.75	8.44	0.94	0.32
Hepatic vein	O_2	8.95	10.06	0.95	0.16
	CO_2	27.20	26.66	2.92	0.81
	Glucose	3.13	2.94	0.26	0.35
	Lactate	0.62	0.73	0.15	0.35
	Ammonia	0.14	0.18	0.05	0.37
	Urea	8.07	8.45	1.21	0.68
Mesenteric artery	O_2	13.76	13.52	1.03	0.77
	CO_2	25.31	24.70	3.02	0.78
	Glucose	2.97	3.00	0.14	0.76
	Lactate	0.67	0.65	0.11	0.80
	Ammonia	0.12	0.17	0.06	0.23
	Urea	7.82	8.46	0.92	0.35
Vena cava	O_2	7.93	8.52	1.45	0.57
	CO_2	26.96	26.84	2.98	0.96
	Glucose	2.82	2.92	0.13	0.31
	Lactate	0.71	0.68	0.09	0.58
	Ammonia	0.13	0.17	0.06	0.24
	Urea	7.79	8.40	0.90	0.38

Table 2.9 Net flux of plasma ammonia (μmol min^{-1}), urea, glucose, lactate and blood oxygen (O_2) and carbon dioxide (CO_2; mmol min^{-1}) across the mesenteric-drained viscera (MDV), portal-drained viscera (PDV), liver, total splanchnic tissues (TSP) and hind limbs of lambs fed fresh lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Positive values represent a net utilisation by the tissue, while a negative value represents a net release. Results are presented as LSmeans the associated pooled standard deviation (SD).

		Control n=6	Parasite n=5	Pooled SD	P
MDV	O_2	2.08	2.46	1.02	0.64
	CO_2	-1.17	-2.22	1.49	0.38
	Glucose	0.04	0.09	0.13	0.64
	Lactate	0.01	-0.12	0.08	0.10
	Ammonia	-314.2	-617.5	325.9	0.26
	Urea	-0.01	0.10	0.18	0.50
PDV	O_2	3.84	4.12	1.41	0.78
	CO_2	-2.62	-3.42	1.53	0.48
	Glucose	-0.10	-0.07	0.15	0.83
	Lactate	0.03	0.01	0.11	0.73
	Ammonia	-929.1	-980.9	411.1	0.86
	Urea	0.09	0.04	0.17	0.66
Liver*	O_2	3.36	3.69	1.00	0.66
	CO_2	-0.64	-0.61	0.67	0.95
	Glucose	-0.24	-0.04	0.25	0.31
	Lactate	0.16	0.08	0.08	0.26
	Ammonia	872.4	797.3	364.6	0.81
	Urea	-0.45	-0.49	0.94	0.95
TSP*	O_2	7.36	7.02	2.09	0.83
	CO_2	-3.17	-3.08	1.36	0.93
	Glucose	-0.38	-0.17	0.28	0.33
	Lactate	0.18	0.01	0.15	0.15
	Ammonia	-56.9	147.7	138.3	0.25
	Urea	-0.35	-0.44	1.05	0.91
Hind limbs	O_2	3.57	3.42	1.26	0.87
	CO_2	-1.08	-1.27	0.60	0.66
	Glucose	0.08	0.02	0.06	0.25
	Lactate	-0.01	-0.01	0.03	0.68
	Ammonia	0.16	3.96	3.48	0.16
	Urea	0.02	0.03	0.10	0.87

* n=4 for the parasite treatment.

The ammonia AV concentration difference was not significantly different from zero in the MDV, PDV, TSP and hind limbs. However, in the liver the AV concentration difference was significantly different from zero ($P<0.05$; data not presented). Ammonia concentration in the mesenteric and portal veins were substantially higher than that of the hepatic vein (Table 2.8) indicating that the liver removed a large proportion of the ammonia from the circulation, and therefore the TSP and hind limbs released a small amount of ammonia. Ammonia concentration in the mesenteric, portal and hepatic veins, mesenteric artery and vena cava were unaffected by the presence of parasitic infection ($P>0.15$; Table 2.8). There was no effect of parasite infection on net ammonia flux across the tissue beds ($P>0.15$; Table 2.9), with ammonia produced by the MDV and PDV.

Urea AV concentration differences were not significantly different from zero across the MDV, PDV, TSP or hind limbs ($P<0.05$; data not presented), whilst they were significantly different from zero in the liver ($P>0.05$; data not presented). Plasma urea concentration was similar in all blood vessels between treatments ($P>0.15$; Table 2.8), and the liver was largely responsible for the production of urea with an overall release from the TSP whilst the PDV and hind limbs had removed urea from the circulation (Table 2.9). There was no effect of parasitic infection on urea flux across any tissues ($P>0.15$).

2.5 Discussion

An established parasitic infection did not result in the partitioning of more AA from the muscle to the GIT and liver, as indicated by the similar net AA fluxes from the hind limbs. Net fluxes of AA from the MDV and PDV were similar between treatments suggesting that infection did not increase the AA requirement by these tissues. With the exception of a few AA, there were relatively small changes in the utilisation of AA by the liver, suggesting that at 48 d post infection, there was no increase in AA requirement by this tissue. Similarly the net fluxes of metabolites were also unaffected by parasitic

infection suggesting that overall energy requirements by the infected lambs were not influenced by the presence of adult parasites in the small intestine.

2.5.1 Parasitology and feed availability

The lambs in this experiment were dosed with approximately 36 000 L3 *T. colubriformis* larvae over the course of six days, with worm burdens of around 22 000 at slaughter. This represents an establishment rate of 60%, which is comparable to other studies utilising a single dose method of infection (Athanasiadou *et al.*, 2000; Chan *et al.*, in preparation). Faecal egg count (eggs per gram of wet faeces; EGF) in this study declined rapidly from day 27 post infection (6 000 EGF) to around 300 EGF on the day of blood sampling (day 48 post infection), which is consistent with the results observed by Roseby (1973).

2.5.2 Plasma nutrients and metabolites

Plasma flows across the MDV, PDV, TSP, hepatic artery and the hind limbs are within the ranges presented elsewhere in the literature for sheep of a similar age and DM intake. Plasma flow through the MDV was reported to be approximately 1 500 mL min^{-1} (Janes *et al.*, 1985), while that of the PDV ranged from 1300 to 1600 mL min^{-1} (Heitmann & Bergman, 1980; Burrin *et al.*, 1989; Lobley *et al.*, 1996; Piccioli Cappelli *et al.*, 1997). The hepatic artery ranged from 69 to 460 mL min^{-1} (Burrin *et al.*, 1989; Lobley *et al.*, 1996), whilst the TSP has been reported to range from 1300 to 1900 mL min^{-1} (Burrin *et al.*, 1989; Lobley *et al.*, 1996). Plasma flow though the hind limbs of sheep ranged from approximately 240 mL min^{-1} (Bird *et al.*, 1981; Wester *et al.*, 2000) to 550 mL min^{-1} (Roy *et al.*, in preparation).

Parasite infection has been reported to increase blood flow to both the MDV and the PDV (Yu *et al.*, 2000), which is consistent with results presented in this study. Increased plasma flow across the PDV was observed in the infected lambs suggesting that there is an increase in plasma flow from the visceral tissues. Parasite infection also increased plasma flow across the TSP, however this is attributed to the increase in the plasma flow at the PDV and the hepatic artery. Reasons for alterations in plasma flow

in the MDV and PDV during parasitic infection are unclear. However, localised control of intestinal blood flow means that plasma flow can increase in some sections of the digestive tract without overall changes in cardiac output (Chou & Kvietys, 1981). These changes are under the control of GIT motility and hormonal changes (Granger *et al.*, 1980), both of which have been observed during parasitic infection (Symons & Hennessy, 1981; Fox *et al.*, 1989). For example, gastrin secretion increased during *Ostertagia* infections (Fox *et al.*, 1989) while cholecystokinin secretion increased during intestinal parasitic infections (Symons & Hennessy, 1981). Both these hormones increase gastric blood flow (Chou, 1982; Richardson & Withrington, 1982; Reynolds *et al.*, 1991), however they were not measured in the current study.

The net fluxes of AA across the MDV and PDV were similar between the control and infected lambs. All AA measured in this study were released from the MDV and the PDV, with the exception of glutamate and carnithine. The results presented in this Chapter are consistent with studies with lambs fed pelleted Lucerne (EAA: 158.3 μmol min^{-1}; BCAA: 83.3 μmol min^{-1}; Heitmann & Bergman, 1980; Lobley *et al.*, 1995; 1996; MacRae *et al.*, 1997; Piccioli Cappelli *et al.*, 1997).

Parasitic infection did not affect the hepatic metabolism of total AA, NEAA, EAA or BCAA. Aspartate and alanine were utilised at higher rates by the liver during the presence of parasite infection. These AA are important in the gluconeogenic pathway in the ruminant. This suggests that parasitism may alter the proportion of glucose coming from different precursors, such as glycogen, lactate, propionate and AA. Lambs given a single dose of *T. colubriformis* larvae (40 000) showed a 30% reduction in ruminal acetate production, compared to animals at a similar feed intake, indicating that parasites may affect rumen fermentation (Steel, 1972). However, these authors did not state the effects of parasitic infection on other volatile fatty acids. Therefore, it is possible that the increase in utilisation of the gluconeogenic AA is due to a reduction in traditional energy substrates in the parasitised lambs. The rates of uptake of AA by the liver in control lambs are consistent with those in uninfected animals reported in other studies

(EAA: 84-113 μmol min^{-1}; BCAA 27-34 μmol min^{-1}: Heitmann & Bergman, 1980; Lobley *et al.*, 1995; 1996).

Parasitic infection increased the amounts of methionine, serine, lysine and BCAA released from the TSP into the peripheral blood. Amino acids such as serine and the BCAA are found in high concentrations in GIT secretions (MacRae & Lobley, 1991) and it is possible that these AA were diverted to the GIT (Biolo *et al.*, 1995; Reeds *et al.*, 1999). However, as there was no effect of infection on the mesenteric supply of these AA, it seems unlikely that these AA were utilised by the MDV for endogenous secretions. The portal supply of these AA were all increased in infected lambs, and this may explain why these AA were increased at the TSP. The release of BCAA by the TSP has been observed previously in sheep (Milano *et al.*, 2000), with previous studies indicating that 30-40% of BCAA oxidation occurs in the peripheral tissues (Harris *et al.*, 1992). Glutamate was utilised by the TSP, which contradicts earlier studies which show that glutamate is released by the liver (Lobley *et al.*, 1996; 2001). Glutamate is involved in many reactions in the liver including transamination of AA and is a by-product of ammonia metabolism (Rodwell, 2000) and urea synthesis (Meijer *et al.*, 1999). Therefore, it is possible that there was an increase in the amount of glutamate being used to form urea in parasitised sheep, although there was no difference in total urea production by the liver of infected lambs.

There was no difference in the net AA flux across the hind limbs between the control and parasite infected lambs. It is possible that there may be an alteration in the way that AA are utilised within the hind limbs or between the various components (fat, skin, muscle) that make up this tissue, with changes in protein synthesis, degradation and/or AA oxidation (and endogenous synthesis for NEAA) resulting in a similar net flux. However, there are no differences in the net fluxes of traditional carriers of N-groups (e.g., alanine) which suggests that there was no effect of parasitic infection on the degradation of skin or muscle protein and/or the release of AA from within this tissue bed. The pattern of uptake or release of AA from the hind limbs were similar to lambs fed pelleted rations in other studies (Hoskin *et al.*, 2001; Roy *et al.*, in preparation), with

release of the EAA (10-37 μmol min^{-1}) and NEAA (1-5 μmol min^{-1}) and utilisation of the BCAA: (1-8 μmol min^{-1}).

Minimal effects of parasitic burden on N and AA metabolism are supported by similar O_2, CO_2, glucose and lactate fluxes across all tissue beds between the control and parasite lambs. Oxygen consumption values reported in this chapter are consistent with those reported elsewhere in the literature (*c.* 350 μmol min^{-1}; Bird *et al.*, 1981; Pell & Bergman, 1983).

The presence of a parasite infection in the GIT did not affect the concentration or flux of glucose across the MDV, PDV, liver, TSP or hind limbs, which is consistent with previous work in parasitic lambs (Roseby, 1973; Coop *et al.*, 1976). Lactate was released by the MDV in parasitised lambs, and less was used in the TSP of parasitised lambs. The reduction in hepatic lactate utilisation in the parasitised lambs is consistent with increased synthesis of glucose from AA rather than lactate in parasitised lambs. The concentration of glucose (*c.* 3 mM) and lactate (*c.* 1 mM), and their fluxes across the tissue beds presented in this study are consistent with those presented elsewhere in the literature for uninfected animals (Bird *et al.*, 1981; Pell & Bergman, 1983; Janes *et al.*, 1985; Teleni *et al.*, 1986; Piccioli Cappelli *et al.*, 1997; Wester *et al.*, 2000).

Parasite infections in this study did not have any effect on the plasma concentration of ammonia in the mesenteric, portal, and hepatic veins and mesenteric artery. These concentrations are consistent with values obtained in non-parasitised lambs fed fresh white clover (Greaney *et al.*, 1996), Lucerne pellets (Lobley *et al.*, 1995) and grass pellets (Milano *et al.*, 2000). Plasma urea concentration reported in the present study is higher than those reported by Lobley *et al.* (1995) and Milano *et al.* (2000), but this is expected given that in our study fresh Lucerne was fed rather than pelleted forages. The effect of parasitic infection on the fluxes of ammonia and urea have not previously been reported, but values presented in this study are consistent with the results of Greaney *et al.* (1996) who demonstrated a net release of ammonia across the MDV (673 μmol min^{-1}) and PDV (1 450 μmol min^{-1}) in uninfected lambs fed fresh forage. Ammonia flux

across the liver followed similar patterns of uptake as those reported by Lobley *et al.* (1995; 450 μmol min^{-1}) and Milano *et al.* (2000; 319 μmol min^{-1}) in uninfected lambs fed dried forages.

In this study a single dose of 36 000 L3 larvae had no effect on plasma urea concentration 48 days post infection. Results in the literature relating to urea concentration in parasitic lambs are contradictory, with Coop *et al.* (1976) reporting that a trickle infection of 2 500 *T. colubriformis* larvae had no effect on the concentration of urea in plasma during 14 weeks of infection, whilst other studies have shown that a single dose of 30 000 *T. colubriformis* resulted in a 18-26% increase in urea concentration 30 d post infection, although there was no effect by day 60 (Roseby, 1973; Roseby & Leng, 1974). Urea was removed by the MDV in both the control and parasitised lambs. However as expected, urea was released by the liver, which is consistent with the release reported by Lobley *et al.* (1995; 314 μmol min^{-1}) and Milano *et al.* (2000; 710 μmol min^{-1}) in uninfected animals.

The similarities between ammonia utilisation and urea production in the liver of lambs with a *T. colubriformis* infection suggests that intestinal parasites do not create an additional demand on the animal for AA and energy for ureagenesis.

2.6 Conclusions

The present data indicate an adult *T. colubriformis* population in the small intestine of lambs imposes little nutritional cost to the host 48 d post-infection. It is possible that any effects of the parasite burden occurred during the earlier stages of the trial and the data highlight a need to identify which stage(s) of intestinal parasitism need to be targeted in metabolic studies. The periods that are most likely to impact on production are the initial larvae challenge and the highest point of egg production. There is little information available on the nutritional demands of the parasite during its reproduction phase (egg production; approximately 3 weeks post infection) and the demands of adult

parasites. These factors should be addressed in future studies, beyond the scope of this thesis.

A major question resulting from these results is how did the lambs manage to cope with the presence of such a large parasite burden without any significant effects on their metabolism? In the future, when investigating these questions it is important to consider the effects of larval challenge *vs.* maintenance of worm burdens, previous exposure to the parasite, immune development and the metabolic activity of adult worms.

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3 FRACTIONAL PROTEIN SYNTHESIS RATES OF TISSUES IN LAMBS FED FRESH LUCERNE (*MEDICAGO SATIVA*) AND INFECTED WITH OR WITHOUT *TRICHOSTRONGYLUS COLUBRIFORMIS*

3.1 Abstract

The effect of a *Trichostrongylus colubriformis* infection on the fractional protein synthesis rate (FSR) in the small intestine (duodenum and ileum), liver, lymphoid tissues (spleen, thymus and mesenteric lymph nodes) and productive tissues (muscle and skin) was determined in lambs fed fresh Lucerne (*Medicago sativa*; 800 g DM d^{-1}) on day 48 post infection. Lambs were dosed with 6 000 L3 *T. colubriformis* larvae for 6 days (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 peaked on day 26 post infection ($P<0.001$). On day 48 post infection, the lambs were continuously infused with [3, 4 -^{3}H]-valine (7.6 MBq h^{-1}) for 8 h. Blood was continuously collected from the mesenteric artery and plasma harvested with the specific radioactivity of valine in plasma determined. After the 8 h infusion the lambs were euthanased and tissue samples collected from the small intestine (duodenum and ileum), liver, lymphoid tissues (spleen, thymus and mesenteric lymph nodes) muscle and skin. Enrichment of both the intracellular pool and protein-bound fraction of the tissues were determined. Intestinal worm burdens on day 48 post infection were significantly higher in the infected lambs ($P<0.001$), however there was no effect of parasitic infection on feed intake or liveweight gain ($P>0.15$). The specific radioactivity of valine in plasma intracellular pools and the protein-bound fraction of tissues was increased in the spleen during parasitic infection ($P<0.10$). Parasitic infection reduced the FSR of whole ileal tissue and skin by 4-13% ($P<0.15$), however no other tissues were affected. These results suggest that the presence of an established parasite burden in the small intestine did not alter the amino acid and energy requirements of tissues in the lamb.

3.2 Introduction

Impaired performance, such as reduced liveweight gain (Niezen *et al.*, 1998), wool production (Steel *et al.*, 1980; 1982) and milk production (Leyva *et al.*, 1982; Thomas & Ali, 1983) and decreased nitrogen (N) retention (Sykes & Coop, 1976) have been observed during parasitic infection in sheep. A reduction in feed intake may partly explain the decreases in animal performance in parasitised lambs, as the dietary supply of amino acids (AA) to the tissues will be reduced. However, reduced

N retention is observed even at similar feed intakes during parasitic infection and therefore reduced feed intake is not the only mechanism behind impaired animal performance. Reduced N retention may be due to increased AA catabolism by the liver, thus increasing urinary N excretion (Sykes & Coop, 1976). Increased losses of endogenous protein in the gastrointestinal tract (GIT; Kimambo *et al.*, 1988) may also account for reductions in N retention.

Increased endogenous protein losses into the GIT during parasitic infection include increased mucus production (Cheema & Scofield, 1982), plasma leakage of serum proteins (Kimambo *et al.*, 1988) and sloughed cells due to the damage caused by the parasites tunnelling into the intestinal crypts and the villi epithelium (Coop & Angus, 1975; Holmes, 1985). Some of these protein losses are likely to affect AA metabolism and protein turnover of the tissues within the GIT and thus increase the AA requirement of the GIT. For example, infection with *Trichostrongylus colubriformis* in guinea pigs increased fractional protein synthesis rates (FSR; proportion of the protein pool re-synthesised per day) in the large intestine by 40% (Symons & Jones, 1983), which may be due to the increased mucus production by this tissue observed in other studies (Cheema & Scofield, 1982). In another study, parasitic infection increased the oxidation of leucine in the GIT of sheep by almost 20-40%, which together with increased leucine sequestration to these tissues, had the net effect of decreasing its availability to other tissues (Yu *et al.*, 2000).

The increase in nutrient requirements for the immune response during parasitic infection are likely to be partly responsible for the increased FSR in the liver which has been observed during a *T. colubriformis* infection (Symons & Jones, 1971; 1978; Jones & Symons, 1982). This increase in FSR has been linked to increased synthesis of export proteins such as the acute phase proteins (Stehle & Furst, 1983) which are necessary for mounting an immune response to the parasitic infection. Increased FSR in the liver may also be due to the increase in synthesis of serum proteins that are secreted into the bloodstream, such as albumin, which leak into the GIT during parasitic infections (Poppi *et al.*, 1986).

Endogenous protein secretions in the GIT are rich in AA such as cysteine (MacRae & Lobley, 1991), threonine, serine and proline (Roberton *et al.*, 1991; Van Klinken

et al., 1998) compared to muscle proteins (MacRae *et al.*, 1993). Similarly, the activated immune tissues (e.g., liver, lymph nodes) have an increased requirement for AA such as cysteine, glutamine, threonine and valine (Stehle & Furst, 1983; Grimble, 1990; Souba, 1991; Reeds *et al.*, 1999) which are also disproportionately lower in muscle protein. The higher AA requirements by the GIT, liver and immune tissues during infection is further exacerbated when dietary intake is reduced (MacRae & Lobley, 1991; MacRae *et al.*, 1993). Therefore, to meet the increased requirements by the GIT, liver and activated immune tissues during infection, proportionally more muscle protein needs to be mobilised to supply these increased demands (MacRae & Lobley, 1991). Amino acids released by muscle protein degradation that are not required for gastrointestinal, hepatic and immune protein synthesis are likely to be catabolised and therefore represent a further loss of AA to the animal.

Increased mobilisation of AA from muscle protein is in agreement with results observed during parasitic infection. For example, muscle protein synthesis decreased and degradation increased in sheep given a gelatin capsule containing 60 000 infective *T. colubriformis* larvae (Symons & Jones, 1975). Decreased muscle protein synthesis also occurs in mice and guinea pigs given *T. colubriformis* (Symons & Jones, 1971; 1972; 1978).

The hypothesis of this study was that parasitic infection alters tissue protein synthesis and the partitioning of AA (and other nutrients) between the GIT, liver and muscle, in order to provide AA to the GIT, liver and activated lymphoid tissues for the repair of damaged GIT tissues and the metabolic and immune responses occurring during parasitic infection (Coop & Kyriazakis, 1999; Butter *et al.*, 2000; Coop & Sykes, 2002). Therefore, the aim of this study was to determine the effects of parasitic infection on the FSR (% d^{-1}) in the small intestine (duodenum and ileum), liver, the productive tissues (muscle and skin) and the lymphoid tissues (spleen, thymus and mesenteric lymph nodes) in order to assess any shifts in protein synthesis that may have occurred. This was achieved by utilising the infusion of [3, 4-^{3}H]-valine. Valine was used as part of the larger study because dietary CT can significantly increase the absorption of this amino acid from the small intestine (Waghorn *et al.*, 1987).

3.3 Materials and Methods

3.3.1 Animals and Feed

Wether lambs, were prepared with permanent indwelling catheters in TSP and hind limb blood vessels for infusion and blood sampling as described in Chapter Two to measure AA metabolism, valine kinetics and protein metabolism in the mesenteric-drained viscera (MDV), portal-drained viscera (PDV), liver, TSP and hind limbs. The FSR data was calculated from the incorporation of [3, 4-^{3}H]-valine into tissue protein and are presented in this chapter.

Four days after surgery, the lambs were offered fresh Lucerne (*Medicago sativa*; *c.* 800 g dry matter (DM) d^{-1}) at hourly intervals until the conclusion of the trial as described in Chapter Two. The lambs were weighed at the same time each week to monitor liveweight change during the experimental period.

The infection protocol in this study is described in Chapter Two, but briefly, one week after surgery (day 1 of the experimental period) six sheep were orally given 6 000 *T. colubriformis* L3 larvae daily for 6 d (parasite treatment) while the remaining six sheep were left untreated to serve as controls (control treatment).

3.3.2 Parasitology

Faecal egg counts (FEC) from individual sheep were determined every second day from day 20 to day 45 of infection using the modified McMaster method (Whitlock, 1948) as described in Chapter Two.

3.3.3 Infusions and sampling

On day 45 the sheep received a continuous 8 h infusion of [3, 4-^{3}H]-valine (7.6 MBq h^{-1}; Amersham Life Science, Buckinghamshire, UK) into the jugular vein, for measuring the specific radioactivity (SRA) of valine in the plasma (mesenteric artery), intracellular pool (tissue free pool) and tissue proteins.

The SRA of valine in plasma was calculated in blood samples (30 mL) that were withdrawn continuously in 2 h increments from the mesenteric artery, the mesenteric, portal and hepatic veins, and the vena cava over the infusion period. After each 2 h

sampling period, the syringes were removed from the collection lines and carefully mixed by gentle rotation. Packed cell volume (haematocrit) and concentrations of O_2 and CO_2 were determined as described in Chapter Two. The remaining whole blood was processed as described in Chapter Two for further analysis as described in section 3.3.4 or in Chapters Two and Four. Valine SRA measured in plasma obtained from the mesenteric artery was used as a precursor pool in the calculation of tissue FSR, and is therefore presented in this chapter. All the other data relating to valine kinetics are presented in Chapter Four. The plasma valine SRA data presented in this chapter represent the average of a 2h-intergrated sample taken from 6 to 8 h after the start of infusion.

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 (300 mg mL^{-1}; 0.5 mL kg^{-1} liveweight). 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. The tissue was washed in 0.9% NaCl to remove traces of digesta or blood, and quickly frozen in liquid nitrogen. A section of whole duodenum and ileum were kept and frozen as described above, with a further section of each tissue separated into the smooth muscle component of duodenal or ileal tissue by gently passing a glass slide over the tissue to remove the mucosal layer. Once frozen the tissue samples were stored at -85°C until analysed. Time from death until storage of all tissues in liquid nitrogen was less than 20 min.

3.3.4 Analytical methods

In order to calculate the FSR of tissue protein, the amount of [3, 4-^{3}H]-valine in the precursor pools (plasma and intracellular SRA valine) and the incorporation of [3, 4-^{3}H]-valine into the protein-bound fraction of the duodenum (whole and scraped), ileum (whole and scraped), mesenteric lymph nodes, spleen, liver, thymus, muscle (*biceps femoris*) and skin were measured.

Total ^{3}H radioactivity was determined in plasma and infusates by mixing 50 μL of sample in 2 mL of scintillation mixture (Starcint, INSUS Systems) and counting for 10 min in a Packard Tricarb Model 1500 Scintillation counter as described by Lee *et*

al. (1999). The proportion of total ^{3}H radioactivity attributed to valine and its breakdown product (H_2O) were determined by inline flow on an online detector through a liquid scintillation counter (Model 2, β-ram, IN/US systems Inc., New Jersey, US) coupled to high performance liquid chromatography (HPLC; LC4A, Shimadzu, Kyoto, Japan) as described in Lee *et al.* (1999). The concentration of valine in plasma was determined as described in Chapter Two.

Subsamples (4-5 g) of frozen tissue were pulverised in liquid N using a modified French Cell press as described by Lee *et al.* (1993). The pulverised tissue was then stored at -85°C until further processing. Approximately 1 g of smashed tissue was homogenised in extraction buffer (20 mM Tris pH 7.8; 2.5 mM EDTA; 0.3% SDS; see appendix C.1 for the extraction method). The homogenate was centrifuged at approximately 28 000 g at 4°C for 30 min, the supernatant containing intracellular peptides, AA and soluble protein-bound AA was removed and the resulting pellet (protein bound fraction) re-washed in extraction buffer and centrifuged for a further 30 min in order to determine the total ^{3}H counts associated with valine in the protein-bound pool as described earlier. The pellet was freeze-dried and stored at room temperature until analysed for AA concentration.

Amino acid concentration in the protein-bound fraction was determined by ion exchange chromatography (Shimadzu Scientific Instruments Ltd., Columbia, MD 21046, USA) with a post-column reaction system using ninhydrin as the derivatising agent after acid hydrolysis of a 50 mg pellet with 6.0 M HCl at 110°C for 22 h (see Chapter Two for full details). The hydrolysates were filtered and rotary evaporated to near dryness, washed in deionised water (DI) and rotary evaporated again before being taken up into 0.2 M sodium citrate buffer (pH 2.2). Total ^{3}H radioactivity of the hydrolysate and the proportion of ^{3}H radioactivity attributed to valine and its breakdown product (H_2O) were determined as described for plasma valine.

The supernatant (free pool AA fraction) removed from the pellet was frozen at -85°C until analysis. Free pool supernatant (2 mL) was mixed with 1 mL of 0.75% (9 mM) SDS/EDTA to break up the protein structures in the supernatant, 200 μL of 80 mM DTT (pH 8.0) as an antioxidant and 100 μL of 3 mM norleucine as an internal standard. The samples were mixed and left to stand at room temperature for 15 min

and then deproteinised with 1 mL of 30% trichloroacetic acid and centrifuged at 4°C at 3270 g for 15 min. The resulting supernatant containing free pool AA was stored at -85°C until analysed for AA concentration and total radioactivity as described for plasma valine.

3.3.5 Calculations

The SRA (dpm μmol^{-1}) of valine in plasma (SRA_P), tissue free pool (SRA_I), and tissue proteins (SRA_B) was calculated by dividing the radioactivity of the sampled pool (dpm mL^{-1}) by the concentration in the sampled pool ($\mu mol\ mL^{-1}$). Both the free pool and arterial plasma valine SRA were used as precursor pools for the estimation of FSR.

The traditional equation for the estimation of tissue protein FSR was not used as this equation requires the estimation of the rate-constant describing the rise to plateau of SRA of free valine in plasma. This can be achieved by performing frequent intermittent blood samples during the course of the labelled-AA infusion (see Appendix C.2). In this current study, plasma samples were harvested continuously over 2-h increments at 2, 4, 6 and 8 h after the start of infusion and the SRA of free valine in each sample represents the average valine SRA over the 2-h collection period. Therefore, the accurate rise to plateau of valine SRA in plasma cannot be estimated. Instead the FSR of proteins in whole and scraped duodenum, whole and scraped ileum, liver, mesenteric lymph nodes, spleen, thymus, muscle and skin samples were determined according to Equation 3.1 (Wykes *et al.*, 1996):

Equation 3.1

$$FSR\ (\%\ d^{-1}) = \frac{SRA\ Valine_{(protein\ bound)}}{SRA\ Valine_{(precursor\ pool)} * \text{period of infusion (d)}} * 100$$

where the period of infusion equals 8 h or 0.33 d.

The SRA of valine in the free pool of each tissue was assumed to reflect the steady-state SRA of the true precursor pool, valine-tRNA, for protein synthesis.

These FSR estimates were also compared to the FSR obtained using the SRA of free valine in arterial plasma (mesenteric artery) as a precursor pool.

As isotopic steady state was achieved in arterial plasma between 6-8 h of infusion, the FSR estimates calculated using Equation 3.1 will underestimate the actual rate of FSR. This equation assumes that the SRA of plasma valine is constant from the start of the labelled AA infusion (which can be achieved by giving a priming dose of the same AA). The extent to which the FSR is underestimated will depend on how rapidly the SRA of free valine reaches plateau.

3.3.6 Statistical analysis

Statistical analysis was performed using a General Linear Model (SAS version 8, 1999) to analyse the data according to a completely randomised block design, with treatment (control or infected) and group (when the animal underwent surgery) used as sources of variation in the model. Probability values lower than 0.05 were considered to indicate a significant difference and values between 0.05 and 0.15 to indicate a trend. Results are presented as least squares means (LS means) and associated pooled standard deviation (SD).

The data was checked for normality using a univariate analysis, whilst the Levene test was used to test for homogeneity of variance. No data showed deviation from normality or homogeneity, thus no transformation was required, with probability less than 0.01 used as the determinant for acceptance or rejection of the normality and homogeneity tests.

3.4 Results

Results relating to the worm burdens, feed intake and liveweight changes are presented in Chapter Two. *Trichostrongylus colubriformis* burdens in the small intestine were significantly higher in the infected lambs (440 *vs.* 21 740 (SD 1 101) worms in the control and parasite lambs, respectively; $P<0.15$). However, the presence of parasites in the small intestine did not have any impact on feed intake over the course of the experiment (826 *vs.* 796 (SD 18) g DM d^{-1} in the control and parasite lambs, respectively; $P=0.15$). There was no adverse affect of parasitic

infection on liveweight gain (240 *vs.* 190 (SD 100) g liveweight d^{-1} in the control and parasite lambs, respectively; P>0.10).

3.4.1 Specific radioactivity of valine

The SRA of valine in plasma (SRA_P) reached plateau between 6 to 8 h after the start of infusion (Figure 3.1). During this period, SRA_P averaged 113.7 *vs.* 106.5 (SD 11.0) dpm $nmol^{-1}$ in the control and parasite lambs, respectively (P>0.10). The SRA of tissue free pool valine (SRA_I) for each tissue are presented in Tables 3.1, 3.2 and 3.3. With the exception of the spleen where SRA_I increased in parasitised lambs (P<0.15; Table 3.2) there was no effect of parasitic infection on the SRA_I of other tissues. The SRA_I was generally lower than the SRA_P in all tissues with the exception of scraped duodenal tissue (Table 3.1) and muscle (Table 3.3), where SRA_I was similar to the SRA_P. The SRA of valine in the protein-bound fraction (SRA_B) for each tissue was unaffected by the presence of parasitic infection (P>0.15; Tables 3.1 and 3.2).

3.4.2 Fractional protein synthesis rates

The presence of parasitic infection in lambs only affected FSR in the whole ileum, muscle and skin (Tables 3.1, 3.2 and 3.3). In whole ileal tissue, FSR_I tended to decrease (P<0.15) in the presence of parasite infection from 49 to 36% (Table 3.1), however there was no effect of parasitic infection (P>0.15) when arterial valine was used as a precursor pool (FSR_P; Table 3.1). Similar trends were observed in the skin, which showed a reduction in FSR_I (P<0.15) but no effect on FSR_P (P>.15; Table 3.3). Muscle FSR_I tended to increase during parasitic infection (P<0.15; Table 3.3), however in FSR_P this was not significant (P>0.15).

The FSR calculated using arterial plasma as the precursor pool was not affected by the presence of parasite infection (P>0.15), but overall FSR_P resulted in a lower estimation of FSR, compared to FSR_I estimates (Tables 3.1 to 3.3).

Figure 3.1 The rise in plasma specific radioactivity (SRA; dpm $nmol^{-1}$) of valine over an 8 hour infusion period in lambs fed Lucerne (*Medicago sativa*). Each point represents the average of plasma valine SRA during a 2 h continuous collection.

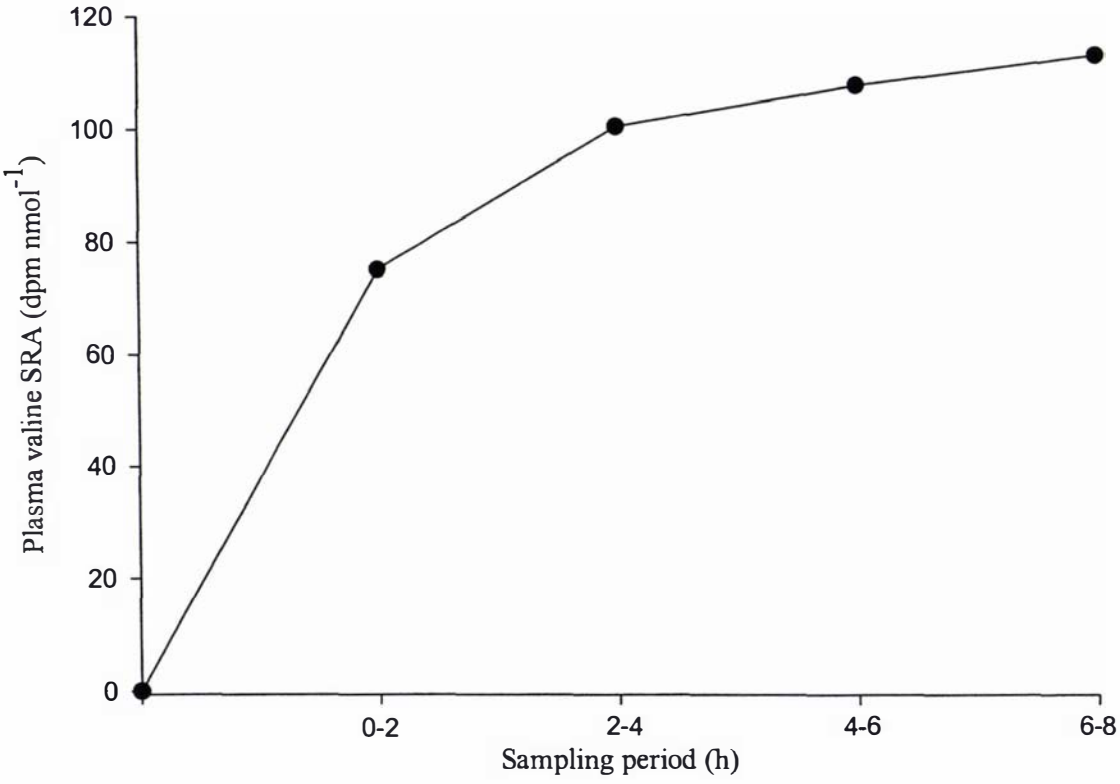

Table 3.1 Specific radioactivity of valine tissue free pool (SRA_I; dpm $nmol^{-1}$) and tissue bound protein (SRA_B; dpm $nmol^{-1}$) and fractional protein synthesis rates (FSR_P or FSR_I; % d^{-1}) in the duodenum and ileum of lambs fed fresh Lucerne (*Medicago sativa*) with a *Trichostrongylus colubriformis* infection or kept as parasite-free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=4	Pooled SD	P
Duodenum scraped	SRA_I	111.7	101.6	55.0	0.81
	SRA_B	3.3	3.5	0.6	0.62
	FSR_P	8.6	9.0	0.9	0.50
	FSR_I	9.2	10.5	3.8	0.64
Duodenum	SRA_I	50.9	85.7	33.6	0.22
	SRA_B	6.7	7.8	1.5	0.35
	FSR_P	18.7	19.8	2.0	0.48
	FSR_I	53.6	32.9	25.9	0.33
Ileum scraped	SRA_I	64.3	102.9	57.7	0.42
	SRA_B	2.0	1.6	0.9	0.59
	FSR_P	5.1	4.2	1.7	0.50
	FSR_I	8.4	6.5	4.2	0.55
Ileum*	SRA_I	65.1	77.3	22.2	0.45
	SRA_B	6.6	5.6	1.7	0.44
	FSR_P	17.8	14.5	3.2	0.18
	FSR_I	48.5	35.6	9.1	0.08

*n=5 for the parasite group

Table 3.2 Specific radioactivity of valine tissue free pool (SRA_I; dpm $nmol^{-1}$) and tissue bound protein (SRA_B: dpm $nmol^{-1}$) and fractional protein synthesis rates (FSR_P or FSRI; % d^{-1}) in the liver and lymphoid tissues of lambs fed fresh Lucerne (*Medicago sativa*) with a *Trichostrongylus colubriformis* infection or kept as parasite-free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=5	Pooled SD	P
Liver	SRA_I	73.1	90.9	51.2	0.63
	SRA_B	5.9	5.6	2.1	0.84
	FSR_P	16.6	14.5	6.0	0.63
	FSR_I	37.4	17.5	24.1	0.27
Mesenteric lymph nodes	SRA_I	57.1	53.0	11.3	0.61
	SRA_B	6.4	5.5	2.1	0.56
	FSR_P	17.6	14.2	4.0	0.26
	FSR_I	34.7	30.8	11.0	0.61
Spleen	SRA_I	35.3	48.5	9.0	0.07
	SRA_B	3.6	3.7	1.6	0.94
	FSR_P	9.7	9.4	3.6	0.90
	FSR_I	31.3	24.2	10.9	0.37
Thymus	SRA_I	68.4	39.6	26.6	0.27
	SRA_B	4.8	5.2	2.1	0.81
	FSR_P	12.8	14.3	4.4	0.69
	FSR_I	24.7	34.2	14.9	0.45

Table 3.3 Specific radioactivity of valine tissue free pool (SRA_I; dpm $nmol^{-1}$) and tissue bound protein (SRA_B: dpm $nmol^{-1}$) and fractional protein synthesis rates (FSR_P or FSRI; % d^{-1}) in the muscle and skin of lambs fed fresh Lucerne (*Medicago sativa*) and with a *Trichostrongylus colubriformis* infection or kept as parasite-free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=5	Pooled SD	P
Muscle	SRA_I	98.4	109.6	51.3	0.76
	SRA_B	0.1	0.2	0.2	0.58
	FSR_P	0.2	0.7	0.4	0.17
	FSR_I	0.1	1.3	0.7	0.12
Skin	SRA_I	76.9	80.6	8.1	0.53
	SRA_B	2.0	1.5	0.9	0.42
	FSR_P	5.3	3.9	2.4	0.44
	FSR_I	12.3	8.7	3.2	0.14

3.4.3 Amino acid concentrations in tissue free pool and protein bound fraction

3.4.3.1 Free pool amino acid concentrations

The concentration of most of AA in the intracellular pool of tissues were not affected by the presence of *T. colubriformis* in the small intestine (P>0.15). Therefore, only the average concentration across the treatments are presented in Table 3.4 and when significant differences between control and parasite animals exist for specific AA those are indicated in the table and described fully in the text below. In the whole duodenal and ileal tissue and the mesenteric lymph nodes, the presence of parasites affected the concentration of several AA in the tissue free pool (P<0.15) and these concentrations are presented in Appendix C Tables C.1 to C.3.

The concentration of branched-chain AA (BCAA) in the free pool of whole duodenal tissue was lower in the parasitised lamb (P<0.15; Table 3.4), which was mainly due to a lower concentration of free pool valine (88.5 *vs.* 63.9 (SD 14.8) μmol L^{-1} in the control and parasite lambs, respectively; P<0.15). Parasitic infection also decreased the concentration of histidine, citrulline and alanine in the intracellular pool of whole duodenal tissue (P<0.15; see Appendix Table C.1).

Taurine concentration was increased in the intracellular pool of scraped duodenal tissue of parasitised lambs compared to that of the controls lambs (324.1 *vs.* 116.8 (SD 128.7) μmol L^{-1}; P<0.15), whilst the concentration of carnithine decreased with parasitic infection (28.8 *vs.* 14.7 (SD 8.9) μmol L^{-1} in the control and parasite lambs, respectively; P<0.15; Table 3.4).

Parasitic infection decreased the concentration of citrulline, alanine, glycine, proline, histidine, leucine, phenylalanine, threonine (Table 3.4 and Appendix Table C.2), essential AA (EAA) and BCAA in the intracellular pool of whole ileal tissue (P<0.15; Table 3.4). In scraped ileal tissue, parasitic infection increased the intracellular pool concentration of serine (72.2 *vs.* 107.6 (SD 12.9) μmol L^{-1} in the control and parasite lambs, respectively; P<0.05), whilst free pool citrulline concentrations were higher in the control lambs, compared to the parasitised lambs (87.1 *vs.* 31.0 (SD 34.4) μmol L^{-1}; P<0.10).

Table 3.4 Non-essential (NEAA) essential (EAA), branched-chain (BCAA) and total amino acid concentrations (μmol L^{-1}) in the intracellular pool in lambs fed fresh Lucerne (*Medicago sativa*). The presence of *Trichostrongylus colubriformis* had no effect on the concentration of most amino acids in the intracellular pool of most tissues so results are presented as pooled means and standard deviations across treatments. Where a significant difference caused by the presence of *Trichostrongylus colubriformis* did occur ($P<0.15$) this is indicated in the table (†).

	Carnithine*	Citrulline*	Hydroxy proline	Ornithine*	Taurine*	Alanine	Asparagine	Glutamine	Glycine
Duodenum	25.1 ± 4.6	60.8 ± 15.2†	28.2 ± 10.0	9.0 ± 2.2	442.7 ± 199.6	183.7 ± 55.4†	55.1 ± 12.8	243.5 ± 53.2	543.8 ± 146.0
Scraped duodenum	21.8 ± 8.9†	57.3 ± 57.3	ND	7.4 ± 4.5	220.4 ± 128.6†	96.3 ± 43.8	30.4 ± 11.3	158.9 ± 66.4	319.6 ± 140.0
Ileum	2477.3 ± 841.8	77.9 ± 27.3†	15.3 ± 5.2	20.2 ± 11.2	1220.1 ± 507.9	362.9 ± 45.0†	25.0 ± 9.7	1207.2 ± 356.8	247.7 ± 50.0†
Scraped ileum	ND*	59.0 ± 34.4†	25.2 ± 6.6	7.8 ± 2.3	483.4 ± 241.2	138.5 ± 49.8	39.1 ± 13.2	139.2 ± 35.8	ND
Liver	112.0● 50.5	134.7 ± 122.1	26.8 ± 20.9	197.4 ± 42.5	400.0 ± 124.7†	140.6 ± 24.7	28.4 ± 10.2	392.8 ± 93.3	1096.6 ± 157.5
Lymph nodes	26.9 ± 10.0	117.6 ± 43.4	18.8 ± 6.5	31.6 ± 35.9†	660.4 ± 191.4	104.2 ± 59.7	52.1 ± 25.6	289.9 ± 71.4	581.0 ± 113.3
Spleen	36.9 ± 12.8	99.1 ± 14.8	15.7 ± 4.6	16.9 ± 7.6†	492.2 ± 128.3	89.8 ± 18.9	38.4 ± 10.1	234.0 ± 41.8	482.3 ± 76.8
Thymus	55.9 ± 14.6	76.9 ± 44.9	18.0 ± 1.5†	8.0 ± 1.3	577.9 ± 355.8	139.9 ± 39.3	51.2 ± 7.8†	270.5 ± 146.0	334.7 ± 185.5
Muscle	ND	32.8 ± 17.5	26.8 ± 13.9	ND	231.9 ± 50.1	43.0 ± 5.4†	14.7 ± 4.9	71.0 ± 18.5	153.6 ± 42.8
Skin	23.4 ± 4.8	31.3 ± 19.0	8.7 ± 3.2	ND	83.6 ± 8.7†	34.6 ± 9.6	17.1 ± 2.6	118.7 ● 11.3†	175.1 ± 23.8
	Proline	Serine	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine
Duodenum	67.2 ± 37.8	130.6 ± 33.0	162.1 ± 50.4	21.3 ± 4.6†	30.2 ± 8.2	78.5 ± 28.7	191.5 ± 69.1	35.1 ± 13.1	46.9 ± 16.0
Scraped duodenum	40.2 ± 13.8	67.4 ± 32.1	67.8 ± 27.9	9.9 ± 3.8	15. ± 5.2	35.7 ± 14.4	84.4 ± 39.2	14.9 ± 4.9	19.9 ± 7.7
Ileum	53.2 ● 5.5†	63.3 ± 7.3	86.1 ± 22.4	27.8 ± 7.2†	24.1 ± 5.5	35.6 ± 8.5†	36.5 ± 10.0	10.9 ± 3.8	12.4 ± 2.9†
Scraped ileum	56.9 ± 17.5	89.9 ± 12.9†	84.8 ± 30.0	12.5 ± 2.5	20.9 ± 5.3	56.7 ± 15.5	145.7 ± 47.0	15.1 ± 5.1	25.3 ± 6.8
Liver	67.0 ± 10.5	65.6 ± 15.3†	ND	62.5 ± 14.6	31.6 ± 8.8	77.7 ± 18.4	93.6 ± 28.1	16.4 ± 9.2	29.1 ± 10.9
Lymph nodes	60.2 ● 21.6	65.4 ± 35.8	75.5 ± 23.3	31.7 ± 36.1†	33.2 ± 7.7	73.9 ± 27.1	227.1 ● 171.2†	25.3 ± 4.3	27.6 ± 9.2†
Spleen	44.9 ± 10.4	19.5 ± 20.4	59.7 ± 22.5	17.9 ± 3.8	24.0 ± 3.4	61.3 ± 10.2	82.0 ± 20.8	20.5 ± 3.3	22.4 ± 3.7
Thymus	70.1 ± 15.3	68.8 ± 32.4	74.0 ± 28.6	30.0 ± 30.3	23.0 ± 9.3	55.6 ± 23.2	113.0 ± 49.8	21.4 ± 4.3	25.6 ± 15.3
Muscle	15.4 ● 4.8	27.6 ± 7.1	26.2 ± 11.9	ND	10.1 ± 2.9	20.1 ± 8.6	42.1 ± 13.6	5.7 ± 1.9	9.9 ± 3.9
Skin	19.3 ± 3.2	47.7 ± 11.0	40.2 ± 7.1	ND	8.7 ± 1.4	14.4 ± 3.1	25.5 ± 6.2	5.8 ● 1.4	8.3 ± 1.7
	Threonine	Tryosine	Valine	NEAA	EAA	BCAA	TOTAL		
Duodenum	75.7 ± 23.9	46.4 ± 17.7	76.2 ± 14.8†	1252.0 ± 348.6	763.8 ± 246.9	184.9 ± 51.8†	2015.8 ± 595.4		
Scraped duodenum	33.2 ± 17.5	18.3 ± 7.5	35.2 ± 14.4	712.9 ± 307.6	345.5 ± 142.8	86.2 ± 34.1	1047.4 ± 450.4		
Ileum	55.0 ± 7.6†	19.0 ± 3.3	66.5 ± 12.2	1974.6 ± 479.9	373.9 ± 83.9†	126.3 ± 26.4†	2348.5 ± 563.8		
Scraped ileum	57.9 ± 17.9	26.0 ● 6.8	53.7 ± 12.5	488.8 ± 136.1	498.5 ± 150	131.3 ± 33.5	987.4 ± 286.0		
Liver	ND	46.2 ± 21.0	80.7 ± 28.4	1817.7 ● 332.2	437.8 ± 139.3	190.0 ± 55.6	1255.57 ± 471.5		
Lymph nodes	42.7 ± 11.9†	38.2 ● 7.9	76.6 ± 21.5†	1711.6 ± 334.2	651.7 ● 320.7	183.6 ± 56.1	1823.4 ● 65.9		
Spleen	25.3 ± 6.2	27.6 ± 5.0	65.8 ● 12.6	942.5 ± 183.5	406.5 ± 92.0	151.1 ± 26.4	1311.0 ± 275.4		
Thymus	83.4 ± 35.8	33.1 ± 9.0	58.0 ± 17.7	953.2 ± 428.0	517.0 ± 223.8	136.6 ± 50.4	1470.3 ±651.8		
Muscle	ND	ND	23.8 ± 8.8	352.0 ± 97.4	137.9 ± 51.5	54.0 ± 20.3	489.9 ± 148.9		
Skin	ND	11.2 ± 2.8	30.3 ± 7.8	421.2 ± 64.6	133.2 ± 28.8	53.4 ± 12.3	544.4 ± 93.4		

ND*: not determined and omitted from NEAA or TOTAL estimates; NEAA = Hydroxy proline, Alanine, Asparagine, Glutamine, Glycine, Proline, and Serine; EAA = Arginine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tyrosine and Valine; BCAA = Isoleucine, Leucine, Valine; TOTAL = NEAA + EAA; † See text for details of what differences were.

In the mesenteric lymph nodes, parasitic infection decreased the intracellular concentration of histidine, threonine, ornithine and lysine, whilst valine and phenylalanine concentration increased (P>0.15; Table 3.4 and Appendix Table C.3). However there was no effect on non-essential (NEAA), EAA, BCAA or total AA concentrations (P>0.15; Table 3.4 and Appendix Table C.3).

In the spleen only the intracellular concentration of ornithine was affected by parasitic infection (10.8 *vs.* 23.1 (SD 7.7) μmol L^{-1} in the control and parasite lambs, respectively; P<0.10), however there was no effect of parasitic infection on NEAA, EAA, BCAA or total AA concentration (P>0.15; Table 3.4). In the thymus there was no effect of parasitic infection on NEAA, EAA, BCAA or total AA concentration (P>0.15; Table 3.4). However, parasitic infection increased the thymus intracellular concentration of hydroxyl-proline (13.3 *vs.* 22.8 (SD 1.5) μmol L^{-1} in the control and infected lambs, respectively; P<0.05) and asparagine (31.0 *vs.* 71.4 (SD 7.9) μmol L^{-1} in the control and parasite lambs, respectively; P<0.05; Table 3.4).

Liver intracellular serine concentration increased during parasitic infection (51.1 *vs.* 80.2 (SD 15.3) μmol L^{-1} in the control and parasite lambs, respectively; P<0.10) while the concentration of taurine was decreased (483.7 *vs.* 316.2 (SD 124.7) μmol L^{-1} in the control and parasite lambs, respectively; P<0.15; Table 3.4). The concentrations of NEAA, EAA, BCAA or total AA were not affected by infection (P>0.15; Table 3.4).

The concentrations of NEAA, EAA, BCAA or total AA in the muscle intracellular pool were not affected by parasitic infection (P>0.15; Table 3.4). However, alanine was increased in parasitised lambs (27.7 *vs.* 58.4 (SD 5.4) μmol L^{-1} in the control and parasite lambs, respectively; P<0.05; Table 3.4). Both the skin intracellular concentration of glutamine (129.5 *vs.* 108.0 (SD 11.3) μmol L^{-1} in the control and parasite lambs, respectively; P<0.10) and taurine (90.8 *vs.* 76.5 (SD 8.7) μmol L^{-1} in the control and infected lambs, respectively; P<0.10; Table 3.4) were reduced by parasitic infection (P<0.15; Table 3.4). The concentrations of NEAA, EAA, BCAA or total AA were not affected (P>0.15; Table 3.4).

3.4.3.2 Protein bound amino acid concentrations

The concentrations of AA in the bound protein of tissues (whole and scraped ileal tissue, liver, spleen or thymus) in most cases were unaffected by the presence of parasitic infection (P>0.15). Therefore, the average AA concentration across treatments are presented in Tables 3.5.

In whole duodenum parasitic infection reduced the concentration of BCAA from 216.7 to 153.2 mg AA g tissue^{-1} (SD 47.8; P<0.15). However, NEAA, EAA and total AA were not affected (P>0.15; Table 3.5). The concentration of glycine (83.4 *vs.* 65.8 (SD 15.0) mg AA g tissue^{-1} in the control and parasite lambs, respectively; P<0.15) and arginine (61.8 *vs.* 47.0 (SD 12.4) mg AA g tissue^{-1} in the control and parasite lambs, respectively; P<0.15) in the protein-bound fraction of whole duodenal tissue were also decreased. In scraped duodenal tissue, the parasitised lambs tended to have a higher concentration of most of the individual AA (P<0.15; Appendix Table C.4), however NEAA, EAA and total AA were unaffected (P>0.15; Table 3.5).

Cysteine concentration in the protein-bound fraction of the mesenteric lymph nodes were increased during parasitic infection (7.0 *vs.* 9.4 (SD 0.9) mg AA g tissue^{-1} in the control and parasite lambs, respectively; P<0.15). However, there was no effect of parasitic infection on the concentration of NEAA, EAA, or BCAA in the protein-bound fraction of the mesenteric lymph nodes.

Threonine, glutamate, isoleucine, lysine, and methionine concentration were all decreased in the protein-bound fraction of muscle in parasitised lambs (P<0.15; Appendix Table C.5). The concentration of NEAA, EAA and total AA in the protein-bound fraction of muscle were unaffected by parasitic infection (P>0.15; Table 3.5). Parasitic infection increased phenylalanine concentration in the skin (20.7 *vs.* 23.2 (SD 2.2) mg AA g tissue^{-1} in the control and parasite lambs, respectively; P<0.15), with no effect on NEAA, EAA, BCAA and total AA concentration (P>0.15; Table 3.5).

Table 3.5 Non-essential (NEAA) essential (EAA), branched-chain (BCAA) and total amino acid concentrations (g AA g tissue DM^{-1}) in the protein-bound fraction of tissues in lambs fed fresh Lucerne (*Medicago sativa*). The presence of *Trichostrongylus colubriformis* had no effect on the concentration of most amino acids in the intracellular pool of most tissues so results are presented as pooled means and standard deviations across treatments. Where a significant difference caused by the presence of *Trichostrongylus colubriformis* did occur ($P<0.15$) this is indicated in the table (†).

	Alanine	Aspartate	Cysteine	Glutamate	Glycine	Serine	Arginine	Histidine	Isoleucine	Leucine
Whole duodenum	47.2 ± 8.7	54.2 ± 10.7	7.2 ± 1.6	85.6 ± 17.5	74.6 ± 15.6†	29.8 ± 5.9	54.4 ± 12.4†	12.9 ± .7.0	19.7 ± 3.8	44.8 ± 12.3
Scraped duodenum	50.8 ± 9.5	55.2 ± 6.3†	6.6 ± 0.8†	89.6 ± 9.9†	92.0 ± 20.4	29.6 ± 3.0†	52.6 ± 7.1	13.0 ± 4.3	19.2 ± 3.5†	40.6 ± 11.6
Whole ileum	50.8 ± 4.7	55.1 ± 5.6	7.1 ± 1.4	87.1 ± 8.1	83.9 ± 12.7	31.1 ± 3.1	55.7 ± 3.7	13.4 ± 3.3	19.8 ± 2.4	44.1 ± 11.4
Scraped ileum	59.9 ± 10.3	58.4 ± 6.3	6.8 ± 1.0	95.5 ± 10.7	112.9 ± 17.8	31.6 ± 3.7	61.7 ± 12.5	12.1 ± 2.6	18.9 ± 2.5	41.5 ± 9.7
Liver	31.8 ± 4.2	47.3 ± 4.5	11.3 ± 2.0	63.4 ± 6.2	33.3 ± 3.2	26.5 ± 2.0	35.1 ± 3.4	14.7 ± 3.2	20.3 ± 2.4	40.8 ± 11.2
Lymph nodes	39.0 ± 6.5	43.9 ± 6.8	8.2 ± 0.9†	67.5 ± 10.2	47.3 ± 8.0	26.6 ± 4.1	48.0 ± 7.6	12.9 ± 3.2	18.5 ± 3.1	40.1 ± 10.9
Spleen	58.1 ± 5.2	62.7 ± 2.5	8.8 ± 0.6	85.2 ± 4.9	54.8 ± 8.1	34.1 ± 1.4	48.2 ± 2.4	24.2 ± 3.4	19.5 ± 2.4	61.4 ± 11.9
Thymus	48.3 ± 7.0	40.9 ± 3.8	5.0 ± 0.2	68.5 ± 5.8	62.4 ± 15.1	26.4 ± 3.2	53.6 ± 4.4	12.5 ± 2.1	17.5 ± 2.1	36.5 ± 7.1
Muscle	55.5 ± 5.9	86.4 ± 7.1	10.1 ± 0.6†	155.6 ± 11.5†	42.6 ± 10.2	39.1 ± 2.4	61.3 ± 3.3	18.6 ± 5.5	34.2 ± 3.5†	69.5 ± 17.7
Skin	62.5 ± 16.4	54.9 ± 11.1	16.6 ± 7.0	98.5 ± 13.9	134.1 ± 47.8	39.2 ± 4.4	64.4 ± 10.7	9.3 ● 3.3	15.9 ± 1.9	39.9 ± 9.9

	Lysine	Methionine	Phenylalanine	Threonine	Valine	NEAA	EAA	BCAA	TOTAL
Whole duodenum	42.5 ± 8.9	12.5 ± 2.7	23.0 ± 5.0	26.5 ± 5.5	24.4 ± 4.2	306.6 ± 62.9	259.5 ± 53.8	88.9 ± 18.5†	566.0 ± 115.3
Scraped duodenum	39.7 ± 4.9†	12.6 ± 1.3†	21.8 ± 2.3†	25.9 ± 2.6†	23.9 ± 3.7†	313.5 ± 46.0	252.0 ± 38.6	83.7 ± 15.6	565.5 ● 81.9
Whole ileum	43.2 ± 5.0	12.3 ± 1.8	22.9 ± 2.7	25.4 ± 2.8	24.9 ± 2.8	320.8 ± 22.2	265.2 ± 33.6	88.8 ± 14.9	586.0 ± 50.0
Scraped ileum	39.9 ± 4.3	12.9 ± 1.8	22.7 ± 2.6	25.1 ± 2.9	24.2 ± 2.8	321.4 ± 36.4	267.1 ± 29.9	84.6 ± 12.3	638.0 ± 74.2
Liver	39.0 ± 3.8	12.1 ± 1.0	25.6 ± 2.5	23.1 ● 2.0	24.8 ± 2.4	215.1 ± 19.0	236.3 ± 25.5	85.8 ± 11.8	451.4 ± 44.1
Lymph nodes	45.1 ± 6.5	10.2 ± 1.6	19.6 ± 3.1	23.6 ± 3.7	23.7 ± 3.8	243.6 ± 36.4	267.1 ± 29.9	84.6 ± 12.3	638.0 ± 74.2
Spleen	55.7 ± 1.6	14.3 ± 1.0	32.7 ± 2.1	31.7 ± 1.2	39.0 ● 4.4	243.6 ± 36.4	255.9 ± 40.6	82.2 ±15.5	499.5 ± 75.9
Thymus	56.0 ± 5.1	7.8 ± 1.8	17.1 ± 1.3	24.1 ± 1.7	22.8 ± 2.5	247.2 ± 35.1	249.4 ± 15.3	76.8 ± 5.7	496.6 ± 49.7
Muscle	76.6 ± 6.3†	25.8 ● 5.4†	34.4 ± 2.2	43.1 ± 3.2†	35.3 ± 3.3	418.2 ± 28.7	414.7 ± 40.8	139 ± 21.2	832.9 ± 64.3
Skin	33.7 ± 4.9	6.9 ± 2.6	21.9 ± 2.2†	24.1 ± 2.7	24.1 ± 3.4	432.2 ± 92.3	250.2 ± 35.4	79.9 ± 13.1	673.3 ± 123.8

NEAA = Alanine, Aspartate, Cysteine, Glutamine, Glycine, and Serine; EAA = Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine and Valine;

BCAA = Isoleucine Leucine, Valine; TOTAL = NEAA + EAA; † See text for details of what differences were.

3.5 Discussion

3.5.1 Fractional synthesis rates during parasitic infection

In the present study, the effects of an established *T. colubriformis* population in the GIT was not enough to elicit an increase in AA requirement of the GIT, liver and lymphoid tissues as indicated by similar rates of FSR between the parasitised and control lambs in most of the tissues measured. Although the presence of an established *T. colubriformis* infection in the GIT reduced FSR_I in whole ileal tissue and the skin, parasitic infection did not seem to result in the repartitioning of AA away from the muscle to the GIT and liver (Coop & Kyriazakis, 1999; Butter *et al.*, 2000; Coop & Sykes, 2002). There are several factors that must be taken into consideration including the infection regime and the period of sampling.

Trickle infections (i.e., a constant exposure to infective larvae), rather than a single large dose, are more representative of what occurs during farming practices (MacRae, 1993). The majority of the studies in sheep reported in the literature have involved long-term constant infection procedures (e.g., Steel *et al.*, 1980; Jones & Symons, 1982; Yu *et al.*, 2000), and this may be a reason for why we did not observe any repartitioning of AA away from the muscle to the GIT, liver and lymphoid tissue. The severity of the infection regimes is known to affect the rate of protein synthesis (Steel *et al.*, 1980), therefore it is possible that the level of infection in this study (36 000 L3 larvae over 6 consecutive d) was not enough to elicit sufficient damage to the GIT, or to increase the immune response to levels that are detectable by the experimental procedure used in this study. It is most probable that alterations in tissue FSR may have been apparent earlier in the infection, with changes in the GIT, liver and lymphoid tissue most likely to occur during parasite establishment in the GIT and the initial stimulation of the immune response (the first three weeks post infection).

Parasites cause damage to the duodenal tissues by tunnelling into the intestinal crypts and the villi epithelium (Coop & Angus, 1975; Holmes, 1985). Despite this, duodenal estimates were similar between the control and parasite lambs. This suggests that the level of parasite establishment in the duodenum was insufficient to cause an increase in the rate of protein turnover of this tissue. Alternatively it is

possible that the damage caused to the intestine occurs with the initial establishment of the larvae, and that the adult worm, once established has little effect on the protein turnover of the tissue.

The FSR_I for whole ileal tissue protein indicate that the synthesis of constitutive proteins in this tissue was reduced by 13% during parasitic infection. As the FSR of smooth muscle protein of ileal tissue was unaffected by the presence of parasites, this suggests less export protein is being re-synthesised per day. However, the latter was not measured in this study. This is in contrast to results by Symons & Jones (1983) who observed no change in small intestine FSR despite a 24% increase in daily protein synthesis in guinea pigs infected with *T. colubriformis*. In the current study, the weight of the small intestine was not measured therefore the rate of absolute protein synthesis could not be measured. Parasitic infection has been seen to increase the weight of the intestine in the guinea pig (Symons & Jones, 1983). However, as the concentration of most AA in the protein-bound fraction of duodenal and ileal tissues was not affected, the rate of absolute protein synthesis in the ileal tissue is likely to be reduced with parasitic infection.

Trichostrongylus colubriformis inhabit the first third of the small intestine, and therefore reasons for the reduction in ileal FSR_I are unclear. However, infections with the nematode *Nippostrongylus brasiliensis*, which also inhabit the proximal small intestine, have resulted in decreased glucose absorption (Scofield, 1980) and altered morphology and increased mucus production in the large intestine (Cheema & Scofield, 1982). These effects in the large intestine may be due to a thymus-dependant immune reaction (Ferguson & Jarrett, 1975), which is initiated by the presence of parasites in the small intestine and results in altered the functional integrity of subsequent sections of the GIT (Symons, 1978). However, this explanation seems unlikely as the FSR of tissues involved in the immune response such as the mesenteric lymph nodes (Tortora & Grabowski, 1996) was unaffected by parasitic infection in the current study.

The FSR in the liver was unaffected by the presence of parasitic infection, 48 d post infection, suggesting that there was no effect of parasitic infection on the synthesis of hepatic constitutive proteins. In previous studies, parasitic infection has increased

the rates of liver protein synthesis in sheep (Symons & Jones, 1975; Jones & Symons, 1982) and guinea pigs (Symons & Jones, 1971; 1978). However, the infection procedures utilised by these studies differed from the current study. For example, in the Jones & Symons (1982) study sheep were exposed to a constant trickle infection, whilst Symons & Jones (1975) gave their sheep a single dose of 60 000 larvae. The effects of increasing larval dosage rates on protein synthesis was investigated by Steel *et al.* (1980), who observed differences in protein synthesis in the liver and muscle only at the highest rates of infection (30 000 larvae per week for 17 weeks). It is possible that alterations in the protein turnover rates in the liver would have been observed at an earlier stage of parasite establishment such as when the lamb was first infected with *T. colubriformis*, and when faecal egg production was at its highest point during the infection (around 26 d post infection; Figure 2.1; Chapter Two).

There was no effect of parasitic infection on liveweight changes over the experiment. However, the presence of parasite infections increased the FSR_I in muscle in this study, which is in contrast to previous observations that indicate a decrease in muscle protein synthesis during parasitic infection (Symons & Jones, 1971; 1972; 1975; 1978). The estimates of muscle FSR in this study are lower (less than 1%) compared to those of other studies (Appendix Table C.6) and this suggests that the infusion length of the ^{3}H-valine may have been too short to enable appreciable incorporation of this AA into skeletal muscle proteins. An 8 h constant infusion of labelled leucine was used in comparable studies in lambs (Davis *et al.*, 1981; Schaefer *et al.*, 1986), resulting in FSR estimate of 2-5%. However, in both these studies the sheep were fed at higher levels of intake compared to the restricted level of feed intake used in the present study, which results in increased estimates of FSR (Oddy *et al.*, 1987; Lobley *et al.*, 1994). Therefore, caution must be taken when interpreting the effect of parasitic infection on muscle FSR in the current study.

In this study, parasitic infection decreased skin FSR_I, which is consistent with previous studies in sheep (Symons & Jones, 1975). Decreases in skin protein FSR may be responsible for the reduction in wool production that have been observed during parasitic infection (Steel *et al.*, 1980; 1982), and may be the result of partitioning of key AA for wool growth (e.g., cysteine) away from skin metabolism

towards the immune response (Miller *et al.*, 2000). However, in this study there was no effect of parasitic infection on the tissues involved in the immune response (e.g., liver, mesenteric lymph nodes, spleen and thymus), so this repartitioning of cysteine away from skin metabolism is unlikely.

3.5.2 Precursor pools for estimating protein fractional synthesis rates

The use of arterial plasma rather than tissue free pool as a precursor pool for calculating FSR resulted in a lower estimation of FSR. This finding has previously been noted in sheep (Schaefer *et al.*, 1986; Liu *et al.*, 2000) and goats (Baracos *et al.*, 1991). For example, the use of plasma leucine SRA as a precursor for calculating protein FSR resulted in an estimate of 12% d^{-1} for the liver, whereas using the free pool leucine SRA increased the FSR estimate to 21% d^{-1} (Schaefer *et al.*, 1986). Similar differences between these two estimates have been observed in this study.

The acylation of AA to AA-tRNA is the immediate precursor for protein synthesis inside the cell (Davis *et al.*, 1999), however tRNA are difficult to measure due to their short half-life and low concentration in some tissues (Ljungqvist *et al.*, 1997). Therefore, more easily sampled pools are often utilised as a precursor pool for estimating tissue protein synthesis. It has been suggested that the free pool of AA during a constant infusion is the best predictor of AA-tRNA, and is therefore the best estimator of the immediate precursor pool (Baumann *et al.*, 1994; Ljungqvist *et al.*, 1997). However, other authors suggest that AA-tRNA enrichment is intermediate of plasma and tissue free pool enrichments (Watt *et al.*, 1991; Young *et al.*, 1994) and using the plasma as a precursor pool will result in an underestimation of protein synthesis, while using the free pool will overestimate protein synthesis.

However, the best prediction of the enrichment of the tRNA precursor pool is a function of the nature of the protein being synthesised i.e., constitutive or export protein. For example, secretory proteins are synthesised on the rough endoplasmic reticulum in the cell, and probably channel AA as they enter the cell from plasma (Connell *et al.*, 1997). Therefore, the plasma pool is likely to be the true precursor for the synthesis of export protein. Tissues such as the duodenum, ileum and liver are involved in the synthesis of export proteins, such as mucin and acute phase

proteins. However, FSR_I estimates only include the rate of synthesis of constitutive proteins and therefore the use of the intracellular precursor pool is likely to underestimate the FSR in these tissues (Davis *et al.*, 1999).

Constitutive proteins are also synthesised within the cell cytosol (Connell *et al.*, 1997) and AA precursors for their synthesis include arterial AA entering the intracellular pool, AA released from the breakdown of tissue protein (Smith & Sun, 1995) and/or from *de novo* synthesis (NEAA only). The muscle and skin synthesise mostly constitutive proteins, and therefore the intracellular pool is likely to be the best predictor of protein synthesis. Therefore, the decrease in FSR_I observed in the skin of parasitised lambs will be the best estimation of FSR.

3.5.3 Tissue protein turnover; comparison with the literature

A summary of FSR estimates from other studies are presented in Appendix Table C.6 showing a range of FSR estimates for various tissues. Values presented in this study, with the exception of muscle FSR, are similar to those reported for the dry goat (Baracos *et al.*, 1991), lamb (Buttery *et al.*, 1977; Davis *et al.*, 1981; Oddy & Lindsay, 1986), sheep (Schaefer *et al.*, 1986; Connell *et al.*, 1997) and cattle (Lobley *et al.*, 1980) using constant infusion of labelled AA such as leucine, lysine and phenylalanine. However, these estimates are lower than those reported for the rat under a constant infusion (Garlick *et al.*, 1973) or flooding dose protocol (Garlick *et al.*, 1980; Appendix Table C.6). Protein turnover is positively correlated to metabolic mass, and therefore the rat has a higher tissue FSR than larger mammals (Waterlow, 1984). Muscle FSR in this study are lower than those presented elsewhere in the literature for similar infusion protocols (e.g., Davis *et al.*, 1981), and reasons for this are unclear, although the restricted feeding (800 g DM d^{-1}) may explain the discrepancies.

Overall, for most tissues, the estimates of FSR vary substantially, and this might be explained by the approach chosen to estimate the protein FSR (i.e., infusion length, constant infusion *vs*. flooding dose) and may also be influenced by the type of tissue, and the nature of the proteins that are synthesised within the tissue. The constant infusion method is more suited to tissues with low rates of protein turnover, such as

muscle and skin because the longer infusion times are required to label the tissues with slow turn over (Davis *et al.*, 1999). The flooding dose, which consists of injecting the animal with a single large dose of labelled AA is more suited to tissues with high rates of protein turnover and/or that secrete large amounts of protein. With this method small changes in protein synthesis can be measured over short periods of time and the effect of label re-cycling within the tissue is negligible (Davis *et al.*, 1999).

For example, the liver exports a large proportion of the protein it synthesises (30-35%; Connell *et al.*, 1997), and therefore estimates of FSR obtained using a constant infusion may underestimate true FSR and will contribute to the large variation in liver FSR observed between experiments (Davis *et al.*, 1981). This occurs because 'labelled' protein lost in the secretory proteins over the 8 h infusion period are not measured using the constant infusion method for estimating FSR. Recycling of tracer from protein degradation which may occur during the constant infusion may also affect the enrichment of the free pool in the tissue (Davis *et al.*, 1999). Tissues with high rates of turnover (e.g., small intestine) may have lower estimates of FSR during continuous infusion due to tracer recycling and the secretion of labelled protein during turnover (James *et al.*, 1971; Baracos *et al.*, 1991), however the latter was not measured in this study.

The heterogeneity of the proteins within the tissue under investigation is also of importance (Schaefer *et al.*, 1986). For example, differences in protein FSR in the same animal have been observed when different tracers of AA have been used, with the infusion of L-[3-^{13}C]-cysteine, L-[ring-d_5]-phenylalanine and L-[2,3,3-d_3]-serine resulting in cysteine giving the largest estimate of FSR_P in the skin of merino lambs, followed by phenylalanine and finally serine ($P<0.05$; Liu *et al.*, 2000). Reasons for these differences were thought to be due to different components in the skin (e.g., skin, wool) not being synthesised in equal amounts (Liu *et al.*, 2000). The AA composition of the skin and wool also differ (MacRae *et al.*, 1993) and this may also impact on the estimate of FSR.

3.6 Conclusions

The presence of an established *T. colubriformis* infection decreased the FSR in the skin and whole ileal tissue. Reasons for the decrease in skin FSR are unclear, but may result from the partitioning of AA to the skin within the hind limbs. The decrease in whole ileal tissue FSR may be due to a decrease in the amount of export protein being synthesised. There was no effect of parasite infection on tissues involved in immune responses (liver, mesenteric lymph nodes, spleen and thymus) which suggests that there was no stimulation of the immune response in these lambs 48 d post infection. As there was no effect of parasitic infection on liveweight gain over the course of the experiment and no reliable effect on muscle FSR, this suggests that an established parasite infection does not result in a repartitioning of AA from the muscle to the GIT liver and/or other tissues involved in metabolic changes including the immune response. These data are supported by those presented in Chapter Two. It is likely that alterations in tissue protein FSR may have been detected at earlier stages of parasite infection, with the most critical periods likely to be the initial infection (days 1-6) or when faecal egg counts were at their highest (day 26 post infection).

3.7 Acknowledgements

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4 THE EFFECT OF A *TRICHOSTRONGYLUS COLUBRIFORMIS* INFECTION ON VALINE KINETICS IN LAMBS FED FRESH LUCERNE (*MEDICAGO SATIVA*).

4.1 Abstract

The effect of a *Trichostrongylus colubriformis* infection on valine kinetics in the mesenteric-drained viscera (MDV), portal-drained viscera (PDV), liver, total splanchnic tissues (TSP) and the hind limbs were determined in lambs fed fresh Lucerne (*Medicago sativa*; 800 g DM d^{-1}) on day 48 post infection. Lambs were dosed with 6 000 L3 *T. colubriformis* larvae for 6 days (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 peaked on day 26 post infection ($P<0.001$). Forty-five d post infection, the lambs received a bolus injection of D_2O (0.61 mL kg^{-1} body weight) into the jugular vein to measure the size of the whole body water pool. On day 48 post infection, the lambs were continuously infused for 8 h with dual isotopes of valine: [3, 4 -^{3}H]-valine (7.6 MBq h^{-1}) into the jugular vein and concurrently, [1-^{13}C]-valine (99 atom percent, 118 mg h^{-1}) was continuously infused into the abomasum. Para-amminohippuric acid (PAH; 750 mg h^{-1} into the mesenteric vein) and indocyanin green (16 mg h^{-1} into the abdominal aorta) were also continuously infused for 8 h in order to measure blood flow across the TSP and hind limbs, respectively. Blood was continuously collected from the mesenteric, portal and hepatic veins, the mesenteric artery and the vena cava. Plasma was harvested, amino acid (AA) and metabolite concentrations measured and the specific radioactivity (SRA) and isotopic enrichment (IE) of valine and water determined. After the 8 h infusion the lambs were euthanased and samples collected from the ileum and muscle for determination of valine SRA and IE in intracellular and protein-bound pools. Intestinal worm burdens on day 48 post infection were significantly higher in the infected lambs ($P<0.0001$), however there was no effect of parasitic infection on feed intake or liveweight gain ($P>0.15$). There was little effect of parasite infection on valine concentration, SRA or IE in plasma, ileal digesta or the muscle free pool. The alterations in the irreversible loss (ILR) of valine across the MDV suggest that the parasite burden did affect AA metabolism and/or protein turnover at the site of the parasitic burden. The alterations in PDV kinetics with parasitic infection reflect mostly the changes seen in the MDV tissues and that is consistent with the small intestine being the region of the gastrointestinal tract (GIT) where *T. colubriformis* reside. The data on valine kinetics in the liver suggest that there may have been follow-on impacts on valine availability to the peripheral tissues as the valine ILR in

the liver was increased in parasitic lambs. However, parasite infection had no effect on the estimates of the rates of net flux and trans-membrane transport of valine and on the rates of protein turnover (estimated from valine ILR) and protein degradation across the hind limbs. These results suggest that the presence of an established parasitic infection in the small intestine did not result in re-partitioning of AA from the posterior hind limbs to the GIT and liver. It is likely that protein turnover is increased in the TSP tissues as a consequence of higher protein synthesis (and/or AA oxidation) and degradation during parasitic infection.

4.2 Introduction

Parasitic infection can increase protein synthesis in the gastrointestinal tract (GIT; Symons & Jones, 1983) and liver (Symons & Jones, 1971; 1978; Jones & Symons, 1982). A decrease in muscle protein synthesis and increase in muscle protein degradation (Symons & Jones, 1971; 1972; 1975; 1978) have also been observed during parasite infection. These alterations suggest that during an intestinal parasite infection, the animal catabolises its own body reserves in order to meet the additional nutrient requirements of the GIT and liver (Parkins & Holmes, 1989; MacRae, 1993; Coop & Kyriazakis, 1999; Butter *et al.*, 2000; Coop & Sykes, 2002). This catabolism of body reserves may partly explain the negative impacts of parasite infection in ruminants. For example, parasite infection has decreased live weight gain (Sykes & Coop, 1976; van Houtert *et al.*, 1995), wool production (Steel *et al.*, 1980; 1982; Leyva *et al.*, 1982) and milk production (Leyva *et al.*, 1982; Thomas & Ali, 1983) in infected animals.

A sub-clinical *Trichostrongylus colubriformis* infection increased the irreversible loss rate (ILR) and oxidation of leucine in the small intestine, thereby decreasing its availability to the peripheral tissues (Yu *et al.*, 2000). Increased amino acid (AA) oxidation in the small intestine (Yu *et al.*, 2000) together with increased endogenous protein secretion into the GIT during infection (Steel *et al.*, 1980; Poppi *et al.*, 1986; Yu *et al.*, 1999), suggest there is a net overall loss of AA from the animal.

Interactions within the whole animal are potentially important due to the significant differences in protein turnover that occur between tissues. For example, the

gastrointestinal tract accounts for only 5% of protein mass but about 25-45% of whole body protein synthesis (Lobley *et al.*, 1980). In contrast the muscle (at approximately 50% of total protein mass) accounts for only 20% of whole body protein synthesis (Lobley *et al.*, 1980). Therefore, any alteration in the AA metabolism and protein turnover rate of the GIT may have significant consequences for other tissues (MacRae *et al.*, 1997; Stoll *et al.*, 1998). Therefore, estimating the simultaneous changes in metabolism within the GIT, liver and hind limbs will be necessary to fully understand any alteration in AA partitioning that occurs in lambs infected with intestinal parasites.

The hypothesis of this study is that an established *T. colubriformis* infection will increase the rate of protein turnover in the mesenteric-drained viscera (MDV; small intestine), portal-drained viscera (PDV; stomachs, small intestine, large intestine, pancreas, spleen), liver, total splanchnic tissues (TSP; PDV + liver) and hind limbs (where the muscle contributes 85% to hind limb kinetics and skin is approximately 15%; Biolo *et al.*, 1992). Therefore, the aim of this study was to firstly determine the rates of whole body protein turnover (estimated from the ILR of valine), oxidation and protein synthesis in infected lambs. Secondly, the ILR of valine across the MDV, PDV, liver, TSP and hind limbs was quantified using a dual infusion of isotopically labelled valine. Isotopes of valine were used as part of the larger study because dietary CT can significantly increase the absorption of this amino acid from the small intestine (Waghorn *et al.*, 1987).

4.3 Materials and Methods

4.3.1 Animals and feed

As part of a larger study (as described in Chapter Two) wether lambs (29 kg) were weaned from their dams and were prepared with permanent indwelling catheters in TSP and hind limb blood vessels for infusion and blood sampling as described in Chapter Two to measure AA metabolism, valine kinetics and protein metabolism in the mesenteric-drained viscera (MDV), portal-drained viscera (PDV), liver, TSP and hind limbs. Infusion of deuterium oxide (D_2O) and [^{13}C]-sodium bicarbonate was performed into the jugular vein on day 45 post infection to measure the water space and the carbon dioxide (CO_2) production in the whole body. To determine the effects

of parasite infection on AA metabolism in the MDV, PDV, liver, TSP and hind limbs two isotopes of valine were infused into the jugular vein ([3, 4-^{3}H]-valine) and abomasum ([1-^{13}C]-valine) on day 48 post infection. This enabled any recycling of the abomasal isotope to the MDV and PDV to be calculated. Radioactive and stable isotopes were purchased from Amersham Life Science (Buckinghamshire, UK) and Mass Trace, Inc. (Woburn, MA, USA), respectively.

The lambs were offered fresh Lucerne (*Medicago sativa*; 800 g dry matter (DM) d^{-1}) commencing four d after surgery (Chapter Two) and were fed at hourly intervals from overhead feeders. The treatments have been described in full in Chapter Two. However, briefly one week after surgery (day 1 of the experimental period) six sheep were given 6 000 *T. colubriformis* L3 larvae per day orally for 6 d (parasite treatment) while the remaining six sheep were drenched with Ivomectin (Ivomec® Merial, New Zealand Ltd) to serve as controls (control treatment).

4.3.2 Parasitology

Faecal egg counts (FEC) from individual sheep were determined every second day from day 20 to day 45 of infection as described in Chapter Two. Total intestinal worm burdens were determined after slaughter (Chapter Two).

4.3.3 Infusions and blood sampling

Forty-five d post infection, the lambs received a bolus injection of D_2O (0.61 mL kg^{-1} body weight) into the jugular vein to measure the size of the whole body water pool. The lambs also received an 8 h continuous infusion of $NaH^{13}CO_3$ (99 atom percent, Mass Trace, Inc., Woburn, MA, USA; 22.9 mg h^{-1}) into the jugular vein to measure the whole body production of carbon dioxide (CO_2). Twenty mL of blood was withdrawn continuously every two hours from the mesenteric artery over the 8 h infusion period. The blood was centrifuged (4°C; 3 270 g for 15 min), and the plasma harvested and stored at -85°C in order to determine plasma DM content and ^{2}H enrichment of water. The concentration and isotopic enrichment of CO_2 was also measured according to the methods described by Read *et al.* (1984). However, at the time this thesis was submitted analysis of blood $NaH^{13}CO_3$ was not completed so these data will not be presented.

On day 48, the lambs received a primed continuous 8 h infusion of [3, 4-^{3}H]-valine (7.6 MBq h^{-1}) into the jugular vein. Concurrently, [1-^{13}C]-valine (99 atom percent, Mass Trace, Inc., Woburn, MA, USA; 118 mg h^{-1}) was continuously infused into the abomasum. In order to measure plasma flow across the MDV, PDV, liver and TSP, para-amminohippuric acid (PAH; see Chapter Two for details) was infused continuously into the mesenteric vein for 8 h. The lambs also received a continuous infusion of indocyanin green (ICG) into the abdominal aorta for 8 h enabling plasma flow across the hind limbs to be calculated (Chapter Two). Blood sampling was performed as described in Chapter Two. Briefly, blood was withdrawn continuously every 2 h from the mesenteric artery, the mesenteric, portal, and hepatic veins, and the vena cava over the infusion period. The data presented in this chapter represent the average of samples taken from the last 2 sampling periods (time 4 to 6 and 6 to 8 h). After each 2-h collection period, blood was processed as described in Chapter Two to measure packed cell volume (haematocrit) and concentration of oxygen (O_2) and CO_2 in whole blood or to harvest plasma that was either processed or stored at -85°C for further analysis as described in Chapter Two or below.

4.3.4 Slaughter

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 (300 mg mL^{-1}; 0.5 mL kg^{-1} liveweight). Tissue samples were rapidly collected from the skin, muscle (*biceps femoris*), liver, duodenum, ileum, spleen, mesenteric lymph nodes and thymus (Chapter Three).

After the tissues had been collected, the ileo-caecal junction of the small intestine was located and sectioned in order to collect ileal digesta from the final 3 m of the ileum for digesta flow (Chapter Two), and measurement of specific activity and valine concentration. Ileal digesta was stored at -20°C until analysis.

4.3.5 Sample processing and chemical analysis

4.3.5.1 Plasma flow across tissue beds

The PAH dye dilution technique was used to determine the flow of plasma through the MDV, PDV, liver and TSP tissues as described in Chapter Two. Plasma flow

across the hind limbs was calculated using the ICG concentration measured in plasma. A full description of this method can be found in Chapter Two.

4.3.5.2 Isotopic activity and concentration of valine

The concentration of valine in plasma was determined as outlined in full in Chapter Two and Appendix B.

Subsamples (4-5 g) of frozen tissue were pulverised in liquid nitrogen (N) using a modified French Cell press as described previously in Chapter Three producing a protein-bound fraction and a free pool fraction. The concentration of valine in these fractions was determined as described in Chapter Three.

Amino acid hydrolysates were prepared from ileal digesta and tissue samples by hydrolysing as described in Chapters Two and Three. Total ^{3}H radioactivity and ^{3}H radioactivity associated with valine in the tissue or digesta hydrolysates were determined as described in Chapter Three.

The isotopic enrichment of valine in plasma, tissue free pool and ileal hydrolysate samples was determined by the method outlined by Calder & Smith (1988). Plasma (1 mL) was deproteinised with 0.5 mL of 20% (w/v) sulphosalicylic acid (SSA) and centrifuged for 5 min at 28 000 g. Deproteinised plasma or free pool supernatant (1 mL), or ileal hydrolysate (2 mL) were transferred onto a preconditioned cation exchange columns (Dowex 50 W X8, 200-400 mesh; H^{+} form), washed with 3 mL of deionised water and then eluted onto anion exchange columns (Dowex 1 X8, 200-400 mesh; acetate form) with 2 mL of 4 M NH_4OH and 1 mL of deionised water. The anion exchange was washed with 3 x 2 mL deionised water then eluted into vials with 3 mL 1 M acetic acid. The sample was then frozen at -85°C and freeze dried. The freeze-dried material was taken up in 100 μL of 0.1 M HCl and dried at 80°C under $N_{2(g)}$.

The dried sample was then derivatised by adding 50 μL of t-butyldimethylsilyl and 50 μL of acetonitrile and incubated at 70°C for 20 min. The isotopic abundance (ratio of ^{13}C:^{12}C) of valine in plasma, tissue free pool or ileal digesta hydrolysate was

measured using a gas chromatograph (GC; Shimadzu GC 17A) equipped with a mass selective detector (MS; Shimadzu QP5050a). The temperature of the GC injector and detector was 260°C. Analysis of ^{13}C-valine was carried out on a 30 m x 0.25 mm ID x 0.25 μm ZB-5 capillary column (Phenomenex, New Zealand Ltd) with the following column temperature programme: initial temperature 150 °C held for 6 min, increased to 240 °C at 35°C min^{-1} and held for 2 min, increased to 260 °C at 35°C min^{-1} and held for 10 min. Sample injections of 1 μL were made in a split mode with a 1:20 split. The carrier gas, helium was at a flow rate of 40 cm^3 min^{-1}. The mass selective detector was operated using electron impact and selected ion monitoring (m/z 186.15 and 187.15) for the $(M-57)^+$ fragment.

4.3.5.3 Isotopic enrichment of deuterium oxide

The isotopic (^{2}H) enrichment of H_2O in plasma samples was quantified using the procedure adapted from Yang *et al.* (1998). The theory behind this method is that there is a rapid exchange of protons from water with hydrogens adjacent to the carbonyl group of acetone under basic conditions (Yang *et al.*, 1998). This equilibrium can be used to determine the isotopic enrichment of water (Equation 4.1). Although all six hydrogens of acetone may be exchanged in this manner, for samples with ^{2}H-enrichments of less than 0.5%, exchange occurs in one position only. This allows quantification from the M/M+1 ratio. At such low enrichments it is advantageous to use 99% U-^{13}C-acetone rather than unlabelled acetone in the reaction, because of its lower natural M/M+1 enrichment.

Equation 4.1

$$C_3H_6O + D_2O + OH^- \rightleftharpoons C_3DH_5O + DHO + OH^-$$

Plasma (1 mL) obtained during the blood sampling on day 45 post-infection was transferred into a glass vial. Universally-labelled ^{13}C-acetone (4 μL in 5% acetonitrile) and 16 μL of 10M NaOH were added to the plasma in quick succession and mixed. The samples were left to incubate at room temperature overnight (a minimum of 5 h) and 1 mL of hexane added and mixed thoroughly. The organic layer (hexane) of the resulting mixture was dried with Na_2SO_4 and then transferred to

an autosampler vial and analysed on a GC (Shimadzu GC 17A) equipped with a MS detector (Shimadzu QP5050a). The temperature of the GC injector and detector was 250°C. Analysis of $^{13}C_3$-acetone was carried out on a 30 m x 0.25 mm ID x 0.25 μm ZB-Wax capillary column (Phenomenex, New Zealand Ltd.) with the target ions eluting between 1.52-1.72 min. The column temperature programme was as follows: initial temperature 70°C, increased to 105°C at 20°C min^{-1} held for 4 min, increased to 250 °C at 35°C min^{-1} and held for 10 min (source temperature 200°C). Sample injections of 1 μL were made in split mode with a 1:30 split. The carrier gas was helium, at a flow rate of 40.9 cm^3 min^{-1}. The mass selective detector was operated using electron impact and selected ion monitoring m/z 61, 62 and 63 for the M, M+1, M+2 ions respectively.

To calculate the DM content in plasma 1 mL of plasma was weighed into pre-weighed containers and then dried at 105°C for 16 h, after which they were re-weighed.

4.3.6 Calculations

In the following equations, several abbreviations are used to specify a sampled pool. These abbreviations are defined as follows:

M = plasma from the mesentery vein
P = plasma from portal vein
H = plasma from hepatic vein
A = plasma from mesentery artery
V = plasma from vena cava
X = plasma from any of the venous drainage (M, P, H or V).
T = tissue free pool
Z = precursor pool for protein synthesis
ABO = abomasal digesta
ILEAL = ileal digesta
I = infusate
[X] = concentration or count of a particular compound
^{13}C-VAL = [1-^{13}C]-valine

^{3}H-VAL = [3,4-^{3}H]-valine

4.3.6.1 Isotopic activity of valine and water

The isotopic activity of valine (VAL IA) refers either to specific radioactivity (VAL SRA) or isotopic enrichment (VAL IE) of valine in plasma obtained from the mesenteric, portal and hepatic veins, the mesenteric artery or the vena cava. The VAL SRA and VAL IE were determined by Equations 4.2 (Wolfe, 1992a) and 4.3 (Wolfe, 1992b), respectively. The isotopic activity of water refers to the isotopic enrichment of H_2O in plasma from the mesenteric artery (H_2O IE_A) and was calculated according to Equation 4.3.

Equation 4.2

$$\text{VAL SRA}_{\text{A or X}}\ (\text{dpm mmol}^{-1}) = \frac{[^{3}\text{H - VAL}]_{\text{A or X}}\ (\text{dpm mL}^{-1})}{[\text{VAL}]_{\text{A or X}}\ (\text{mmol mL}^{-1})}$$

Equation 4.3

$$\text{Val IE}_{\text{A or X}}\ (\text{APE \%}) = \frac{(R_S - R_B)}{1 - (R_S - R_B)} \times 100$$

where: R = the ratio of the abundance of the mass fragment M-57 (VAL) one carbon-^{13}C over the mass containing no carbon-^{13}C.

R_S = the ratio of the abundance measured for each sample taken from 2-h continuous period for plasma only (time 4 to 6; time 6 to 8) or at slaughter (8 h; tissue free pool and ileal digesta hydrolysate) after the start of the tracer infusion.

R_B = the ratio of the background natural abundance which was estimated in plasma samples taken before the beginning of tracer infusion. The background sample for the tissue free pool and ileal digesta hydrolysate was estimated to be equal to the natural abundance of the plasma.

The rise of plasma VAL IE or VAL SRA to plateau was described using the following exponential model (Equation 4.4). This equation is shown only for VAL IE. In this current study, plasma samples were harvested continuously over 2-h increments at 2, 4, 6 and 8 h after the start of infusion and the isotopic enrichment of free valine in each sample represents the average valine isotopic enrichment over the 2-h collection period.

Equation 4.4

$$VAL\ IE_{A\ or\ X} = VAL\ IE_{MAX} \times (1 - e^{-kt})$$

where: $VAL\ IE_{MAX}$ = plateau value of VAL IE or VAL SRA assuming the radioactivity or isotopic enrichment increases at a rate k over time t (Waterlow *et al.*, 1978).

Following the injection of D_2O, the isotopic enrichment of water follows an exponential decay curve. Therefore, to determine the actual body water volume using a D_2O bolus, an exponential decay curve was plotted with the plasma water enrichments on the y-axis and time of sampling (0 to 2 h, 2 to 4 h, 4 to 6 h and 6 to 8 h) in relation to the administration of the D_2O on the x-axis. The values extrapolated back to time zero is the enrichment of water at the time of the injection. The latter is used to calculate the volume of body water as described in the section 4.3.6.2.

4.3.6.2 Whole body kinetics of valine

The whole body model for AA kinetics described by Waterlow *et al.* (1978) was used to estimate whole body valine kinetics. Whole body irreversible loss of valine (WB VAL ILR) estimated from the ^{3}H-valine infusion was determined according to Equation 4.5 (Harris *et al.*, 1992).

Equation 4.5

$$WB\ VAL\ ILR\ (mmol\ h^{-1}) = \frac{^3H\text{ - }VAL\ infusion\ rate\ (dpm\ h^{-1})}{Plateaued\ VAL\ SRA\ \ (dpm\ mmol^{-1})}$$

The whole body valine ILR estimated from the ^{13}C-valine infusion was calculated as described in Equation 4.4 after replacing in this equation the appropriate parameters (e.g., VAL SRA by VAL IE) and subtracting the amount of ^{13}C-valine infused to correct for infusion of non tracer quantities of ^{13}C-valine (Equation 4.6).

Equation 4.6

$$\text{WB VAL ILR (mmol h}^{-1}) = \frac{^{13}\text{C - VAL infusion rate (mmol h}^{-1}) \text{ x VAL IE}_I \text{ (APE\%)}}{\text{Plateaued VAL IE (APE\%)}} - {}^{13}\text{C - VAL infusion rate (mmol h}^{-1})$$

In order to calculate the whole body valine oxidation from the infusion of ^{3}H-valine, the total amount of $^{3}H_2O$ produced from valine over the period of infusion must be quantified. This requires the determination of the water pool size in the whole body which was calculated according to Equation 4.7 (Beckett *et al.*, 1992).

Equation 4.7

$$\text{WB Water pool size (g)} = \frac{\text{D}_2\text{O volume injected (g)} \times \frac{\text{D}_2\text{O IE}_I \text{ (APE\%)}}{\text{D}_2\text{O MW (g mol}^{-1})}}{\frac{\text{H}_2\text{O MW (g mol}^{-1})}{\text{H}_2\text{O IE}_A \text{ (APE\%)}}}$$

where: D_2O MW = molecular weight of D_2O = 20.02

H_2O MW = molecular weight of H_2O = 18.02

The whole body oxidation of valine was then calculated using Equations 4.8 to 4.11 (Beckett *et al.*, 1992):

Equation 4.8

Total $^{3}H_2O$ produced from valine (dpm) = [$^{3}H_2O$] (dpm g^{-1}) x WB water pool size (g)

Equation 4.9

Total dose of ^{3}H-VAL infused (dpm) = ^{3}H-VAL infusion rate (dpm h^{-1}) x 8 h

where: 8 h = length of infusion of ^{3}H-valine

Equation 4.10

$$\text{WB VAL oxidation (\%)} = \frac{\text{Total } {}^{3}H_2O \text{ produced from VAL (dpm)}}{\text{Total dose of } {}^{3}\text{H - VAL infused (dpm)}}$$

Equation 4.11

$$\text{WB VAL oxidation (mmol h}^{-1}) = \frac{\text{WB VAL oxidation (\%)}}{100} \text{ x WB VAL ILR (mmol h}^{-1})$$

Whole body valine used for protein synthesis (WB VAL PS) was calculated according to Equation 4.12, and was converted to protein synthesis (g d^{-1}) assuming a valine concentration of 36 mg g^{-1} tissue (MacRae *et al.*, 1993).

Equation 4.12

$$\text{WB VAL PS (mmol h}^{-1}) = \text{WB VAL ILR (mmol h}^{-1}) - \text{WB VAL oxidation (mmol h}^{-1})$$

4.3.6.3 Plasma flow

Plasma flow (Pf) across the tissue beds (Tissue Pf or MDV Pf, PDV Pf, TSP Pf and hind limbs Pf) was determined by Equation 4.13. The hepatic artery plasma flow was determined by difference between the TSP Pf and PDV Pf. A full description of these calculations can be found in Chapter Two.

Equation 4.13

$$\text{Tissue Pf (mL min}^{-1}) = \frac{[\text{PAH}]_I \text{ (mg mL}^{-1}) \text{ x infusion rate (mL h}^{-1})}{([\text{PAH}]_X - [\text{PAH}]_A) \text{ (mg mL}^{-1})}$$

4.3.6.4 Net flux of valine across tissue beds

Net flux of valine across the MDV, PDV, TSP and hind-limb tissues (Tissue net VAL flux) was calculated using an arterial-venous (AV) approach as outlined in Equation 4.14 (Lobley *et al.*, 1995). This calculation for the liver (Liver net VAL flux) was done as described in Equation 4.15 (Lobley *et al.*, 1995). A full description of these equations can be found in Chapter Two.

Equation 4.14

Tissue net VAL flux (mmol min^{-1}) = ($[VAL]_A - [VAL]_X$) (mmol mL^{-1}) x Tissue Pf (mL min^{-1})

Equation 4.15

Liver net VAL flux (mmol min^{-1})
= ($[VAL]_A$ x ART Pf) + ($[VAL]_P$ x PDV Pf) – ($[VAL]_H$ x TSPT Pf)

Arterial flow of valine (Arterial VAL inflow) into the tissue bed was calculated according to Equation 4.16. Additionally, apparent valine absorption by the MDV was calculated using Equation 4.17.

Equation 4.16

Arterial VAL inflow (mmol d^{-1}) = $[VAL]_A$ (mmol mL^{-1}) x Tissue Pf (mL min^{-1})

Equation 4.17

MDV apparent VAL absorption (mmol d^{-1}) =
($[VAL]_{ABO}$ x $Flow_{ABO}$) (mmol h^{-1}) – ($[VAL]_{ILEAL}$ x $Flow_{ILEAL}$) (mmol h^{-1})

where: $Flow_{ABO}$ = dry matter flow through the abomasum
$Flow_{ILEAL}$ = dry matter flow through the ileum

4.3.6.5 Kinetics of valine across the gastrointestinal tract

The valine kinetics in the MDV was calculated using Equations 4.18 to 4.32. The equations are similar to those of Neutze *et al.* (1997) and MacRae *et al.* (1997) but have been re-arranged for clarification. To determine the effects of parasite infection on AA metabolism in the MDV two isotopes of valine were infused; ^{3}H-valine into the jugular and ^{13}C-valine into the abomasum. This enabled any recycling of the abomasal valine isotope to the MDV and PDV to be calculated. The underlying assumption of this method is that the two isotopes of valine behave in an identical manner.

The amount of ^{3}H-valine in plasma collected from the mesenteric, portal or hepatic vein, mesenteric artery or vena cava was determined by Equation 4.18. The amount of ^{13}C-valine ([^{13}C-VAL]) in plasma collected from these vessels (mmol L^{-1}) was calculated similarly by replacing VAL $SRA_{A\ or\ X}$ in Equation 4.18 by VAL $IE_{A\ or\ X}$ (APE%).

Equation 4.18

$$[^{3}H\text{–VAL}]_{A\ or\ X}\ (dpm\ mL^{-1}) = VAL\ SRA_{A\ or\ X}\ (dpm\ mol^{-1}) \times [VAL]_{A\ or\ X}\ (mol\ mL^{-1})$$

The arterial ^{3}H-valine inflow to the MDV (Arterial ^{3}H-VAL inflow) was calculated as described in Equation 4.19. The arterial recycled ^{13}C-valine inflow to MDV (mmol d^{-1}) was calculated similarly by replacing [^{3}H-VAL] in Equation 4.19 by [^{13}C-VAL].

Equation 4.19

$$\text{Arterial } ^{3}\text{H-VAL inflow } (dpm\ d^{-1}) = [^{3}\text{H-VAL}]_{A}\ (dpm\ mL^{-1}) \times \text{MDV Pf } (mL\ d^{-1})$$

The extraction or loss of ^{3}H-valine into the MDV from arterial sources (MDV arterial ^{3}H-VAL extraction) was determined using the values derived from the intravenous infusion of ^{3}H-valine (Equation 4.20). This parameter is also called extraction of the infused tracer into the jugular vein in Neuzte *et al.* (1997; Equation 15).

Equation 4.20

$$\text{MDV arterial } ^{3}\text{H - VAL extraction } (\%) = \frac{([^{3}\text{H - VAL}]_{A} - [^{3}\text{H - VAL}]_{M})\ (mmol\ mL^{-1}) \times \text{MDV Pf } (mL\ d^{-1})}{[^{3}\text{H - VAL}]_{A}\ (mmol\ mL^{-1}) \times \text{MDV Pf } (mL\ d^{-1})} \times 100$$

The valine ILR across the MDV (MDV VAL ILR) from arterial sources based on intravenous infusion of ^{3}H-valine was calculated by Equation 4.21.

Equation 4.21

$$\text{MDV VAL ILR from arterial sources } (mmol\ d^{-1}) = [VAL]_{A}\ (mmol\ mL^{-1}) \times \text{MDV Pf } (mL\ d^{-1}) \times \text{MDV arterial } ^{3}\text{H-VAL extraction } (\%)$$

The calculation of the recovery of digesta-derived VAL into the mesenteric (M) drainage (Equation 4.22) was made on the assumption that the ^{13}C-valine that disappeared from the MDV mixed with the liberated valine peptides that were absorbed from the intestine.

Equation 4.22

Luminal ^{13}C-VAL recovery in M (mmol d^{-1})
= ([^{13}C-VAL]$_M$ – [^{13}C-VAL]$_A$) (mmol mL^{-1}) x MDV Pf (mL d^{-1})

The extraction of re-circulated ^{13}C-valine from the mesenteric artery into the MDV tissue was calculated according to the Equation 4.23 using the MDV arterial ^{3}H-valine extraction estimated from the intravenous infusion of ^{3}H-valine (Equation 4.19). This parameter calculated using Equation 4.23 is also called true net uptake of tracer infused into the MDV from blood (Neuzte *et al.*, 1997; Equation 16) and assumes that ^{13}C-valine entry into the mesenteric vein comes from arterial and lumen sources.

Equation 4.23

Arterial recycled ^{13}C - VAL extraction by MDV (mmol d^{-1}) =
[^{13}C - VAL$_A$] (mmol mL^{-1}) x MDV arterial ^{3}H - VAL extraction (%) x MDV Pf (mL d^{-1})

The corrected recovery of ^{13}C-valine (from abomasal infusion) into the mesenteric (M) drainage was obtained as follows (Equations 4.24 and 4.26). The fractional recovery is equal to the total recovery expressed as a proportion of ^{13}C-valine apparently absorbed from the MDV (MDV apparent ^{13}C-VAL absorption; Equation 4.25).

Equation 4.24

Luminal ^{13}C-VAL corrected recovery in M (mmol d^{-1}) =
Luminal ^{13}C-VAL recovery in M (mmol d^{-1}) + Arterial recycled ^{13}C-VAL extraction by MDV (mmol d^{-1})

Equation 4.25

MDV apparent ^{13}C-VAL absorption (mmol d^{-1}) =
^{13}C-VAL infusion rate$_{ABO}$ (mmol d^{-1}) – ^{13}C-VAL flow$_{ILEAL}$ (mmol d^{-1})

Equation 4.26

$$\text{Luminal } {}^{13}\text{C - VAL corrected recovery in M (\%)} = \frac{\text{Luminal } {}^{13}\text{C - VAL corrected recovery in M (mmol d}^{-1}\text{)} \times 100}{\text{MDV apparent } {}^{13}\text{C - VAL absorption (mmol d}^{-1}\text{)}}$$

On this basis, the fraction of ^{13}C-valine that was absorbed from the MDV but not appearing in the mesenteric drainage was assumed to be used by the MDV tissues for either protein synthesis or valine oxidation. The fraction of ^{13}C-valine that is extracted or lost into the MDV tissue was calculated according to Equation 4.27.

Equation 4.27

$$\text{MDV luminal } {}^{13}\text{C-VAL extraction (\%)} = 100\,(\%) - \text{Luminal } {}^{13}\text{C-VAL corrected recovery in M (\%)}$$

The valine ILR in the MDV based on luminal supply of ^{13}C-valine to MDV (Equation 4.31) is the summation of the ^{13}C-valine apparently absorbed from the MDV (Equations 4.25) that is extracted by the MDV (Equations 4.28) and the ^{13}C-valine that leaves the MDV (i.e., endogenous loss; Equations 4.29; Yu *et al.*, 1999) but extracted (reabsorbed) by the MDV during the infusion of ^{13}C-valine (Equation 4.30).

Equation 4.28

$$\text{MDV absorbed VAL extraction (mmol d}^{-1}\text{)} = \text{MDV apparent VAL absorption (mmol d}^{-1}\text{)} \times \frac{\text{MDV } {}^{13}\text{C-VAL extraction (\%)}}{100}$$

Equation 4.29

$$\text{MDV endogenous VAL loss (mmol d}^{-1}\text{)} = \text{VAL flow}_{\text{ILEUM}}\ (\text{mmol d}^{-1}) \times \frac{\text{SRA VAL}_{\text{ILEUM}}\ (\text{dpm mmol}^{-1})}{\text{SRA VAL}_{\text{A}}\ (\text{dpm mmol}^{-1})}$$

Equation 4.30

$$\text{MDV VAL loss into lumen extraction (mmol d}^{-1}\text{)} = \text{MDV endogenous VAL loss (mmol d}^{-1}\text{)} \times \frac{\text{MDV [1-}{}^{13}\text{C]-VAL fractional extraction (\%)}}{100}$$

Equation 4.31

MDV VAL ILR from luminal sources (mmol d^{-1}) =
MDV absorbed VAL extraction (mmol d^{-1}) + MDV VAL loss into lumen extraction (mmol d^{-1})

Total ILR of valine across the MDV (MDV total VAL ILR) was calculated using Equation 4.32.

Equation 4.32

MDV total VAL ILR (mmol d^{-1}) =
MDV VAL ILR from arterial sources (mmol d^{-1}) + MDV ILR from luminal sources (mmol d^{-1})

The kinetics of valine across the PDV were determined similarly to the equations described previously for the MDV.

4.3.6.6 Kinetics of valine across the liver and total splanchnic tissues

The fractional extraction or loss of ^{13}C-valine across the liver (Liver ^{13}C-VAL extraction) was calculated according to Equation 4.33 and used to determine the valine ILR across the liver (Equation 4.34). These equations are based on the same principles as those for the MDV and PDV, using the equations outlined by MacRae *et al.* (1997) and Yu *et al.* (2000).

Equation 4.33

$$\text{Liver } ^{13}\text{C - VAL extraction (\%)} = \frac{([^{13}\text{C} - \text{VAL}]_A \text{ (mmol mL}^{-1}\text{) x ART Pf (mL d}^{-1}\text{))} + ([^{13}\text{C - VA}]_P \text{ (mmol mL}^{-1}\text{) x PDV Pf (mL d}^{-1}\text{))} - ([^{13}\text{C - VAL}]_H \text{ (mmol mL}^{-1}\text{) x TSP Pf (mL d}^{-1}\text{))}}{([^{13}\text{C - VAL}]_A \text{ (mmol mL}^{-1}\text{) x ART Pf (mL d}^{-1}\text{))} + ([^{13}\text{C - VAL}]_P \text{ (mmol mL}^{-1}\text{) x PDV Pf (mL d}^{-1}\text{))}}$$

Equation 4.34

Liver VAL ILR (mmol d^{-1}) =
Liver ^{13}C-VAL extraction (%) x {([VAL]$_A$ (mmol mL^{-1}) x ART Pf (mL d^{-1})) + ([VAL]$_P$ (mmol mL^{-1}) x PDV Pf (mL d^{-1}))}

The ILR of valine across the TSP was calculated according to Equation 4.35.

Equation 4.35

$$\text{TSP VAL ILR (mmol d}^{-1}) = \text{PDV total VAL ILR (mmol d}^{-1}) + \text{Liver VAL ILR (mmol d}^{-1})$$

4.3.6.7 Kinetics of valine across the hind limbs

The fractional extraction of ^{13}C-valine by the hind limbs (Hind limb arterial ^{13}C-VAL extraction) was calculated according to Equation 4.36 and used to calculate valine ILR across this tissue in Equation 4.37. The arterial ^{3}H-valine extraction by the hind limbs was calculated similarly by replacing [^{13}C-VAL] in Equation 4.36 by [^{3}H-VAL].

Equation 4.36

$$\text{Hind limb arterial } {}^{13}\text{C - VAL extraction(\%)} = \frac{([{}^{13}\text{C - VAL}]_A - [{}^{13}\text{C - VAL}_V]\,(\text{mmol mL}^{-1})) \times \text{Hind limb Pf (mL d}^{-1})}{[{}^{13}\text{C - VAL}_A]\,(\text{mmol mL}^{-1}) \times \text{Hind limb Pf (mL d}^{-1})}$$

Equation 4.37

$$\text{Hind limbs VAL ILR (mmol d}^{-1}) = \text{Hind limbs arterial } {}^{13}\text{C-VAL extraction (\%)} \times [\text{VAL}]_A\,(\text{mmol mL}^{-1}) \times \text{Hind limb Pf (mL d}^{-1})$$

Additionally to the traditional AV kinetic model, valine kinetics across the hind limbs including the transport of valine was calculated according to a three-compartmental model described by Biolo *et al.* (1992). This model is depicted in Figure 4.1 and described in Equations 4.38 to 4.47. This model assumes that the tissue free pool represents a common precursor pool for inward and outward transfers of valine and that the muscle represents the average AA composition for the hind limb (Biolo *et al.*, 1992). These assumptions will be discussed in detail in the discussion. Equations 4.40 to 4.46 are described for ^{13}C-valine kinetics, however, replacing VAL IE for VAL SRA enabled the ^{3}H-valine kinetics to be calculated.

Flow of valine into the cell (F_{IN}) and out of the cell (F_{OUT}) was calculated according to Equations 4.38 and 4.39.

Equation 4.38

$$F_{in}\ (\text{mmol d}^{-1}) = [VAL]_A\ (\text{mmol mL}^{-1}) \times \text{Hind limb Pf}\ (\text{mL d}^{-1})$$

Equation 4.39

$$F_{out}\ (\text{mmol d}^{-1}) = [VAL]_V\ (\text{mmol mL}^{-1}) \times \text{Hind limb Pf}\ (\text{mL d}^{-1})$$

The net movement (or transport) of valine from arterial plasma to the tissue (F_{TA}) was calculated according to Equation 4.40.

Equation 4.40

$$F_{TA}\ (\text{mmol d}^{-1}) = \left(\frac{VALIE_T - VALIE_V}{VALIE_A - VALIE_T} \times [VAL]_V + [VAL]_A\right)(\text{mmol mL}^{-1}) \times \text{Hind limb Pf}\ (\text{mL min}^{-1})$$

The transport of valine from muscle free pool to the venous drainage (F_{VT}) was calculated according to the Equation 4.41.

Equation 4.41

$$T_{VT}\ (\text{mmol d}^{-1}) = \left(\frac{VALIE_T - VALIE_V}{VALIE_A - VALIE_T} \times [VAL]_V + [VAL]_V\right)(\text{mmol mL}^{-1}) \times \text{Hind limb Pf}\ (\text{mL d}^{-1})$$

By-pass of valine (F_{VA}) from the arterial pool directly to the venous drainage (ie., no entry into the cells) was calculated according to Equation 4.42.

Equation 4.42

$$F_{VA}\ (\text{mmol d}^{-1}) = \left(-\left[\frac{VALIE_T - VALIE_V}{VALIE_A - VALIE_T}\right] \times [VAL]_V\right)(\text{mmol mL}^{-1}) \times \text{Hind limb Pf}\ (\text{mL d}^{-1})$$

Transfer of valine from the muscle free pool to the protein bound pool and other pools (i.e., protein synthesis plus oxidation; F_{OT}) was calculated according to Equation 4.43 and converted to daily protein turnover by Equation 4.44 (Harris *et al.*, 1992).

Equation 4.43

$$F_{OT}\ (\text{mmol d}^{-1}) = \left(\frac{[VAL]_A \times VAL\ IE_A - [VAL]_V \times VAL\ IE_V}{VAL\ IE_T}\right)(\text{mmol mL}^{-1}) \times \text{Hind limb Pf}\ (\text{mL d}^{-1})$$

Equation 4.44

$$\text{Hind limb protein turnover}\ (\text{g d}^{-1}) = F_{OT}\ (\text{mmol d}^{-1}) \times \left(\frac{VAL\ MW\ (\text{g mmol}^{-1})}{[VAL]_{muscle}\ (\text{g g}^{-1})}\right)$$

where: $[VAL]_{muscle}$ = 0.00049 g per g of muscle tissue (MacRae *et al.*, 1993)

VAL MW = 117.1 g mol^{-1}

Transfer of valine to the muscle free pool via protein degradation (PD) was calculated according to Equation 4.45 and converted to daily protein degradation as described for protein turnover in Equation 4.44.

Equation 4.45

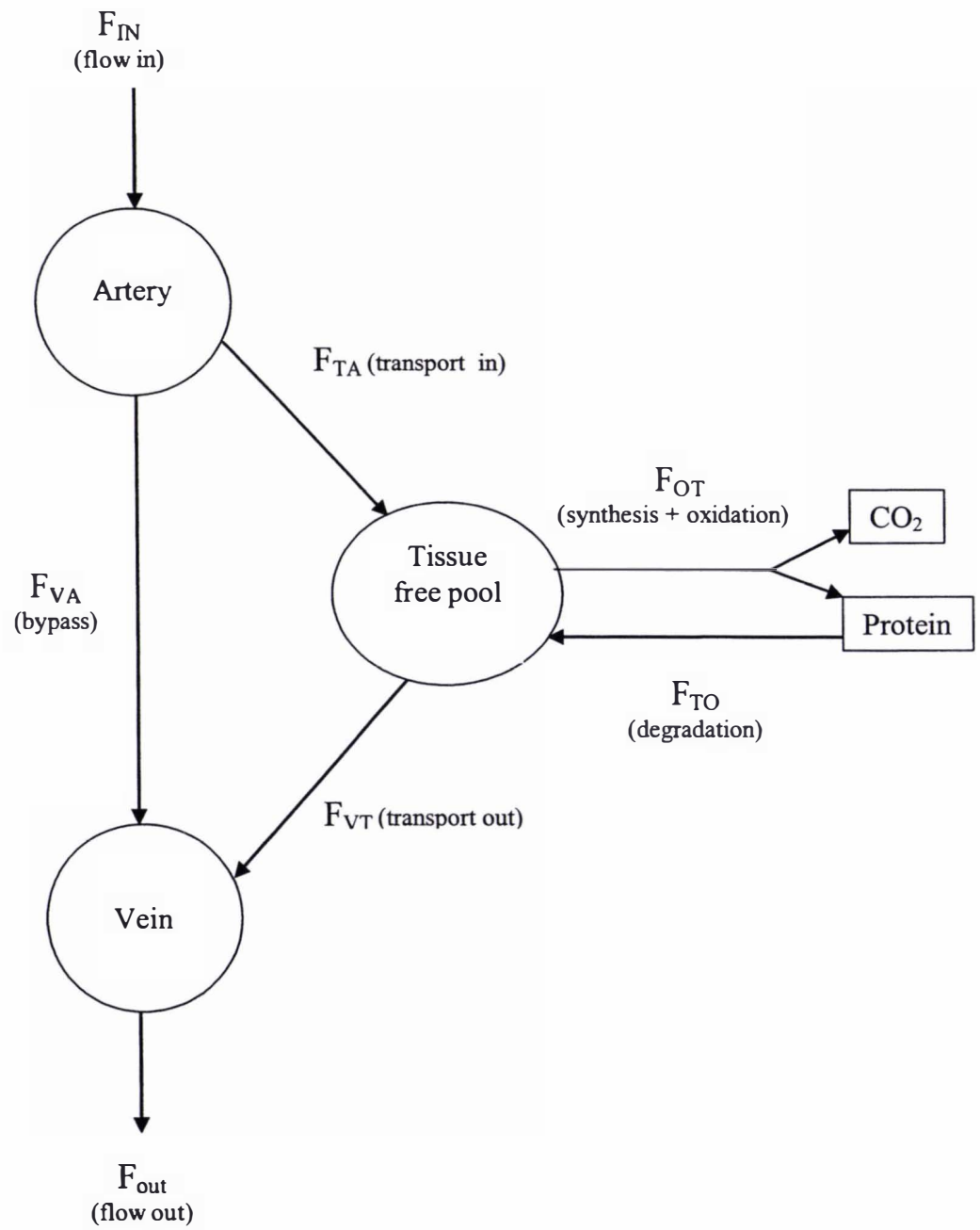
$$F_{TO}\ (\text{mmol d}^{-1}) = \left(\frac{[[VAL]_A \times (VAL\ IE_A - VAL\ IE_T) - [VAL]_V \times (VAL\ IE_V - VAL\ IE_T)]}{VAL\ IE_T}\right)(\text{mmol mL})^{-1} \times \text{Hind limb Pf}\ (\text{mL d}^{-1})$$

The sum between inward transport of valine (F_{TA}) and that appearing in the intracellular pool from endogenous sources (F_{TO}) represents the total intracellular appearance of valine (R_{AT}) and was calculated according to equation 4.46.

Equation 4.46

$$R_{AT}\ (\text{mmol d}^{-1}) = F_{TA}\ (\text{mmol d}^{-1}) + F_{TO}\ (\text{mmol d}^{-1})$$

Figure 4.1 Valine kinetics (F) in the muscle of lambs according to the model described by Biolo *et al.* (1992).



4.3.7 Statistical analysis

Statistical analysis for all variables was performed using a General Linear Model (SAS version 8, 1999), with treatment and group (the week that the lamb underwent surgery) used as sources of variation in the model. Additionally, feed intake, faecal egg counts and liveweight were analysed using Proc Mix repeated measures. The data were checked for normality and the presence of outliers by plotting residuals versus the predicted residuals.

Probability values lower than 0.10 were considered to indicate a significant difference and values between 0.10 and 0.15 to indicate a trend. Results are presented as least squares means (LS means) and associated pooled standard deviation (SD).

One sheep from the parasite treatment was omitted from all statistical analysis as the Captec^{TM} Alkane CRC capsule blocked the reticulo-rumen orifice and prevented feed intake 2 d prior to the blood sampling period.

4.4 Results

Results relating to the faecal egg counts and intestinal worm burdens are presented in Chapter Two. Briefly, intestinal worm burdens were significantly higher in the parasitised lambs on day 48 post infection (440 *vs.* 21 740 (SD 1 101) worms in the control and parasite lambs, respectively; $P<0.001$).

Feed intake and liveweight changes have been described fully in Chapter Two. In summary, the presence of parasites in the small intestine did not have any impact on feed intake over the course of the experiment (826 *vs.* 796 (SD 18) g DM d^{-1} in the control and parasite lambs, respectively; $P=0.15$). Change in liveweight gain (g d^{-1}) was also unaffected by parasitic infection (240 *vs.* 190 (SD 100) g liveweight d^{-1} in the control and parasite lambs, respectively; $P>0.15$).

4.4.1 Valine concentration and isotopic activity

Catheter patency was approximately 99%, with only one catheter in the hepatic vein failing. Parasite infection increased the concentration of valine in the mesenteric

artery and vena cava (P<0.15; Table 4.1). However, the concentration of valine in the mesenteric vein, portal vein or hepatic vein was unaffected by infection (P>0.15; Table 4.1). Valine isotopic enrichment and specific radioactivity in the mesenteric, portal or hepatic veins, the mesenteric artery and vena cava (P>0.15; Table 4.1) were similar between control and parasitised lambs. The valine concentration and specific radioactivity in ileal digesta was similar between control and parasitised lambs (Table 4.1). However, valine enrichment in ileal digesta was slightly higher in the infected sheep (P<0.15; Table 4.1). The concentration, specific activity and isotopic enrichment of valine in the free pool of muscle was similar between control and parasitised lambs (P>0.15; Table 4.1).

4.4.2 Whole body valine kinetics

The size of the whole body water pool in the lambs was unaffected by the parasite infection (P>0.15; Table 4.2). Whole body valine ILR, oxidation and valine used for protein synthesis calculated from the ^{3}H-valine infused into the jugular vein were also similar between treatment groups (P>0.15; Table 4.2). Whole body ILR (calculated from the infusion of ^{13}C-valine into the abomasum) was similar between the control and parasite lambs (316.9 *vs.* 324.8 (SD 124.6) mmol d^{-1}; P>0.15). However, this estimate was much higher than that estimated from the ^{3}H-valine infusion. Whole body protein synthesis was slightly decreased by parasitic infection however this was not significant at the 15% level. Whole body valine oxidation accounted for 12-14% of whole body valine ILR and was unaffected by infection (P>0.15; Table 4.2). Similarly, the proportion of whole body ILR that was used for whole body protein synthesis was unaffected by the presence of *T. colubriformis* in the small intestine (86-88%; P>0.15; Table 4.2).

4.4.3 Valine flux and kinetics in the mesenteric-drained viscera

The flow of valine through the abomasum and ileum and apparent absorption of valine from the MDV were unaffected by parasite infection (P>0.15; Table 4.3). Infection did not affect the ratio of valine SRA in ileal digesta over the valine SRA in arterial plasma (P>0.15; Table 4.3). Consequently, the endogenous flow of valine was unaffected by infection (P>0.15; Table 4.3). Infection also had no effect on the apparent ^{13}C-valine absorption from the small intestine (P>0.15; Table 4.3).

The flow of plasma across and inflow of arterial valine to the MDV were not changed by the presence of parasite infection (P>0.15; Table 4.3). Net valine flux into the MDV was however increased in parasitised lambs (P<0.15; Table 4.3).

Valine ILR in the MDV was estimated using both jugular (^{3}H-valine) and luminal infusions (^{13}C-valine). The extraction of arterial ^{3}H-valine by the MDV was more than doubled by the infection from 1.3 to 3.5 mmol d^{-1} in the infected lambs (P<0.02; Table 4.3). Consequently, the estimates of valine ILR from arterial sources in the MDV using either the arterial or venous free valine precursor pool were increased by 2-3 fold in parasitised lambs (P<0.05; Table 4.3).

Recovery of luminal ^{13}C-valine to the mesenteric vein was lower in parasitised lambs (P<0.10; Table 4.3). This recovery was corrected by accounting for the fractional uptake of ^{13}C-valine from the arterial supply by the MDV tissues (arterial recycled ^{13}C-valine). The latter was estimated using the fractional uptake of ^{3}H-valine obtained during the ^{3}H-valine infusion in the jugular vein and higher extraction was observed in the infected lambs (P<0.01; Table 4.3). The corrected recovery was unaffected by infection (P>0.15; Table 4.3). Thus, the fractional extraction of luminal ^{13}C-valine by the MDV was increased from 72% to 100% in the infected lambs but this was not statistically significant. Neither the valine being reabsorbed by the MDV from the pool of endogenous valine secreted into lumen nor the extraction of apparently absorbed valine by the MDV was affected by the infection (P>0.15; Table 4.3). Consequently, the valine ILR from all luminal sources was similar between control and infected lambs (P<0.15; Table 4.3). Overall, the total valine ILR (from arterial and lumen sources) in the MDV was approximately 2-fold higher in the parasitised lambs whether the arterial or venous free valine precursor pool was used for estimating valine ILR (P<0.05; Table 4.3).

Table 4.1 Valine concentration ([VAL]), specific radioactivity (VAL SRA) and isotopic enrichment (VAL IE) in plasma and digesta of lambs fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=5	Pooled SD	P
Mesentery vein					
[VAL]	μmol L^{-1}	267	260	52	0.56
VAL SRA	dpm $nmol^{-1}$	77.8	97.3	12.7	0.33
VAL IE	APE%	4.6	4.8	1.5	0.87
Portal vein					
[VAL]	μmol L^{-1}	234	253	25	0.29
VAL SRA	dpm $nmol^{-1}$	87.7	89.7	6.3	0.66
VAL IE	APE%	5.0	5.4	1.5	0.74
Hepatic vein					
[VAL]	μmol L^{-1}	228	257	27	0.19
VAL SRA	dpm $nmol^{-1}$	88.1	86.9	10.5	0.88
VAL IE	APE%	5.0	5.2	1.4	0.89
Mesentery artery					
[VAL]	μmol L^{-1}	223	252	25	0.14
VAL SRA	dpm $nmol^{-1}$	97.3	103.3	10.2	0.23
VAL IE	APE%	4.7	4.9	1.3	0.69
Vena cava					
[VAL]	μmol L^{-1}	214	241	18	0.07
VAL SRA	dpm $nmol^{-1}$	92.8	96.9	10.6	0.59
VAL IE	APE%	4.0	4.5	1.3	0.62
Ileal digesta					
[VAL]	mg g DM^{-1}	1.8	2.0	0.4	0.72
VAL SRA	dpm $nmol^{-1}$	12.6	15.7	3.4	0.29
VAL IE	APE%	1.0	1.3	0.2	0.14
Free pool muscle					
[VAL]	μmol L^{-1}	23	24	9	0.89
VAL SRA	dpm $nmol^{-1}$	98.4	109.6	51.3	0.76
VAL IE	APE%	2.7	2.9	1.0	0.33

Table 4.2 Whole-body valine (VAL) and protein kinetics of lambs (day 48 post infection) fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=5	Pooled SD	P
VAL ILR[1]	mmol d^{-1}	106.8	98.6	9.4	0.24
Water pool size[2]	L	19.5	19.6	1.6	0.76
Total $^3H_20$ from VAL	dpm x 10^7	40.9	48.4	7.7	0.19
Total ^{3}H-VAL infused	dpm x 10^8	43.9	44.9	24.9	0.58
VAL OX[3]	%	11.7	13.6	2.0	0.23
	mmol d^{-1}	12.5	13.0	2.2	0.71
VAL PS[4]	mmol d^{-1}	94.6	85.7	8.4	0.17
VAL PS : VAL ILR	%	88.5	86.9	2.1	0.28
VAL OX : VAL ILR	%	11.5	13.1	1.9	0.28
VAL OX : VAL PS	%	13.2	15.2	2.2	0.16
PS	g d^{-1}	307.5	278.7	27.0	0.17

1. Valine irreversible loss rate.
2. Calculated from the injection of deuterated water on day 45 post infection.
3. Valine oxidation.
4. Protein synthesis.

Table 4.3 Valine (VAL) kinetics in the mesenteric-drained viscera (MDV) of lambs (day 48 post infection) fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD). Positive values for net valine flux represent a net utilisation by the tissue, while a negative value represents a net release.

		Control n=6	Parasite n=5	Pooled SD	P
Digesta VAL flux					
Abomasal VAL flux	mmol d^{-1}	42.7	44.4	2.6	0.35
Ileal VAL flux	mmol d^{-1}	15.8	16.8	3.7	0.73
Apparent VAL absorption	mmol d^{-1}	27.2	27.3	4.8	0.99
Net plasma VAL flux					
Plasma flow	mL min^{-1}	545	772	221	0.22
Arterial VAL inflow	mmol d^{-1}	185.3	273.6	83.3	0.21
Net VAL flux	mmol d^{-1}	-26.9	-51.4	4.8	0.03
VAL kinetics calculated using jugular ^{3}H-VAL infusion					
Arterial ^{3}H-VAL inflow x 10^{9}	dpm d^{-1}	19.8	30.8	9.6	0.18
Arterial ^{3}H-VAL extraction x 10^{9}	dpm d^{-1}	1.3	3.5	0.9	0.02
Arterial ^{3}H-VAL extraction	%	7.9	14.6	4.8	0.12
VAL ILR from arterial sources[1]	mmol d^{-1}	11.5	30.4	5.0	0.004
VAL ILR from arterial sources[2]	mmol d^{-1}	13.8	30.3	6.2	0.02
Ileal VAL SRA: Arterial VAL SRA	%	11.3	14.4	2.7	0.20
Endogenous VAL loss	mmol d^{-1}	1.9	2.5	0.8	0.36
VAL kinetics calculated using abomasal ^{13}C-VAL infusion					
Arterial recycled ^{13}C-VAL inflow	mmol d^{-1}	9.0	13.5	5.5	0.32
Luminal ^{13}C-VAL recovery in M[3]	mmol d^{-1}	2.3	-0.3	2.0	0.08
Apparent ^{13}C-VAL absorption	mmol d^{-1}	8.6	9.4	1.3	0.49
VAL kinetics calculated using abomasal ^{13}C-VAL and jugular ^{3}H-VAL infusions					
Arterial recycled ^{13}C-VAL extraction	mmol d^{-1}	0.5	1.5	0.3	0.004
Luminal ^{13}C-VAL corrected recovery in M[3]	mmol d^{-1}	2.8	1.0	2.0	0.29
Luminal ^{13}C-VAL corrected recovery in M[3]	%	28.0	-0.6	23.3	0.23
Luminal ^{13}C-VAL extraction	%	71.9	100.6	23.2	0.23
Absorbed VAL extraction	mmol d^{-1}	20.6	28.4	6.2	0.22
Extracted VAL loss into lumen	mmol d^{-1}	1.2	2.1	0.9	0.32
VAL ILR from luminal sources	mmol d^{-1}	17.2	25.9	6.5	0.19
Total VAL ILR (arterial + luminal)	mmol d^{-1}	29.1	53.0	8.8	0.04

1. Irreversible loss rate (ILR) of VAL calculated using the arterial free VAL as precursor pool.
2. Irreversible loss rate (ILR) of VAL calculated using the mesenteric vein free VAL as precursor pool.
3. M = mesenteric vein.

The ratio of arterial to total valine ILR was unaffected by parasitic infection (46.0 *vs.* 50.5 (SD 11.7) % in the control and parasitised lambs, respectively). Similarly, the luminal contributions to total ILR were also similar between treatments (53.9 *vs.* 49.6 (SD 11.7) % in the control and parasitised lambs, respectively; P>0.15).

4.4.4 Valine flux and kinetics in the portal-drained viscera

Parasite infection increased plasma flow and arterial valine inflow across the PDV tissues (P<0.10; Table 4.4), however net release of valine by these tissue beds was similar between treatments and averaged 20 mmol d^{-1} (P>0.15; Table 4.4).

The ILR of valine in the PDV was obtained using both jugular (^{3}H-valine) and lumen sources of valine (^{13}C-valine; Table 4.4). The inflow of arterial ^{3}H-valine to the PDV tissues was higher in the parasitised lambs as was ^{3}H-valine extraction by the PDV (P<0.10; Table 4.4). Consequently, the valine ILR from arterial sources in the PDV tissues was increased 48 d after infection (P<0.10; Table 4.4). A similar effect of treatment was observed whether the estimate of valine ILR was calculated using the arterial or venous free valine precursor pool.

The recovery of luminal ^{13}C-valine into the portal drainage was similar between the control and infected lambs and averaged 3 mmol d^{-1} (P>0.15; Table 4.4). This recovery was corrected by accounting for the fractional uptake of recycled ^{13}C-valine estimated using parameters calculated from the ^{3}H-valine infusion in the jugular vein. This arterial extraction of recycled ^{13}C-valine by the PDV was doubled in parasitised lambs (P<0.15). However, the recovery of luminal ^{13}C-valine in the portal drainage corrected for this extraction was not significantly altered (P>0.15; Table 4.4), but when presented as a proportion of ^{13}C-valine absorption, there was more valine recovered in the portal drainage and thus less valine taken up by the PDV in the infected lambs (P<0.10; Table 4.4). Therefore, the extraction of luminal ^{13}C-valine by the PDV (from the pools of absorbed valine) and the valine ILR from all luminal sources were decreased by infection (P<0.10). Overall, parasite infection increased the total valine ILR (arterial + lumen) sources across the PDV (P<0.05; Table 4.4).

Table 4.4 Valine (VAL) kinetics in the portal-drained viscera (PDV) of lambs (day 48 post infection) fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD). Positive values for net valine flux represent a net utilisation by the tissue, while a negative value represents a net release.

		Control n=6	Parasite n=5	Pooled SD	P
Net flux parameters					
Plasma flow	mL min^{-1}	1182	1353	112	0.06
Arterial VAL inflow	mmol d^{-1}	385.5	491.0	59.0	0.04
Net VAL flux	mmol d^{-1}	-23.3	-15.6	29.7	0.72
VAL kinetics calculated using jugular ^{3}H-VAL infusion					
Arterial ^{3}H-VAL inflow x 10^{9}	dpm d^{-1}	37.9	52.1	7.1	0.03
Arterial ^{3}H-VAL extraction x 10^{9}	dpm d^{-1}	3.0	6.6	2.4	0.08
Arterial ^{3}H-VAL extraction	%	7.0	13.1	4.0	0.07
VAL ILR from arterial sources[1]	mmol d^{-1}	29.2	63.1	22.9	0.07
VAL ILR from arterial sources[2]	mmol d^{-1}	31.3	64.6	25.5	0.10
VAL kinetics calculated using abomasal ^{13}C-VAL infusion					
Arterial recycled ^{13}C-VAL inflow	mmol d^{-1}	17.7	23.3	7.8	0.33
Luminal ^{13}C-VAL recovery in P[3]	mmol d^{-1}	2.9	2.8	1.3	0.87
Apparent ^{13}C-VAL absorption	mmol d^{-1}	8.6	9.4	1.3	0.50
VAL kinetics calculated using abomasal ^{13}C-VAL and jugular ^{3}H-VAL infusions					
Arterial recycled ^{13}C-VAL extraction	mmol d^{-1}	1.3	2.8	1.2	0.11
Luminal ^{13}C-VAL corrected recovery P[3]	mmol d^{-1}	4.2	5.8	2.4	0.36
Luminal ^{13}C-VAL corrected recovery P[3]	%	34.7	61.1	15.1	0.07
Luminal ^{13}C-VAL extraction	%	65.3	38.9	15.1	0.07
Absorbed VAL extraction	mmol d^{-1}	9.5	6.1	1.9	0.07
Extracted VAL loss into lumen	mmol d^{-1}	1.1	0.9	0.6	0.59
VAL ILR from luminal sources	mmol d^{-1}	15.7	8.6	4.0	0.07
TOTAL VAL ILR (arterial + luminal)	mmol d^{-1}	42.1	85.4	20.0	0.04

1. Irreversible loss rate (ILR) of VAL calculated using the arterial free VAL as precursor pool.
2. Irreversible loss rate (ILR) of VAL calculated using the portal vein free VAL as precursor pool.
3. P = portal vein.

The ratio of arterial to total ILR was increased by parasitic infection (53.8 *vs.* 91.5 (SD 11.2) % in the control and parasitised lambs, respectively; $P<0.05$). Therefore, the luminal contribution to total ILR was also lower in the infected lambs (46.2 *vs.* 8.5 (SD 1.2) % in the control and parasitised lambs, respectively; $P<0.05$).

The contribution of the MDV to the PDV valine kinetics was similar between the control and parasitised lambs (70.5 *vs.* 43.2 (SD 23.6) % in the control and parasitised lambs, respectively; $P>0.15$).

4.4.5 Valine kinetics in the splanchnic tissues

Despite a 3-fold increase in plasma flow through the hepatic artery ($P<0.15$; Table 4.5), there was no effect after 48 d of infection on the net flux of valine across the liver (Table 4.5; $P>0.15$). Fractional hepatic extraction of ^{3}H-valine was increased from 6 to 18% during parasite infection ($P<0.01$; Table 4.5). The valine ILR estimated from the ^{3}H-valine infusion using either the arterial and venous free valine precursor pool was significantly increased with infection ($P<0.01$; Table 4.5). However, the extraction of ^{13}C-valine by the liver was similar between treatments and averaged 5% and therefore, valine ILR obtained using parameters calculated from the infusion of ^{13}C-valine were not affected by infection ($P>0.15$; Table 4.5).

Net valine flux across the TSP tissues was similar between control and infected lambs ($P>0.15$), despite an increase in plasma flow across these tissues ($P<0.10$; Table 4.5). Valine was released by the TSP tissues at an averaged rate of 11 mmol d^{-1}. Fractional hepatic extraction of ^{3}H-valine by the TSP tissues was doubled by parasitic infection ($P<0.01$; Table 4.5). Consequently, valine ILR across the TSP tissues calculated from the ^{3}H-valine infusion was increased during infection. In contrast, the estimates of valine ILR calculated from the infusion of ^{13}C-valine into the abomasum was not affected by the presence of parasites in the MDV ($P>0.15$; Table 4.5).

Table 4.5 Valine (VAL) kinetics in liver and total splanchnic tissues (TSP) of lambs (day 48 post infection) fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD). Positive values for net valine flux represent a net utilisation by the tissue, while a negative value represents a net release.

		Control	Parasite	Pooled	
		n=6	n=5	SD	P
LIVER					
Net flux parameters					
Plasma flow through hepatic artery	mL min^{-1}	101	346	199	0.14
Net VAL flux	mmol d^{-1}	10.1	-1.8	6.1	0.71
VAL kinetics calculated using jugular ^{3}H-VAL infusion					
^{3}H-VAL extraction$_{A+P}$ [1,2]	%	6.4	18.2	2.1	0.01
ILR_{A+P} [1,2,3]	mmol d^{-1}	21.3	101.2	16.5	0.01
ILR_H [4]	mmol d^{-1}	27.8	145.2	15.8	0.01
VAL kinetics calculated using abomasal ^{13}C-VAL infusion					
^{13}C-VAL extraction $_{A+P}$ [1,2]	%	4.4	6.3	3.5	0.43
ILR_{A+P} [1,2,3]	mmol d^{-1}	23.7	40.3	25.3	0.46
ILR_H [4]	mmol d^{-1}	24.5	46.3	23.7	0.31
TOTAL SPLANCHNIC TISSUES					
Net flux parameters					
Plasma flow through TSP	mL min^{-1}	1281	1676	238	0.07
Net VAL flux	mmol d^{-1}	-9.2	-12.1	13.8	0.43
VAL kinetics calculated using jugular ^{3}H-VAL infusion					
^{3}H-VAL extraction$_{A}$ [1]	%	10.3	19.7	3.5	0.01
ILR_A [1,5]	mmol d^{-1}	45.8	153.1	32.6	0.04
ILR_H [5]	mmol d^{-1}	53.5	197.3	39.6	0.01
VAL kinetics calculated using abomasal ^{13}C-VAL infusion					
ILR [6]	mmol d^{-1}	58.6	122.0	33.9	0.63

1. A = mesenteric artery.
2. P = portal vein.
3. ILR_{A+P}= irreversible loss rate calculated using the arterial and portal free valine as precursor pool.
4. ILR_H calculated using the hepatic vein free valine as precursor pool.
5. ILR_A= irreversible loss rate calculated using the free valine as precursor pool.
6. TSP ILR calculated from PDV ILR + Liver ILR.

Table 4.6 Valine kinetics across the muscles of the hind limbs of lambs fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD). Positive values for net valine flux represent a net utilisation by the tissue, while a negative value represents a net release.

		Control n=6	Parasite n=5	Pooled SD	P
Plasma flow	mL min^{-1}	489	464	141	0.81
F_{IN}[1]	mmol d^{-1}	111.2	122.8	36.5	0.66
F_{OUT}[2]	mmol d^{-1}	106.1	115.6	30.6	0.66
Net flux	mmol d^{-1}	6.3	7.3	8.1	0.72
VAL kinetics calculated using jugular ^{3}H-VAL infusion					
Arterial ^{3}H-VAL extraction	%	11.1	15.7	5.0	0.23
ILR_A[3]	mmol d^{-1}	18.2	29.5	17.7	0.41
ILR_V[4]	mmol d^{-1}	20.6	34.3	21.3	0.38
F_{TA}[5]	mmol d^{-1}	23.5	33.8	15.3	0.47
F_{VT}[6]	mmol d^{-1}	21.7	28.8	16.8	0.64
F_{VA}[7]	mmol d^{-1}	88.9	101.5	37.5	0.71
F_{TO}[8]	mmol d^{-1}	40.7	35.5	26.3	0.83
	g d^{-1}	140.1	122.3	90.9	0.83
F_{OT}[9]	mmol d^{-1}	42.5	40.5	26.4	0.93
	g d^{-1}	146.4	139.4	90.9	0.93
R_{AT}[10]	mmol d^{-1}	64.2	69.3	24.3	0.82
VAL kinetics calculated using abomasal ^{13}C-VAL infusion					
Arterial ^{13}C-VAL extraction	%	15.0	12.3	4.0	0.28
ILR_A[3]	mmol d^{-1}	21.9	21.6	11.4	0.96
ILR_V[4]	mmol d^{-1}	25.7	23.9	8.1	0.83
F_{TA}[5]	mmol d^{-1}	34.6	31.9	20.8	0.85
F_{VT}[6]	mmol d^{-1}	29.5	24.6	17.7	0.70
F_{VA}[7]	mmol d^{-1}	76.5	90.9	33.6	0.55
F_{TO}[8]	mmol d^{-1}	19.0	19.5	6.3	0.90
	g d^{-1}	63.6	72.9	23.2	0.58
F_{OT}[9]	mmol d^{-1}	24.1	26.7	11.5	0.75
	g d^{-1}	81.9	96.3	36.9	0.59
R_{AT}[10]	mmol d^{-1}	53.6	51.4	18.9	0.87

1. F_{IN}: arterial valine inflow into the hind limbs.
2. F_{OUT}: venous valine outflow from the hind limbs.
3. ILR calculated using the arterial free valine as precursor pool.
4. ILR calculated using the vena cava free valine as precursor pool.
5. F_{TA}: valine inwards transport from artery to muscle.
6. F_{VT}: valine outward transport from muscle to vein.
7. F_{VA}: valine by pass flow from the artery to the vein.
8. F_{TO}: valine released from muscle degradation.
9. F_{OT}: valine used for muscle protein synthesis and oxidation.
10. R_{AT}: total appearance of valine in intracellular pool of muscle.

4.4.6 Valine kinetics across the hind limbs

Parasite infection had no effect on plasma flow across the hind limbs (P>0.15; Table 4.6). Fractional extraction of ^{13}C- or ^{3}H-valine across these tissues was also similar between treatments (P>0.15; Table 4.6). The valine ILR across the hind limbs estimated from both infusions was unchanged by the infection when either arterial or venous valine isotopic activity was used as precursor pool (P>0.15; Table 4.6).

Estimates of transmembrane fluxes of valine across the muscles of the hind-limbs, estimated from intra-venous ^{3}H-valine and intra-luminal ^{13}C-valine infusions, were unaffected by parasite infection (P>0.15; Table 4.6; Appendix D.1 and D.2). Neither the arterial inflow (F_{IN}) nor outflow (F_{OUT}) of valine was affected by the infection. As a result of F_{IN} being greater than F_{OUT}, a net uptake of valine across the hind limbs was observed in both groups of lambs. Transport of valine into (F_{TA}) and out of (F_{VT}) muscle cells was unaffected by the presence of parasite infection (P>0.15; Table 4.6). Valine bypass flow (F_{VA}) was unaffected by parasitic infection. Estimates of valine used for protein synthesis and oxidation (F_{OT}) and released from degradation (F_{TO}) were also similar between control and parasitised lambs (P>0.15; Table 4.6).

4.5 Discussion

The hypothesis of this study was that during parasitic infection there is a repartitioning of AA from the muscles of the hind limbs to the GIT and other tissues such as the liver for assisting the GIT in combating the parasite burden (e.g., for the repair of damaged GIT tissues). Despite no effect of parasitic infection on the whole body valine kinetics, change in valine kinetics across some tissue beds was observed in parasitised lambs. The alterations in the ILR of valine across the MDV suggest that the parasite burden did affect AA metabolism and/or protein turnover at the site of the infection. The alterations in PDV kinetics with parasitic infection reflect mostly the changes seen in the MDV tissue which is consistent with the small intestine being the region of the GIT where *T. colubriformis* reside. The data on valine kinetics in the liver suggest that there may have been flow-on impacts on valine availability to the peripheral tissues as the valine ILR in the liver was increased in parasitic lambs. However, parasite infection had no effect on the

estimates of net flux and trans-membrane transport of valine and on the rates of protein turnover (estimated from valine ILR) and protein degradation across the hind limbs. Therefore, it seems unlikely that the presence of an established parasite infection resulted in a repartitioning of AA from the posterior hind limbs to the GIT or liver. The similar net flux of valine and higher valine ILR across the MDV, PDV and liver at 48 d post infection suggest that the increase in utilisation of valine for protein synthesis and/or oxidation is matched by an increase in the release of valine from protein degradation in these tissues.

4.5.1 Whole body valine kinetics

The similar whole body valine ILR between the control and infected lambs presented in the current study are consistent with the results of Yu *et al.* (2000) where whole body leucine flux was not altered during a trickle-infection of *T. colubriformis*. Similar whole body valine ILR estimates between control and infected lambs are in agreement with reports of similar energy intake and expenditure during infection (MacRae *et al.*, 1982).

Whole body valine ILR presented in this study is in agreement with that reported by others (Teleni *et al.*, 1986; Lobley *et al.*, 1996; Savary-Auzeloux *et al.*, 2003). Valine oxidation estimates in the whole body obtained during the infusion of ^{3}H-valine agree with those presented for pigs and rats (13-20% of the total dose given; Beckett *et al.*, 1992) and for a range of AA in sheep (Lobley *et al.*, 2003). Protein synthesis estimates are in agreement with results in lambs fed similar intake and of liveweight presented in the literature (196-320 g d^{-1}; Davis *et al.*, 1981; Teleni *et al.*, 1986; Harris *et al.*, 1992; Lobley *et al.*, 1996; 2003; Connell *et al.*, 1997; MacRae *et al.*, 1997).

The estimate of whole body valine ILR calculated from the intravenous ^{3}H-valine infusion is approximately 70% lower than the ILR calculated from the intra-luminal ^{13}C-valine infusion (*c.* 102 *vs.* 300 mmol d^{-1}). This discrepancy between valine ILR estimated from different sites of infusion has been observed previously in humans where the ILR estimate from intravenous infusion of labelled AA was about 30% lower than that obtained with an intra-luminal infusion of the same AA (Hoerr *et al.*, 1991). Lower ILR estimates from an intra-luminal infusion of labelled AA is a

consequence of the labelled AA infused going through additional dilution by the free valine present in the GIT and consequently, resulting in a lower plasma isotopic activity of valine in the peripheral blood. However, the extent of the difference between the estimates of valine ILR obtained between the sites of infusions in the current study was surprising.

4.5.2 Tissue valine kinetics

Whilst determining the net flux of AA across the tissue beds may provide some clues about the partitioning of AA throughout the body, net flux in itself is not a descriptive measurement as the end fate of the AA is not determined. Therefore, by measuring the fate of labelled AA we were able to gain insight into what is happening in the tissue bed of interest.

Commonly, a single tracer infused into the jugular vein has been used to estimate protein turnover in tissues (e.g., liver and muscle) where the entry of AA from the blood circulation is from the arterial supply (e.g., Harris *et al.*, 1992; Lobley *et al.*, 1995; Lapierre *et al.*, 1999). For these tissues an estimate of the fate of the AA can be obtained using this technique. However, tissues such as the MDV and PDV utilise AA from both arterial (*c.* 80%) and luminal (*c.* 20%) supply (MacRae *et al.*, 1997; Stoll *et al.*, 1998; Yu *et al.*, 2000; Rémond *et al.*, 2003). Therefore, in these tissues a dual-tracer technique can provide important insights into the AA requirements by the tissue bed and the source of the AA used to meet their requirements. This can determine whether there is any repartitioning of AA from other tissues to the GIT during intestinal parasite infection as indicated by an increase in arterial loss of AA to the tissue bed. This is especially important during parasitic infection as AA absorption may be reduced (Poppi *et al.*, 1986; Bown *et al.*, 1991) due to the alterations in GIT morphology caused by the parasite (Coop & Angus, 1975). The use of dual labels of AA has previously been used in the sheep (MacRae *et al.*, 1997; Yu *et al.*, 2000), pig (Bush *et al.*, 2003) and human (Hoerr *et al.*, 1991). It is assumed when using dual tracers the metabolism of the different isotopic-labels of the AA are similar (Bush *et al.*, 2003).

4.5.2.1 Valine kinetics in the mesenteric- and portal-drained viscera

Parasite infection increased the fractional extraction of ^{3}H-valine from the mesenteric artery by the MDV. Consequently, the ILR of valine from arterial sources was increased during parasitic infection. The ILR of valine from luminal sources was unaffected by the parasitic infection and thus, the higher total valine ILR (arterial and luminal sources) in infected lambs was mainly attributed to the increased extraction from arterial sources. These results agree with those from Yu *et al.* (2000) who observed an increase in the leucine sequestration (or ILR) from arterial sources by the MDV during a trickle infection. In Yu's study, there was no report of leucine ILR from luminal sources by the MDV because it was assumed that ILR from luminal sources from the PDV was representative of those in the MDV. Leucine ILR across the MDV from arterial sources in uninfected lambs was 8 mmol d^{-1} (Yu *et al.*, 2000). This is similar to the results observed in the control lambs in the current study (*c.* 11 mmol d^{-1}).

As observed for MDV, valine ILR from arterial sources in the PDV was increased by parasite infection. This agrees with results by Yu *et al.* (2000) who observed a 24% increase in fractional extraction of arterial ^{13}C-leucine in parasitised lambs and thereby higher leucine ILR across the PDV in these lambs. Values for ILR of valine across the PDV from arterial sources are within the range presented for other labelled AA in the uninfected lamb (*c.* 39-70 mmol d^{-1}; Lobley *et al.*, 1995; MacRae *et al.* 1997; Yu *et al.* 2000). Irreversible loss of valine across the PDV based on luminal supply are also within the range presented in the literature (10-15 mmol d^{-1}; MacRae *et al.* 1997; Yu *et al.* 2000; Bush *et al.* 2003).

In the uninfected lamb, the contribution of valine ILR from arterial sources to the total valine ILR in the MDV were lower than those from the luminal sources. The presence of an established parasitic infection did not alter this ratio (luminal: 54 *vs.* 50% and arterial: 46 *vs.* 51%, for the control and parasitised lambs, respectively). This is in contrast to the increase in the contribution of valine from arterial sources to the total valine ILR in the MDV that was observed during a trickle infection of *T. colubriformis* in another study (Yu *et al.*, 2000). However, the value presented by Yu *et al.* (2000) was calculated assuming that the luminal contribution to the ILR of

leucine across the PDV was equivalent to the luminal contribution to leucine ILR across the MDV.

In the PDV the contribution of valine ILR from arterial sources to total ILR was increased from 54 to 92% during infection and consequently the luminal contribution was reduced from 46 to 8%. The larger contribution of arterial AA to ILR in the PDV tissues observed in infected lambs agrees with the results published by MacRae *et al.* (1997) and Yu *et al.* (2000). Furthermore, our study together with MacRae *et al.* (1997) and Yu *et al.* (2000) confirms that the major supply of AA to the PDV is from the arterial supply. In the current study, total ILR of valine (arterial and luminal sources) by the MDV accounted for 70% of whole gastrointestinal tract losses in the control lambs, and this ratio was unaffected by the infection. This decrease contradicts the data presented by Yu *et al.* (2000), where MDV valine ILR from arterial and luminal sources represented only 26% of PDV ILR and with a higher proportion observed in infected lambs (32%).

As valine oxidation in the PDV tissues was not measured in the current study, absolute protein synthesis could not be calculated from the valine ILR estimates. In this study, protein fractional synthesis rates (FSR) in MDV (duodenum and ileum) and parts of the PDV (MDV, spleen and mesenteric lymph nodes) were similar between treatments (see Chapter Three). Similar FSR together with higher valine ILR in the MDV and PDV suggests that there might be an increase in valine oxidation in these tissues. While this parameter was not measured for MDV and PDV tissues in the current study, Yu *et al.* (2000) observed an increase in the oxidation of leucine by the MDV and PDV tissues of infected lambs. It must be noted that the constant infusion procedure of a labelled AA used to estimate FSR is not optimal for tissues that secrete a large amount of protein or have high rates of protein turnover (Davis *et al.*, 1999). Therefore, the estimates of FSR in the MDV and PDV tissues are only indicative of the synthesis of constitutive proteins whilst the ILR estimates include both synthesis of secreted and constitutive proteins and oxidation of AA. It is plausible that the synthesis of proteins secreted by these tissues might have increased during the parasitic infection with or without an increased AA oxidation. Since the availability of valine to the PDV tissues was similar between the control and infected lambs it is likely that the increased valine

ILR is accompanied with an increased release of valine from protein degradation in these tissues.

4.5.2.2 Valine kinetics in the liver and splanchnic tissues

This is the first report of the effects of an established parasitic infection on the ILR of valine across the liver and TSP. In both tissue beds the presence of parasite infection increased the ILR of valine calculated from the data obtained from the intravenous ^{3}H-valine infusion. However, estimates of valine turnover in the liver and TSP tissues obtained from the intra-luminal ^{13}C-valine infusion were unaffected during infection, although higher but non significant ILR estimates were obtained in infected animals. The discrepancy between these two estimates is unclear. However, Krempf *et al.* (1990) suggested that differences in whole body kinetics using several labels (e.g., $^{2}H_{5}$-, ^{15}N- and ^{13}C-phenylalanine) of phenylalanine infused intragastrically was due to differences in their splanchnic metabolism. This suggestion is supported by the findings of Yu *et al.* (1990) who observed a higher whole body leucine flux calculated from an infusion of ^{15}N-leucine into the jugular vein compared to a similar infusion protocol with ^{13}C-leucine. These authors suggested that a faster rate of N removal from leucine compared to the rates of decarboxylation of leucine might be responsible. Similarly, in the current study, differences between the metabolism of ^{3}H and ^{13}C-isotopes of valine in the liver may also explain the discrepancy between the hepatic valine ILR estimates obtained although these isotopes were not administered at the same site of infusion.

Higher ILR together with similar FSR in the liver during parasitic infection (Chapter Three) suggests that oxidation in the liver might be increased in infected lambs. However, as discussed in Chapter Three and in the previous section, the constant infusion procedure for estimating FSR may not be optimal for studying tissues which have a relatively high rate of protein turnover or secrete exported proteins such as the liver. In contrast, the valine ILR estimates include both an estimation of synthesis of constitutive and exported proteins. It is possible that only the hepatic synthesis of secreted proteins was increased in infected lambs in the current study and thus, the higher valine ILR in the liver could be explained by either increased utilization of

valine for synthesis of secreted proteins in the liver, increased valine oxidation or both.

Parasite infection increases the leakage of serum proteins (e.g., albumins) into the GIT (Poppi *et al.*, 1986). Therefore, it is likely that the synthesis and secretion of serum proteins by the liver into the peripheral circulation increased in parasitised lambs and more of these secreted proteins can leak into the GIT lumen during the parasitic infection. Furthermore, similar net flux of valine across the liver between the control and infected lambs suggests that the increased valine ILR was accompanied by an increased release of valine from protein degradation in the liver of infected lambs as suggested for the MDV and PDV tissues.

4.5.2.3 Valine kinetics in the hind limbs

Valine ILR was estimated in the hind limbs using the traditional arterio-venous balance of ^{3}H-valine or ^{13}C-valine as described by Harris *et al.* (1992). These arterio-venous data was combined with intracellular valine kinetics. Therefore, transmembrane transport, intracellular production and intracellular disposal of valine from the muscle free pool of valine were estimated using the three-compartment model of leg amino acid kinetics developed by Biolo *et al.* (1992).

The assumptions of Biolo's model include that the tissue free pool of AA is the precursor pool for protein synthesis and that those released from protein degradation pass though this pool. It also assumes that the isotopic activity and concentration of an AA for muscle is representative of the whole hind limb preparation. The hind limb preparation includes the contributions from muscle, skin, fat and bone. Approximately 70% of the blood flow in the hind limb can be attributed to muscle, with 10% to both the skin and bone (Biolo *et al.*, 1992). Furthermore muscle accounts for 85-90% of the hind limb preparation (Biolo *et al.*, 1995), while skin accounts for 10-15% of the hind limb protein kinetics (Biolo *et al.*, 1994). Skin and muscle enrichment were found to be comparable for most AA (Biolo *et al.*, 1992). However, this model does assume that there is no oxidation or *de novo* synthesis of AA (Biolo *et al.*, 1992). Therefore, the chosen labelled AA for our study (valine) does not meet all these criteria because branched-chain AA undergo the first step of

their oxidation in the muscle (Rodwell, 2000). Thus, the estimates of intracellular disposal of valine from the muscle free pool do not represent an estimate of valine used for protein synthesis but rather an estimate of valine ILR (protein synthesis and oxidation). The rate of intracellular production of valine from the muscle free pool does estimate protein degradation as valine, an essential AA, does not undergo *de novo* synthesis.

Parasite infection did not affect the ILR of valine and the transmembrane transport of valine across the hind limbs either from intravenous or intra-luminal infusion, with both isotopes giving similar estimates (20-34 *vs.* 21-26 mmol d^{-1} for the ^{3}H-valine and ^{13}C-valine, respectively). Values presented for the valine transport kinetics in the muscle of the hind limbs are in agreement with those presented for sheep of a similar liveweight and feed intake using ^{13}C-labelled valine (*c.* 14-24 mmol d^{-1}; Hoskin *et al.*, 2003). However, protein turnover estimated from valine ILR and degradation estimates using ^{3}H-labelled valine (present study) are much higher than the results presented for ^{13}C-valine in the present study and that of Hoskin *et al.* (2003). Reasons for this may include differences in the metabolism of the isotope within the hind limb as discussed in Section 4.5.2.2 and by Yu *et al.* (1990).

The estimation of protein synthesis and oxidation (estimated from valine ILR) and protein degradation in the hind limbs using the Biolo's model (current study; Hoskin *et al.*, 2003) are 2-4 times higher than those obtained from the traditional arterio-venous approach for sheep of a similar age, liveweight and dry matter intake (e.g., Harris *et al.*, 1992; Hoskin *et al.*, 2003). Estimates of protein turnover across the hind limbs using the traditional arterio-venous technique range between 24-30 g protein d^{-1} (based on labelled phenylalanine and leucine, respectively; Harris *et al.*, 1992) to 46 g protein d^{-1} (based on valine; Hoskin *et al.*, 2003) and 80 g protein d^{-1} (current study). However, the traditional arterio-venous approach does not take by-pass of AA into consideration and therefore will underestimate protein synthesis if there is a significant by-pass of AA directly from the artery to the venous blood compartment without entering the intracellular pool. This flow is significant and ranged from 53-60 mmol d^{-1} (current study) to 110 mmol d^{-1} (Hoskin *et al.*, 2003).

4.6 Conclusions

In conclusion an established *T. colubriformis* infection increased the ILR of valine in the MDV, PDV and liver and consequently in the TSP tissues. As FSR in the TSP tissues were unaffected by infection this might suggest that there was an increase in the oxidation of valine in this tissue bed. The TSP tissues are tissues synthesising a large amount of proteins that are secreted into the lumen (MDV and PDV) or peripheral blood (liver). The FSR estimates of these tissues do not account for this contribution whilst the ILR of an essential AA across these tissues include the utilisation of this AA for oxidation and synthesis of constitutive and secreted proteins. Therefore, the increase in valine ILR in the TSP tissues could be a consequence of more secreted proteins being synthesised with or without more AA being oxidised. Despite the increase in ILR of valine into the TSP tissues, there was no net affect on the availability of valine to the rest of the tissues as indicated by similar net flux of valine across these tissues. The ILR and trans-membrane transport of valine across and the protein turnover within the posterior hind limbs were unaffected by infection. This suggests that there is no re-partitioning of AA from the posterior hind limbs to the GIT and liver during an established infection. However, the increase in the arterial contribution to total valine ILR in the PDV tissues suggests that there is a diversion of AA from sources other than the tissues of the posterior hind-limbs. Alternatively, and more likely, protein turnover is increased in the TSP tissues as a consequence of higher protein synthesis (and/or AA oxidation) and degradation during parasitic infection. This suggests that an established parasitic infection triggers localised alterations in AA metabolism and/or protein turnover in the affected tissue without changing significantly the metabolism of AA and proteins in tissues peripheral to the TSP tissues and without impacting negatively on the growth of the parasitised lambs.

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5 AMINO ACID AVAILABILITY AND NUTRIENT FLUXES IN LAMBS FED FRESH SULLA (*HEDYSARUM CORONARIUM*) DURING A *TRICHOSTRONGYLUS COLUBRIFORMIS* INFECTION.

5.1 Abstract

The effect of a *Trichostrongylus colubriformis* infection on amino acid (AA) availability to the small intestine and the partitioning of AA and nutrients across the mesenteric-drained viscera (MDV), portal-drained viscera (PDV), liver, total splanchnic tissues (TSP; PDV and liver) and hind limbs were determined in lambs 48 d post infection. The lambs were fed fresh Sulla (*Hedysarum coronarium*; 800 g DM d^{-1}). Lambs were dosed with 6 000 L3 *T. colubriformis* larvae for 6 d (n=6) or kept as parasite free controls (n=6) and faecal egg production was monitored every second day from day 22 to day 48. A nitrogen (N) balance was conducted on days 35 to 43 post infection, and digesta flow measurements made on day 44. On day 48 post infection, the lambs were continuously infused with para-aminohippuric acid (720 mg h^{-1}) and indocyanin green (15 mg h^{-1}) for 8 h in order to measure blood flow across the splanchnic tissues and hind limbs, respectively. Blood was continuously collected from the mesenteric, portal and hepatic veins, the mesenteric artery and the vena cava and plasma harvested and AA and metabolite concentrations determined. At the end of the infusion period the lambs were euthanased and digesta in the proximal intestine was sampled for intestinal worm burdens. Ileal contents were also sampled for digesta flow measurements. Faecal egg production peaked on day 26 post infection ($P<0.001$) and intestinal worm burdens on day 48 post infection were significantly higher in the infected lambs ($P<0.05$). Parasitic infection in the small intestine decreased feed intake ($P<0.15$) and liveweight gain ($P<0.15$). The digestibility of dry matter (DM) and N were unaffected by parasitic infection ($P>0.15$). Despite a reduction in both AA intake and abomasal flow of AA ($P<0.05$) during infection, apparent AA digestibility in the small intestine was unaffected ($P>0.15$). Plasma flow across the tissue beds were unaffected by parasitic infection ($P>0.15$), however less total-AA were released from the MDV ($P<0.11$) and PDV ($P<0.05$). With the exception of the branched-chain AA which were released in control lambs and utilised in infected lambs ($P<0.10$), the majority of non-essential, essential, sulphur and total AA utilisation were similar in the liver ($P<0.15$). In the TSP, all AA were released by the control lambs and utilised by the infected lambs ($P<0.10$). However, there was no effect of parasitic infection on AA

utilisation across the hind limbs ($P<0.15$). Parasitic infection altered the utilisation of glucose and lactate in the liver, TSP and hind limbs ($P<0.10$), however the MDV and PDV fluxes were unaffected ($P>0.15$). Despite a reduction in the release of AA from the TSP of infected lambs, there was no effect of parasitic infection on the release of AA from the hind limbs. However, liveweight gain was adversely affected over the last 20 d of the experiment suggesting that there may be alterations in either protein synthesis and/or degradation in the hind limbs which would cause a reduction in liveweight but have no effect on net AA flux across the tissue bed. These results also suggest that feeding Sulla (compared to control lambs fed Lucerne; *Medicago sativa*; Chapter Two) did not result in increased supply of AA from the small intestine for productive purposes in the lamb infected with intestinal parasites.

5.2 Introduction

Parasitic infection results in decreased liveweight gain (43-66%; Sykes & Coop, 1976; van Houtert *et al.*, 1995), wool production (20-77%; Steel *et al.*, 1980; 1982; Leyva *et al.*, 1982) and milk production in ewes (17-23%; Leyva *et al.*, 1982; Thomas & Ali, 1983). This may be due to the animal diverting nutrients away from the productive tissues by catabolising body reserves, such as skeletal muscle. These alterations are likely to occur to meet the additional metabolic demands of the gastrointestinal tract (GIT) and liver during parasitic infection due to the increase in amino acid (AA) requirements by these tissues for repair of damaged tissue and the immune response (Coop & Kyriazakis, 1999; Butter *et al.*, 2000; Coop & Sykes, 2002).

Forages that contain condensed tannins (CT) have been reported to improve liveweight gain (Neizen *et al.*, 1993) and to decrease faecal egg counts (FEC; Robertson *et al.*, 1995; Niezen *et al.*, 1998) during parasitic infection. When comparisons of different forages containing CT were made, the infected lambs fed Sulla (*Hedysarum coronarium*) had consistently higher liveweight gains than *L. corniculatus* (Robertson *et al.*, 1995; Niezen *et al.*, 1998). In these field trials only lambs fed Sulla had reduced intestinal worm burdens (Robertson *et al.*, 1995; Niezen *et al.*, 1998). Feeding Sulla

improved lean carcass gain and wool production (Terrill *et al.*, 1992a; Burke *et al.*, 2002) when compared to pasture-based diets. High intakes of Sulla at both low (4-5%; Terrill *et al.*, 1992a) and high (7%; Stienezen *et al.*, 1996) concentrations of CT have been reported in sheep. It has been suggested that feeding Sulla during parasitic infection may affect the resistance and resilience of sheep to parasites due to improvements in AA flow to the small intestine (Coop & Kyriazakis, 2001). The importance of improved protein supply during parasitic infection is well documented in the literature (Bown *et al.*, 1991; Donaldson *et al.*, 1997) and the CT present in Sulla increased the absorption of AA from the small intestine of sheep (Bermingham *et al.*, 2001). Therefore, an integrated strategy that involves feeding Sulla may be an alternative to the sole reliance on chemical drenching as a means to control intestinal parasites in sheep.

The positive impact of some CT-containing forages during parasitic infection may be due to either a direct effect on the parasite (Athanasiadou *et al.*, 2001; Butter *et al.*, 2001; Molan *et al.*, 2000a; 2000b; 2002) or by indirect mechanisms such as improving AA availability to the animal (Waghorn *et al.*, 1987b; Bermingham *et al.*, 2001). This would reduce the need for the animal to divert nutrients away from the productive tissue and to catabolise body reserves. Direct effects of CT on parasite larvae movement and development have been demonstrated *in vitro* (Molan *et al.*, 2000a; 2000b; 2002), however there has been no attempt to measure the effects of CT present in fresh forages on the availability of AA to the parasite-infected animal.

The hypothesis of this study was that feeding Sulla during an established parasite infection in lambs would result in no re-partitioning of AA from the hind limbs to the small intestine, due to the inclusion of CT present in Sulla resulting in an increase in the availability of dietary AA to the lamb. Therefore, the aim of this study was to determine the apparent absorption of dietary AA and their partitioning from the small intestine to the hind limbs (muscle, skin and fat) in the lamb when fed Sulla during an intestinal parasite infection. This was achieved by measuring the flux of nutrients and metabolites across the mesenteric-drained viscera (MDV; small intestine), portal drained viscera

(PDV; rumen, small intestine, large intestine, pancreas, and spleen), liver, total splanchnic tissues (TSP; PDV and liver) and hind limbs in lambs fed fresh Sulla.

5.3 Materials and Methods

5.3.1 Animals and feeds

The experimental procedures for this trial were reviewed and approved by the Crown Research Institute Animal Ethics Committee in Palmerston North, New Zealand (NZ) according to the Animals Protection Act (1960) and Animals Protection Regulations (1987) and amendments.

Wether lambs (33 kg) were weaned from their dams and were transported to AgResearch Limited, Grasslands Research Centre, Palmerston North. Upon arrival the lambs were drenched on consecutive days with Ivomectin (Ivomec® Merial, NZ Ltd.) and treated for external parasites using Wipeout (Coopers, Schering-Plough Animal Health Limited, NZ). The lambs were shorn and maintained on Lucerne pellets (800 g dry matter (DM) d^{-1}) and chaffed Lucerne hay (200 g DM d^{-1}) on feed pads for approximately 3 weeks. One week before surgery the sheep were brought indoors and housed in individual metabolism crates and maintained on the same diet.

Three weeks prior to the start of surgery, catheters were prepared using Tygon® tubing (Scientific supplies Ltd., Wellington, New Zealand), adapted from the method outlined by Huntington *et al.* (1989) and described in Appendix B.1. Catheters were treated with tridodecylmethylammonium chloride heparin (Polysciences Inc., Warrington, PA, USA) to prevent blood clotting between sampling periods (Huntington *et al.*, 1989). The catheters were sterilised in ethylene gas at 55°C for 2.5 h, with an 8 h aeration cycle. Catheters were left for a minimum of 1 week prior to surgery.

Twelve lambs were fasted for 24 hours before surgery. Due to the invasive nature of the surgical preparation, the 12 sheep were prepared over a 3 week period, with a maximum of 4 sheep in each week. Anaesthesia was induced with an intravenous injection of

ketamine (Phoenix; 100 mg mL^{-1}; 0.04 mL kg^{-1} liveweight (IV); Vetworks, Cambridge. NZ) and diazepam (DBL Diazepam; 5 mg mL^{-1}; 0.2 mL kg^{-1} liveweight; Vetworks, Cambridge. NZ) and maintained by isoflurane (1.5%; Vetworks, Cambridge, NZ) administered through an endotracheal tube. Permanent indwelling catheters were placed in the mesenteric artery, and the mesenteric, portal and hepatic veins (Huntington *et al.*, 1989) and vena cava (Ortigues & Durand, 1995) for blood sampling (see Appendix B.1 for catheter placement). Additional permanent catheters were placed in the mesenteric vein (upstream from the sampling catheter) and abdominal aorta for infusion of ρ-aminohippuric acid (PAH) and indocyanin green (ICG), respectively, to measure plasma flow across the TSP and the hind limbs. A permanent Teflon cannula was fitted in the abomasum (see Appendix B.1 for details) for the infusion of [1-^{13}C]-valine and [^{35}S]-cysteine on day 48 post infection to measure the kinetics of these AA across the MDV, PDV, liver, TSP and hind limbs. A temporary catheter was inserted into the jugular vein 2 d before the start of blood sampling for the infusion of [^{35}S]-sulfate, deuterium oxide and [^{13}C]-sodium bicarbonate on day 45 post infection, and [3, 4-^{3}H]-valine on day 48 post infection. Results relating to these parameters are presented in Chapters Six and Seven. All radioactive and stable isotopes were purchased from Amersham Life Science (Buckinghamshire, UK) and Mass Trace, Inc. (Woburn, MA, USA), respectively.

After surgery, the catheters were filled with sterile 250 iu mL^{-1} heparinised saline, containing 1% procaine penicillin (Bomacillin, Bomac Laboratories Ltd., Auckland, New Zealand). Catheters were flushed every 14 d with a sterile solution of porcine heparin (50 iu mL^{-1}) containing 1% procaine penicillin (Bomacillin, Bomac Laboratories Ltd., Auckland, NZ) in isotonic saline and then filled with sterile heparinised saline (250 iu mL^{-1}; containing 1% procaine penicillin) to maintain their patency. The lambs received a daily intramuscular injection of procaine penicillin (3 mL; Bomacillin, Bomac Laboratories Ltd., Auckland, NZ) for 4 d post-surgery.

The lambs were fed Lucerne pellets (800 g DM d^{-1}) and chaff (200 g DM d^{-1}) for 3 d after surgery and then were offered approximately 800 g DM d^{-1} of fresh Sulla (Table

5.1) until the conclusion of the trial. The Sulla was harvested every 2 d with a sickle bar mower by 10.00 am and stored at 4°C. Dry matter content of the Sulla was determined daily in order to adjust the amount of wet forage given to maintain DM offered at approximately 800 g DM d^{-1}. The lambs were fed at hourly intervals from overhead feeders and water was available *ad libitum*. The lambs were weighed weekly to monitor liveweight changes during the experimental period.

One week after surgery (day 1 of the experimental period) six sheep were given 6 000 *T. colubriformis* L3 larvae per day orally for 6 d (parasite treatment) while the remaining six sheep were drenched with Ivomectin (Ivomec® Merial, NZ Ltd) to serve as controls (control treatment). The lambs were assigned to either the control or parasite group according to a completely randomised block design. The L3 larvae were prepared from the faeces of sheep infected with pure cultures of *T. colubriformis* which were incubated at 20°C for 14 d in high humidity, with larvae extracted over a 24 h period using the standard Baermannisation procedure (Jørgensen *et al.*, 1998).

Table 5.1 Nutrient composition of Sulla (*Hedysarum coronarium*) fed to lambs over a 48 day infection period. Values based on feed samples taken during the nitrogen balance period (day 35 to 42) and are presented on a dry matter (DM) basis.

Nutrient	Concentration (g kg DM^{-1})
Total condensed tannin	26
Total non-essential amino acid	86
Total essential amino acid	61
Total amino acid	147
Nitrogen	27
Lipid	19
Ash	122
Acid detergent fibre	229
Neutral detergent fibre	207
Soluble sugars and starch	209
Organic matter digestibility	84
Metabolisable energy (MJ kg DM^{-1})*	12

* based on samples analysed by near infra red spectroscopy.

5.3.2 Parasitology

Faecal egg counts (FEC) from individual sheep were measured every second day from day 20 to day 45 of infection using the modified McMaster method (Whitlock, 1948), where the presence of one egg represents 50 eggs per gram of wet faeces. Total intestinal worm burdens were determined after slaughter. The proximal 10 m of the small intestine was detached from the abomasum, ligated and refrigerated. The contents of the intestine were washed and a 10% subsample of the washings was passed through a 38 μm sieve to collect worms for counting (Sutherland *et al.*, 1999b).

5.3.3 Digesta flow

On day 32 of the experiment the sheep were dosed with a pre-weighed, slow release alkane capsule (Captec^{TM} Alkane CRC; Captec NZ Ltd) into the rumen. The plant alkanes (odd-numbered carbons) present in the Sulla and the synthetic alkanes present in the Captec^{TM} capsules (even-numbered carbons) were used to estimate digesta flow by using natural plant and synthetic alkanes. On day 44, abomasal digesta (approximately 100 g) was sampled four times during a 24 h period and bulked in order to estimate digesta flow. The bulked digesta was freeze dried and ground (0.5 mm sieve) and analysed for DM, nitrogen (N), alkane and AA concentrations.

5.3.4 Nitrogen balance

Dry matter and N digestibility and N retention were measured between days 35 and 43. A sub-sample of feed offered and refused was taken daily and bulked to give one sample per group. Faecal collection harnesses were placed on the sheep on day 32 and the total daily output of faeces and urine were measured from day 35 to day 43 to measure DM and N digestibility and N retention. Urine was collected by gravity into a container containing 150 mL of 50% HCl and mixed several times a day to minimise ammonia losses. Faeces and urine were collected and weighed daily during the N balance period with a 10% aliquot collected and stored at -20°C for analysis.

5.3.5 Infusions and blood sampling

On day 48, in order to measure plasma flow across the MDV, PDV, liver and TSP, PAH (723 mg h^{-1}; 0.14 mmol L^{-1} Na form, dissolved in water and then autoclaved) was infused continuously into the mesenteric vein for 8 h. The lambs also received a continuous infusion of ICG for 8 h into the abdominal aorta (14.6 mg h^{-1}; 0.83 mmol L^{-1} ICG) to estimate plasma flow across the hind limbs (Wester *et al.*, 2000). The ICG was dissolved in deionised water and 1% albumin and 0.9% NaCl were added to stabilise the ICG and maintain isotonic molarity, respectively. The resultant infusate was filter-sterilised (0.2 μm) immediately prior to infusion. To prevent blood clotting during the continuous sampling, 6000 iu porcine heparin h^{-1} was infused into the jugular vein with the [^{3}H]-valine (see Chapter Six for details) over the 8 h infusion period. Sampling lines and syringes were kept in an ice-water bath in order to minimise the degradation of blood constituents (Lobley *et al.*, 1995). As part of the larger study, 30 mL of blood was withdrawn continuously every 2 h from the mesenteric artery, the mesenteric, portal and hepatic veins, and the vena cava over the infusion period. The data presented in this chapter represent the average of samples taken from the last 2 sampling periods (time 4 to 6 and 6 to 8 h of infusion).

After each 2-h collection period, the syringes were removed from the collection lines and carefully mixed by gentle rotation. Packed cell volume (haematocrit) was determined. Immediately following the collection of blood samples approximately 0.2 mL of blood was injected into a blood gas analyser (ABL 615, Radiometer Pacific Limited, Copenhagen) to determine the concentration of oxygen (O_2) and carbon dioxide (CO_2) in whole blood. The remaining 25 mL of whole blood was centrifuged (4°C; 3 270 g for 15 min) and the plasma harvested and either processed or stored at -85°C for further analysis as described below or in Chapter Seven.

5.3.6 Slaughter

On day 48 of the experiment, the sheep were euthanased by an intravenous overdose of sodium pentobarbitone (300 mg mL^{-1}; 0.5 mL kg^{-1} liveweight). The duodenum (comprising the proximal 3 m of the small intestine) was isolated in order to sample

duodenal digesta. The ileo-caecal junction of the small intestine was located and sectioned in order to collect ileal digesta from the final 3 m of the ileum for digesta flow measurements. Ileal digesta was stored at -20°C until analysis. The alkane capsule was recovered from the rumen of each sheep, dried overnight and weighed to determine the release rate of C32 and C36 alkanes over the 15 d period in the rumen.

5.3.7 Sample processing and chemical analysis

Dry matter of feeds and refusals were determined daily after oven drying at 85°C for 24 h. Faeces and digesta samples were dried at 85°C for 48 h at the completion of the experiment. Feed, feed refusals, faeces and digesta samples were freeze-dried and ground (0.5 mm sieve) for alkane, N, CT and AA analysis. The concentration of CT in the feed offered was determined by the method outline by Terrill *et al.* (1992b). Nitrogen content in feed, feed refusals, faeces, urine and digesta samples were determined by automated analysis of ammonia following Kjeldahl digestion (Williams & Twine, 1967). In order to determine the fibre content of the diet a sub-sample (0.5 g) of feed offered was submitted for near infra-red reflectance spectroscopy analysis (NIRSystems, Foss Ltd, New Zealand), where the sample was scanned between 1100 nm and 2500 nm wavelengths at 2 nm intervals.

The alkanes present in freeze-dried feed and digesta samples were determined by gas chromatography (Hewlett Packard 5890 series II Gas Chromatograph; Avondale, CA, USA) according to the method of Mayse *et al.* (1986).

5.3.7.1 Amino acids

Amino acid hydrolysates were prepared from feed and digesta samples by hydrolysing approximately 50 mg of freeze-dried material in 6.0 M HCl at 110°C for 22 h. The hydrolysates were filtered, rotory evaporated to near dryness, washed in deionised water and reconcentrated before being dissolved in 0.2 M sodium citrate buffer (pH 2.2). Amino acids in the hydrolysates were analysed by ion exchange chromatography (Shimadzu Scientific Instruments Limited, Columbia, MD 21046, USA) with a post-column reaction using ninhydrin as the derivitising agent.

To determine the concentration of AA in plasma (with the exception of cysteine), 0.5 mL of plasma was treated with 80 mM dithiothreitol (DTT) as an antioxidant and 3 mM norleucine (in 0.1% phenol; as an internal standard) and stored at -85°C. The samples were thawed and transferred to a Centrisart® filter (10 000 molecular weight cut off), and then centrifuged at approximately 28 000 g for 60 min, with the filtrate containing free AA removed and stored at -85°C for analysis. A 50 μL aliquot of the filtrate was dried under vacuum before the addition of 20 μL redry solution (2:2:1, methanol: 1M Na acetate: triethylamine (TEA; under $N_{2(g)}$)) mixed by vortex and again dried down. The derivatisation reagent (20 μL containing 7:1:1:1 ratio of methanol, MilliQ water, TEA (under $N_{2(g)}$), phenylisothiocyanate (PITC; under $N_{2(g)}$)) was added to each dried sample which was then vortexed and incubated at room temperature for 10 min and then dried down under vacuum. Dried derivatised samples were resuspended in 200 μL diluent containing 5 % CH_3CN and 95 % phosphate buffer (5 mM Na_2HPO_4 adjusted to pH 7.40 using 10 % (v/v) H_3PO_4). Samples were vortexed and transferred to 1.5 mL microfuge tubes and centrifuged at approximately 14 000 g for 5 min. The supernatant was transferred to autosampler vials. A stock standard solution containing 0.5 mM AA was prepared using 200 μL Pierce A/N (Pierce C Chemicals, Lab Supply Pierce (NZ) Ltd, Auckland, New Zealand) and 200 μL Pierce B (Pierce Chemicals, Lab Supply Pierce (NZ) Ltd, Auckland, New Zealand) standard solutions together with 0.5 mM norleucine in a final volume of 1 mL 0.1 M HCl. Stock standards were diluted 10-fold using 0.1 M HCl before use. Derivitised plasma samples (50 μL) were injected onto a Picotag C_{18} reverse phase column in an oven set to 46°C, with a 90 min run time between each injection (Appendix B.3).

Measurement of AA in plasma (with the exception of cysteine) were determined using a method modified from Bidlingmeyer *et al.* (1984). The samples were analysed after reverse-phase high performance liquid chromatography (HPLC) separation of PITC derivatives, using a Waters Pico-Tag® column (3.9 x 300 mm, Waters Corporation, Milford, NMA 01757, USA) and a Shimadzu LC-10/A HPLC system (Shimadzu Scientific Instruments Ltd., Columbia, MD 21046, USA).

In order to determine plasma cysteine concentration, 2 mL of plasma was added to a pre-weighed centrifuge tube and weighed. In order to denature the protein in the plasma, 1 mL of a solution containing 0.75% w/v sodium dodecyl sulphate (SDS) and 9 mM EDTA, 200 μL DTT (80 mM) was added. 100 μL of norleucine (3 mM in 0.1% phenol) was added as an internal standard. The samples were re-weighed after each addition, and then left at room temperature for 15 min before adding 1 mL of trichloroacetic acid (TCA; 30% w/v) to precipitate plasma protein. The tubes were reweighed and centrifuged (3 270 g, 15 min at 4°C) with the resulting supernatant filtered (0.45 μm) and stored at -85°C. The supernatant was reacted with acid ninhydrin after reduction using DTT, and cysteine concentration determined photospectrometrically at 570 nm using a continuous flow analyser (Technicon Autoanalyser II) as described by Gaitonde (1967).

5.3.7.2 Ammonia, urea, glucose and lactate

Plasma (1.5 mL) was mixed with TCA (30% w/v) and the resulting solution was centrifuged at 3 270 g for 15 min at 4°C on the day of sampling. The supernatant was filtered and stored at -85°C until it was injected onto a Tecator FIAstar Flow Injection 5010 Analyser (Tecaort Ltd., Höganäs, Sweden) to determine the absorbance at 590 nm in order to calculate the concentration of ammonia in plasma.

Urea concentration in plasma was determined using a commercial assay (Catalogue number 07 3685 6; Roche Diagnostics Ltd, Basel, Switzerland) that utilised the enzymes urease and glutamate dehydrogenase, and followed the production of NADH at 340 nm. This assay measures urea concentration in 0.5 mL of plasma by converting it to ammonia using urease. The urea concentration was corrected for plasma ammonia using a second assay (Catalogue number 171C; Sigma Diagnostics Ltd, St Louis, Missouri, USA) that lacked urease, where the ammonia underwent reductive amination using L-glutamate dehydrogenase. The urea and ammonia assays were performed on a Cobas

Fara II analyser (Hoffmann la Roche, Basel Switzerland) using protocols recommended by the kit manufacturers.

The concentration of O_2, CO_2, glucose and lactate in blood were determined on a blood gas analyser (ABL 615, Radiometer Pacific Limited, Copenhagen) and a metabolite analyser (EML 105, Radiometer Pacific Limited, Copenhagen). Oxygen concentration was determined by measuring the decrease in electric potential caused as O_2 diffuses across the membrane of the electrode. Carbon dioxide concentration was determined by measuring the change in pH caused by the CO_2 as it dissolves in the electrolyte. Glucose and O_2 present in whole blood sample were metabolised by glucose oxidase (which is present in the electrode membrane) to form hydrogen peroxide (H_2O_2) and gluconic acid. The breakdown of H_2O_2 creates a flow of electrons which produces a current that is proportional to the concentration of glucose in the sample. Lactate concentration was determined in a similar manner, with lactate oxidase present in the lactate electrode producing H_2O_2 and pyruvate.

5.3.7.3 Plasma flow

Para-aminohippuric acid dye dilution was used to determine the flow of plasma though the MDV, PDV, liver and TSP tissues as described by Katz & Bergman (1969) with an additional deacylation step as described by Lobley *et al.* (1995). This method is described fully in Appendix B.4. Plasma (0.5 mL) that had been stored at -85°C was deproteinised with 12% (w/v) TCA (5 mL), mixed and centrifuged at 3 270 g for 15 min. Four mL of the resulting supernatant was mixed with 0.5 mL of 1.2 N HCl, capped and heated for 60 min at 90°C in order to deacylate the PAH. After cooling to room temperature, 0.5 mL of sodium nitrite (1 mg mL^{-1}), 0.5 mL of ammonium sulphamate (5 mg mL^{-1}) and 0.5 mL of N-1-napthyl-ethylene-diamino-dihydrochloride (NEDC; 1 mg mL^{-1}) were added sequentially at intervals of 4-5 min in order to form an azo dye, with the weight recorded at the end. This mixture was left at room temperature for at least 30 min and the absorbance of samples read at 540 nm using a spectrophotometer (GBC UV/VIS 918, GBC Scientific Equipment Pty Ltd., Victoria, Australia).

Standards for each lamb were prepared by gravimetrically diluting a sample of the PAH (1:200) that was infused into that lamb. Aliquots of 1:200 infusate were added at 10, 25, 50 and 100 μL to deproteinised plasma which contained no PAH. The standards were prepared in the same manner as the plasma samples and read at 540 nm in a spectrophotometer (GBC UV/VIS 918, GBC Scientific Equipment Pty Ltd., Victoria, Australia).

Plasma flow across the hind limbs was calculated using the ICG concentration measured in plasma. One mL of plasma was thawed and then centrifuged at 3 270 g for 15 min at 4°C before measuring the absorbance at 790 nm in a spectrophotometer (GBC UV/VIS 918, GBC Scientific Equipment Pty Ltd., Victoria, Australia). Plasma concentration of ICG was determined from a standard curve generated from known concentrations of ICG and their corresponding absorbance (Wester *et al.*, 2000).

5.3.8 Calculations

5.3.8.1 Digesta flow

The calculations relating to the flow of DM through the abomasum and ileum have been reported in full in Chapter Two (Section 2.3.8.1). Briefly, DM flow was calculated by the dilution of natural plant alkanes and synthetic plant alkanes in the abomasum and ileum.

Amino acid flows at the abomasum and ileum were calculated by multiplying AA concentration in the digesta by the corresponding DM flow at the abomasum or ileum. Apparent absorption of AA from the small intestine was calculated as the difference between AA flux at the abomasum and ileum, and adjusted for AA intake in order to give apparent AA digestibility (Chapter Two, Section 2.3.8.1).

5.3.8.2 Plasma flow

Plasma flow (Pf) across the tissue beds (Tissue Pf or MDV Pf, PDV Pf, TSP Pf and hind limbs Pf) was determined by Equation 5.1. The hepatic artery plasma flow was determined by difference between the TSP Pf and PDV Pf. A full description of these calculations can be found in Chapter Two (Section 2.3.8.2).

Equation 5.1

$$\text{Tissue Pf } (\text{mL min}^{-1}) = \frac{[\text{PAH}]_I\ (\text{mg mL}^{-1}) \times \text{infusion rate } (\text{mL h}^{-1})}{([\text{PAH}]_X - [\text{PAH}]_A)\ (\text{mg mL}^{-1})}$$

5.3.8.3 Metabolite flux across the tissue beds

Arterio-venous (AV) concentration differences (data not presented) across the tissue beds were calculated as the difference between the concentration in the arterial supply (mesenteric artery; A) and that of the venous drainage (X) of the tissue bed (mesenteric (M), portal (P) and hepatic veins (H)), and the vena cava (V)). Nutrient fluxes across the MDV, PDV, liver, TSP and hind-limb tissues were calculated using an AV approach as outlined in Chapter Two (Section 2.3.8.3). The Z refers to the concentration of the nutrient in question (e.g., AA, glucose etc.).

Equation 5.2

$$\text{Tissue net Z flux } (\text{mmol min}^{-1}) = ([Z]_A - [Z]_X)\ (\text{mmol mL}^{-1}) \times \text{Tissue Pf } (\text{mL min}^{-1})$$

Equation 5.3

$$\text{Liver net VAL flux } (\text{mmol min}^{-1})$$
$$= ([\text{VAL}]_A \times \text{ART Pf}) + ([\text{VAL}]_P \times \text{PDV Pf}) - ([\text{VAL}]_H \times \text{TSPT Pf})$$

5.3.9 Statistical analysis

Statistical analysis was performed using a General Linear Model (SAS version 8, 1999), with treatment and group (the week that the lamb underwent surgery) used as sources of variation in the model. Feed intake, FEC and liveweight were analysed using Proc Mix

repeated measures. The data was checked for normality and the presence of outliers by plotting residuals versus the predicted residuals. The FEC were transformed by In (x+1) before analysis in order to ensure symmetry in the data and to standardise variances across the treatments (Sutherland *et al.*, 1999a).

Probability values lower than 0.10 were considered to indicate a significant difference and values between 0.10 and 0.15 to indicate a trend. Results are presented as least squares means (LSmeans) and associated pooled standard deviation (SD). Statistical difference from zero for AV concentration difference of nutrients and metabolites was determined using the T-statistic, with P>T values greater than 0.05 considered as non significant. Unless otherwise indicated in the results section, all AV values presented were statistically different from zero.

5.4 Results

5.4.1 Feed intake and liveweight

The presence of *T. colubriformis* in the small intestine of lambs fed fresh Sulla decreased DM intake over the course of the experiment (769 *vs.* 689 (SD 47) g DM d^{-1} in the control and parasite lambs, respectively; P<0.15). Consequently, liveweight was adversely affected during the trial (34.9 *vs.* 33.4 (SD 0.7) kg in the control and parasite lambs, respectively; P<0.10) with parasitised lambs weighing less than the control lambs from day 17 onwards (Figure 5.1). Parasitic infection reduced liveweight gain, however this was not significant over the 48 day infection period (75 *vs.* -17 (SD 50) g d^{-1} in the control and parasite lambs, respectively; P>0.15). However, differences in liveweight became significant over the last 20 d of the infection period (50 *vs.* -50 (SD 70) g d^{-1} in the control and parasite lambs, respectively; P<0.15).

5.4.2 Parasitology

Dosing lambs with infective L3 *T. colubriformis* larvae was effective in producing a parasite burden in the intestine of lambs fed fresh Sulla (P<0.01; Table 5.2). Faecal egg

counts were monitored over the course of the experiment and peaked around day 26 in the infected lambs (*c.* 1 800 eggs per g faeces; P<0.001; Figure 5.2).

Table 5.2 Parasite burdens in the small intestine of lambs fed fresh Sulla (*Hedysarum coronarium*) and with a *Trichostrongylus colubriformis* infection or kept as parasite-free controls. Results are presented as LSmeans and their associated standard deviation (SD).

	Control n=6	Parasite n=6	Pooled SD	P
Male	118	8197	3438	0.004
Female	127	9363	3653	0.002
Total	245	17560	7072	0.003

Figure 5.1 Liveweight (kg) of lambs fed fresh Sulla (*Hedysarum coronarium*) with a *Trichostrongylus colubriformis* infection (n=6) or kept as parasite-free controls (n=6) on days 0 to 6 of the experimental period. Results are presented as LSmeans, with the error bar representing the pooled standard deviation.

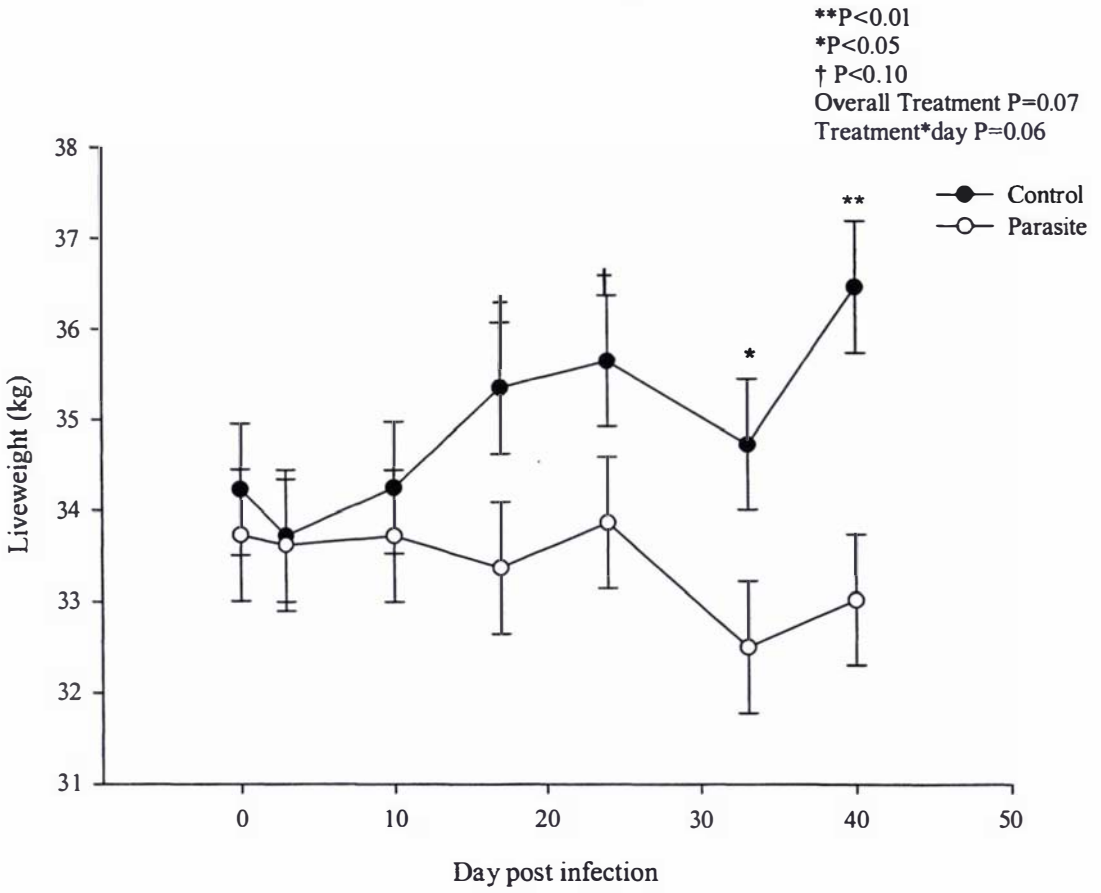
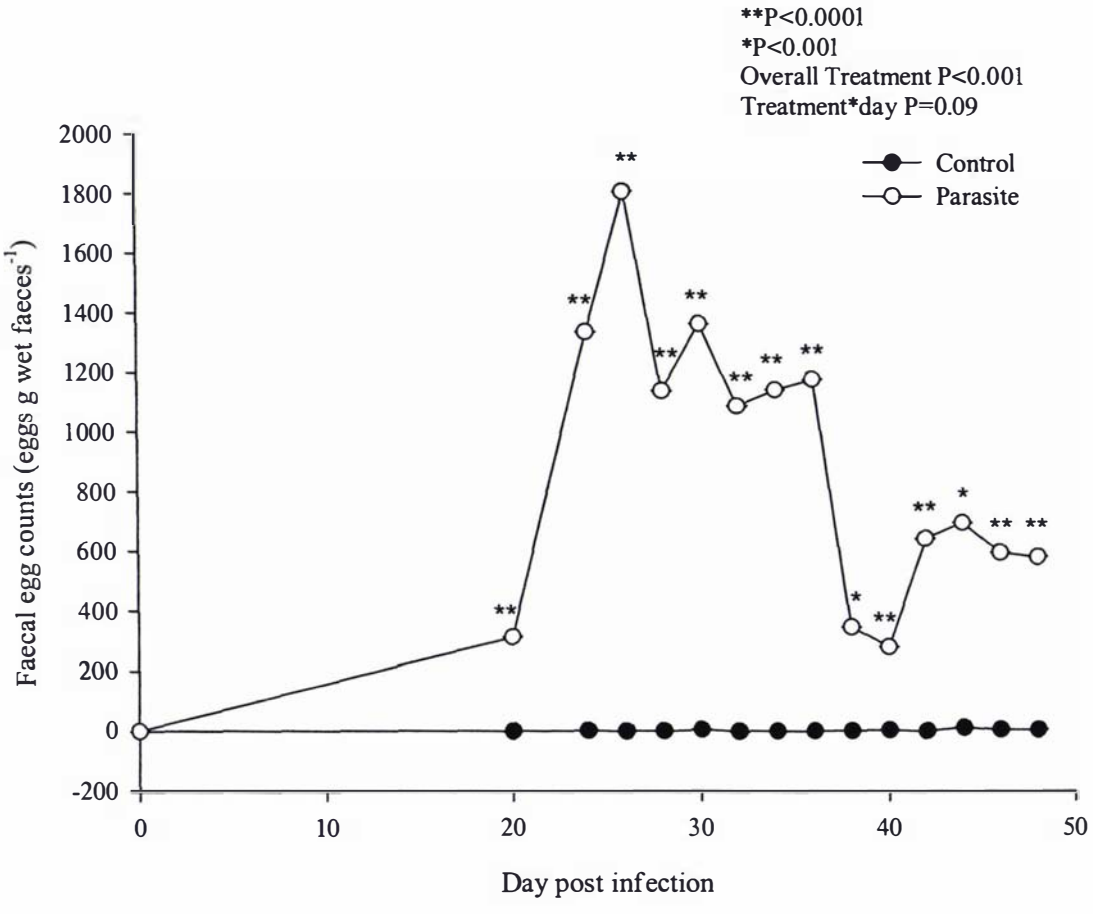


Figure 5.2 Faecal egg counts in lambs fed fresh Sulla (*Hedysarum coronarium*) with *Trichostrongylus colubriformis* infection (n=6) or kept as parasite-free controls (n=6). Results are presented as means, with the error bar representing the pooled standard deviation.



5.4.3 Nitrogen balance

Lambs in the parasite infected group had a lower DM and N intake during the N balance on days 32 to 40 compared to control lambs ($P<0.05$; Table 5.3). However, the excretion of N in urine (12 *vs.* 20 (SD 11) g N d^{-1} in the control and parasite lambs, respectively; $P>0.15$) and faeces (6 *vs.* 6 (SD 3) g N d^{-1} in the control and parasite lambs, respectively; $P>0.15$) was similar between treatment groups. Consequently, the digestibility of DM and N and the retention of N was unaffected by parasitic infection ($P>0.15$). Parasitic infection reduced the flux of DM and N through the abomasum ($P<0.15$; Table 5.3) however there was no effect of parasite infection on these parameters at the ileum ($P>0.15$; Table 5.3).

5.4.4 Amino acid digestibility

Intake of individual AA ($P<0.05$; Appendix Table E.1) and total AA ($P<0.05$; Table 5.4) were significantly lower in the infected lambs. With the exception of cysteine, valine ($P>0.15$; Appendix Table E.2) and the total sulphur AA (SAA; $P=0.15$; Table 5.4) which were similar between the control and infected lambs, the flux of AA at the abomasum were lower in the infected lamb ($P<0.10$; Table 5.4). However, ileal AA flux was not affected by the presence of the parasite infection ($P>0.15$; Table 5.4 and Appendix Table E.3). There was a reduction in the apparent net absorption of essential (EAA), branched-chain (BCAA) and total AA ($P<0.15$) from the small intestine of lambs infected with *T. colubriformis*. Among the EAA the absorption of arginine, isoleucine, leucine, methionine, phenylalanine and valine were reduced in the infected lambs, while lysine was increased ($P<0.15$; Appendix Table E.4). However, as a proportion of AA intake, there was no effect of parasitic infection on AA digestibility in the small intestine ($P>0.15$; Table 5.4 and Appendix Table E.5).

Table 5.3 Dry matter (DM) and nitrogen (N) intake, digestibility and fluxes in the abomasum and ileum (g d^{-1}) and N retention (g d^{-1}) in lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free contorls. Results are presented as the LSmeans and associated pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
Intake	DM	757	616	103	0.05
	N	21	17	3	0.05
Abomasum flux	DM	354	281	60	0.07
	N	21	17	4	0.15
Ileum flux	DM	218	196	107	0.73
	N	10	8	4	0.50
Digestibility	DM	0.74	0.67	0.15	0.46
	N	0.82	0.77	0.09	0.35
Retention	N	1.4	-6.9	14.6	0.36

Table 5.4 Non-essential (NEAA), essential (EAA), branched-chained (BCAA) and sulphur (SAA) and total amino acid intake and flux (g d^{-1}) through the abomasum and ileum and apparent absorption from the small intestine (SI) of lambs fed fresh Sulla (*Hedysarum coronarium*) with a *Trichostrongylus colubriformis* infection or kept as parasite-free controls. Results are presented as LSmeans and the pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
Intake[1]	NEAA	65.1	52.7	9.2	0.05
	EAA	45.9	37.0	6.6	0.05
	BCAA	17.8	14.4	2.6	0.05
	SAA	4.1	3.3	0.6	0.04
	Total	110.0	89.7	15.7	0.05
Abomasal flux[2]	NEAA	62.3	48.8	10.4	0.06
	EAA	54.0	41.7	9.0	0.05
	BCAA	21.6	16.8	3.9	0.07
	SAA	4.4	3.6	0.9	0.15
	Total	116.3	90.5	19.3	0.05
Ileal flux[3]	NEAA	21.2	25.3	14.2	0.64
	EAA	15.7	20.9	11.8	0.49
	BCAA	6.7	6.1	5.0	0.45
	SAA	1.9	2.4	1.4	0.59
	Total	36.9	46.2	25.9	0.57
Apparent absorption of amino acids in the SI	NEAA	41.1	32.6	9.1	0.23
	EAA	38.3	27.8	6.7	0.07
	BCAA	14.9	10.2	2.5	0.03
	SAA	2.5	1.0	0.6	0.16
	Total	79.4	44.4	15.8	0.14
Proportion of amino acids digested in the SI	NEAA	0.62	0.66	0.16	0.74
	EAA	0.71	0.71	0.13	0.96
	BCAA	0.70	0.68	0.13	0.85
	SAA	0.55	0.58	0.21	0.86
	Total	0.67	0.69	0.14	0.87

1. Based on data obtained during the nitrogen balance (days 35-43).
2. Based on digesta samples taken on day 44.
3. Based on samples taken at slaughter (day 48); n=5 for the P treatment.

NEAA: alanine, aspartate, glutamate, glycine, proline, serine and tyrosine.
EAA: arginine, cysteine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine.
BCAA: isoleucine, leucine and valine.
SAA: cysteine and methionine.
Total = NEAA + EAA.

5.4.5 Plasma amino acid fluxes

Four catheters (2 in the mesenteric vein, and 1 in the portal vein and vena cava) failed due to fibrous sheaths forming over the end of the catheter reducing the patency to 95%. Plasma flow through the MDV, PDV, TSP and hind limbs was unaffected by the presence of parasitic infection (Table 5.5; P>0.15).

Table 5.5 Plasma flow (mL min^{-1}) across the mesenteric-drained viscera (MDV), portal-drained viscera (PDV), total splanchnic tissues (TSP), hepatic artery and hind limbs of lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Results are presented as LSmeans and associated pooled standard deviation (SD).

	Control n=6	Parasite n=6	Pooled SD	P
MDV	533	663	280	0.51
PDV	1518	1398	369	0.61
TSP	1687	1560	434	0.63
Hepatic artery	256	163	180	0.43
Hind limbs	637	502	171	0.24

The presence of an adult *T. colubriformis* population in the small intestine did not affect the concentration of AA in the mesenteric vein with the exception of SAA, which were lower in the infected animals (P<0.15; Table 5.6). Parasites also decreased the concentration of histidine, alanine and cysteine (P<0.15), while citrulline concentration was increased in the mesenteric vein (P<0.15; Appendix Table E.6). Non-essential AA (NEAA; P<0.15; Table 5.6) and glycine (P<0.10; Appendix Table E.7) were increased in the portal vein of parasitised lambs, however histidine and alanine were decreased (P<0.05; Appendix Table E.7). Parasitic infection increased the concentration of NEAA (P<0.15; Table 5.6), serine, glycine and ornithine in the hepatic vein, whilst SAA (P<0.15; Table 5.6), histidine and alanine were decreased (P<0.15; Appendix Table E.8). The concentration of aspartate, serine, glycine, citrulline, ornithine, and lysine, NEAA and total AA in the mesenteric artery were higher in the parasitised lamb (P<0.15; Table 5.6 and Appendix Table E.9). Histidine concentration was lower in the mesenteric artery of the parasitised lambs (Appendix Table E.9). In the vena cava parasitised lambs had a higher concentration of serine, glycine, ornithine (P<0.10; Appendix Table E.10), NEAA and Total AA compared to control lambs (P<0.15; Table

5.6). Parasite infection decreased the concentration of cysteine in the vena cava (P<0.10; Appendix Table E.10).

Across the MDV all AV concentration differences of AA were significantly different from zero (P<0.05; data not presented). With the exception of glutamine, all AA were released by the MDV in both the control and parasite lambs (Table 5.7 and Appendix Table E.11). Parasitic infection resulted in a greater utilisation of glutamine by the MDV (10 *vs.* 30 (SD 18) μmol min^{-1} in the control and parasite lambs, respectively; P<0.15; Appendix Table E.11). Threonine was also affected, with a lower release by the MDV (-31 *vs.* -10 (SD 14) μmol min^{-1} in the control and parasite lambs, respectively; P<0.15; Appendix Table E.11). Net fluxes of EAA and Total AA across the MDV were lower in the parasitised lambs (P<0.15; Table 5.7).

All AV concentration differences of AA across the PDV were significantly different from zero (P<0.05; data not presented). The presence of parasitic infection resulted in less NEAA, EAA, BCAA, SAA and total AA being released from the PDV (P<0.05; Table 5.7). Individual AA were affected by parasitic infection with the exception of aspartate, glutamate, glycine, taurine, lysine and cysteine which were similar between treatments (P>0.15; Appendix Table E.12). Threonine and citrulline were both utilised by the PDV of infected lambs, compared to a net release in the control lambs. Hydroxyl-proline was released by infected lambs compared to a net uptake in the control lambs (P<0.10; Appendix Table E.12). Parasitic infection resulted in a greater utilisation of glutamine by the PDV (P<0.05; Appendix Table E.12).

Across the liver, all AV concentration differences of AA were significantly different from zero (P<0.05; data not presented). Parasitic infection decreased the release of glutamate (P<0.15; Appendix Table E.13), while it decreased the utilisation of histidine and methionine in this tissue (P<0.15; Appendix Table E.13). Threonine was released by the liver of infected lambs compared to a net utilisation in the control lambs (P<0.05; Appendix Table E.13). Parasitic infection resulted in the utilisation of BCAA across the liver, whereas in the control lambs, the BCAA were released (P<0.10; Table 5.8). The

change in BCAA flux across the liver was mostly due to a significant change in the flux of valine across the liver ($P<0.10$), although there was a trend for increased leucine and isoleucine utilisation by the liver in the parasitised lambs ($P=0.16$; Appendix Table E.13).

In the TSP, AV concentration differences of glutamate, glutamine, valine, isoleucine and leucine were significantly different from zero ($P<0.05$; data not presented). Net fluxes of individual AA are presented in Appendix Table E.14. Of the NEAA, only alanine, asparagine and proline were affected by parasitic infection, with utilisation of these AA by the TSP of infected lambs compared to their release in control lambs ($P<0.15$; Appendix Table E.14). The NEAA, EAA, SAA and total AA were utilised by the TSP in infected lambs, whilst in the control lambs they were released. The net release of BCAA and lysine were decreased by parasitic infection ($P<0.15$; Appendix Table E.14). The release of BCAA from the TSP was 85% lower in the parasitised lambs ($P<0.05$) as a consequence of the reduced appearance in BCAA in the portal drainage and alteration in BCAA utilisation by the liver.

Across the hind limbs only glutamate, glutamine and alanine were significantly different from zero ($P<0.05$; data not presented). The presence of *T. colubriformis* in the small intestine had no effect on the net flux of AA across the hind limbs ($P<0.10$) with the exception of aspartate ($P<0.10$; Appendix Table E.15) and citrulline ($P<0.15$; Appendix Table E.15) which were utilised in the parasitised lambs but released in the control lambs.

5.4.6 Nutrient Fluxes

The AV concentration difference of metabolites showed that urea was not significantly different from zero across the PDV, TSP and hind limbs, while glucose, lactate and ammonia were not significantly different from zero across the MDV and hind limbs ($P>0.05$). In the liver, the AV concentration difference for lactate was not significantly different from zero ($P>0.05$).

The concentration of O_2, CO_2, ammonia and urea in all vessels were not affected by parasitic infection ($P>0.15$; Table 5.8). Glucose concentration was significantly lower in the mesenteric and portal veins and the mesenteric artery in the infected lambs ($P<0.10$). Lactate concentrations were also lower in the portal and hepatic veins, the mesenteric artery and vena cava of parasitised lambs ($P<0.10$).

Nutrient fluxes across the MDV, PDV, liver, TSP and hind limbs were generally unaffected by the presence of adult *T. colubriformis* in the small intestine (Table 5.9). Lactate was utilised by the liver in parasitised lambs, whilst it was released in control lambs ($P<0.10$; Table 5.9). Infection decreased the utilisation of glucose by the hind limbs ($P<0.05$; Table 5.9).

5.5 Discussion

Parasitic infection reduced feed intake and liveweight gain in lambs fed fresh Sulla. Consequently, the net flux of AA across the portal drainage was lower in infected lambs which resulted in a notable uptake of AA from the TSP in the control lambs compared to a release in the infected lambs. However, there was no effect of decreased AA availability to the peripheral tissues, as the net flux of AA across the hind limbs was similar between treatments.

5.5.1 Parasitology and feed availability

The presence of an established adult *T. colubriformis* population in the small intestine (*c.* 17 000 adult worms) of lambs fed fresh Sulla reduced feed intake by 10%, and had detrimental effects on apparent AA absorption from the small intestine and liveweight gain over the latter stages of infection. These results agree with earlier studies which indicated reduced performance in parasitised lambs (e.g., Steel *et al.*, 1982; van Houtert *et al.*, 1995; Niezen *et al.*, 1998). However, they contrast with the findings from Chapter Two. In that experiment, Lucerne (*Medicago sativa*; no CT) was fed to infected lambs using the same experimental protocol with no resulting effect on intake.

Table 5.6 Non-essential (NEAA), essential (EAA), branched-chain (BCAA), sulphur (SAA) and total amino acid concentration (μmol L^{-1}) in the mesenteric, portal and hepatic veins, the mesenteric artery and the vena cava of lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with a *Trichostrongylus colubriformis* infection or kept as parasite-free controls. Results are presented as LSmeans and their pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
Mesenteric vein	NEAA	1475	1488	204	0.92
	EAA	1125	1007	319	0.57
	BCAA	579	538	177	0.72
	SAA	73	56	15	0.11
	Total AA	2600	2495	486	0.74
Portal vein	NEAA	1131	1306	166	0.13
	EAA	823	794	199	0.83
	BCAA	439	435	104	0.95
	SAA	53	44	9	0.16
	Total AA	1931	2100	288	0.41
Hepatic vein	NEAA	1056	1223	139	0.07
	EAA	715	719	217	0.98
	BCAA	389	420	123	0.67
	SAA	50	41	8	0.11
	Total AA	1747	1986	310	0.28
Mesenteric artery	NEAA	1010	1263	159	0.04
	EAA	672	730	212	0.69
	BCAA	354	415	116	0.39
	SAA	48	42	9	0.34
	Total AA	1687	2047	318	0.14
Vena cava	NEAA	1046	1287	179	0.08
	EAA	669	761	191	0.49
	BCAA	341	410	109	0.34
	SAA	49	43	9	0.31
	Total AA	1715	2048	300	0.14

NEAA: alanine, asparagine, aspartate, glutamate, glutamine, glycine, hydroxyl-proline, proline, serine and taurine.
EAA: arginine, cysteine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine and valine.
BCAA: isoleucine, leucine and valine.
SAA: cysteine and methionine.
Total = EAA + NEAA.

Table 5.7 Net flux (μmol min^{-1}) of non-essential (NEAA), essential (EAA), branched-chain (BCAA), sulphur (SAA) and total amino acids across the mesenteric-drained viscera (MDV), portal-drained viscera (PDV), liver, total splanchnic tissues (TSP) and hind limbs in lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with or without *Trichostrongylus colubriformis*. Positive values represent a net utilisation by the tissue, while a negative value represents a net release. Results are presented as LSmeans and associated pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
MDV	NEAA	-209.5	-179.0	143.6	0.76
	EAA	-302.3	-80.3	135.2	0.11
	BCAA	-122.6	-99.8	86.2	0.70
	SAA	-13.7	-11.8	9.5	0.77
	Total AA	-539.1	-146.2	234.1	0.11
PDV	NEAA	-123.2	-26.0	47.6	0.01
	EAA	-163.5	-44.1	65.8	0.04
	BCAA	-72.9	-29.3	28.2	0.04
	SAA	-9.4	-3.2	3.1	0.01
	Total AA	-286.7	-70.1	113.1	0.03
Liver	NEAA	70.0	61.4	60.3	0.83
	EAA	50.8	55.8	45.2	0.88
	BCAA	-3.5	19.1	18.1	0.08
	SAA	4.7	4.3	1.5	0.67
	Total AA	120.6	127.3	109.3	0.46
TSP	NEAA	-47.5	36.4	56.8	0.07
	EAA	-112.8	11.7	58.4	0.03
	BCAA	-76.4	-10.2	29.0	0.01
	SAA	-4.8	1.1	2.5	0.01
	Total AA	-91.3	65.5	81.1	0.06
Hind limbs	NEAA	-35.2	-10.1	40.5	0.38
	EAA	7.4	-1.6	46.8	0.80
	BCAA	6.8	4.6	20.6	0.87
	SAA	0.3	-0.1	2.1	0.74
	Total AA	-27.3	-13.5	86.5	0.83

NEAA: alanine, asparagine, aspartate, glutamate, glutamine, glycine, hydroxyl-proline, proline, serine and taurine.
EAA: arginine, cysteine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine and valine.
BCAA: isoleucine, leucine and valine.
SAA: cysteine and methionine.
Total = EAA + NEAA.

Table 5.8 The concentration of oxygen (O_2), carbon dioxide (CO_2), glucose and lactate (mmol L^{-1}) in whole blood and ammonia (μmol L^{-1}) and urea (mmol L^{-1}) concentrations in plasma collected from the mesenteric, portal and hepatic veins, the mesenteric artery and the vena cava in lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with or without *Trichostrongylus colubriformis*. Results are presented as LSmeans and associated pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
Mesenteric vein	O_2	3.27	3.23	0.38	0.88
	CO_2	26.60	26.37	1.12	0.76
	Glucose	3.84	3.53	0.25	0.09
	Lactate	0.80	0.68	0.19	0.35
	Ammonia	0.68	0.76	0.20	0.56
	Urea	5.70	5.50	1.61	0.85
Portal vein	O_2	3.83	3.80	0.37	0.91
	CO_2	26.32	26.50	0.92	0.76
	Glucose	3.81	3.41	0.18	0.01
	Lactate	0.97	0.68	0.18	0.03
	Ammonia	0.88	0.86	0.23	0.90
	Urea	5.09	5.42	1.41	0.71
Hepatic vein	O_2	2.93	3.10	0.33	0.42
	CO_2	26.73	26.70	1.03	0.96
	Glucose	3.88	3.64	0.28	0.22
	Lactate	0.87	0.54	0.26	0.08
	Ammonia	0.23	0.23	0.05	0.95
	Urea	6.14	5.54	1.43	0.52
Mesenteric artery	O_2	4.74	4.83	0.75	0.85
	CO_2	25.70	25.49	0.90	0.70
	Glucose	3.84	3.50	0.24	0.04
	Lactate	0.78	0.55	0.20	0.08
	Ammonia	0.20	0.21	0.03	0.56
	Urea	5.69	5.40	1.50	0.75
Vena cava	O_2	2.67	2.69	0.25	0.89
	CO_2	27.04	26.97	0.99	0.90
	Glucose	3.61	3.46	0.20	0.25
	Lactate	0.83	0.59	0.21	0.10
	Ammonia	0.20	0.20	0.03	0.86
	Urea	5.92	5.37	1.32	0.52

Table 5.9 Net flux of oxygen (O_2), carbon dioxide (CO_2), glucose and lactate (mmol min^{-1}) in blood and ammonia (μmol min^{-1}) and urea (mmol min^{-1}) in plasma across the mesenteric-drained viscera (MDV), portal-drained viscera (PDV), liver, total splanchnic tissues (TSP) and hind limbs of lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with or without *Trichostrongylus colubriformis*. Positive values represent a net utilisation by the tissue, while a negative value represents a net release. Results are presented as LSmeans and associated pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
MDV	O_2	1.24	1.43	0.55	0.62
	CO_2	-0.82	-0.76	0.25	0.72
	Glucose	-0.01	-0.04	0.10	0.67
	Lactate	-0.02	-0.05	0.05	0.31
	Ammonia	-269.94	-372.68	170.90	0.39
	Urea	-0.01	0.062	0.07	0.18
PDV	O_2	1.82	1.78	1.49	0.97
	CO_2	-1.91	-1.74	1.20	0.82
	Glucose	0.22	0.16	0.15	0.53
	Lactate	-0.23	-0.22	0.08	0.76
	Ammonia	-1070.38	-887.75	392.58	0.47
	Urea	0.20	0.02	0.28	0.33
Liver	O_2	1.95	1.60	0.72	0.46
	CO_2	-0.88	-0.32	0.59	0.17
	Glucose	-0.42	-0.38	0.26	0.79
	Lactate	-0.08	0.22	0.20	0.05
	Ammonia	968.65	884.29	423.82	0.75
	Urea	-0.39	0.2	0.35	0.43
TSP	O_2	3.76	3.38	1.99	0.77
	CO_2	-2.78	-2.06	1.37	0.42
	Glucose	-0.21	-0.22	0.26	0.92
	Lactate	-0.32	0.00	0.24	0.07
	Ammonia	-97.30	-38.92	68.45	0.24
	Urea	-0.19	-0.19	0.48	0.99
Hind limbs	O_2	1.51	1.33	0.62	0.65
	CO_2	-1.21	-0.93	0.55	0.44
	Glucose	0.14	0.03	0.08	0.04
	Lactate	-0.02	-0.02	0.05	0.99
	Ammonia	-8.17	10.92	28.46	0.31
	Urea	0.13	0.03	0.15	0.31

The reduction in DM intake in the Sulla fed lambs are surprising as Sulla has traditionally been considered to be highly palatable. The concentration of CT (2.6 % of DM) in the present study is lower than the range reported elsewhere in the literature (Terrill *et al.*, 1992a; Stienezen *et al.*, 1996; Bermingham *et al.*, 2001; Burke *et al.*, 2002). Increased levels of gut hormones such as gastrin and cholecystokinin are thought to be the cause for the reduction in DM intake in parasitic infections (Symons & Hennessy, 1981; Fox *et al.*, 1989; Fox, 1997). However, these were not measured in the current study. The Sulla fed in the current study was very lush (approximately 10% DM) so it may have been difficult for the parasitised lambs to maintain a high intake throughout the experiment.

The lambs in this experiment were dosed with similar numbers of infective L3 *T. colubriformis* larvae as those used in Chapter Two, however the establishment rate was lower in this experiment (48% establishment) compared to the Lucerne fed lambs (60% establishment). The lower establishment of an adult population in the Sulla-fed lambs resulted in a lower FEC in the parasitised lambs compared to the parasitised lambs fed Lucerne (Chapter Two), with peak egg production of around 1 800 eggs per gram of faeces (EFG) at day 26 post infection. The overall pattern of FEC was similar to that of the Lucerne-fed lambs with a peak at day 26, followed by an overall decrease to 600 EGF at day 48 post infection. The apparent reduction in larvae establishment observed in the Sulla-fed lambs may have been due to the presence of a low concentration of CT in the Sulla.

Reductions in FEC have been reported in trickle-infected lambs fed Quebracho tannin (from the *Schinopsis* species; QT; 3-6%) both in the initial establishment phase of infection (weeks 1-5) and during established infection (weeks 6-10; Athanasiadou *et al.*, 2000b). In previous studies feeding CT-containing forages such as *L. corniculatus*, *L. pedunculatus* and Sulla have decreased FEC (Niezen *et al.*, 1993; 1998; Robertson *et al.*, 1995). However, only Sulla was reported to reduce intestinal worm burdens at slaughter (Robertson *et al.*, 1995; Niezen *et al.*, 1998). The concentration of CT in those studies were not directly measured, so direct comparisons are not possible because the

concentration of CT in the forage is likely to be a major factor affecting parasite infection (Molan *et al.*, 2000b).

Condensed tannins fed to uninfected rats and chickens resulted in increases in mucus production (Sell *et al.*, 1985). Sheep fed QT (5%) showed changes in the morphology and functional integrity of the small intestine (Dawson *et al.*, 1999). However, when similar levels of CT from *L. pedunculatus* (5.5%) were fed to uninfected sheep there was no effect on intestinal morphology (Walton *et al.*, 2001). Therefore, it is possible that CT also alter the chemical and physical environment of the GIT, which can impact on larval survival. Extracted CT can inhibit parasite movement through sieves (Molan *et al.*, 2000a; 2000b) and disrupt the lifecycle of *T. colubriformis* (Molan *et al.*, 2002). An experiment conducted by Butter *et al.* (2001) showed that the numbers of lumen-dwelling intestinal nematodes was reduced when QT was included in the diet of lambs, whilst the number of mucosal dwelling nematodes was unaffected. Direct interactions of CT from Sulla with parasites present in the GIT may contribute to the differences in FEC and intestinal worm counts that have been observed (Niezen *et al.*, 1993; 1998; Robertson *et al.*, 1995).

Feeding forages that contain CT during parasitic infections has generally improved liveweight gain. For example, when parasitised lambs were fed either *L. pedunculatus* or grass (Neizen *et al.*, 1993), the liveweight gain was four times higher in *L. pedunculatus* compared to the grass fed lambs (184 *vs.* 33 g d^{-1}, respectively). Differences within the CT-containing forages have also been noted in parasitised lambs, with the liveweight gain of Sulla and *L. pedunculatus* fed lambs twice that of lambs fed *L. corniculatus* (approximately 200 *vs.* 86 g d^{-1}, respectively; Robertson *et al.*, 1995; Niezen *et al.*, 1998). In comparison to the lambs in Chapter Two, the lambs in this study had a reduced liveweight gain, with the infected lambs in this study losing weight over the 48 day infection period (17 g d^{-1}). This result may be due to the lower feed intake by the lambs in this study (810 *vs.* 730 g DM d^{-1}, in the Lucerne and Sulla fed lambs, respectively). Care is needed when interpreting these liveweight changes as these present studies were not designed to rigorously test the effect of these diets on changes

in liveweight as there were not enough animals per treatment, the lambs were on a restricted feed intake, and the studies were undertaken in different years.

Despite reductions in feed intake, DM and N digestibility were not adversely affected by the presence of adult *T. colubriformis* in the small intestine, with values consistent with those presented elsewhere for Sulla (Stienezen *et al.*, 1996; Bermingham *et al.*, 2001). The fluxes of AA at the ileum were not different between the control and infected sheep despite a reduction in AA intake and abomasal AA flux. This suggests that there may have been a decrease in AA absorption and /or an increase in endogenous AA losses. Endogenous losses include increased intestinal secretions or an increase in plasma leakage, both of which have been previously documented during parasitic infection (e.g., Steel *et al.*, 1982; Kimambo *et al.*, 1988; Bown *et al.*, 1991). Apparent absorption of EAA, BCAA, SAA and total AA from the small intestine decreased during parasitic infection. However, apparent digestibility of AA in the small intestine was not affected by parasitic infection, which is similar to results presented in Chapter Two. The amount of AA digested in the small intestine, as a proportion of feed intake, when Lucerne was fed to parasitised lambs was 60%, however this increased to approximately 68% when Sulla was fed. Although a direct comparison cannot be made between these two experiments, it does appear that feeding Sulla results in more AA being absorbed by the GIT of lambs, despite a reduction in DM intake.

5.5.2 Plasma nutrients

5.5.2.1 Amino acids

Plasma flow was unaffected by parasitic infection and was consistent with those presented in Chapter Two and else where in the literature (Heitmann & Bergman, 1980; Bird *et al.*, 1981; Janes *et al.*, 1985; Burrin *et al.*, 1989; Lobley *et al.*, 1996; Piccioli Cappelli *et al.*, 1997; Wester *et al.*, 2000; Roy *et al.*, in preparation). Despite no effect of parasitic infection on plasma flow, there were substantial effects on the net AA flux across the MDV, PDV, liver and TSP. The pattern of fluxes across all the tissue beds were in agreement with those presented in the literature (Heitmann & Bergman, 1980;

Lobley *et al.*, 1995; 1996; MacRae *et al.*, 1997; Piccioli Cappelli *et al.*, 1997; Hoskin *et al.*, 2001; Roy *et al.*, in preparation).

The MDV of parasitised lambs released less EAA and total AA. This may be due to either the decrease in apparent absorption of AA from the GIT, or more AA being utilised within the small intestine. The latter explanation agrees with results presented by Yu *et al.* (2000) who observed an increase in the oxidation and sequestration of leucine to the small intestine.

Glutamine is a major fuel for the intestinal mucosa (Souba, 1991) and immune response (Wu *et al.*, 1991; Calder, 1995). The increased utilisation of glutamine in the MDV of parasitised lambs may be due to increased requirements by the GIT for the repair of damaged tissue and local immune response. Glutamine is important for GIT structure, function and metabolism during illness when the gut mucosal barrier is compromised (Souba *et al.*, 1990). Intestinal glutamine requirement has also been linked to the activation of the immune response (Obled, 2002). Threonine release from the MDV was 30% lower in parasitised lambs, suggesting that there may have been an increase in the use of threonine in the small intestine. Threonine is present in high concentrations in GIT secretions (MacRae & Lobley, 1991; Roberton *et al.*, 1991; Van Klinken *et al.*, 1998), and as mucus production may have increased as a consequence of parasitic infection (Stephenson *et al.*, 1980; Holmes, 1985), and it is possible that more threonine was utilised for the synthesis of these products.

During parasitic infection the PDV released less NEAA, EAA, BCAA, SAA and Total AA. The fates of these AA are not known, as they could either be retained by the tissues in the PDV for protein synthesis, oxidised or used for endogenous AA synthesis (NEAA only). The tissues that contribute to the PDV include the rumen, small intestine, mesenteric lymph nodes, large intestine, pancreas and spleen. In MDV (small intestine) parasitic infection only affected the release of EAA and Total AA, therefore it is possible that other tissues within the PDV affected the overall pattern of AA utilisation. For example, the spleen and mesenteric lymph nodes participate in the immune response

and parasitic infection may have increased their utilisation of AA resulting in a decrease in net release of AA from the PDV. Parasitic infection increased protein turnover (Symons & Jones, 1983) and mucus production (Cheema & Scofield, 1982) in the large intestine. These changes may increase the requirements of the PDV for AA and also contribute to the reduction in the release of AA from this tissue.

Threonine was released from the liver of infected lambs compared to a net uptake for the control lambs. Threonine is an important AA for both acute phase protein (APP) synthesis in the liver during the immune response (Obled, 2002) and GIT secretions (MacRae & Lobley, 1991; Roberton *et al.*, 1991; Van Klinken *et al.*, 1998). The release of threonine from the liver during parasitic infection suggests that the requirement for the GIT was more important than for the immune response. Therefore, there seems to be a diversion of threonine to the small intestine at 48 d post infection to meet the increased demands of this tissue. It would be interesting to measure the pattern of AA utilisation at earlier stages of the parasite infection to determine whether the pattern of AA utilisation would change when the requirements of the immune response would be higher. Alternatively, other AA may have been used for the immune response, and this reduced the requirement for threonine. Histidine and methionine were used in smaller amounts by the liver of parasitised lambs.

The release of BCAA by the liver of control lambs is contradictory to results published in the literature (e.g., Heitmann & Bergman, 1980; Lobley *et al.*, 1995; 1996; Milano *et al.*, 2000). This is mainly due to a release of valine by the liver whilst the other BCAA were utilised by this tissue. However, 30-40% of BCAA are oxidised in the peripheral tissues in uninfected sheep (Harris *et al.*, 1992). Increased utilisation of the BCAA in the parasitised lambs may be linked to the role that BCAA have in the immune response (Grimble, 1990).

Net AA fluxes in the TSP were significantly affected by parasitic infection, with NEAA, EAA and SAA being released in the control lambs and utilised by parasitised lambs. It is possible that these AA in the parasitised lambs were used for the synthesis of

components of the immune system (e.g., acute phase proteins) and export proteins from the liver, both of which were not measured in this study. However, the effect on the TSP was largely due to less AA being released by the PDV, as in most cases AA utilisation by the liver was similar between treatments. Despite less AA being released by the TSP into the peripheral circulation, there was no effect of parasitic infection on the utilisation of AA across the hind limbs.

The pattern of AA fluxes across the MDV, PDV, liver, TSP and hind limbs presented in this study are consistent with those observed in lambs fed Lucerne (Chapter Two). In both experiments there was a net release of most AA from the MDV and PDV. However, the presence of parasites in the small intestine significantly reduced the release of AA from both these tissues in the Sulla fed lambs, whilst in the Lucerne fed lambs there was no effect of parasitic infection. This may have been a consequence of the lower feed intake in the Sulla experiment. The liver in both Lucerne (Chapter Two) and Sulla-fed lambs utilised AA, with no affect of parasitic infection on overall flux with the exception of the BCAA, histidine, methionine and glutamine in the Sulla-fed lambs. Reasons for these differences in AA groups are unclear but seems to be related to their specific requirements within the liver. Amino acid utilisation by the TSP was unaffected in the Lucerne-fed lambs, however in the Sulla fed lambs, more AA were utilised during parasitic infection. The utilisation of AA within the hind limbs of both Lucerne fed (Chapter Two) and Sulla-fed lambs (Table 5.7) was unaffected by parasitic infection, and this suggests that there may have been an alteration in the way in which AA were partitioned within the hind limbs (i.e., to the skin, muscle or bone) of the Sulla-fed lambs in order to compensate for the decrease in AA being released by the TSP.

5.5.2.2 Metabolites

There were no significant effects of parasitic infection on the net fluxes of O_2 and CO_2 across the MDV, PDV, liver, TSP and hind limbs. Values presented in this chapter are consistent with those presented for Lucerne-fed lambs (Chapter Two) and those elsewhere in the literature (Bird *et al.*, 1981; Pell & Bergman, 1983).

Glucose metabolism was similar between control and parasitised lambs across the MDV, PDV, liver and TSP. In the hind limbs however, less glucose was utilised by the parasitised lambs possibly indicating a sparing of glucose for other tissues. Lactate is formed by the breakdown of glucose in muscle, which is then transported to the liver where it can be re-synthesised into glucose via the Cori cycle (Mayes, 2000) or by the oxidation of glutamine (Calder, 1995). In the liver lactate was utilised by parasitised lambs, however both glucose concentration in the hepatic vein and hepatic fluxes across this tissue were unaffected by parasitism. This suggests that parasitic infection increased the lambs dependence on lactate for energy production in the liver.

As was the case for Lucerne-fed lambs (Chapter Two), the presence of a parasitic infection had no impact on ammonia or urea utilisation within the lamb suggesting no additional requirements for AA or energy for the formation of urea. The inclusion of CT in the diet of ruminants reduces the amount of ammonia produced in the rumen (Waghorn *et al.*, 1987a; 1994a; 1994b; Waghorn & Shelton, 1995; Stienezen *et al.*, 1996; Burke *et al.*, 2002). Inclusion of QT in the diet of sheep was also seen to reduce rumen ammonia concentration (Salawu *et al.*, 1999) and when QT was included in the diet of parasitised lambs, plasma urea concentration decreased for 3 weeks post infection, however by week 4 they had recovered (Athanasiadou *et al.*, 2000b). A reduction in plasma urea concentration was also observed when QT was given as a drench to parasitised sheep (Athanasiadou *et al.*, 2000a). In contrast, in this study, feeding Sulla did not affect the concentration of urea in plasma during parasitic infection, however plasma urea concentration was only measured at 48 d post-infection, therefore any possible changes that may have occurred earlier in the infection period would have been missed.

5.6 Conclusions

It was the aim of this study to determine the partitioning of AA and other metabolites in lambs infected with *T. colubriformis*. Feed intake was reduced in this study, and this would have had 'carry-over' effects on the availability and flux of nutrients within the

parasitised lamb. This leads to the following question; why do intestinal parasites cause sheep to eat less? From the results it is clear that feeding Sulla during an established parasite burden did not improve the availability of AA to the lamb 48 d post infection. However, any benefits of the inclusion of CT in the diet in the earlier stages of the infection cycle need to be determined. Parasite infection reduced the release of Total AA across the MDV, which was also the case in the PDV. The utilisation of AA in the liver was similar between control and parasitised lambs, despite this there were significant effects of infection across the TSP. There was no apparent repartitioning of AA from the hind limbs during infection, as indicated by the similar net flux across this tissue bed.

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5.8 References

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6 FRACTIONAL SYNTHESIS RATES OF TISSUE PROTEINS IN LAMBS FED FRESH SULLA (*HEDYSARUM CORONARIUM*) DURING A *TRICHOSTRONGYLUS COLUBRIFORMIS* INFECTION.

6.1 Abstract

The fractional protein synthesis rates (FSR % d^{-1}) in the small intestine (duodenum and ileum), liver, lymphoid tissues (spleen, thymus and mesenteric lymph nodes) and productive tissues (muscle and skin) during an established *Trichostrongylus colubriformis* infection were determined in lambs fed Sulla (*Hedysarum coronarium*; 800 g DM d^{-1}; 22 g condensed tannins (CT) kg DM^{-1}). Additionally the FSR in this study were compared to the results obtained from Chapter Three where Lucerne (*Medicago sativa*; no CT) was fed in order to make comments about the possible the effects of CT on FSR in parasitised lambs. Lambs were dosed with 6 000 L3 *T. colubriformis* larvae for 6 d (n=6) or kept as parasite free controls (n=6). Faecal egg production was monitored every second day from day 22 until 48 d post infection. On day 48 post infection, the lambs were continuously infused with [3, 4 -^{3}H]-valine (5.8 MBq h^{-1}) for 8 h. Blood was continuously collected from the mesenteric artery and the specific radioactivity (SRA) of valine in plasma determined. After the 8 h infusion the lambs were euthanased and tissue samples collected from the small intestine (duodenum and ileum), liver, lymphoid tissues, muscle and skin. The presence of parasitic infection in the small intestine decreased feed intake ($P<0.15$) and liveweight gain ($P<0.15$). The SRA of valine in plasma was unaffected by parasitic infection ($P>0.15$). However, the SRA of valine in the intracellular pool in whole ileal tissue was higher in the infected lambs ($P<0.15$). Parasitic infection increased the SRA of protein-bound valine in scraped duodenal and ileal tissues, the mesenteric lymph nodes and the liver ($P<0.15$). Parasitic infection doubled the FSR estimates when plasma valine was used as a precursor (FSR_P) in scraped duodenal tissue ($P<0.15$) although when intracellular valine was used (FSR_I) this increase was not significant ($P>0.15$). Similarly, the presence of *T. colubriformis* in the small intestine of lambs increased estimates of FSR_P in scraped ileal tissue by 50% ($P<0.15$). Infection increased FSR_I ($P<0.15$) in the liver and FSR_P in the mesenteric lymph nodes ($P<0.15$). As there was no effect of parasitic infection on muscle FSR ($P>0.15$) this suggests that AA must have been sourced from other tissue pools to support the increase in FSR in the small intestine and liver of infected lambs. The inclusion of CT resulted in similar FSR estimates in most tissues with the exception of the duodenum and ileum which were higher in the Sulla-fed lambs compared to the Lucerne-fed lambs.

6.2 Introduction

The effects of intestinal parasitic infection on the fractional protein synthesis rates (FSR; % d^{-1}) of the gastrointestinal tract (GIT), liver and muscle protein have been documented to some extent (Symons & Jones, 1971; 1972; 1978; Jones & Symons, 1982). The FSR data from these studies indicate a diversion of amino acids (AA) from growth to the repair of damaged tissue in the GIT. Reasons for the alteration in GIT protein synthesis during parasitic infection include increased mucus production (Cheema & Scofield, 1982) and increased cell turnover and altered morphology of the GIT (Symons & Jones, 1970; Coop & Angus, 1975). Increased FSR in the liver elicited by parasites in the GIT may be due to stimulation of the immune response (Grimble, 1990; Reeds *et al.*, 1999; Butter *et al.*, 2000; Coop & Sykes, 2002). These increases in FSR in the GIT and liver have the net effect of increasing the AA requirement of these tissues.

Feed intake is often reduced during parasitic infection (Sykes *et al.*, 1988) thus any additional AA required as a result of the infection must be sourced within the GIT or from other tissues such as skeletal muscle. The concentration of key AA (e.g., cysteine) are often low in the muscle compared to the AA profile in the GIT and liver (MacRae & Lobley, 1991; MacRae *et al.*, 1993). Therefore, proportionally more muscle protein needs to be mobilised in order to meet the increase in demands for AA by the GIT and liver during parasitic infection.

Feeding forages containing condensed tannins (CT; e.g., Sulla; *Hedysarum coronarium*) has improved animal performance (Terrill *et al.*, 1992; Wang *et al.*, 1996a; 1996b; Min *et al.*, 1998; Burke *et al.*, 2002). The improved performance that results from feeding some CT-containing forages can probably be attributed to increased amino acid (AA) supply and absorption from the small intestine (Waghorn *et al.*, 1987; Wang *et al.*, 1996c; Bermingham *et al.*, 2001). However, the effects of feeding CT-containing forages on FSR have not been reported despite these improvements in animal production.

Feeding some forages that contain CT has beneficial impacts on animal production during parasitic infection (Neizen *et al.*, 1993; 1998; Robertson *et al.*, 1995), however the mechanisms behind this action are unclear. It is likely that the increased supply of AA to the intestine alleviates some of the negative impact of parasitic infection on liveweight gain and tissue FSR. However, *in vitro* (Molan *et al.*, 2000a; 2000b; Athanasiadou *et al.*, 2001) and *in vivo* experiments (Butter *et al.*, 2001) have shown that that a direct action of CT on the parasite may also be partly responsible.

The hypothesis of this study was that feeding CT during the presence of an established parasite infection would not increase the FSR of the GIT and liver due to increased AA availability to the infected lamb. Consequently, the FSR of the productive tissues will not be affected as they would not need to supply the additional AA required for FSR in the GIT and liver. Therefore, the aim of this study was to determine the effects of an established parasitic infection on FSR in the small intestine (duodenum and ileum), liver, lymphoid tissues (spleen, mesenteric lymph nodes and thymus), muscle and skin of lambs fed fresh Sulla during a parasitic infection. This was achieved by using an infusion of [3, 4-^{3}H]-valine. Valine was used as part of the larger study because dietary CT can significantly increase the absorption of this amino acid from the small intestine (Waghorn *et al.*, 1987).

6.3 Materials and Methods

6.3.1 Animals and Feed

Wether lambs weaned from their dams at 33 kg were treated prior to surgery as described in Chapter Five. Twelve lambs were prepared with permanent indwelling catheters in the mesenteric artery and the mesenteric, portal and hepatic veins (Huntington *et al.*, 1989) and vena cava (Ortigues & Durand, 1995) for blood sampling as described in Chapter Five. A temporary catheter was inserted into the jugular vein two d before the start of blood sampling for the infusion of [3, 4-^{3}H]-valine (Amersham Life Science, Buckinghamshire, UK). The FSR was calculated from the incorporation of [3, 4-^{3}H]-valine into tissue protein.

The diet and treatments were previously described in Chapter Five. Briefly, four d after surgery, the lambs were offered fresh Sulla (*Hedysarum coronarium*; 800 g dry matter (DM) d^{-1}) until the conclusion of the trial. The lambs were fed at hourly intervals from overhead feeders and water was available *ad libitum*. One week after surgery (day 1 of the experimental period) six sheep were orally given 6 000 *T. colubriformis* L3 larvae daily for 6 d (parasite treatment) while the remaining six sheep were left untreated to serve as controls (control treatment).

6.3.2 Parasitology

Faecal egg counts (FEC) from individual sheep were determined every second day from day 20 to day 45 of infection (Chapter Five).

6.3.3 Infusions and sampling

On day 45 the sheep received a continuous 8 h infusion of [3, 4-^{3}H]-valine (5.8 MBq h^{-1}; Amersham Life Science, Buckinghamshire, UK) into the jugular vein for measuring the specific radioactivity (SRA) of valine in the plasma (mesenteric artery), tissue intracellular pool (tissue free pool) and tissue proteins.

The SRA of valine in plasma was measured in blood samples (30 mL) that were withdrawn continuously in 2 h increments from the mesenteric artery, the mesenteric, portal, and hepatic veins, and the vena cava over the infusion period. After each 2 h sampling period, the syringes were removed from the collection lines and carefully mixed by gentle rotation. Packed cell volume (haematocrit) and the concentration of oxygen (O_2) and carbon dioxide (CO_2) were determined as described in Chapter Five. The remaining 25 mL of whole blood was processed as described in Chapter Five and the plasma harvested and either processed or stored at -85°C for further analysis as described in section 6.3.5 of this chapter or in Chapters Five and Seven. Valine SRA measured in plasma obtained from the mesenteric artery was used as a precursor pool in the calculation of tissue FSR, and is therefore presented in this chapter. The plasma valine SRA presented in this chapter represents the average of a 2 h-intergrated sample taken from 6 to 8 h after the start of infusion. All the other data relating to valine kinetics are presented in Chapter Seven.

Upon 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 (300 mg mL^{-1}; 0.5 mL kg^{-1} liveweight). 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. The tissues were washed in 0.9% NaCl to remove traces of digesta or blood and quickly frozen in liquid nitrogen (N). A section of whole duodenum and ileum were kept and frozen in liquid N. A further section of each intestinal tissue was separated into the smooth muscle component of duodenal or ileal tissue by gently passing a glass slide over the tissue to remove the mucosal layer. The mucosal layer was frozen in liquid nitrogen however no analysis was completed on this sample. Once frozen the tissue samples were stored at -85°C until analysed. Time from death until storage of all tissues in liquid nitrogen was less than 20 min.

After the tissues had been collected, the ileo-caecal junction of the small intestine was located and sectioned in order to collect ileal digesta from the final 3 m of the ileum for digesta flow measurements (Chapter Five). Ileal digesta was stored at -20°C until analysis.

6.3.4 Analytical methods

In order to calculate the FSR of tissue protein in this experiment, the amount of [3, 4-^{3}H]-valine in the precursor pools (plasma and intracellular SRA valine) and the incorporation of [3, 4-^{3}H]-valine into the protein-bound fraction of the duodenum (whole and scraped), ileum (whole and scraped), mesenteric lymph nodes, spleen, liver, thymus, muscle (*biceps femoris*) and skin were measured.

Total radioactivity of [3, 4-^{3}H]-valine was determined in plasma and infusates by mixing 50 μL of sample in 2 mL of scintillation mixture (Starcint, INSUS Systems) and counting in a Packard Tricarb Model 1500 Scintillation counter for 10 min as described by Lee *et al.* (1999). The proportion of total radioactivity attributed to valine and its breakdown product (H_2O) were determined using an inline flow detector attached to a liquid scintillation counter (Model 2, β-ram, IN/US systems Inc., New Jersey, US) coupled to high performance liquid chromatography (HPLC; LC4A, Shimadzu, Kyoto, Japan) as described in Lee *et al.* (1999).

Valine concentration in plasma was determined by treating plasma (0.5 mL) with 80 mM DTT (80 mM in 0.1% phenol) as an antioxidant, and 3mM norleucine as an internal standard, with the resulting solution stored at -85°C. Analysis of AA in plasma, tissue free pool and standards involved pre column derivatisation and chromatography modified from that outlined by Bidlingmeyer *et al.* (1984). A full description of the method can be found in Chapter Two.

Subsamples (4-5 g) of frozen tissue were pulverised in liquid N using a modified French Cell press as described by Lee *et al.* (1993). The pulverised tissue was then stored at -85°C until homogenised in an extraction buffer (20 mM Tris pH 7.8; 2.5 mM EDTA; 0.3% SDS; See Appendix C.1 for extraction method). This method is described in full in Chapter Three. Total ^{3}H counts associated with valine in the protein-bound pool and the intracellular (free) pool were determined as described previously in Chapter Three. Total radioactivity of the protein-bound and intracellular pools and the proportion of radioactivity attributed to valine and its breakdown product (H_2O) was determined as described above for plasma valine.

Amino acid concentration in the plasma, tissue free pool and protein-bound fraction was determined by using methodologies described in Chapter Five (plasma) or Chapter Three (tissue).

6.3.5 Calculations

Specific radioactivity (SRA; dpm $nmol^{-1}$) of valine in plasma (SRA_P), tissue free pool (SRA_I), and tissue-bound proteins (SRA_B) was calculated by dividing the radioactivity of the sampled pool (dpm mL^{-1}) by the concentration in the sampled pool (nmol mL^{-1}). Both the tissue free pool and arterial plasma valine SRA were used as precursor pools for the estimation of FSR.

The equation used in the current study for the estimation of tissue protein FSR and its underlying assumptions is described in Chapter Three and presented in Equation 6.1 (Wykes *et al.*, 1996):

Equation 6.1

$$\text{FSR } (\%\,d^{-1}) = \frac{\text{SRA Valine}_{(protein\ bound)}}{\text{SRA Valine}_{(precursor\ pool)} * \text{period of infusion (d)}} * 100$$

where the period of infusion equals 8 h or 0.33 d.

6.3.6 Statistical analysis

Statistical analysis was performed using a General Linear Model (SAS version 8, 1999) to analyse the data according to a completely randomised block design, with treatment (control or parasite) and group (when the animal underwent surgery) used as sources of variation in the model. Probability values lower than 0.05 were considered to indicate a significant difference and values between 0.05 and 0.15 to indicate a trend. Results are presented as least squares means (LSmeans) and associated pooled standard deviation (SD).

The data was checked for normality using a univariate analysis, whilst the Levene test was used to test for homogeneity of variance. No data showed deviation from normality or homogeneity, thus no transformation was required, with a probability less than 0.01 used as the determinant for acceptance or rejection of the normality and homogeneity tests.

6.4 Results

Results relating to the feed intake and liveweight change are presented in Chapter Five. Briefly, the Sulla contained 22.1 g kg^{-1} DM of total CT. The presence of parasites in the small intestine (240 *vs.* 18 000 (SD 7 000) worms in the control and parasite lambs, respectively; $P<0.001$) reduced DM intake (769 *vs.* 689 (SD 47) g DM d^{-1} in the control and parasite lambs, respectively; $P<0.15$) over the course of the experiment. Differences in liveweight were apparent over the last 20 d of the infection period (50 *vs.* -50 (SD 70) g d^{-1} in the control and parasite lambs, respectively; $P<0.15$).

6.4.1 Specific radioactivity and concentration of valine

The SRA_P in the blood samples continuously withdrawn between 6 to 8h after the start of the ^{3}H-valine infusion averaged 68.9 *vs.* 74.6 (SD 23.3) dpm $nmol^{-1}$ in the control and parasite lambs, respectively (P>0.15). The concentration of valine in arterial plasma was unaffected by parasitic infection (203 *vs.* 235 (SD 64) $\mu mol\ L^{-1}$ in the control and parasite lambs, respectively; P>0.15).

The presence of *T. colubriformis* did not affect the concentration of most AA in the intracellular and protein-bound pools with a few exceptions. Parasitic infection significantly increased the intracellular concentration of valine in the liver (105.9 *vs.* 129.3 (SD 18.2) $\mu mol\ L^{-1}$ in the control and parasite lambs, respectively; P<0.15; Table 6.1). However, there was no effect of parasitic infection on intracellular concentration of this AA in the other tissues, therefore the concentration of valine was averaged across treatments (P>0.15; Table 6.1).

The protein-bound concentration of valine was unaffected by the presence of parasitic infection in the lambs (P>0.15) with the exception of duodenal tissues and the skin (Table 6.1). The concentration of valine in the protein-bound fraction of scraped duodenal (19.8 *vs.* 22.8 (SD 1.2) mg g DM^{-1} in the control and parasite lambs, respectively; P<0.15) and whole duodenal tissue (21.1 *vs.* 22.3 (SD 1.2) mg g DM^{-1} in the control and parasite lambs, respectively; P<0.15) was increased by parasitic infection. However, skin protein-bound valine was decreased with parasitic infection (25.8 *vs.* 21.4 (SD 2.7) mg g DM^{-1} in the control and parasite lambs, respectively; P<0.15).

The SRA_I of valine for each tissue is presented in Tables 6.2 to 6.4 and in all cases SRA_I was lower than SRA_P. The SRA_I of valine increased in whole ileal tissue (P<0.15; Table 6.2) of parasitised lambs, however no effect of parasitic infection on valine SRA_I was observed in the other tissues (P>0.15). Parasite infection increased SRA_B of valine in the scraped duodenum, scraped ileum (P<0.15; Table 6.2), liver and the mesenteric lymph nodes (P<0.05; Table 6.3).

6.4.2 Fractional Synthesis Rates

With the exception of skeletal muscle in control lambs, the FSR_P estimates were lower than the FSR_I (Table 6.2 to 6.4). Parasitic infection significantly increased FSR_P in scraped duodenal and ileal tissue ($P<0.15$; Table 6.2). Parasitic infection did not affect both whole duodenal and ileal tissue estimates of FSR_P and FSR_I ($P>0.15$; Table 6.2).

In the liver, FSR_I was doubled during parasitic infection (Table 6.3; $P<0.15$). The mesenteric lymph nodes showed a significant increase in FSR_P in the infected lambs ($P<0.05$; Table 6.3), however FSR_I (although higher) was not affected ($P>0.15$). In the spleen and thymus both FSR_P and FSR_I estimates were unaffected by parasitic infection ($P>0.15$).

Infected lambs had larger estimates of FSR_I in the muscle ($P<0.15$; Table 6.3), however FSR_P was unaffected. Parasitic infection had no effect on skin FSR estimates (Table 6.4; $P>0.15$).

Table 6.1 The concentration of valine in the intracellular pool (μmol L^{-1}) and protein-bound fraction (mg g DM^{-1}) of tissues in lambs fed fresh Sulla (*Hedysarum coronarium*). The presence of *Trichostrongylus colubriformis* had no effect on the concentration of most amino acids in the intracellular pool of most tissues so results are presented as pooled means and standard deviations across treatments. Where a significant difference caused by the presence of *Trichostrongylus colubriformis* did occur ($P<0.15$) this is indicated in the table (†).

	Free pool μmol L^{-1}	Protein Bound mg g DM^{-1}
Duodenum scraped	31.9 ± 8.2	21.2 ± 1.1†
Duodenum unscraped	97.0 ± 35.1	21.6 ± 1.1†
Ileum scraped	35.8 ± 11.8	22.7● 0.9
Ileum unscraped	97.4 ± 19.9	24.1 ± 1.6
Liver	117.5 ± 18.1†	22.3 ± 3.2
Lymph nodes	115.3 ± 37.4	20.6 ± 2.
Muscle	34.5 ± 10.9	28.5 ± 3.4
Skin	40.1 ± 12.2	23.5 ± 2.7†
Spleen	68.3 ± 21.6	32.1 ± 6.6
Thymus	31.7 ± 9.5	21.2 ± 1.1

† see text for treatment differences.

Table 6.2 Specific radioactivity of tissue free pool (SRA_I; dpm $nmol^{-1}$) and protein-bound (SRA_B; dpm $nmol^{-1}$) and fractional protein synthesis rates (FSR_P or FSR_I; % d^{-1}) in the small intestine in lambs infected with or without *Trichostrongylus colubriformis* and fed fresh Sulla (*Hedysarum coronarium*). Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
Duodenum scraped	SRA_I	38.3	36.2	25.2	0.89
	SRA_B	0.8	2.0	0.8	0.03
	FSR_P	3.42	7.40	2.5	0.03
	FSR_I	8.3	26.8	22.9	0.20
Whole duodenum	SRA_I	21.6	31.5	11.6	0.18
	SRA_B	5.2	5.6	1.8	0.74
	FSR_P	23.9	23.8	9.0	0.98
	FSR_I	87.3	54.1	39.1	0.21
Ileum scraped	SRA_I	23.7	18.0	15.3	0.54
	SRA_B	0.5	1.0	0.3	0.03
	FSR_P	2.4	4.0	1.0	0.03
	FSR_I	15.8	19.3	16.2	0.72
Whole ileum	SRA_I	11.1	34.3	9.9	0.01
	SRA_B	2.5	3.1	1.4	0.45
	FSR_P	11.6	12.0	5.8	0.92
	FSR_I	65.9	28.9	35.1	0.16

Table 6.3 Specific radioactivity of tissue free pool (SRA_I; dpm $nmol^{-1}$) and protein-bound (SRA_B; dpm $nmol^{-1}$) and fractional protein synthesis rates (FSR_P or FSR_I; % d^{-1}) in the liver and lymphoid tissues in lambs infected with or without *Trichostrongylus colubriformis* and fed fresh Sulla (*Hedysarum coronarium*). Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
Liver	SRA_I	22.6	28.5	14.7	0.97
	SRA_B	2.3	3.8	1.5	0.12
	FSR_P	11.4	14.7	6.3	0.38
	FSR_I	21.3	54.1	11.5	0.01
Mesenteric lymph nodes	SRA_I	13.6	17.8	6.9	0.32
	SRA_B	2.3	5.1	1.3	0.01
	FSR_P	10.9	20.8	5.6	0.00
	FSR_I	67.0	92.0	39.2	0.30
Spleen	SRA_I	14.9	30.7	16.6	0.19
	SRA_B	1.1	1.5	0.6	0.30
	FSR_P	5.1	5.6	2.4	0.95
	FSR_I	27.4	17.5	17.6	0.39
Thymus	SRA_I	19.6	24.7	15.3	0.61
	SRA_B	3.7	3.9	1.8	0.84
	FSR_P	15.9	15.6	5.9	0.94
	FSR_I	52.8	49.7	28.8	0.86

Table 6.4 Specific radioactivity of tissue free pool (SRA_I; dpm $nmol^{-1}$) and protein-bound (SRA_B; dpm $nmol^{-1}$) and fractional protein synthesis rates (FSR_P or FSR_I; % d^{-1}) in the muscle and skin in lambs infected with or without *Trichostrongylus colubriformis* and fed fresh Sulla (*Hedysarum coronarium*). Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
Muscle	SRA_I	37.8	38.8	17.5	0.93
	SRA_B	0.1	0.2	0.1	0.37
	FSR_P	0.5	0.6	0.6	0.84
	FSR_I	0.3	1.4	0.9	0.11
Skin	SRA_I	52.5	43.8	41.2	0.76
	SRA_B	0.8	0.8	0.3	0.82
	FSR_P	3.8	3.3	1.5	0.57
	FSR_I	7.9	4.4	5.0	0.27

6.5 Discussion

6.5.1 Effect of parasitic infection on protein fractional synthesis rates

The presence of an established parasite infection in the small intestine of lambs fed Sulla increased FSR_P in scraped duodenal and ileal tissues and the mesenteric lymph nodes despite a reduction in feed intake. Similarly, estimates of FSR_I were increased in the liver of the parasitised lambs. This indicates that there was an increase in the AA requirements by the intestinal tissues and the tissues supporting the alterations in GIT metabolism including the immune response (liver and mesenteric lymph nodes). As feed intake was also reduced during parasitic infection, these additional requirements were not met by an increase in dietary AA availability to the lamb, suggesting that there was a mobilisation of AA from proteins stores elsewhere in the body. Reduced liveweight gain over the last 20 d of the experiment agrees with this explanation. However, as the net total AA flux across the hind limbs was similar between the control and parasitised lambs this suggests that the skeletal muscles are unlikely to be the source of additional AA.

Parasitic infection doubled the FSR_P estimates in scraped duodenal tissue and FSR_I estimates were also increased although this did not achieve significance ($P>0.15$). Similarly, the presence of *T. colubriformis* in the small intestine increased estimates of FSR_P in scraped ileal tissue by at least 50%. Scraped intestinal tissue represents the smooth muscle component of the intestine, and the increases in FSR of these tissues indicate that more constitutive proteins are being re-synthesised per d. These changes in both duodenal and ileal tissues contradict data in the literature relating to parasitic infection, which suggest that parasitic infection increased the absolute rates of protein synthesis in the small intestine, with no effect on FSR (Symons & Jones, 1983). However, absolute protein synthesis was not measured in the current study, as the weight and N content of tissues were not measured. Increased ileal smooth muscle FSR has been observed in septic rats, however the authors noted that different models of infection would be likely to have different effects on tissue FSR (Breuillé *et al.*, 1994).

Trichostrongylus colubriformis inhabit the proximal section of the small intestine (Vlassoff & McKenna, 1994; Pomroy, 1997) and may cause damage to the duodenal

tissue as the parasite burrows into the epithelial layers (Coop & Angus, 1975; Holmes, 1985). Damage to duodenal tissue is likely to increase the protein turnover as tissue integrity needs to be re-established. This explanation agrees with the increased scraped ileum FSR reported in the current study. However, contrary to the present study, similar infection protocols did not affect the FSR in the duodenum of lambs fed fresh Lucerne (Chapter Three).

Studies where tannin extracted from the bark of the *Schinopsis* species (QT) were fed to uninfected rats (4% of DM) have shown that there was no effect of the tannin on FSR in the duodenum (Dawson *et al.*, 1999). While different CT have variable impacts on animal performance due to differences in the chemical structure and concentration (McNabb *et al.*, 1998; Aerts *et al.*, 1999), it is unlikely that the increase in duodenal FSR in the parasitised lambs is due to the presence of CT *per se*.

The infection protocol in the current study resulted in a lower establishment rate of parasites in the proximal small intestine (48% *vs.* 60% in the Sulla and Lucerne fed lambs, respectively). This may be due to a direct action of the CT on the parasite which has been documented in *in vitro* (Molan *et al.*, 2000a; 2000b; Athanasiadou *et al.*, 2001) and *in vivo* studies (Butter *et al.*, 2001). Increasing protein and/or energy supply post-ruminally improves the immune status of the animal (Roberts & Adams, 1990; Bown *et al.*, 1991; Donaldson *et al.*, 1997). This may be due to an increase in the circulating levels of eosinophils (Valderrábano *et al.*, 2002). It is likely that the reduction in feed intake in the infected lambs in the current study meant that the lambs were more susceptible to the presence of parasites despite the reduction in the intestinal worm burden when compared to the lambs in Chapter Three. Therefore, the increased FSR in the duodenum in parasitised lambs is likely to be due to an interaction between the parasite, CT and the immune status of the lamb. However, the nature of this interaction is unclear, and at this stage is purely speculative.

It has been proposed that the parasite initiates a general immune response which results in altered functional integrity of subsequent sections of the GIT (Symons, 1978), and this may account for the increase of FSR in the scraped ileal tissue observed in the current study. The ileal AA flux was similar between control and

infected lambs despite a reduction in both DM intake and abomasal AA flux (Chapter Five). This suggests that there was indeed an increase in the endogenous losses of AA in the parasitised lamb. These losses may be due to an increase in protein turnover (i.e., an increase in sloughed intestinal cells as indicated by an increase in FSR in scraped ileal tissue), the leakage of plasma proteins into the GIT and/or mucus production which have previously been observed during parasitic infection (Poppi *et al.*, 1986).

Parasite infection has increased liver FSR in sheep (Jones & Symons, 1982) and guinea pigs (Symons & Jones, 1971; 1978) and this is in agreement with the results presented in this study. However, under similar experimental methodology, parasitic infection did not affect FSR in the liver of lambs fed Lucerne (Chapter Three). The increase in liver FSR_P in the current study suggests that there was an increase in the amount of constitutive protein synthesised by the liver. Some of the constitutive proteins might be required to support the hepatic synthesis and secretion of acute phase proteins used for the immune response during parasitic infection. An increase in hepatic protein synthesis to support the immune response is in agreement with the increase in FSR_P in the mesenteric lymph nodes observed in parasitised lambs.

Feed intake in the current study was decreased by approximately 10% in the parasitised lambs, and subsequently the AA supply in the portal drainage to the liver was also decreased (Chapter Five). The liver FSR was unaffected by parasitic infection in a similar study where there was no adverse affect of parasitic infection on intake (Chapter Three) and total AA utilisation in the liver was also unaffected (Chapter Two). This suggests that an established intestinal worm burden in the GIT alone may not be enough to elicit an increase in AA requirement for the immune response.

The increase in FSR of intestinal tissue and liver combined with reduced intake in the infected lambs suggest that AA must be sourced from other protein stores. Although the parasitised lambs in this study lost weight, an increase in muscle protein FSR_I estimates was observed in these lambs. This latter result supports the data presented in Chapter Three, but contradicts observations in the literature which indicate a decrease in muscle FSR during parasitic infection (Steel *et al.*, 1980; Jones

& Symons, 1982). Reduced protein synthesis in parasitised animals is often accompanied by an increase in protein degradation (Symons & Jones, 1971; 1972; 1975; 1978). In the current study, the net flux of total AA across the hind limbs was similar between control and infected animals (Chapter Five) and this indicates that no additional AA mobilisation from the hind limbs occurred in the infected lambs. Studies indicating changes in muscle protein metabolism (synthesis and/or degradation) in the lamb during parasite infection have used more severe infection protocols than the one used in the current study. For example, Jones & Symons (1982) dosed their lambs with 18 000 L3 larvae per week for 11 weeks, while Steel *et al.* (1980) infected their lambs with 30 000 L3 larvae per week for 24 weeks (Appendix Table A.1). Therefore, these infection regimes may account for the altered muscle protein turnover that was observed in those studies.

The muscle FSR estimates in this study are comparable with those presented in Chapter Three using a similar infusion protocol, but are low compared to the literature (e.g., Davis *et al.*, 1981; Schaefer *et al.*, 1986). Reasons for these differences may be due to the infusion length, or the level of nutrition (Chapter Three). Therefore, caution must be taken when interpreting the effect of parasitic infection on muscle FSR in the current study and in Chapter Three.

6.5.2 Does feeding condensed tannins increase protein fractional synthesis rates?

Improvements in liveweight gain (Terrill *et al.*, 1992; Burke *et al.*, 2002), wool growth (Wang *et al.*, 1996b; Min *et al.*, 1998) and milk production (Wang *et al.*, 1996a) have been observed when forages containing CT are fed to sheep. However, little is known about how the CT may result in providing more nutrients to fulfil the metabolic requirements of the different tissues. A direct comparison between the lambs fed Lucerne (no CT; Chapter Three) and Sulla (contains CT; current study) cannot be made statistically in order to elucidate the effects of CT on tissue protein FSR. Feed intake differed between the parasitised lambs fed Lucerne (800 g DM d^{-1}) and parasitised lambs fed Sulla (690 g DM d^{-1}) however, the control lambs of both forages had similar feed intakes (830 *vs.* 770 g DM d^{-1} in the control lambs of the Lucerne and Sulla experiments, respectively) thus, it is of interest to compare the

'control' lambs fed Lucerne to the control lambs fed Sulla (Table 6.5) in order to indicate some possible effects of feeding CT on tissue FSR.

In whole duodenum and ileum tissue, the average FSR_P and FSR_I estimates in the lambs fed Sulla containing 2% CT were generally higher than those fed Lucerne. Previous studies involving rats showed that the inclusion of QT in the diet (4% CT) had no effect on duodenal mucosa FSR (Dawson *et al.*, 1999), however they did not investigate any effects of QT on whole intestinal duodenal tissue. In lambs, the inclusion of QT in the diet (5% CT), resulted in epithelial degeneration and ulceration in the jejunum and ileum, however there was no morphological changes in the duodenum, possibly due to the alkaline environment of the jejunum and ileum (Wang *et al.*, 1996c; Dawson *et al.*, 1999). Similar levels of CT present in fresh *Lotus pedunculatus* (5.5% CT) had no effect on the morphology of the duodenum, jejunum or ileum in lambs (Walton *et al.*, 2001). This suggests that various types of CT may differ in their effects within the digestive tract as well as on animal production, due to variations in their chemical structure (McNabb *et al.*, 1998; Aerts *et al.*, 1999).

The FSR_P of the liver ranged from 16% to 40% and were slightly lower in the Sulla-fed lambs. In contrast, in the thymus and mesenteric lymph nodes the FSR_I estimates were 2-3 times greater in the Sulla-fed lambs. It is possible that the CT present in Sulla resulted in a localised immune response in the GIT due to harmful interactions with the GIT mucosa, however as the effects of the CT in Sulla on the histology of the GIT have not been determined, this remains speculative.

In the peripheral tissues, the estimates of muscle protein FSRP in the Sulla-fed lambs were similar to those of the Lucerne-fed lambs (c. 0.2 % d^{-1}). However, both these estimates are quite low compared to the results in the literature (Chapter Four) and this suggests that the infusion length of the ^{3}H-valine may have been too short to enable sufficient incorporation of ^{3}H-valine into the skeletal muscle protein pool (Chapter Four). The skin estimates of protein FSR_I (10 to 12 % d^{-1}) were fairly consistent between the Lucerne and Sulla-fed lambs. Therefore, it seems unlikely that feeding CT improves performance in the muscle and skin by increasing the amount of AA available for production, however in both studies intake was restricted

to approximately maintenance requirements (800 g DM d^{-1}) and therefore a larger difference between the two studies may become apparent only at higher intakes.

6.6 Conclusions

Parasitic infection increased the FSR in scraped duodenal and ileal tissue, liver, mesenteric lymph nodes and muscle. Increased FSR in the intestine suggest an increased requirement for constitutive proteins during parasitic infection. This could be attributed to an increase in sloughed cells caused by the establishment of parasites in the intestinal tissue. Increased intestinal FSR will be required to support the re-establishment of intestinal integrity. The increases in the FSR of liver and mesenteric lymph nodes suggest an increased requirement for AA for the synthesis of constitutive proteins involved in mounting and controlling the immune response during parasitic infection. It is likely that this response is due to the decreased AA availability to the portal drainage observed in the infected lambs. Muscle FSR increased during parasitic infection, however this effect may be unreliable due to the length of infusion used being unsuitable for FSR measurements in slow turnover tissues. Feed intake was decreased by parasitic infection, therefore it seems likely that there was an increase in AA mobilisation from protein sources other than the hind limbs in order to provide the intestinal tissues, liver and mesenteric lymph nodes with the additional AA the lambs required.

Although the effects of CT on tissue FSR can only be speculated, it seems that the presence of CT in the diet has little effect on the turnover rates of the intestine, liver, muscle and skin. However, the mesenteric lymph nodes and spleen were significantly higher in the Sulla-fed lambs, and this may be due to the CT present in the Sulla having a negative interaction with the components of the GIT involved in the immune response. These interactions – if any, are of interest due to the impacts that individual tissues may have on whole body AA requirements.

Table 6.5 A comparison of fractional protein synthesis rates in different tissues using either the specific radioactivity of valine in plasma (FSR_P; % d^{-1}) or tissue free pool (FSR_I; % d^{-1}) in lambs fed fresh Lucerne (n=6; *Medicago sativa*; no condensed tannin) or Sulla (n=6; *Hedysarum coronarium*; 2.2% condensed tannin). Results are presented as LSmeans and pooled standard deviations (SD) for each forage.

		Lucerne Mean	Pooled SD	Sulla Mean	Pooled SD
Duodenum scraped	FSR_P	8.6	0.9	3.4	2.5
	FSR_I	9.2	3.8	8.3	22.9
Whole duodenum	FSR_P	18.7	2.0	23.9	9.0
	FSR_I	53.6	25.9	87.3	39.1
Ileum scraped	FSR_P	5.1	1.7	2.4	1.0
	FSR_I	8.4	4.2	15.8	16.2
Whole ileum	FSR_P	17.8	3.2	11.6	5.8
	FSR_I	48.5	5.1	65.9	35.1
Mesenteric lymph nodes	FSR_P	17.6	4.0	10.9	5.6
	FSR_I	34.7	11.6	92.0	39.2
Spleen	FSR_P	9.7	3.6	5.1	4.8
	FSR_I	31.3	10.9	27.4	17.6
Thymus	FSR_P	12.8	4.4	15.9	5.9
	FSR_I	24.7	14.9	52.8	22.8
Liver	FSR_P	16.6	6.0	11.4	6.3
	FSR_I	37.4	24.1	21.3	11.5
Muscle	FSR_P	0.2	0.4	0.5	0.6
	FSR_I	0.1	0.7	0.3	0.9
Skin	FSR_P	5.3	2.4	3.8	1.5
	FSR_I	12.3	3.2	9.9	5.0

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6.8 References

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7 THE EFFECT OF A *TRICHOSTRONGYLUS COLUBRIFORMIS* INFECTION ON VALINE AND CYSTEINE KINETICS IN LAMBS FED FRESH SULLA (*HEDYSARUM CORONARIUM*).

7.1 Abstract

The effect of a *Trichostrongylus colubriformis* infection on cysteine and valine kinetics in the mesenteric-drained viscera (MDV), portal-drained viscera (PDV), liver, total splanchnic tissues (TSP) and the hind limbs were determined in lambs fed fresh Sulla (*Hedysarum coronarium*; 800 g DM d^{-1}) on day 48 post infection. Lambs were dosed with 6 000 L3 *T. colubriformis* larvae for 6 d (n=6) 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 peaked on day 26 post infection ($P<0.0001$). Forty-five d post infection, the lambs were continuously infused for 8 h with [^{35}S]-sulfate (4.6 MBq h^{-1}) to measure whole body cysteine kinetics. They also received a bolus injection of D_2O (0.45 mL kg^{-1} body weight) into the jugular vein to measure the size of the whole body water pool. On day 48 post infection, the lambs were continuously infused for 8 h with dual isotopes of valine: [3, 4 -^{3}H]-valine (5.8 MBq h^{-1}) into the jugular vein and [1-^{13}C]-valine (99 atom percent, 101 mg h^{-1}) into the abomasum. Concurrently, [^{35}S]-cysteine (2.4 MBq h^{-1}) was also continuously infused into the abomasum. Para-aminohippuric acid (783 mg h^{-1} into the mesenteric vein) and indocyanin green (14.6 mg h^{-1} into the abdominal aorta) were also continuously infused for 8 h in order to measure blood flow across the splanchnic tissues and hind limbs, respectively. Blood was continuously collected from the mesenteric, portal and hepatic veins, the mesenteric artery and the vena cava. Plasma was harvested, AA and metabolite concentrations measured and the specific radioactivity (SRA) and isotopic enrichment (IE) of valine and/or cysteine determined. After the 8 h infusion the lambs were euthanased and samples collected from the ileum and muscle for determination of valine SRA and IE. Intestinal worm burdens on day 48 post infection were significantly higher in the infected lambs ($P<0.0001$). Feed intake was reduced in the infected lambs, and as a consequence liveweight was also reduced over the last 20 days of the infection period. There was little effect of parasite infection on cysteine and valine concentration, SRA or IE in plasma, ileal digesta or the muscle free pool. The alterations in the ILR of valine across the MDV were due to the reduction in the apparent absorption of valine and this had a carry -over effect in the PDV. However, despite the alterations in the GIT tissues

there was no effect of parasitic infection on either cysteine or valine kinetics across the liver or TSP. There was no apparent repartitioning of AA from the muscle of the hind limbs to the visceral tissues with both cysteine and valine kinetics being largely unaffected by the parasites. The reduction in liveweight observed in infected lambs was attributed to the decreased appearance of AA in the portal vein.

7.2 Introduction

Increased endogenous protein losses into the gastrointestinal tract (GIT) during parasitic infection include increased mucus production (Cheema & Scofield, 1982), increased plasma leakage into the GIT (Kimambo *et al.*, 1988) and increased sloughing of cells (Coop & Angus, 1975; Holmes, 1985). These are likely to affect protein turnover of the tissues within the GIT and increase the amino acid (AA) requirements of the GIT for the production of these proteins. This is supported by an increase in protein synthesis rate in the small and large intestines, as observed during a *Trichostrongylus colubriformis* infection in guinea pigs (Symons & Jones, 1983). In another study, parasitic infection increased the oxidation of leucine in the GIT of sheep by almost 20-40%, which together with increased leucine sequestration to these tissues, had the net effect of decreasing its availability to other tissues (Yu *et al.*, 2000).

The liver has a pivotal role in the metabolism of AA, and possibly regulates their supply to the peripheral tissues (Lobley, 2002). During a *T. colubriformis* infection increased fractional protein synthesis rate (FSR) in the liver has been observed (Symons & Jones, 1971; 1978; Jones & Symons, 1982). Synthesis of serum and export proteins (e.g., albumin; Vary & Kimball, 1992) and of immune proteins such as the acute phase proteins (Stehle & Furst, 1983) occur in the liver and their synthesis can increase during infection (Poppi *et al.*, 1986).

The increased AA requirements by the GIT, liver and immune tissues during infection can be further exacerbated as dietary intake may also reduce (Sykes *et al.*, 1988). Therefore, to meet the increased requirements of these tissues, proportionally more

muscle protein needs to be mobilised to supply these additional demands (MacRae & Lobley, 1991; MacRae *et al.*, 1993). The AA released from muscle protein through degradation which are not required for gastrointestinal, hepatic and immune protein synthesis, are likely to be catabolised and therefore represent a further loss of AA to the animal. The negative impacts of a parasitic infection on ruminant productivity have been well documented and include decreased liveweight gain (Sykes & Coop, 1976; van Houtert *et al.*, 1995). This change has been attributed to a decrease in muscle protein synthesis and an increase in muscle protein degradation (Symons & Jones, 1971; 1972; 1975; 1978) suggesting that AA are mobilised from muscle. This might be triggered by the increased demand for AA in tissues such as the GIT and liver during the parasitic infection.

Feeding forages containing condensed tannins (CT) has lessened the negative impact of parasites on liveweight gain (Niezen *et al.*, 1995; 1998a). A direct effect of CT on parasites has been reported (Molan *et al.*, 2000a; 2000b; 2002; Athanasiadou *et al.*, 2001), and CT also improves AA supply to the small intestine (Waghorn *et al.*, 1987; 1994; Bermingham *et al.*, 2001). Therefore, improved liveweight gain in the infected lamb may also be due to increased availability of dietary AA to the animal which decreases the demand on the skeletal muscle to mobilise its reserves to supply the GIT and liver with the additional AA that they require. However, the effects of feeding CT on AA kinetics and protein metabolism during parasitic infection have not been reported despite the improvements in animal production that have been observed.

The hypothesis of this study is that an established *T. colubriformis* infection will increase the irreversible loss rate (ILR = oxidation and proteins synthesis) of valine and cysteine across the mesenteric-drained viscera (MDV), portal-drained viscera (PDV), liver, total splanchnic tissues (TSP) of lambs fed fresh Sulla. However, the increases in AA requirements by these tissues will not be met by alteration of valine ILR within the muscles of the hind limbs but instead arise from a greater reliance on dietary AA to meet the increased demand by the GIT and liver. Therefore, the aim of this study was to firstly investigate the effects of parasitic infection on whole body metabolism of valine

and cysteine. Secondly the ILR of valine and cysteine across the MDV, PDV, liver, TSP and hind limbs will be quantified. Valine kinetics will be estimated using two isotopes of valine: [3, 4-^{3}H]-valine infused into the jugular vein and [1-^{13}C]-valine infused into the abomasum. This will enable any recycling of the abomasal isotope to the MDV and PDV to be calculated as well as the contribution of arterial and luminal AA supply to tissue turnover. Cysteine kinetics will be determined using an abomasal infusion of [^{35}S]-cysteine. In order to elucidate the potential advantages of feeding forages that contain CT in sheep exposed to parasites, the data from the current chapter and from Chapter Four will also be compared. Isotopes of valine were used for the larger study because dietary CT can significantly increase the absorption of this amino acid from the small intestine (Waghorn *et al.*, 1987). Cysteine was used because of its role in immune responses and the repair of the GIT (MacRae, 1993).

7.3 Materials and Methods

7.3.1 Animals and feed

As part of a larger study (Chapters Five and Six) wether lambs (33 kg) were weaned from their dams and were transported to AgResearch Limited, Grasslands Research Centre, Palmerston North. The lambs were prepared with permanent indwelling catheters in the mesenteric artery, and the mesenteric, portal and hepatic veins (Huntington *et al.*, 1989) and vena cava (Ortigues & Durand, 1995) for blood sampling. Additional permanent catheters were placed in the mesenteric vein (upstream from the sampling catheter) and abdominal aorta for infusion of ρ-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 [^{35}S]-cysteine and [1-^{13}C]-valine on day 48 post infection to measure cysteine and valine kinetics across the MDV, PDV, liver, TSP 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_2O), [^{35}S]-sulfate and [^{13}C]-sodium bicarbonate on day 45 post infection. To determine the effects of parasite infection on AA metabolism in the MDV, PDV, liver, TSP and hind limbs two isotopes

of valine were infused into the jugular vein ([3, 4-^{3}H]-valine) and abomasum ([1-^{13}C]-valine) on day 48 post infection. This enabled any recycling of the abomasal isotope to the MDV and PDV to be calculated. Radioactive and stable isotopes were purchased from Amersham Life Science (Buckinghamshire, UK) and Mass Trace, Inc. (Woburn, MA, USA), respectively.

The lambs were offered fresh Sulla (*Hedysarum coronarium*; 800 g dry matter (DM) d^{-1}) four d after surgery (Chapter Five) and were fed at hourly intervals from overhead feeders.

The parasitological aspects of the current chapter have been described in full in Chapter Five. However, briefly one week after surgery (day 1 of the experimental period) six sheep were given 6 000 *T. colubriformis* L3 larvae per day orally for 6 d (parasite treatment) while the remaining six sheep were drenched with Ivomectin (Ivomec® Merial, New Zealand Ltd) to serve as controls (control treatment).

7.3.2 Parasitology

Faecal egg counts (FEC) from individual sheep were determined every second day from day 20 to day 45 of infection as described in Chapter Five. Total intestinal worm burdens were determined after slaughter (Chapter Five).

7.3.3 Infusions and blood sampling

Forty-five days post infection, the lambs received a bolus injection of D_2O (0.45 mL kg body weight^{-1}) into the jugular vein to measure the size of the whole body water pool. The lambs also received an 8 h continuous infusion of $NaH^{13}CO_3$ (99 atom percent, Mass Trace, Inc., Woburn, MA, USA; 205 mg h^{-1}) and ^{35}S-sulfate (4.56 MBq h^{-1}) into the jugular vein to measure the whole body production of carbon dioxide (CO_2) and sulfate, respectively so that the oxidation of valine and cysteine could be estimated. Twenty mL of blood was withdrawn continuously every two hours from the mesenteric artery over the 8 h infusion period. The blood was centrifuged (4°C; 3 270 g for 15 min), and the plasma harvested and stored at -85°C in order to determine plasma total

count, specific activity and concentration of sulfate, DM content of plasma and ^{2}H enrichment of water. The concentration and isotopic enrichment of CO_2 was measured according to the methods described by Read *et al.* (1984). However, at the time this thesis was submitted analysis of blood $NaH^{13}CO_3$ was not completed so this data will not be presented.

On day 48, the lambs received a primed continuous 8 h infusion of [3, 4-^{3}H]-valine (5.8 MBq h^{-1}) into the jugular vein. Concurrently, [1-^{13}C]-valine (99 atom percent, Mass Trace, Inc., Woburn, MA, USA; 101 mg h^{-1}) and ^{35}S-cysteine (2.4 MBq h^{-1}) were continuously infused into the abomasum. In order to measure plasma flow across the MDV, PDV, liver and TSP, para-ammonihuppuric acid (PAH) was infused continuously into the mesenteric vein for 8 h (see Chapter Five for details). The lambs also received a continuous infusion of indocyanin green (ICG) into the abdominal aorta for 8 h enabling plasma flow across the hind limbs to be calculated (Chapter Five). To prevent blood clotting during the continuous sampling, 6 000 iu ovine heparin h^{-1} was infused into the jugular vein of the lambs over the 8 h infusion period. Sampling lines and syringes were kept in an ice-water bath to minimise the degradation of blood constituents (Lobley *et al.*, 1995). As part of the larger study, 30 mL of blood was withdrawn continuously every 2 h from the mesenteric artery, the mesenteric, portal, and hepatic veins, and the vena cava over the infusion period. The data presented in this chapter represent the average of samples taken from the last 2 sampling periods (time 4 to 6 and 6 to 8 h).

After each 2-h collection period, the syringes were removed from the collection lines and carefully mixed by gentle rotation. Packed cell volume (haematocrit) was also determined. Approximately 0.2 mL of whole blood was injected into a blood gas analyser (ABL3, Radiometer Pacific Limited, Copenhagen) in order to determine the concentration of O_2 and CO_2 in whole blood. The remaining 25 mL of whole blood was centrifuged (4°C; 3 270 g for 15 min), and the plasma harvested and either processed or stored at -85°C for further analysis as described below.

7.3.4 Slaughter

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 (300 mg mL^{-1}; 0.5 mL kg^{-1} liveweight). Tissue samples were rapidly collected from the skin, muscle (*biceps femoris*), liver, duodenum, ileum, spleen, mesenteric lymph nodes and thymus (Chapter Six).

After the tissues had been collected, the ileo-caecal junction of the small intestine was located and sectioned in order to collect ileal digesta from the final 3 m of the ileum for digesta flow (Chapter Five), and measurement of specific activity and valine concentration. Ileal digesta was stored at -20°C until analysis.

7.3.5 Sample processing and chemical analysis

7.3.5.1 Plasma flow across tissue beds

The PAH dye dilution technique was used to determine the flow of plasma through the MDV, PDV, liver and TSP tissues as described by Katz & Bergman (1969) with an additional deacylation step as described by Lobley *et al.* (1995; Chapter Five). Plasma flow across the hind limbs was calculated using the ICG concentration measured in plasma. A full description of these methods can be found in Chapter Five (Section 5.3.7.3).

7.3.5.2 Isotopic activity and concentration of valine

To determine the concentration of valine in plasma, 0.5 mL of plasma was treated with 80 mM dithiothreitol (DTT) and 3 mM norleucine (in 0.1% phenol; as an internal standard) and stored at -85°C. This method is described in full in Chapter Five and Appendix B.

Subsamples (4-5 g) of frozen tissue were pulverised in liquid nitrogen (N) using a modified French Cell press as described previously in Chapter Four to produce a

protein-bound fraction and a free pool fraction. The concentration of valine in these fractions were determined as described in Chapter Four (Section 4.3.5.2).

The AA hydrolysates were prepared from ileal digesta and tissue samples by hydrolysing approximately 50 mg of freeze-dried material in 6.0 M HCl at 110°C for 22 h (Chapters Five and Six). Total ^{3}H radioactivity and ^{3}H radioactivity associated with valine in the tissue or digesta hydrolysates were determined as described below.

Total ^{3}H radioactivity in infusates, plasma, tissue free pool and ileal digesta and tissue hydrolysates was determined by mixing 50 μL of sample in 2 mL of scintillation mixture (Starcint, INSUS Systems) and counting for 10 min (Packard Tricarb Model 1500 Scintillation counter) as described by Lee *et al.* (1999). The proportion of total ^{3}H radioactivity attributed to valine and its breakdown product (H_2O) in these sampled pools was determined by inline flow through an online liquid scintillation detector (Model 2, β-ram, IN/US systems Inc., New Jersey, US) coupled to high performance liquid chromatography (HPLC; LC4A, Shimadzu, Kyoto, Japan) as described in Lee *et al.* (1999).

The isotopic enrichment of valine in plasma, tissue free pool and ileal hydrolysate samples was determined by the method outlined by Calder & Smith (1988). Plasma (1 mL) was deproteinised with 0.5 mL of 20% (w/v) sulphosalicylic acid (SSA) and centrifuged for 5 min at 28 000 g. Deproteinised plasma or free pool supernatant (1 mL), or ileal hydrolysate (2 mL) were transferred onto a preconditioned cation exchange column (Dowex 50 W X8, 200-400 mesh; H^+ form), washed with 3 mL of deionised water and then eluted onto an anion exchange column (Dowex 1 X8, 200-400 mesh; acetate form) with 2 mL of 4 M NH_4OH and 1 mL of deionised water. The anion exchange was washed with 3 x 2 mL deionised water then eluted into vials with 3 mL 1 M acetic acid. The sample was then frozen at -85°C and freeze dried. The freeze-dried material was taken up in 100 μL of 0.1 M HCl and dried at 80°C under $N_{2(g)}$.

The dried sample was then derivatised by adding 50 μL of t-butyldimethylsilyl and 50 μL of acetonitrile and incubated at 70°C for 20 min. The isotopic abundance (ratio of ^{13}C:^{12}C) of valine in plasma, tissue free pool or ileal digesta hydrolysate was measured using a gas chromatograph (GC; Shimadzu GC 17A) equipped with a mass selective detector (MS; Shimadzu QP5050a). The temperature of the GC injector and detector was 260°C. Analysis of ^{13}C-valine was carried out on a 30 m x 0.25 mm ID x 0.25 μm ZB-5 capillary column (Phenomenex, New Zealand Ltd) with the following column temperature programme: initial temperature 150 °C held for 6 min, increased to 240 °C at 35°C min^{-1} and held for 2 min, increased to 260 °C at 35°C min^{-1} and held for 10 min. Sample injections of 1 μL were made in a split mode with a 1:20 split. The carrier gas was helium, at a flow rate of 40 cm^3 min^{-1}. The mass selective detector was operated using electron impact and selected ion monitoring (m/z 186.15 and 187.15) for the $(M-57)^+$ fragment.

7.3.5.3 Isotopic enrichment of deuterium oxide

The isotopic (^2H) enrichment of H_2O in plasma samples was quantified using the procedures adapted from Yang *et al.* (1998) using the rapid exchange of protons from water with the hydrogens adjacent to the carbonyl group of acetone under basic conditions. Plasma (1 mL) obtained during the blood sampling on day 45 post-infection was transferred into a glass vial and reacted with universally-labelled ^{13}C-acetone (4 μL in 5% acetonitrile) and 10M NaOH (16 μL) as described in Chapter Four (Section 4.3.5.3). To calculate the DM content in plasma 1 mL of plasma was weighed into pre-weighed containers and then dried at 105°C for 16 h, after which they were re-weighed.

7.3.5.4 Concentration and specific activity of cysteine and sulfate

Plasma samples from day 42 and 45 were thawed and 0.5 mL transferred to a Vivaspin (cut off 10 000 molecular weight; Viviascience Ltd, Auckland, NZ) in order to measure the concentration of sulfate. The samples were spun at approximately 12 000 g for 45 min, and 50 μL of the resulting filtrate removed and placed into an autosampler vial and

stored at -20°C until analysis for sulfate concentration. For plasma samples from day 48 post-infection, analysis for sulfate concentration was completed on the filtrate produced when preparing plasma for measuring valine concentration (see Section 7.3.5.2).

Plasma concentration of sulfate was obtained by correcting back to a sulfate standard. The standard was made using K_2SO_4 stock solution (1 mmol L^{-1} in buffer), with both plasma samples and standards analysed on a HPLC (Shimadzu 10 AVP system). The temperature of the column was 35°C. Analysis of the samples was carried out on a 4.1 mm x 250 mm column with guard column (Wescan, All Tech), with a conductivity detector sample without suppression. Injections of 5μL were made with a mobile phase buffer of p-hydroxybenzoic acid (5 mmol L^{-1}; pH 8.4) with a flow rate of 1.6 mL min^{-1}.

In order to determine plasma cysteine concentration at day 45, 2 mL of plasma was added to a pre-weighed centrifuge tube, re-weighed and then mixed with 1 mL of a solution containing 0.75% w/v sodium dodecyl sulphate (SDS) and 9 mM EDTA, 200 μL DTT (80 mM). 100 μL of norleucine (3 mM in 0.1% phenol) was added as an internal standard. The samples were mixed and re-weighed after each addition and then left at room temperature for 15 min before adding 1 mL of trichloroacetic acid (TCA; 30% w/v) to precipitate plasma protein. The tubes were reweighed and centrifuged (3 270 g, 15 min at 4°C) with the resulting supernatant filtered (0.45 μm) and stored at -85°C. The supernatant was reacted with acid ninhydrin after reduction using DTT, and cysteine concentration determined photospectrometrically at 570 nm using a continuous flow analyser (Technicon Autoanalyser II) as described by Gaitonde, (1967).

Total ^{35}S radioactivity in infusates and plasma from days 42 and 45 was determined by mixing 50 μL of sample in 2 mL of scintillation mixture (Starcint, INSUS Systems) and counting for 10 min (Packard Tricarb Model 1500 Scintillation counter) as described by Lee *et al.*, (1999). The proportion of total ^{35}S radioactivity attributed to cysteine and its breakdown product (sulphate) in these sampled pools was determined by inline flow through an online liquid scintillation counter (Model 2, β-ram, IN/US systems Inc., New

Jersey, US) coupled to a HPLC (LC4A, Shimadzu, Kyoto, Japan) as described in Lee *et al.*, (1999).

7.3.6 Calculations

In the following equations, several abbreviations are used to specify a sampled pool. These abbreviations are defined as follows:

M = plasma from the mesentery vein
P = plasma from portal vein
H = plasma from hepatic vein
A = plasma from mesentery artery
V = plasma from vena cava
X = plasma from any of the venous drainage (M, P, H or V).
T = tissue free pool
Z = precursor pool for protein synthesis
ABO = abomasal digesta
ILEAL = ileal digesta
I = infusate
[Y] = concentration or count of a particular compound
^{13}C-VAL = [1-^{13}C]-valine
^{3}H-VAL = [3, 4-^{3}H]-valine
^{35}S-CYS = [^{35}S]-cysteine

7.3.6.1 Isotopic activity of valine, cysteine and water

The isotopic activity of valine (VAL IA), cysteine (CYS IA) or sulfate (Sulfate IA) refers either to specific radioactivity of valine, cysteine or sulfate (VAL SRA, CYS SRA or Sulfate SRA) or isotopic enrichment of valine (VAL IE) in plasma obtained from the mesenteric, portal and hepatic veins, the mesenteric artery or the vena cava. The VAL SRA, CYS SRA, and sulfate SRA were determined by Equation 7.1 (Wolfe, 1992a). This equation is shown for valine only, but CYS SRA and sulfate SRA were calculated in a similar manner. The VAL IE were determined by Equation 7.2 (Wolfe, 1992b),

respectively. The isotopic activity of water refers to the isotopic enrichment of ^{2}H in plasma from the mesenteric artery (D_2O IE_A) and was calculated according to Equation 7.2.

Equation 7.1

$$\text{VAL SRA}_{\text{A or X}}\ (\text{dpm mmol}^{-1}) = \frac{[^{3}\text{H - VAL}]_{\text{A or X}}\ (\text{dpm mL}^{-1})}{[\text{VAL}]_{\text{A or X}}\ (\text{mmol mL}^{-1})}$$

7.3.6.2 Whole body kinetics of valine, cysteine and sulfate

The whole body (WB) model for AA kinetics described by Waterlow *et al.* (1978) was used to estimate whole body valine kinetics. A full description of these calculations can be found in Chapter Four (Section 4.3.6.2). Additionally, WB cysteine kinetics was determined according to a 2-pool model as described by Nolan *et al.* (1976) and described by Equation 7.2 to Equation 7.15 (Figure 7.1). In the steady-state, CYS ILR is equal to inflow-to or outflow-of the CYS plasma pool (Equation 7.2). Cysteine inflow was calculated according to Equation 7.3.

Equation 7.2

$$\text{CYS ILR (mmol h}^{-1}) = \text{CYS inflow (mmol h}^{-1}) = \text{Cys outflow (mmol h}^{-1})$$

Equation 7.3

$$\text{CYS inflow (mmol h}^{-1}) = \text{CYS entry (mmol h}^{-1}) + \text{Sulfate recycled to CYS (mmol h}^{-1})$$

Cysteine entry from plasma includes cysteine from transulphuration of methionine, protein degradation and absorption from the gastrointestinal tract. Plasma re-assimilation of sulfate to cysteine (Sulfate recycled to CYS) was assumed to equal zero as it does not occur in mammalian tissue over the time course of this experiment. Therefore, Equation 7.3 was simplified to Equation 7.4 and cysteine irreversible loss (CYS ILR) was obtained using Equation 7.7.

Equation 7.4

$$\text{CYS inflow (mmol h}^{-1}\text{)} = \text{CYS entry (mmol h}^{-1}\text{)} = \text{CYS ILR (mmol h}^{-1}\text{)}$$

Cysteine outflow from the plasma cysteine pool was calculated using Equation 7.5 and Equation 7.6, respectively.

Equation 7.5

$$\text{CYS outflow (mmol h}^{-1}\text{)} = \text{CYS exit (mmol h}^{-1}\text{)} + \text{CYS oxidation (mmol h}^{-1}\text{)}$$

where CYS exit represents the amount of cysteine used for protein synthesis (CYS for PS) and was calculated according to Equation 7.11 and cysteine oxidation obtained using Equation 7.10. These two equations were derived as described in Equation 7.6 to Equation 7.9.

Equation 7.6

$$\text{CYS ILR (mmol h}^{-1}\text{)} = \text{CYS for PS (mmol h}^{-1}\text{)} + \text{CYS oxidation (mmol h}^{-1}\text{)}$$

Whole body ILR was calculated for cysteine according to Equation 7.7.

Equation 7.7

$$\text{WB CYS ILR (mmol h}^{-1}\text{)} = \frac{\text{Infusion rate of } ^{35}\text{S-CYS (dpm h}^{-1}\text{)}}{\text{Plateaued CYS SRA (dpm mmol}^{-1}\text{)}}$$

The transfer quotient of cysteine to sulphate (TQ CYS) was measured at plateau, and is defined as the proportion of radioactivity in the secondary pool (sulfate) which originated from the infusion into a primary pool (cysteine) as described by McNabb *et al.* (1993) and shown in Equation 7.8. The whole body cysteine oxidation was calculated using Equation 7.8 and Equation 7.9 and is shown in Equation 7.10.

Equation 7.8

$$\text{TQ CYS} = \frac{\text{SRA of Sulfate (dpm nmol}^{-1})}{\text{CYS SRA (dpm nmol}^{-1})}$$

Equation 7.9

$$\text{WB Sulfate ILR (mmol h}^{-1}) = \frac{\text{Infusion rate of Sulfate (dpm h}^{-1})}{\text{Plateaued Sulfate SRA (dpm mmol}^{-1})}$$

Equation 7.10

$$\text{WB CYS oxidation (mmol h}^{-1}) = \text{TQ CYS} * \text{WB Sulfate ILR (mmol h}^{-1})$$

Cysteine leaving the plasma pool for productive purposes (CYS PS) was calculated according to Equation 7.11 and then converted to protein synthesis (g d^{-1}) assuming a cysteine concentration of 5 mg g^{-1} tissue (MacRae *et al.*, 1993).

Equation 7.11

$$\text{CYS PS (mmol h}^{-1}) = \text{WB CYS ILR (mmol h}^{-1}) - \text{WB CYS oxidation (mmol h}^{-1})$$

In steady-state, sulfate ILR is equaled to inflow to or outflow of the sulfate plasma pool (Equation 7.12). Sulfate inflow was calculated using Equation 7.13.

Equation 7.12

$$\text{Sulfate ILR (mmol h}^{-1}) = \text{Sulfate inflow (mmol h}^{-1}) = \text{Sulfate outflow (mmol h}^{-1})$$

Equation 7.13

$$\text{Sulfate inflow (mmol h}^{-1}) = \text{Sulfate entry (mmol h}^{-1}) + \text{CYS oxidation (mmol h}^{-1})$$

where the sulfate entering the plasma (Sulfate entry) is from the oxidation of methionine and absorption from the gastrointestinal tract.

Sulfate outflow was calculated using Equation 7.14, where sulphate exit represents the sulphate leaving plasma in urine and via saliva or the sulfate recycled to CYS.

Equation 7.14

Sulfate outflow (mmol h^{-1}) = Sulfate exit (mmol h^{-1}) + Sulfate recycled to CYS (mmol h^{-1})

As mentioned previously in this section, plasma re-assimilation of sulfate to cysteine (Sulfate recycled to CYS) was assumed to equal zero as it does not occur in mammalian tissue over the time course of this experiment. Therefore Equation 7.14 was simplified into Equation 7.15 and sulfate outflow obtained using Equation 7.9.

Equation 7.15

Sulfate outflow (mmol h^{-1}) = Sulfate exit (mmol h^{-1}) = Sulfate ILR (mmol h^{-1})

7.3.6.3 Plasma flow

Plasma flow (Pf) across the tissue beds (Tissue Pf or MDV Pf, PDV Pf, TSP Pf and hind limbs Pf) was determined by Equation 7.16. The hepatic artery plasma flow was determined by difference between the TSP Pf and PDV Pf. A full description of these calculations can be found in Chapters Two and Five.

Equation 7.16

$$\text{Tissue Pf } (\text{mL min}^{-1}) = \frac{[PAH]_I\ (\text{mg mL}^{-1}) \times \text{infusion rate } (\text{mL min}^{-1})}{([P\ H]_X - [P\ H]_A)\ (\text{mg mL}^{-1})}$$

7.3.6.4 Net flux of valine and cysteine across tissue beds

Net flux of valine and cysteine across the MDV, PDV, TSP and hind-limb tissues (Tissue net VAL flux or Tissue net CYS flux) was calculated using an arterial-venous approach as outlined in Equation 7.28 (Lobley *et al.*, 1995). This calculation for the liver (Liver net VAL flux or Liver net CYS flux) was done as described in Equation 7.29 (Lobley *et al.*, 1995). A full description of these equations can be found in Chapters Two

and Five. These equations are shown for the valine flux, and cysteine flux was calculated by replacing [VAL] with [CYS] in the appropriate blood vessel.

Equation 7.17

Tissue net VAL flux (mmol d^{-1}) = ($[VAL]_A - [VAL]_X$) (mmol mL^{-1}) x Tissue Pf (mL d^{-1})

Equation 7.18

Liver net VAL flux (mmol d^{-1}) = ($[VAL]_A$ x ART Pf) + ($[VAL]_P$ x PDV Pf) – ($[VAL]_H$ x TSPT Pf)

Arterial flow of valine (Arterial VAL inflow) or cysteine (Arterial CYS inflow) into the tissue bed was calculated according to Equation 7.19. Additionally, apparent valine or cysteine absorption by the MDV was calculated using Equation 7.20. These equations are presented as valine parameters, but by replacing the [VAL] with [CYS], the corresponding cysteine values were calculated.

Equation 7.19

Arterial VAL inflow (mmol d^{-1}) = $[VAL]_A$ (mmol mL^{-1}) x Tissue Pf (mL d^{-1})

Equation 7.20

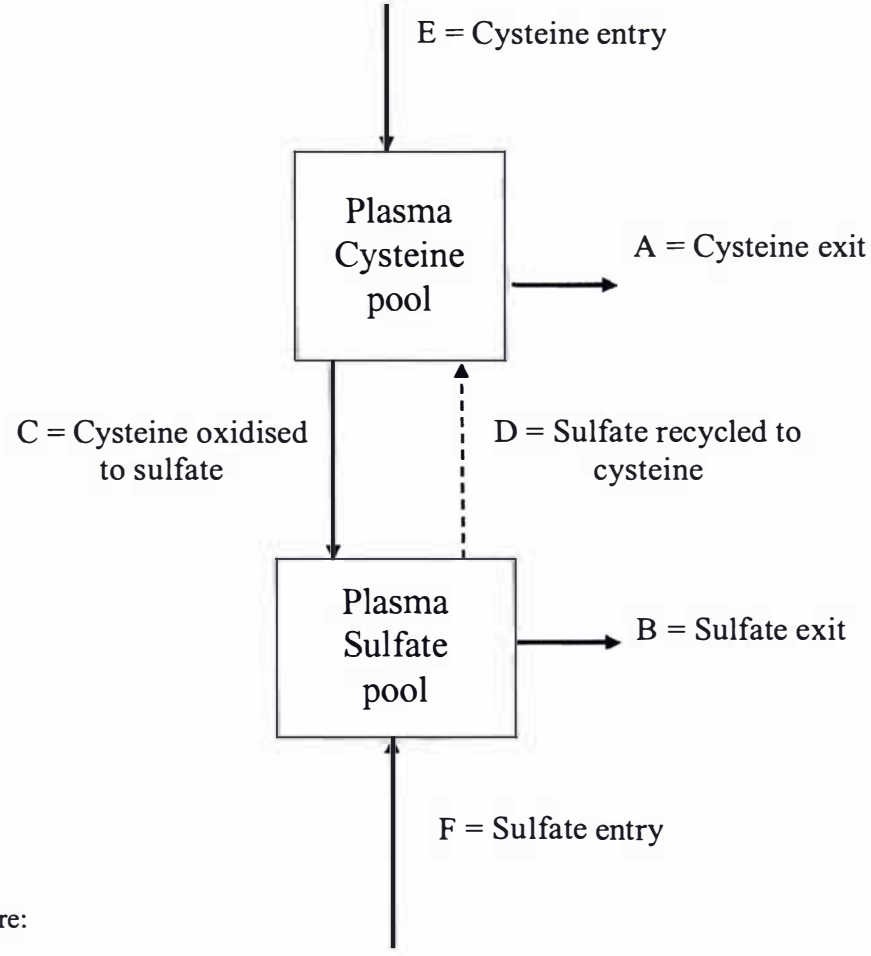
MDV apparent VAL absorption (mmol d^{-1}) =

($[VAL]_{ABO}$ x $Flow_{ABO}$) (mmol d^{-1}) – ($[VAL]_{ILEAL}$ x $Flow_{ILEAL}$) (mmol d^{-1})

where: $Flow_{ABO}$ = dry matter flow through the abomasum

$Flow_{ILEAL}$ = dry matter flow through the ileum

Figure 7.1 Fluxes (mmol h^{-1}) through the cysteine and sulfate pools following the infusion of [^{35}S]-cysteine and [^{35}S]-sulfate using equations (Eqn) from Nolan *et al.* (1976).



Where:

A = Cysteine leaving the plasma pool for productive purposes: CYS ILR (Eqn. 7.7) - C
B = Sulfate leaving plasma primarily in urine: Sulfate ILR (Eqn. 7.14)
C = Cysteine oxidised to sulfate: CYS TQ (Eqn. 7.8) x B
D = Plasma sulfate re-assimilated to cysteine; within time course of infusion it is assumed that there is zero flux in mammalian tissues: 0
E = Cysteine entry to plasma (includes cysteine from transulphuration of methionine, protein degradation, and absorption from the gastrointestinal tract): A + C
F = Sulfate entering plasma (sulfate from all sources including the oxidation of methionine, and absorption from the gastrointestinal tract): B - C

7.3.6.5 Kinetics of valine and cysteine across the gastrointestinal tract

Kinetics of valine across the GIT were calculated according to the Equations described in Chapter Four (Section 4.3.6.5). The equations are similar to those of Neutze *et al.* (1997) and MacRae *et al.* (1997) but have been re-arranged for clarification as described in Chapter Four. To determine the effects of parasite infection on AA metabolism in the MDV two isotopes of valine were infused; ^{3}H-valine into the jugular and ^{13}C-valine into the abomasum. This enabled any recycling of the abomasal valine isotope to the MDV and PDV to be calculated. The underlying assumption of this method is that the two isotopes of valine behave in an identical manner. Additionally, cysteine kinetics across the MDV and PDV were also determined based on the equations described by Neutze *et al.* (1997) and MacRae *et al.* (1997) using the infusion of ^{35}S-cysteine (^{35}S-CYS) into the abomasum and most of these equations have been presented in full in Chapter Four (Section 4.3.6.5).

In the current study only one label of cysteine was infused into the sheep therefore the arterial ^{35}S-cysteine extraction (%) by the MDV were estimated from Greaney (2001) using Equation 7.34. This value was estimated from 2 growing sheep of similar weight and fed a similar intake of fresh forage to our study (800 g white clover DM d^{-1}) and averaged 12%. The ILR of cysteine from arterial sources for the MDV was calculated using Greaney's value for arterial ^{35}S-cysteine extraction and the values obtained in this experiment for arterial plasma concentration of cysteine and MDV plasma flow according to Equation 7.35. No values for the arterial ^{35}S-cysteine extraction by the PDV were available so we assumed that the value for CYS in the MDV and PDV were similar.

The corrected recovery of the ^{35}S-cysteine infused into the abomasum (luminal ^{35}S-CYS) that appears in the mesenteric vein was calculated according to Equation 7.38 described in the previous section. This equation assumed that the tracer that was not recovered in the mesenteric vein was utilised for oxidation and protein synthesis in the MDV. The non-absorbed ileal ^{35}S-cysteine was unavailable in this dataset because the

total ^{35}S counts associated with CYS and SO_4^{2-} were not determined and thus no correction for ^{35}S- cysteine infusion rate was made. Therefore, the apparent ^{35}S-cysteine absorption was approximated to be equal to the ^{35}S-cysteine infusion rate. The fractional recovery of ^{35}S-cysteine in the mesenteric vein was then calculated using the Equation 7.21. This recovery will be overestimated because there is less ^{35}S-cysteine absorbed than the amount infused.

Equation 7.21

$$\text{Luminal } ^{35}\text{S - CYS corrected recovery in M (\%)} = \frac{\text{Luminal } ^{35}\text{S - CYS corrected recovery in M (dpm d}^{-1}\text{)} \times 100}{\text{Abomasum } ^{35}\text{S - CYS infusion rate (dpm d}^{-1}\text{)}}$$

The luminal ^{35}S-cysteine extraction in the MDV (%) was calculated using the equations described for ^{13}C-valine (Equations 7.41). The ^{13}C-valine parameter was replaced by the ^{35}S-cysteine parameter in these equations. The endogenous loss of valine was calculated according to Equation 7.43 using the ratio of $[^3H\text{-VAL}]_{ileum}$: $[^3H\text{-VAL}]_A$ as that of ^{35}S-cysteine was not measured. All other kinetic parameters calculated for ^{13}C-valine using Equations 7.44 to 7.46 were also calculated for ^{35}S-cysteine.

7.3.6.6 Kinetics of valine and cysteine across the liver and splanchnic tissues

The fractional extraction or loss of ^{13}C-valine, ^3H-valine and ^{35}S-cysteine across the liver was calculated according to Equation 7.22, and used to determine the valine ILR across the liver (Equation 7.23). These equations are based on the same principles as those for the MDV and PDV, using the equations outlined by MacRae *et al.* (1997) and Yu *et al.* (2000). Only the equations for ^{13}C-valine are presented.

Equation 7.22

$$\text{Liver } ^{13}\text{C - VAL extraction (\%)} = \frac{\{([^{13}\text{C - VAL}]_A \text{ (mmol mL}^{-1}\text{) x ART Pf (mL d}^{-1}\text{))} + ([^{13}\text{C - VAL}]_P \text{ (mmol mL}^{-1}\text{) x PDV Pf (mL d}^{-1}\text{))}\} - ([^{13}\text{C - VAL}]_H \text{ (mmol mL}^{-1}\text{) x TSP Pf (mL d}^{-1}\text{))}}{([^{13}\text{C - VAL}]_A \text{ (mmol mL}^{-1}\text{) x ART Pf (mL d}^{-1}\text{))} + ([^{13}\text{C - VAL}]_P \text{ (mmol mL}^{-1}\text{) x PDV Pf (mL d}^{-1}\text{))}}$$

Equation 7.23

Liver VAL ILR (mmol d^{-1}) =

Liver ^{13}C-VAL extraction (%) x {([VAL]$_A$ x ART Pf) (mmol d^{-1}) + ([VAL]$_P$ x PDV Pf) (mmol d^{-1})}

The ILR of valine across the TSP was calculated according to Equation 7.24.

Equation 7.24

TSP VAL ILR (mmol d^{-1}) = PDV total VAL ILR (mmol d^{-1}) + Liver VAL ILR (mmol d^{-1})

7.3.6.7 Kinetics of valine and cysteine across the hind limbs

The fractional extraction of ^{13}C-valine, ^{3}H-valine and ^{35}S-cysteine by the hind limbs was calculated according to Equation 7.25 and used to calculate valine ILR across this tissue in Equation 7.26. The arterial ^{3}H-valine or ^{35}S-cysteine extraction by the hind limbs was calculated similarly by replacing [^{13}C-VAL] in Equation 7.25 by [^{3}H-VAL] or [^{35}S-cysteine]. Only the equations for ^{13}C-valine are presented.

Equation 7.25

$$\text{Hind limb arterial } {}^{13}\text{C-VAL extraction (\%)} = \frac{(([{}^{13}\text{C-VAL}]_A - [{}^{13}\text{C-VAL}_V]\ (\text{mmol mL}^{-1})) \times \text{Hind limb Pf (mL d}^{-1})}{[{}^{13}\text{C-VAL}_A]\ (\text{mmol mL}^{-1}) \times \text{Hind limb Pf (mL d}^{-1})}$$

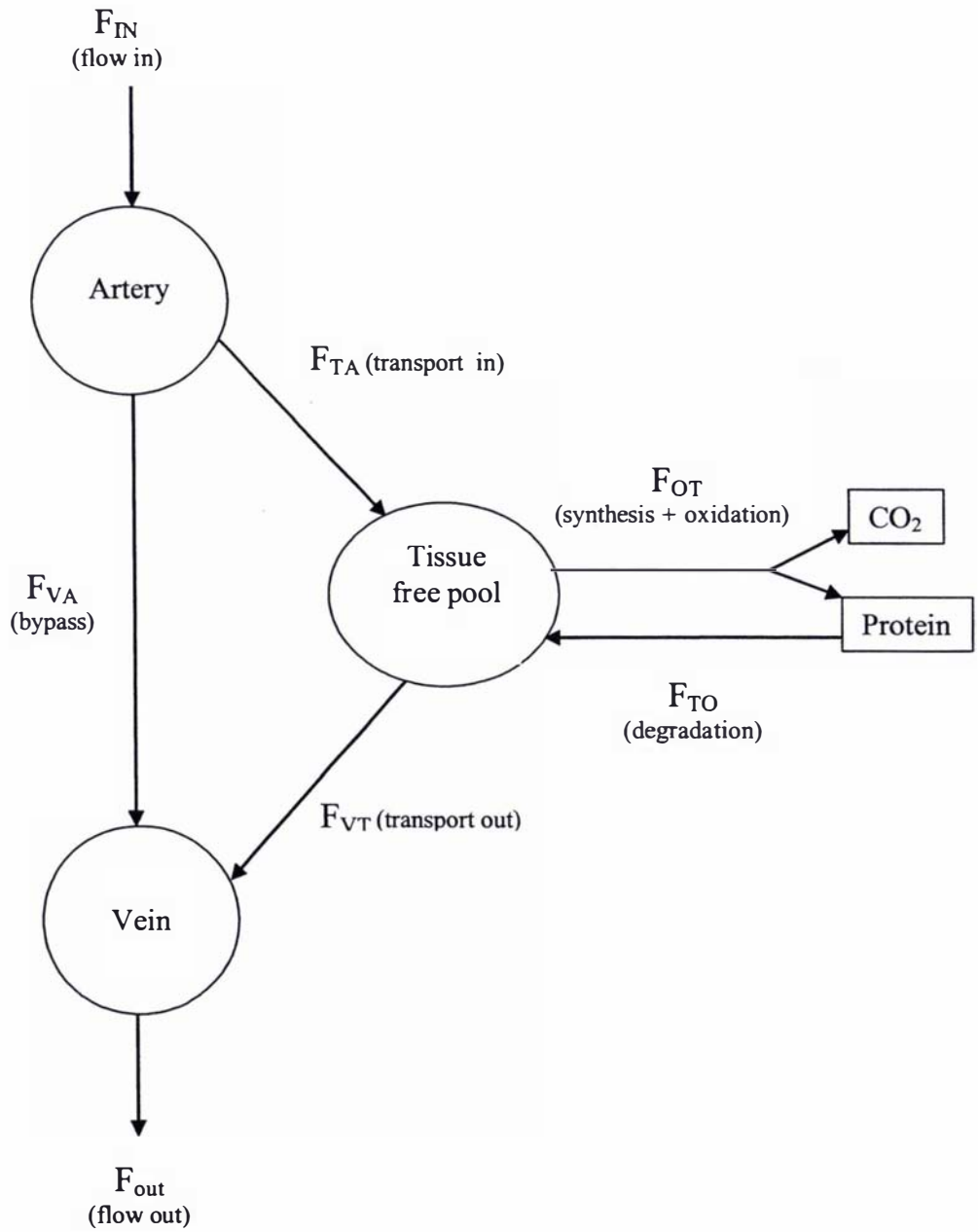
Equation 7.26

Hind limbs VAL ILR (mmol d^{-1}) =

Hind limbs arterial ^{13}C-VAL extraction (%) x [VAL]$_A$ (mmol mL^{-1}) x Hind limb Pf (mL d^{-1})

Additional to the traditional arterio-venous kinetics model, valine kinetics across the hind limbs including the transport of valine across the hind limb muscle was calculated according to a three-compartmental model described by Biolo *et al.* (1992). This model is depicted in Figure 7.2. Equations relating to this model are described in full in Chapter Four (Section 4.3.6.7).

Figure 7.2 Valine kinetics (F) in the muscle of lambs according to the model described by Biolo *et al.*, (1992).



7.3.7 Statistical analysis

Statistical analysis for all variables was performed using a General Linear Model (SAS version 8, 1999), with treatment and group (the week that the lamb underwent surgery) used as sources of variation in the model. Additionally, feed intake, faecal egg counts and liveweight were analysed using Proc Mix repeated measures. The data was checked for normality and the presence of outliers by plotting residuals versus the predicted residuals.

Probability values lower than 0.10 were considered to indicate a significant difference and values between 0.10 and 0.15 to indicate a trend. Results are presented as least squares means (LSmeans) and associated pooled standard deviation (SD).

7.4 Results

Full details of the results relating to the faecal egg counts, intestinal worm burdens, feed intake and liveweight changes are presented in Chapter Five. In summary, intestinal worm burdens were significantly higher in the parasitised lambs on day 48 post infection (240 *vs.* 18 000 (SD 7 000) worms in the control and parasite lambs, respectively; $P<0.001$). The presence of parasites in the small intestine reduced DM intake over the course of the experiment (769 *vs.* 689 (SD 47) g DM d^{-1} in the control and parasite lambs, respectively; $P<0.15$). While liveweight gain over the 48 day period was not affected by parasite infection (75 *vs.* -17 (SD 50) g d^{-1} in the control and parasite lambs, respectively; $P>0.15$) there was a significant decrease in liveweight gain in the infected lambs over the last 20 d of the experiment (50 *vs.* -50 (SD 70) g d^{-1} in the control and parasite lambs, respectively; $P<0.15$). Despite a decrease in feed intake, dry matter flow at the ileum was unaffected by the presence of parasites (217 *vs.* 167 (SD 87) g DM d^{-1} in the control and parasite lambs, respectively; $P>0.15$).

7.4.1 Plasma amino acid concentration and isotopic activity

Catheter patency was reduced to 95% with four catheters (2 in the mesenteric vein, and 1 in the portal vein and vena cava) failing due to fibrous sheaths forming over the end of

the catheter. The concentration and SRA of cysteine and sulfate on day 48 post infection are presented in Table 7.1. Parasite infection had no effect on the concentration or SRA of sulfate in all vessels ($P>0.15$; Table 7.1). Cysteine concentration was lower in the mesenteric and hepatic veins and vena cava of parasitised lambs ($P<0.15$; Table 7.1), however specific radioactivity of cysteine in these vessels was similar between control and parasitised lambs. Plasma concentration and specific radioactivity of cysteine in the portal vein and mesenteric artery was not affected by the infection.

The concentration, SRA and isotopic enrichment of valine in plasma collected from all vessels was similar between control and parasitised lambs ($P>0.15$; Table 7.2) with the exception of the portal vein which had higher SRA of valine in the infected lambs ($P<0.10$; Table 7.2). In ileal digesta, the concentration of valine was increased during parasitic infection ($P<0.05$; Table 7.2) however, SRA of valine was similar between control and infected lambs ($P>0.15$; Table 7.2). In the muscle-free pool there was no effect of parasitic infection on concentration, SRA or isotopic enrichment of valine ($P>0.15$; Table 7.2).

Table 7.1 Cysteine ([CYS]) and sulfate concentration ([Sulfate]) and specific radioactivity (SRA) in blood vessels of lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Results are presented as LSmeans and associated pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
Mesenteric vein					
[CYS]	μmol L^{-1}	37.0	30.0	6.0	0.10
CYS SRA	dpm $nmol^{-1}$	163.0	146.9	113.8	0.83
[Sulfate]	mmol L^{-1}	1.4	1.2	0.3	0.56
Sulfate SRA	dpm $nmol^{-1}$	13.2	16.0	11.9	0.72
Portal vein					
[CYS]	Mmol L^{-1}	31.0	28.0	6.0	0.37
CYS SRA	dpm $nmol^{-1}$	147.8	123.7	66.1	0.57
[Sulfate]	mmol L^{-1}	1.5	1.2	0.3	0.24
Sulfate SRA	dpm $nmol^{-1}$	13.3	17.8	11.7	0.47
Hepatic vein					
[CYS]	Mmol L^{-1}	32.0	22.0	6.0	0.12
CYS SRA	dpm $nmol^{-1}$	120.5	123.0	76.9	0.96
[Sulfate]	mmol L^{-1}	1.4	1.2	0.4	0.46
Sulfate SRA	dpm $nmol^{-1}$	11.2	18.8	11.2	0.28
Mesenteric artery					
[CYS]	μmol L^{-1}	31.0	26.0	6.0	0.20
CYS SRA	dpm $nmol^{-1}$	110.7	117.7	63.2	0.85
[Sulfate]	mmol L^{-1}	1.4	1.2	0.4	0.37
Sulfate SRA	dpm $nmol^{-1}$	10.7	17.6	10.9	0.30
Vena cava					
[CYS]	μmol L^{-1}	33.0	26.0	6.0	0.09
CYS SRA	dpm $nmol^{-1}$	102.2	109.2	67.3	0.87
[Sulfate]	mmol L^{-1}	1.5	1.2	0.4	0.40
Sulfate SRA	dpm $nmol^{-1}$	10.2	19.2	12.8	0.29

Table 7.2 Valine concentration ([VAL]), specific radioactivity (VAL SRA) and isotopic enrichment (VAL IE) in plasma, ileal digesta and free pool muscle of lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Results are presented as LSmeans and associated pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
Mesentery vein					
[VAL]	μmol L^{-1}	289.0	280.0	82.0	0.87
VAL SRA	dpm $nmol^{-1}$	46.5	55.5	16.8	0.40
VAL IE	APE%	5.9	3.9	4.7	0.50
Portal vein					
[VAL]	μmol L^{-1}	241.0	243.0	55.0	0.95
VAL SRA	dpm $nmol^{-1}$	48.0	66.4	13.8	0.07
VAL IE	APE%	4.4	5.2	3.9	0.76
Hepatic vein					
[VAL]	μmol L^{-1}	217.0	237.0	66.0	0.62
VAL SRA	dpm $nmol^{-1}$	55.6	65.5	17.7	0.36
VAL IE	APE%	4.8	5.0	3.7	0.92
Mesentery artery					
[VAL]	μmol L^{-1}	202.0	235.0	64.0	0.41
VAL SRA	dpm $nmol^{-1}$	68.9	74.6	23.3	0.68
VAL IE	APE%	4.6	4.1	3.6	0.82
Vena cava					
[VAL]	μmol L^{-1}	199.0	232.0	62.0	0.43
VAL SRA	dpm $nmol^{-1}$	57.6	67.2	20.0	0.38
VAL IE	APE%	4.5	3.5	3.4	0.66
Ileal digesta					
[VAL]	mg g DM^{-1}	7.9	11.9	2.4	0.03
VAL SRA	dpm $nmol^{-1}$	26.1	23.4	15.8	0.80
VAL IE	APE%	2.1	1.2	1.2	0.26
Free pool muscle					
[VAL]	μmol L^{-1}	30.9	38.1	10.9	0.29
VAL SRA	dpm $nmol^{-1}$	37.8	38.8	17.5	0.93
VAL IE	APE%	2.7	3.2	1.5	0.64

7.4.2 Whole body amino acid kinetics

7.4.2.1 Valine

The valine kinetics in the whole body based on the intra-venous infusion of ^{3}H-valine are described in Table 7.3. Parasitic infection decreased the size of the whole body water pool ($P<0.10$; Table 6.3). There was no effect of parasitic infection on whole body valine ILR, oxidation or protein synthesis estimated from ^{3}H-valine infusion ($P>0.15$; Table 7.3). The proportion of valine ILR used for either protein synthesis (*c.* 84%) or oxidised (*c.* 16%) was similar between treatments ($P>0.15$; Table 7.3).

Whole body valine ILR calculated from the infusion of ^{13}C-valine into the abomasum was reduced by the presence of parasite infection (438.2 *vs.* 294.1 (SD 80.4) mmol d^{-1} in the control and parasite lambs, respectively; $P<0.10$).

7.4.2.2 Cysteine

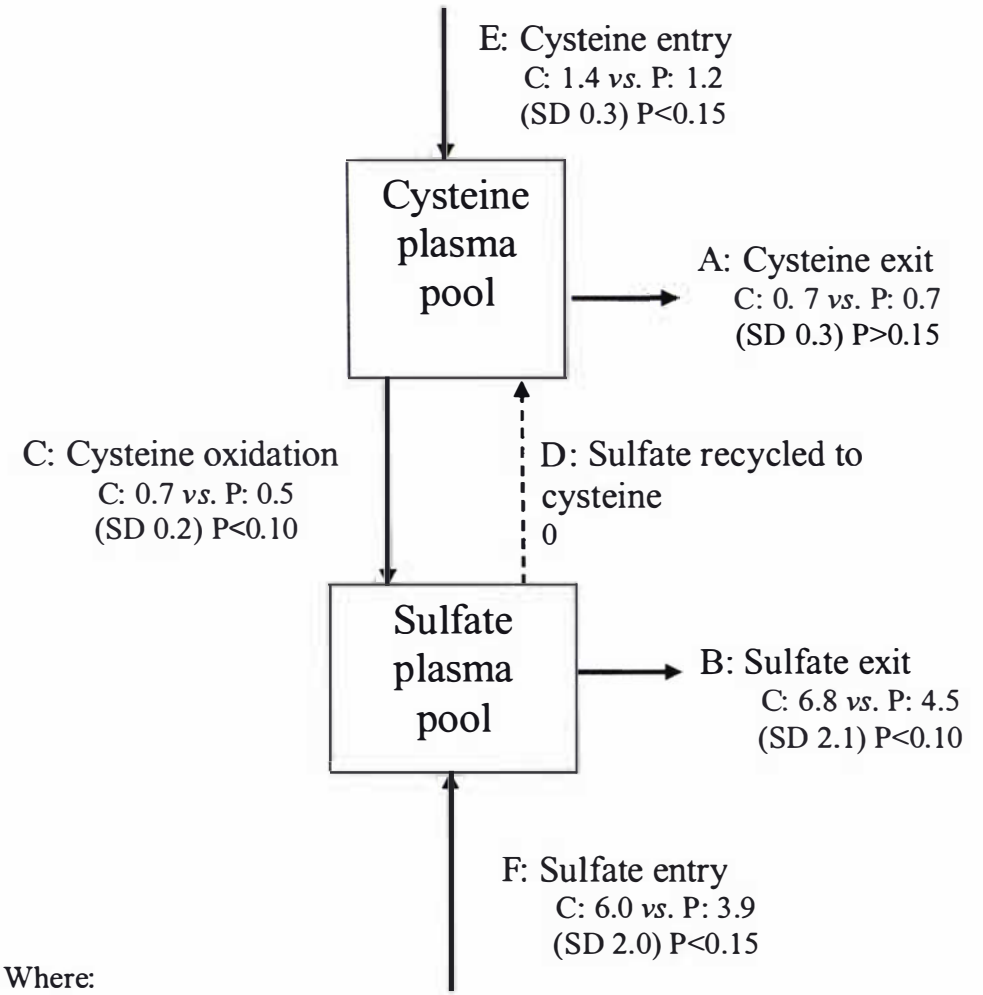
Whole body cysteine kinetics based on a two-pool model are depicted in Figure 7.3 and presented in Table 7.3. Whole body sulfate ILR was decreased during infection ($P<0.10$; Figure 7.3). The ILR of cysteine was similar between control and parasitised lambs ($P>0.15$; Table 7.3). Cysteine oxidation was reduced during infection ($P<0.10$; Figure 7.3 and Table 7.3). However, whole body protein synthesis estimates based on cysteine kinetics were unaffected by parasitic infection ($P>0.15$; Table 7.3). The proportion of cysteine ILR used for either protein synthesis (*c.* 50%) or oxidised (*c.* 50%) was similar between treatments ($P>0.15$; Table 7.3). These proportions were higher than the estimates obtained from the infusion of ^{3}H-valine.

Table 7.3 Whole-body cysteine (CYS) and valine (VAL) irreversible loss rate (ILR), oxidation (OX) and protein synthesis (PS) in lambs fed fresh Sulla (*Hedysarum coronarium*) with or without a *Trichostrongylus colubriformis* infection. Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
Cysteine					
ILR[1]	mmol h^{-1}	1.4	1.1	0.2	0.17
OX[2]	mmol h^{-1}	0.7	0.5	0.2	0.13
CYS for PS[3]	mmol h^{-1}	0.7	0.7	0.3	0.92
CYS for PS: CYS ILR	%	47.9	55.5	19.2	0.48
CYS OX: CYS ILR	%	53.1	44.5	19.2	0.48
CYS OX: CYS PS	%	1.7	1.3	1.8	0.77
PS	g d^{-1}	46.8	35.5	12.7	0.32
Valine					
ILR[1]	mmol h^{-1}	4.9	4.4	1.3	0.62
Water pool size[4]	L	21.7	19.4	1.9	0.07
Total ^3H_2O from VAL	dpm x 10^7	39.2	39.5	2.9	0.85
Total ^{3}H-VAL infused	dpm x 10^8	31.2	32.0	3.9	0.72
VAL OX[2]	%	15.9	15.6	2.4	0.82
	mmol h^{-1}	0.8	0.7	0.2	0.48
VAL for PS[3]	mmol h^{-1}	4.1	3.8	1.1	0.66
VAL for PS: VAL ILR	%	84.0	85.0	1.9	0.82
VAL OX: VAL ILR	%	16.2	15.3	1.5	0.73
VAL OX: VAL PS	%	19.2	18.1	3.0	0.87
PS	g d^{-1}	320.6	297.4	88.5	0.66

1. Irreversible loss rate.
2. Oxidation.
3. Protein synthesis.
4. Calculated from the injection of deuterated water on day 45 post infection.

Figure 7.3 Whole body cysteine kinetics (mmol h^{-1}) based on the infusion of [^{35}S]-sulfate (day 45 post infection) and [^{35}S]-cysteine (day 48 post infection) in lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with *Trichostrongylus colubriformis* (P; n=6) or kept as parasite free controls (C; n=6). Results are presented as LSmeans and associated pooled standard deviation (SD).



Where:

A = Cysteine leaving the plasma pool for productive purposes: CYS ILR (Eqn. 7.7) - C
B = Sulfate leaving plasma primarily in urine: Sulfate ILR (Eqn.7.14)
C = Cysteine oxidised to sulfate: CYS TQ (Eqn. 7.8) x B
D = Plasma sulfate re-assimilated to cysteine; within time course of infusion it is assumed that there is zero flux in mammalian tissues: 0
E = Cysteine entry to plasma (includes cysteine from transulphuration of methionine, protein degradation, and absorption from the gastrointestinal tract): A + C
F = Sulfate entering plasma (sulfate from all sources including the oxidation of methionine, and absorption from the gastrointestinal tract): B - C

7.4.3 Valine and cysteine kinetics across the mesenteric-drained viscera

Valine flow at both the abomasum and ileum were unaffected by parasitic infection, (P>0.15; Table 7.4), however the apparent absorption of valine was decreased by 34% in the parasitised lambs (P<0.05; Table 7.4). Parasite infection did not have any impact on the ratio of valine SRA in ileal digesta over the valine SRA in arterial plasma and subsequently, the endogenous valine loss into the small intestine was also similar between treatments (P>0.15; Table 7.4).

Plasma flow across the MDV was similar between the control and parasitised lambs (P>0.15; Table 7.4). Despite the reduction in apparent intestinal absorption, net valine flux across the MDV was similar between treatments and averaged 32 mmol d^{-1} (P>0.15; Table 7.4). Parasite infection had no effect on the ^{3}H-valine arterial extraction ratio nor on the valine ILR from arterial sources across the MDV. The latter averaged 20 mmol d^{-1} when arterial precursors were used (P>0.15; Table 7.4).

Both the inflow and the extraction of arterial recycled ^{13}C-valine by the MDV was unaffected by parasitic infection (P>0.15; Table 7.4). The corrected recovery of luminal ^{13}C-valine into the MDV drainage and thereby its extraction by the MDV were also similar between treatments (P>0.15; Table 7.4). The apparent absorption of ^{13}C-valine was also similar between treatments (c. 24 mmol d^{-1}; Table 7.4), however the extraction of absorbed valine was lower in the parasitised lambs, mainly because of lower apparent absorption of valine in these sheep (P<0.01). No effect of infection was observed on the reabsorption of valine lost into the MDV lumen. Despite a 45% reduction in ILR from luminal sources in the infected lambs (P<0.01), there was no significant affect on total valine ILR across the MDV, averaging 76 mmol d^{-1} (P>0.15; Table 7.4).

The ratio of arterial to total valine ILR in the MDV was unaffected by parasitic infection (23.4 *vs.* 76.6 (SD 26.7) % in the control and parasite lambs, respectively; P>0.15). Similarly, the luminal contribution to total valine ILR was also similar between treatments (76.6 *vs.* 32.7 (SD 26.7) % in the control and parasite lambs, respectively; P>0.15).

There was no effect of infection on either cysteine flow through the abomasum or ileum and subsequently apparent absorption of cysteine from the small intestine was also similar between treatments (*c.* 14 mmol d^{-1}; P>0.15; Table 7.5). Plasma flow across and cysteine inflow to the MDV were similar between control and parasitised lambs, resulting in a similar net flux of cysteine across this tissue bed (P>0.15; Table 7.5).

Arterial extraction of ^{35}S-cysteine was assumed to equal 0.12 based on the values reported by Greaney (2002). Cysteine ILR estimates from arterial sources based on both arterial and venous precursor pools were similar between treatments (P>0.15; Table 7.5). Endogenous loss of cysteine into the MDV was estimated from the ratio of ileal valine SRA to arterial valine SRA (Table 7.4) and was similar between the control and parasitised lambs. However, luminal recovery of ^{35}S-cysteine in the mesenteric drainage was decreased by almost 60% in the infected lambs (P<0.15; Table 7.5). Apparent ^{35}S-cysteine absorption in the MDV and the extraction of recycled ^{35}S-cysteine were similar between treatments. Luminal ^{35}S-cysteine corrected recovery was unaffected by parasitic infection and averaged 79%. Consequently, extraction of the absorbed cysteine and the reabsorption of cysteine loss into the MDV lumen were similar between the control and parasitised lambs. The ILR of cysteine based on luminal supply was similar in both groups of lambs, similarly total ILR was unaffected by the parasite infection and averaged 9 mmol d^{-1} (P>0.15; Table 7.5).

The contribution of arterial ILR to total cysteine ILR in the MDV was similar between the control and infected lambs (69.1 *vs.* 76.8 (SD 17.7) % in the control and parasite lambs, respectively; P>0.15). Luminal contribution to total ILR followed a similar pattern, with the contribution being 30.9 and 23.2 % (SD 17.7) in the control and parasite lambs, respectively (P>0.15).

Table 7.4 Valine (VAL) kinetics in the mesenteric-drained viscera (MDV) of lambs (day 48 post infection) fed fresh Sulla (*Hedysarum coronarium*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD). Positive values for net valine flux represent a net utilisation by the tissue, while a negative value represents a net release.

		Control	Parasite	Pooled	
		n=6	n=6	SD	P
Digesta VAL flux					
Abomasal VAL flux	mmol d^{-1}	50.4	42.7	16.2	0.45
Ileal VAL flux	mmol d^{-1}	15.0	16.2	7.5	0.37
Apparent VAL absorption	mmol d^{-1}	35.0	26.2	4.4	0.01
Net plasma flux parameters					
Plasma flow	mL min^{-1}	533	663	286	0.51
VAL arterial inflow	mmol d^{-1}	149.0	243.5	110.8	0.23
Net VAL flux	mmol d^{-1}	-38.3	-26.3	15.8	0.37
VAL kinetics calculated using jugular ^{3}H-VAL infusion					
Arterial ^{3}H-VAL inflow x10^{7}	dpm d^{-1}	11.5	17.0	9.7	0.45
Arterial ^{3}H-VAL extraction x10^{7}	dpm d^{-1}	3.4	2.5	5.4	0.86
Arterial ^{3}H-VAL extraction x10^{7}	%	6.8	11.4	2.6	0.21
VAL ILR from arterial sources[1]	mmol d^{-1}	8.9	32.3	15.6	0.25
VAL ILR from arterial sources[2]	mmol d^{-1}	9.3	37.7	19.2	0.23
Ileal VAL SRA: Arterial VAL SRA	%	34.5	30.8	12.8	0.59
Endogenous VAL loss	mmol d^{-1}	4.93	5.74	3.03	0.69
VAL kinetics calculated using abomasal ^{13}C-VAL infusion					
Arterial recycled ^{13}C-VAL inflow	mmol d^{-1}	6.7	7.6	7.7	0.87
Luminal ^{13}C-VAL recovery in M	mmol d^{-1}	5.0	2.8	5.4	0.62
Apparent ^{13}C-VAL absorption	mmol d^{-1}	25.4	22.0	4.6	0.29
VAL kinetics calculated using abomasal ^{13}C-VAL and jugular ^{3}H-VAL infusions					
Arterial recycled ^{13}C-VAL extraction	mmol d^{-1}	0.3	1.4	1.5	0.48
Luminal ^{13}C-VAL corrected recovery in M[3]	mmol d^{-1}	4.0	4.9	4.2	0.82
Luminal ^{13}C-VAL corrected recovery in M[3]	%	18.7	17.7	17.2	0.95
Luminal ^{13}C-VAL extraction	%	81.3	82.3	17.2	0.95
Absorbed VAL extraction	mmol d^{-1}	26.0	14.9	1.0	0.01
Extracted VAL loss into lumen	mmol d^{-1}	3.1	5.7	3.6	0.46
VAL ILR from luminal sources	mmol d^{-1}	29.1	15.7	1.3	0.01
Total ILR (arterial + luminal)	mmol d^{-1}	38.0	48.0	16.9	0.69

1. Irreversible loss rate (ILR) of VAL calculated using the arterial free VAL as precursor pool. 2. Irreversible loss rate (ILR) of VAL calculated using the mesenteric vein free VAL as precursor pool. 3. M = mesenteric vein.

Table 7.5 Cysteine (CYS) kinetics in the mesenteric-drained viscera (MDV) of lambs (day 48 post infection) fed fresh Sulla (*Hedysarum coronarium*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD). Positive values for net cysteine flux represent a net utilisation by the tissue, while a negative value represents a net release.

		Control	Parasite	Pooled	
		n=6	n=6	SD	P
Digesta CYS flux					
Abomasal CYS flux	mmol d^{-1}	34.5	29.3	8.1	0.30
Ileal CYS flux	mmol d^{-1}	21.4	16.0	7.1	0.55
Apparent CYS absorption	mmol d^{-1}	13.1	14.7	6.2	0.71
Net plasma flux parameters					
Plasma flow	mL min^{-1}	533	663	286	0.51
CYS arterial inflow	mmol d^{-1}	55.7	52.3	31.6	0.88
Net CYS flux	mmol d^{-1}	-10.1	-10.6	5.6	0.91
CYS kinetics					
CYS ILR from arterial sources[1]	mmol d^{-1}	6.7	6.3	3.8	0.88
CYS ILR from arterial sources[2]	mmol d^{-1}	7.9	7.6	3.8	0.88
Endogenous CYS loss[3]	mmol d^{-1}	7.4	9.3	7.4	0.68
Luminal ^{35}S-CYS recovery in M[4] x 10^7	dpm d^{-1}	41.3	17.6	15.5	0.13
Apparent ^{35}S-CYS absorption x 10^7	dpm d^{-1}	36.9	30.4	16.7	0.52
Arterial recycled ^{35}S-CYS extraction x 10^7	dpm d^{-1}	6.2	5.6	3.6	0.82
Luminal ^{35}S-CYS corrected recovery in M[4] x 10^7	dpm d^{-1}	47.5	23.0	24.1	0.17
Luminal ^{35}S-CYS corrected recovery in M[4]	%	79.8	78.3	6.6	0.86
Luminal ^{35}S-CYS extraction	%	20.3	21.7	6.6	0.86
Absorbed CYS extraction	mmol d^{-1}	1.3	1.1	0.7	0.81
Extracted CYS loss into lumen	mmol d^{-1}	1.7	2.3	2.7	0.86
CYS ILR from luminal sources	mmol d^{-1}	3.0	1.9	2.0	0.68
TOTAL CYS ILR (arterial + luminal)	mmol d^{-1}	9.7	8.2	2.9	0.35

1. ILR of CYS based on arterial free CYS as a precursor pool and assuming that ^{35}S-CYS extraction by the artery is 0.12 (Greany, 2002).
2. ILR of CYS based on venous free CYS as a precursor pool and assuming that ^{35}S-CYS extraction by the artery is 0.12 (Greany, 2002).
3. Estimated using the ration of ileal VAL SRA: arterial VAL SRA.
4. M = mesenteric vein.

7.4.4 Cysteine and valine kinetics across the portal-drained viscera

Plasma flow and valine supply to the PDV were similar between control and parasitised lambs and averaged 1 400 mL min^{-1} and 490 mmol d^{-1}, respectively (P>0.15; Table 7.6). However, the net flux of valine was decreased in infected animals by approximately 50% (P<0.05; Table 7.6).

While ^{3}H-valine inflow to the PDV was similar between treatments (P>0.15; Table 7.6), arterial extraction of ^{3}H-valine was decreased from 14% to 9% by the presence of an established parasite infection (P<0.15; Table 7.6). However, the ILR based on arterial sources were comparable between the control and parasitised lambs (*c.* 45-64%; P>0.15).

Valine kinetics based on the infusion of ^{13}C-valine into the abomasum were largely unaffected by the presence of parasites in the small intestine (P>0.15; Table 7.6). The only exception was the extraction of absorbed valine which was 30% lower in the infected lambs (P>0.10; Table 7.6). Despite this decrease, valine ILR based on luminal sources was similar between treatments, as was total ILR (arterial + luminal sources) which averaged *c.* 100 mmol d^{-1} (P>0.15; Table 7.6).

The ratio of arterial to total valine ILR in the PDV was unaffected by parasitic infection (63.2 *vs.* 63.9 (SD 20.2) % in the control and parasitised lambs, respectively; P>0.15). Similarly, the luminal contribution to total valine ILR in the PDV was also similar between treatments (33.8 *vs.* 31.1% (SD 20.2) in the control and parasitised lambs, respectively; P>0.15). The MDV comprised of approximately 37.2 to 70.2% of the PDV ILR in the control and parasitised lambs, respectively (SD 35.4; P>0.15).

Arterial cysteine supply across the PDV was similar between control and parasitised lambs (P>0.15; Table 7.7). However, the net flux of cysteine was reduced by 35% in the parasitised lambs (P<0.05; Table 7.7). The ^{35}S-cysteine sequestration across the PDV from arterial sources was assumed to equal 0.12 (Greaney, 2002) and cysteine ILR from arterial sources was unaffected by parasitic infection when free cysteine in arterial and

venous plasma was used as a precursor (P>0.15; Table 7.7). This was due in part to similar cysteine inflow to the PDV (P>0.15; Table 7.7). The recovery of ^{35}S-cysteine in the portal drainage was similar between the control and parasitised lambs (P>0.15; Table 7.7) as was apparent ^{35}S-cysteine absorption by the PDV (*c.* 30 dpm d^{-1}; P>0.15; Table 7.7). While luminal ^{35}S-cysteine corrected recovery in the portal drainage was unaffected by parasitic infection (*c.* 40 dpm d^{-1}; P>0.15; Table 7.7), when expressed as a proportion of the apparent absorption of ^{35}S-cysteine across the PDV, there was a 20% decrease in the infected lambs (P<0.10; Table 7.7). Consequently, the luminal ^{35}S-cysteine extraction by the PDV was increased by 20% in the infected lambs (P<0.10; Table 7.7) as was the extraction of absorbed cysteine (P<0.15; Table 7.7). Similar reabsorption of cysteine lost into the PDV lumen was observed between treatments. Despite this, there was no effect of parasite infection on cysteine ILR from luminal sources (*c.* 3 mmol d^{-1}) nor total cysteine ILR across the PDV (*c.* 17 mmol d^{-1}).

When expressed as a proportion of total ILR, the arterial contribution of cysteine to total PDV ILR was unaffected by parasitic infection (95.6 *vs.* 91.1 (SD 7.6) %, in the control and parasitised lambs, respectively; P>0.15). Luminal contribution of cysteine to total ILR was also similar between treatments (4.4 *vs.* 8.9 (SD 7.6) %, in the control and parasitised lambs, respectively; P>0.15). The MDV comprised of approximately 50 % of the PDV ILR in the control and parasitised lambs, respectively (SD 15.4; P>0.15).

Table 7.6 Valine (VAL) kinetics in the portal-drained viscera (PDV) of lambs (day 48 post infection) fed fresh Sulla (*Hedysarum coronarium*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD). Positive values for net valine flux represent a net utilisation by the tissue, while a negative value represents a net release.

		Control n=6	Parasite n=6	Pooled SD	P
Net flux parameters					
Plasma flow	mL min^{-1}	1518	1398	369	0.61
Arterial VAL inflow	mmol d^{-1}	498.8	485.1	209.1	0.92
Net VAL flux	mmol d^{-1}	-39.5	-16.5	16.0	0.05
VAL kinetics calculated using jugular ^{3}H-VAL infusion					
Arterial ^{3}H-VAL inflow x 10^{9}	dpm d^{-1}	35.1	37.0	16.9	0.87
Arterial ^{3}H-VAL extraction	dpm d^{-1}	8.9	3.4	6.2	0.22
	%	14.4	8.9	5.1	0.14
VAL ILR from arterial sources[1]	mmol d^{-1}	64.6	43.7	31.9	0.34
VAL ILR from arterial sources[2]	mmol d^{-1}	77.9	49.1	39.7	0.31
VAL kinetics calculated using abomasal ^{13}C-VAL					
Arterial recycled ^{13}C-VAL inflow	mmol d^{-1}	23.4	23.7	20.7	0.98
Luminal ^{13}C-VAL recovery into P	mmol d^{-1}	2.4	4.1	3.9	0.54
Apparent ^{13}C-VAL absorption	mmol d^{-1}	25.4	22.2	4.3	0.25
VAL kinetics calculated using abomasal ^{13}C-VAL and jugular ^{3}H-VAL infusions					
Arterial recycled ^{13}C-VAL extraction	mmol d^{-1}	2.1	2.1	2.1	0.99
Luminal ^{13}C-VAL corrected recovery P[3]	mmol d^{-1}	5.4	5.2	5.2	0.94
	%	18.4	15.7	7.1	0.82
Luminal ^{13}C-VAL extraction	%	81.5	84.3	7.1	0.82
Absorbed VAL extraction	mmol d^{-1}	27.7	19.8	4.5	0.09
Extracted VAL loss into lumen	mmol d^{-1}	4.6	5.3	3.2	0.78
VAL ILR from luminal sources	mmol d^{-1}	32.3	24.5	6.7	0.25
Total ILR (arterial + luminal)	mmol d^{-1}	102.2	68.3	38.6	0.45

1. Irreversible loss rate (ILR) of VAL calculated using the arterial free VAL as precursor pool.
2. Irreversible loss rate (ILR) of VAL calculated using the portal vein free VAL as precursor pool.
3. P = portal vein.

Table 7.7 Cysteine (CYS) kinetics portal-drained viscera (PDV) of lambs (day 48 post infection) fed fresh Sulla (*Hedysarum coronarium*) and infected with or without *Trichostrongylus colubriformis*. Results are presented as LS means and their associated pooled standard deviation (SD). Positive values represent a net utilisation by the tissue, while a negative value represents a net release.

		Control n=6	Parasite n=6	Pooled SD	P
Net plasma flux parameters					
Plasma flow	mL min^{-1}	1518	1398	369	0.61
CYS arterial inflow	mmol d^{-1}	149.5	119.2	49.6	0.41
Net CYS flux	mmol d^{-1}	-7.7	-5.0	1.3	0.03
CYS kinetics					
CYS ILR from arterial sources[1]	mmol d^{-1}	17.5	14.4	7.7	0.67
CYS ILR from arterial sources[2]	mmol d^{-1}	18.0	14.9	7.5	0.67
Luminal ^{35}S-CYS recovery in P^3 $x10^7$	dpm d^{-1}	33.9	74.7	50.6	0.42
Apparent ^{35}S-CYS absorption $x10^7$	dpm d^{-1}	36.9	25.4	17.6	0.36
Arterial recycled ^{35}S-CYS extraction	mmol d^{-1}	16.7	14.7	7.4	0.77
Luminal ^{35}S-CYS corrected recovery in P^3 $x10^7$	dpm d^{-1}	47.3	40.6	34.7	0.92
Luminal ^{35}S-CYS corrected recovery in P^3 $x10^7$	%	87.8	64.3	8.1	0.09
Luminal ^{35}S-CYS extraction	%	12.2	35.8	8.1	0.09
Absorbed CYS extraction	mmol d^{-1}	1.0	3.1	0.3	0.12
Extracted CYS loss into lumen	mmol d^{-1}	0.8	1.4	0.5	0.51
CYS ILR from luminal sources	mmol d^{-1}	1.7	4.5	0.9	0.23
TOTAL CYS ILR (arterial + luminal)	mmol d^{-1}	18.3	15.8	5.9	0.55

1. ILR of CYS based on arterial free CYS as a precursor pool and assuming that ^{35}S-CYS extraction by the artery is 0.12 (Greany, 2002).
2. ILR of CYS based on venous free CYS as a precursor pool and assuming that ^{35}S-CYS extraction by the artery is 0.12 (Greany, 2002).
3. P = portal vein.

7.4.5 Cysteine and valine kinetics across the liver and splanchnic tissues

Plasma flow across the hepatic artery was unaffected by parasitic infection (P>0.15; Table 7.8), however there were treatment differences for net valine flux across the liver with a net utilisation in the infected lambs compared to a net release in the control lambs (P>0.05; Table 7.8). Fractional hepatic extraction of both ^{3}H-valine (*c.* 8%) and ^{13}C-valine (*c.* 10%) by the liver were similar between treatments (P>0.15; Table 7.8). Parasite infection had no effect on valine ILR across the liver (P>0.15; Table 7.8) based on estimates from either ^{3}H-valine or ^{13}C-valine and averaged 40 mmol d^{-1}.

Net uptake of cysteine across the liver was unaffected by parasitic infection and averaged 2 mmol d^{-1} (P>0.15; Table 7.9). Fractional hepatic extraction of ^{35}S-cysteine by the liver (*c.* 19%) and cysteine ILR across the liver were also similar between treatments (P>0.15; Table 7.9).

Plasma flow across the TSP was unaffected by infection, however the TSP released less valine in the infected animals (P<0.15). Fractional extraction of ^{3}H-valine by the TSP was lowered by infection and averaged 16%. However, valine ILR across the TSP based on ^{3}H-valine was unaffected by parasitic infection (P>0.15; Table 7.8). Similar results were obtained using the ^{13}C-valine data although theses estimates were higher than those obtained using the ^{3}H-valine data.

In the TSP, parasitic infection resulted in a net utilisation of cysteine compared to the net release of cysteine from this tissue in control lambs (P<0.15; Table 7.9). There was no effect of infection on the cysteine ILR (P>0.15; Table 7.9).

Table 7.8 Valine (VAL) kinetics across the liver and total splanchnic tissues (TSP) of lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with or without *Trichostrongylus colubriformis*. Results are presented as LS means and their associated pooled standard deviation (SD). Positive values for net valine flux represent a net utilisation by the tissue, while a negative value represents a net release.

		Control n=6	Parasite n=6	Pooled SD	P
LIVER					
Net flux parameters					
Hepatic artery plasma flow	mL min^{-1}	256	163	180	0.43
Net VAL flux	mmol d^{-1}	-8.9	10.7	13.5	0.05
VAL kinetics calculated using a jugular infusion of ^{3}H-VAL					
^{3}H-VAL extraction$_{A+P}$ [1, 2]	%	10.2	6.8	6.9	0.45
ILR_{A+P} [1, 2, 3]	mmol d^{-1}	42.9	32.8	33.12	0.63
ILR_{H} [4]	mmol d^{-1}	45.8	37.6	29.52	0.71
VAL kinetics calculated using abomasal infusion of ^{13}C-VAL					
^{13}C-VAL extraction $_{A+P}$ [1, 2]	%	8.2	11.1	15.2	0.91
ILR_{A+P} [1, 2, 3]	mmol d^{-1}	31.6	59.4	32.2	0.65
ILR_{H} [4]	mmol d^{-1}	38.0	67.0	32.2	0.66
TOTAL SPLANCHNIC TISSUES					
Net flux parameters					
Plasma flow through TSP	mL min^{-1}	1687	1560	434	0.63
Net VAL flux	mmol d^{-1}	-38.0	-12.1	25.8	0.12
VAL kinetics calculated using jugular infusion of ^{3}H-VAL					
^{3}H-VAL extraction$_{A}$ [1]	%	21.3	11.1	9.8	0.11
ILR_{A} [1, 5]	mmol d^{-1}	98.4	80.6	23.3	0.58
ILR_{H} [4]	mmol d^{-1}	117.4	91.9	25.9	0.42
VAL kinetics calculated using abomasal infusion of ^{13}C-VAL					
ILR [6]	mmol d^{-1}	133.8	151.0	30.9	0.47

1. A = mesenteric artery.
2. P = portal vein.
3. ILR_{A+P} = irreversible loss rate calculated using the arterial and portal free valine as precursor pool.
4. ILR_{H} calculated using the hepatic vein free valine as precursor pool.
5. ILR_{A} = irreversible loss rate calculated using the arterial free valine as precursor pool.
6. TSP ILR calculated from PDV ILR + Liver ILR.

Table 7.9 Cysteine (CYS) kinetics across the liver and total splanchnic tissues (TSP) of lambs fed fresh Sulla (Hedysarum *coronarium*) and infected with or without *Trichostrongylus colubriformis*. Results are presented as LS means and their associated pooled standard deviation (SD). Positive values for cysteine flux represent a net utilisation by the tissue, while a negative value represents a net release.

		Control n=6	Parasite n=6	Pooled SD	P
LIVER					
Net flux parameters					
Hepatic artery plasma flow	mL min^{-1}	256	163	180	0.43
Net CYS flux	mmol d^{-1}	1.3	2.9	2.6	0.32
CYS kinetics calculated using an abomasal infusion of ^{35}S-CYS					
^{35}S-CYS extraction $_{A+P}$ [1, 2]	%	24.8	13.3	15.9	0.54
ILR_{A+P} [1, 2, 3]	mmol d^{-1}	18.9	22.9	13.3	0.85
ILR_H [4]	mmol d^{-1}	26.7	32.1	19.0	0.85
TOTAL SPLANCHNIC TISSUES					
Net flux parameters					
Plasma flow TSP	mL min^{-1}	1687	1560	434	0.63
Net CYS flux	mmol d^{-1}	-2.1	0.3	1.6	0.03
CYS kinetics calculated using an abomasal infusion of ^{35}S-CYS					
ILR [5]	mmol d^{-1}	45.1	53.3	19.9	0.88

1. A = mesenteric artery.
2. P = portal vein.
3. ILR_{A+P} = irreversible loss rate calculated using the arterial and portal free cysteine as precursor pool.
4. ILR_H calculated using the hepatic vein free cysteine as precursor pool.
5. TSP ILR calculated from PDV ILR + Liver ILR.

7.4.6 Metabolism of amino acids across the hind limbs

Plasma flow, valine inflow (F_{IN}), valine outflow (F_{OUT}) and net valine flux across the hind limbs were not affected by an established intestinal parasite burden (Table 7.10; P>0.15). Fractional extraction of ^{3}H- (*c.* 21%) and ^{13}C-valine (*c.* 14%) were similar between treatments (P>0.15; Table 7.10). Valine ILR across the hind limbs calculated from both arterial (using arterial and venous precursors) and intra-gastric infusions was unaffected by parasitic infection and averaged 30 mmol d^{-1} for both tracers (P>0.15; Table 7.10).

Transmembrane fluxes of valine across the muscle in the hind limbs were estimated from infusions of ^{13}C-valine and ^{3}H-valine and are presented in Table 7.10 and Appendix Figures F.1 and F.2. Whilst valine transmembrane transport rates (F_{TA} and F_{VT}) calculated from the infusion of ^{13}C-valine were numerically lower in the infected lambs, this was not significant. However, those calculated from the ^{3}H-valine infusion did show significant treatment differences. Inwards (F_{TA}) and outwards (F_{VT}) transport of valine to and from the muscle free pool were significantly lower in the infected lambs (P<0.10; Table 7.10). By-pass of valine flow (F_{VA}) from arterial to venous blood was unaffected by infection (P>0.15; Table 7.10). Estimates of valine (based on ^{3}H-valine data) used for protein synthesis and oxidation (F_{OT}) and released from muscle degradation (F_{TO}) were also lower in the infected lambs (P<0.10; Table 7.10).

Cysteine inflow into the hind limbs was lower in the infected lambs (P<0.15; Table 7.11). The parasite infection did not affect the net uptake of cysteine by the hind limbs (P>0.15; Table 7.11). The arterial extraction of ^{35}S-cysteine by this tissue was not affected by parasitic infection (*c.* 14%). However, cysteine ILR across by the hind limbs was lower but this did not reach statistical significance (P>0.15; Table 7.11).

Table 7.10 Valine kinetics across the muscles of the hind limbs of lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with or without *Trichostrongylus colubriformis*. Results are presented as LS means and their associated pooled standard deviation (SD). Positive values for net valine flux represent a net utilisation by the tissue, while a negative value represents a net release.

		Control n=6	Parasite n=6	Pooled SD	P
Net flux parameters					
Plasma flow	mL min^{-1}	637	502	171	0.24
F_{IN}[1]	mmol d^{-1}	205.2	153.3	101.8	0.47
F_{OUT}[2]	mmol d^{-1}	193.4	152.5	93.8	0.53
Net flux	mmol d^{-1}	11.8	0.8	11.5	0.19
VAL kinetics calculated using jugular infusion of ^{3}H-VAL					
Arterial ^{3}H-VAL extraction	%	21.1	13.6	10.2	0.27
ILR_A[3]	mmol d^{-1}	37.4	23.3	21.4	0.32
ILR_V[4]	mmol d^{-1}	31.7	24.2	15.4	0.48
F_{TA}[5]	mmol d^{-1}	55.9	21.8	24.1	0.08
F_{VT}[6]	mmol d^{-1}	54.2	20.2	24.6	0.08
F_{VA}[7]	mmol d^{-1}	136.1	171.8	112.6	0.62
F_{TO}[8]	mmol d^{-1}	60.8	35.6	20.6	0.11
	g d^{-1}	145.3	85.1	49.2	0.11
F_{OT}[9]	mmol d^{-1}	60.8	39.1	14.0	0.06
	g d^{-1}	145.3	93.4	33.5	0.06
R_{AT}[10]	mmol d^{-1}	122.0	62.2	28.5	0.03
VAL kinetics calculated using abomasal infusion of ^{13}C-VAL					
Arterial ^{13}C-VAL extraction	%	15.3	12.9	12.1	0.78
ILR_A[3]	mmol d^{-1}	28.9	15.3	15.8	0.22
ILR_V[4]	mmol d^{-1}	35.5	22.5	13.9	0.39
F_{TA}[5]	mmol d^{-1}	73.5	48.2	35.7	0.32
F_{VT}[6]	mmol d^{-1}	62.9	47.6	37.3	0.55
F_{VA}[7]	mmol d^{-1}	127.2	107.1	85.0	0.73
F_{TO}[8]	mmol d^{-1}	44.8	35.8	35.6	0.71
	g d^{-1}	107.1	85.0	85.2	0.71
F_{OT}[9]	mmol d^{-1}	55.3	36.4	40.0	0.50
	g d^{-1}	132.3	87.0	95.5	0.50
R_{AT}[10]	mmol d^{-1}	118.3	84.0	62.7	0.44

1. F_{IN}: arterial valine inflow into the hind limbs 2. F_{OUT}: venous valine outflow from the hind limbs 3. ILR calculated using the arterial free valine as precursor pool. 4. ILR calculated using the vena cava free valine as precursor pool. 5. F_{TA}: valine inwards transport from artery to muscle free pool. 6. F_{VT}: valine outward transport from muscle free pool to vein 7. F_{VA}: valine by pass flow directly from the artery to the vein 8. F_{TO}: valine released from muscle protein degradation 9. F_{OT}: valine used for muscle protein synthesis and oxidation. 10. R_{AT}: total appearance of valine in intracellular pool of muscle.

Table 7.11 Cysteine (CYS) kinetics across the muscles of the hind limbs of lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with or without *Trichostrongylus colubriformis*. Results are presented as LS means and their associated pooled standard deviation (SD). Positive values fro net cysteine flux represent a net utilisation by the tissue, while a negative value represents a net release.

		Control n=6	Parasite n=6	Pooled SD	P
Net flux parameters					
Plasma flow	mL min^{-1}	637	502	171	0.24
CYS arterial inflow to HL	mmol d^{-1}	61.6	44.1	8.8	0.13
Net CYS flux	mmol d^{-1}	0.1	0.4	0.4	0.37
CYS kinetics calculated using abomasal infusion of ^{35}S-CYS					
Arterial ^{35}S-CYS extraction	%	15.2	12.9	11.0	0.78
ILR_A[1]	mmol d^{-1}	13.9	5.9	5.7	0.18
ILR_V[2]	mmol d^{-1}	10.5	8.9	2.1	0.18

1. ILR_A = irreversible loss rate calculated using the arterial free cysteine as precursor pool.
2. ILR_V calculated using the vena cava free cysteine as precursor pool.

7.5 Discussion

This study showed that an established parasitic infection in lambs lowered feed intake and triggered downstream metabolic shifts in the MDV and PDV, such as a decrease in apparent absorption of valine, extraction of absorbed valine and consequently, reduced valine ILR from luminal sources. The metabolism of valine in the liver was relatively unchanged by infection. Contrary to the hypothesis, there was no apparent repartitioning of AA from the muscle of the hind limbs to the visceral tissues and the reduction in liveweight observed in infected lambs was attributed to the decreased appearance of AA in the portal vein and in the peripheral circulation. These changes obtained from the valine data were, in most cases, not mirrored by the cysteine data.

7.5.1 Whole body estimates of protein turnover

Parasite infection reduced the whole body valine ILR when calculated from the intra-gastric infusion of both ^{13}C-valine and ^{35}S-cysteine. In contrast, whole body valine ILR calculated from the infusion of ^{3}H-valine into the jugular vein was unaffected by parasite infection, consistent with that reported by Yu *et al.* (2000) with labeled leucine. The data presented in this chapter is in contrast to those found in Chapter Four which showed no effect of treatment between the two sites of infusion. However, in the present study feed intake was reduced by approximately 20% during the infection. Feed intake has a linear relationship with whole body ILR (e.g., Savary-Auzeloux *et al.*, 2003) and therefore it is possible that the decrease in feed intake in the infected lambs in the present study is responsible for the alterations in whole body AA kinetics during infection. However, this does not explain the difference in the treatment effects observed due to the site of infusion of labeled AA.

Estimates of whole body ILR were four times larger when the abomasal infusion of ^{13}C-valine was used (100 *vs.* 400 mmol d^{-1} for the ^{3}H-valine and ^{13}C-valine, respectively), which is consistent with data presented in Chapter Four. Similarly, in studies involving the use of dual-tracers, whole body leucine ILR was 30% lower when based on the intravenous infusion in humans, compared to an intragastric infusion

(Hoerr *et al.*, 1991). In contrast, in the uninfected pig there was no difference in estimated whole body kinetics calculated from an intra-gastric or intra-jugular phenylalanine infusion (Bush *et al.*, 2003). This discrepancy in the literature may be due to the choice of labeled AA used – phenylalanine is not metabolized by tissues other than the liver, while branched-chain AA are metabolised by the tissues of the GIT (e.g., Yu *et al.*, 2000). Reasons for differences between the site of infusion may also be due to the free AA in the digesta diluting the labeled AA infused intra-luminally, thereby resulting in a decreased isotopic activity.

The whole body estimates of valine kinetics using both ^{13}C-valine and ^{3}H-valine presented in this study are in agreement with those presented in Chapter Four and the literature (Davis *et al.*, 1981; Teleni *et al.*, 1986; Harris *et al.*, 1992; Lobley *et al.*, 1996; Connell *et al.*, 1997). Estimates of whole body cysteine kinetics are lower than reported previously in the literature for sheep fed fresh forages (McNabb *et al.*, 1993; Wang *et al.*, 1994). This may either be due to differences in the level of feed intake (*c.* 800 (current study) *vs.* 900-1300 g DM d^{-1} from Wang *et al.*, 1994 and McNabb *et al.*, 1993, respectively) and/or to the route of ^{35}S-cysteine infusion. The ^{35}S-cysteine was infused into the abomasum in the current study, whilst the kinetics in the published studies were based on intravenous infusions.

7.5.2 Tissue amino acid kinetics

The advantages of using labeled AA to determine the fate of specific AA has been discussed previously in Chapter Four. Briefly, the net flux of an AA is not a descriptive measurement as the intermediate and end fates of the AA is not determined. Therefore, by measuring the disappearance of labeled AA into other metabolic pools (oxidation and synthesis) we were able to gain insight into what is happening in the tissue bed of interest. A single tracer infused into the systemic circulation can be used to estimate AA kinetics and protein turnover in tissues (e.g., liver and muscle) where the only entry of AA from the blood circulation is from the arterial supply (Harris *et al.*, 1992; Lobley *et al.*, 1995; Lapierre *et al.*, 1999). However, tissues such as the MDV and PDV utilise AA from both arterial (*c.* 80%) and luminal (*c.* 20%) sources (MacRae *et al.*, 1997; Stoll

et al., 1998; Yu *et al.*, 2000; Rémond *et al.*, 2003). Therefore, the use of a dual-tracer will provide additional insights into the AA requirements by the tissue bed. The use of dual labels of AA has been used previously in the sheep (MacRae *et al.*, 1997; Yu *et al.*, 2000), pig (Bush *et al.*, 2003) and human (Hoerr *et al.*, 1991) and it has been assumed that the metabolism of the different isotopic-labels of AA is similar (Bush *et al.*, 2003).

7.5.2.1 Amino acid kinetics in the mesenteric- and portal- drained viscera

The presence of intestinal parasites in the small intestine had no effect on the kinetics of valine and cysteine across the MDV and PDV, with the exception of a decrease in valine ILR from luminal sources. This is in contrast to the results presented in Chapter Four which showed an increase in valine ILR across the MDV and PDV from both arterial and luminal sources. However, in the current study, the infection resulted in a reduction in feed intake. Indeed the apparent absorption of valine was lower in the infected lambs and this decrease is likely to be responsible for the lowered valine ILR from luminal sources in the parasitised animals.

Despite lower extraction of absorbed valine by the MDV and PDV, net valine appearance in the mesenteric drainage was not affected by the parasite infection although there was a numerical decrease (25%) with infection. Similarly, the PDV data suggest a decrease in net appearance of valine (58%) and cysteine (35%) in the portal drainage.

As the oxidation of valine or cysteine was not estimated in the current study, absolute protein synthesis in the MDV was not calculated. However, the FSR of constitutive proteins in the duodenum and ileum were not affected by the presence of an established parasite infection (Chapter Six). Therefore, it is likely that the lower valine ILR from luminal sources in the infected lambs is due to an increased oxidation within this tissue bed, although ILR estimates also include synthesis of both secreted and constitutive proteins (see Chapter Four for detailed discussion). An increase in valine oxidation in the MDV, agrees with the results by Yu *et al.* (2000) who observed an increase in leucine oxidation in the MDV despite a decrease in feed intake.

The contribution of cysteine ILR from arterial sources to total ILR in the MDV (*c.* 69-77%) were unaffected by the presence of an established parasite infection in the MDV and confirms the results from Chapter Four that the artery is the major source of AA to the MDV. However, the contribution of valine ILR from arterial sources to total ILR was increased numerically by 44% with infection in the MDV. This suggests that dietary cysteine may be preferentially spared from metabolism within the tissues of the GIT, possibly due to its roles elsewhere in the body, such as the immune system (Grimble, 1990). Cysteine is also a major component of both skin and wool (MacRae *et al.*, 1993). Estimates of the contribution of arterial ILR to total ILR in the PDV tissues were similar between control and infected lambs for both cysteine and valine.

The contribution of valine MDV ILR to PDV ILR was numerically larger in the infected lambs (70.6%) compared to the control (37.3%) mainly because total valine ILR in the PDV was 33% lower in the parasitised lambs. However the cysteine MDV ILR: PDV ILR ratios were similar between treatments (*c.* 50%). The values presented in this study are in agreement with those presented elsewhere in the literature (e.g., MacRae *et al.*, 1997; Yu *et al.*, 2000) and in Chapter Three.

7.5.2.2 Amino acid kinetics in the total splanchnic tissues

Despite the lower appearance of both valine and cysteine in the portal circulation, and the numerical decrease in valine ILR from luminal sources there was no significant effect of the presence of intestinal parasites on the ILR of either cysteine or valine in the liver. However, the FSR of liver constitutive protein was increased during infection (Chapter Six) therefore it may be possible that there was a reduction in valine oxidation or concomitant decrease in synthesis of export protein in the liver, resulting in similar valine ILR. Similarly, the valine ILR in the TSP tissues was unaffected by parasitic infection. However, the TSP of infected lambs released less valine and cysteine, and this was largely due to the reduction in their supply to the liver by the PDV.

7.5.2.3 Amino acid kinetics in the hind limbs

Amino acid kinetics were estimated in the hind limbs using a two-compartmental approach as described by Harris *et al.* (1992) and using a three-compartmental transmembrane model described by Biolo *et al.* (1992; 1994; 1995). The assumptions behind the use of this transmembrane model and its limitations have been discussed in Chapter Four.

Parasite infection did not have any effect on valine or cysteine ILR across the hind limbs for either the intra-venous or intra-luminal infusions. There was no effect of parasite infection on transmembrane kinetics based on the infusion of ^{13}C-valine into the abomasum. However, this was not the case when valine kinetics were calculated from ^{3}H-valine infused into the jugular vein. With the exception of valine by-pass which was similar between treatments, parasite infection reduced the transfer of valine into and out of the muscle free pool, and consequently protein synthesis and degradation estimates were also lower. Larger variation in the measurement of ^{13}C-valine kinetics may be masking possible effects of the infection. Indeed, higher coefficient of variation has been observed for ^{13}C-valine kinetics (58%) compared to percentage of difference between treatments (34%). This compares to approximately 60% for both parameters for the ^{3}H-valine kinetics.

It is likely that the changes observed in valine kinetics in the hind limbs in the current study may be due to the reduction in feed intake caused by the presence of the parasite, as the kinetics in Chapter Four were unaffected by the presence of the parasites when feed intake was similar. Results by Hoskin *et al.* (2003) showed a significant relationship between food intake and transfer kinetics, with increasing intake resulting in increased AA kinetics within the muscle of the hind limbs. Estimates of valine transfer kinetics, protein synthesis and degradation across the hind limbs in the current study were similar to those presented in Chapter Four and Hoskin *et al.* (2003).

The reduction in liveweight gain associated with parasite infection could be accounted for by alteration in protein turnover (i.e., protein degradation being larger than protein

synthesis) in the hind limbs, based on the ^{3}H-valine estimates. Previous studies (Coop *et al.*, 1982) have shown that *Ostertagia circumcincta* reduced total body protein by 10-17%, depending on the size of the infective dose given, which supports this claim. However, in Coop's study body fat was also reduced by 25-36%. Therefore, it is conceivable that the infected lambs in the present study also mobilised adipose tissue, resulting in a decrease in liveweight gain.

7.5.3 Does feeding condensed tannins affect amino acid kinetics in the lamb during parasitic infection?

The feeding of forages that contain CT increased animal performance in the uninfected animal. While the presence of some CT (e.g., *L. corniculatus*, Sulla and Sainfoin) does increase the flow of AA to the small intestine and increase apparent availability of AA to the animal (Waghorn *et al.*, 1987; Bermingham *et al.*, 2001), the carry-over effects on AA availability to the tissues have not been investigated. In the current study and Chapter Four, the infusion of ^{13}C-valine into the abomasum in combination with the jugular infusion of ^{3}H-valine enables the determination of the contribution from both luminal (dietary) and arterial AA to different tissues.

The effects of CT in ruminant nutrition are usually determined by daily drenching or by infusing of polyethylene glycol (PEG) into the rumen. Polyethylene glycol preferentially binds to CT minimising the binding between CT and plant protein (Kumar & Singh, 1984). Therefore, animals infused with PEG can be considered to be free of the actions of CT. However, PEG is inappropriate to use in determining the effects of CT in parasitised lambs (Niezen *et al.*, 1998b), therefore we have used Lucerne, which is a forage of similar chemical composition to Sulla (Chapter Two and Five, respectively) as a CT-free comparison.

A direct statistical comparison between the control lambs from Chapter Four (Lucerne fed lambs; no CT) and the infected lambs in the current study (Sulla fed lambs; CT) cannot be made to determine the effects of CT on an established parasite infection as the experiemnts were not conducted at the same time. Also, feed intake was different

between the control lambs fed Lucerne (*c.*830 g DM d^{-1}) and parasitised lambs fed Sulla (*c.* 690 g DM d^{-1}) therefore any differences in valine kinetics between these lambs will be confounded by the reduction in intake.

However, we are still able to gain some insight into the effects of feeding CT in the diet on the availability of AA to the lamb by comparing the control lambs of both experiments as feed intakes were similar between these animals (Table 7.12). While the valine ILR from arterial sources is similar between the control lambs fed Lucerne and Sulla, the luminal ILR of the Sulla-fed lambs is double that of the lucerne fed lambs. Consequently, the total valine ILR in the Sulla-fed lambs is 23% higher than that of the Lucerne-fed lambs (Table 7.12). In the control lambs fed Sulla the luminal contribution of valine ILR to total ILR in the MDV increases by 23% when compared to the estimates obtained in the control animals fed Lucerne. This suggests that there is an increase in the dietary AA available to the tissues of the MDV and this may also result in less need for protein degradation in the peripheral tissues to support the AA requirements of the GIT. The endogenous loss of valine in the GIT of lambs fed Sulla were twice that of the control lambs fed Lucerne. Condensed tannins are thought to interact with the lining of the MDV and this may promote an increase in mucin secretion in the digestive tract which has been observed in monogastrics (Ortiz *et al.*, 1994). However, the effects in ruminants are not well documented.

In the PDV, the valine ILR estimates from arterial and luminal sources in the lambs fed Sulla are double that of Lucerne-fed lambs (Table 7.12). However, as a proportion of total ILR, both arterial and luminal contributions are similar between the two forages.

Overall the contribution of the MDV to the PDV valine ILR in the Lucerne-fed lambs is double that of the control lambs fed Sulla, and is most likely due to the increased ILR of valine across the PDV.

Table 7.12 A comparison of the mean valine irreversible loss (ILR) in the mesenteric-drained viscera (MDV) and portal-drained viscera (MDV), arterial and luminal contributions (%) to total valine irreversible loss and endogenous valine losses (mmol d^{-1}) in control lambs fed either lucerne (n=6; *Medicago sativa*; no condensed tannin) or Sulla (n=6; *Hedysarum coronarium*; 2.2% condensed tannin). Results are presented as LSmeans for each forage and their pooled standard deviation (SD).

		Lucerne Mean	Pooled SD	Sulla Mean	Pooled SD
Intake	g DM d^{-1}	830	16.0	770	47.0
MDV Arterial ILR	mmol d^{-1}	11.5	5.0	8.9	15.6
MDV Luminal ILR	mmol d^{-1}	17.2	6.5	29.1	1.3
MDV Total ILR	mmol d^{-1}	29.1	8.8	38.0	16.9
MDV Arterial: Total ILR	%	46.0	11.7	23.4	26.7
MDV Luminal: Total ILR	%	53.9	11.7	76.6	26.7
PDV Arterial ILR	mmol d^{-1}	29.2	22.9	64.6	31.9
PDV Luminal ILR	mmol d^{-1}	15.7	4.0	32.3	6.7
PDV Total ILR	mmol d^{-1}	42.1	20.0	102.2	38.6
PDV Arterial: Total ILR	%	53.8	11.2	63.2	20.2
PDV Luminal: Total ILR	%	46.2	11.2	33.8	20.2
MDV:PDV[1]	%	70.5	23.6	37.2	35.4
Endogenous valine loss	mmol d^{-1}	1.9	0.8	4.9	3.0

1. MDV total ILR: PDV total ILR

7.6 Conclusions

In conclusion, whole-body ILR and oxidation (for cysteine only) of valine and cysteine using the intra-gastric infusion of ^{13}C-valine and ^{35}S-cysteine were lowered by the parasitic infection. This suggests that the first pass metabolism of these AA was affected by the parasitic infection and is likely to be attributable to lowered feed intake and reduced apparent valine absorption in these animals. This was reflected by a reduction in MDV and PDV valine ILR from luminal sources estimated from intra-gastric ^{13}C-valine infusion. These reductions are likely to have contributed to the decreased whole body valine turnover observed in these lambs. However, no major effects of the infection were observed on the valine ILR in the liver and TSP tissues although a lower release of valine by the TSP tissues into the peripheral blood circulation was observed. This correlates with lower net uptake of valine and valine

kinetics such as inward and outward transport, ILR and valine released from protein degradation in the hind-limb tissues. This might explain the reduction in liveweight and N retention that was observed in parasitised lambs. Thus, partitioning of AA from the hind limbs to the GIT or liver was not increased during an established parasite infection and the reduction in valine kinetics observed in the whole body and tissue beds such as MDV, PDV and hind limbs were mainly a consequence of the reduction in feed intake.

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8 GENERAL DISCUSSION

8.1 Introduction

Parasite infections impose significant costs to both producers and the infected animal. These costs are economic with increased expenditure on anthelmintic chemicals and decreased animal production, as well as metabolic in terms of increased requirements for nutrients such as amino acids (AA) for the repair of the damaged tissue and mounting an immune response towards the parasite. While there have been studies that have focused on the effects of infection on protein synthesis during parasite infection in laboratory animals such as the guinea pig (e.g., Symons & Jones, 1971) or animals fed pelleted rations (Symons & Jones, 1975; Yu *et al.*, 2000) there is little knowledge on the effects of an established parasite infection on the lamb fed fresh forages, which is more representative of the New Zealand situation.

It was hypothesised at the start of this study that the parasitised animal re-prioritises its metabolic activities to the extent of breaking down its own body reserves (e.g., skeletal muscle) in order to meet the additional demands of specific tissues (e.g., gastrointestinal tract and liver) during parasitic infection (Chapters Two, Three, Four, Five, Six and Seven). This hypothesis was tested in two experiments: in the first experiment (Year One), fresh Lucerne (*Medicago sativa*) was fed to a group of uninfected lambs and a group of parasitised lambs, whilst in the second experiment (Year Two), fresh Sulla (*Hedysarum coronarium*) was fed to another group of control and parasitised lambs. Secondly, we hypothesised that, when the lambs were fed forages containing condensed tannins (CT) there may be a reduced impact of the parasite infection due to an increase in the AA supply to productive tissues (Chapters Five, Six and Seven).

Therefore, the present study was conducted in order to firstly determine the effects of an established parasite infection on nutrient fluxes across the mesenteric-drained viscera (MDV), portal-drained viscera (PDV), liver, total splanchnic tissues (TSP) and hind limbs (Chapter Two), and the effects on protein synthesis using the labelled AA incorporation techniques (Chapter Three) and on AA and protein metabolism using the labelled AA arterio-venous dilution techniques (Chapter Four). Secondly, the effects of

feeding a forage containing CT on these parameters were also determined (Chapters, Five, Six, and Seven, respectively).

This discussion will firstly review the major findings of the experimental chapters and any questions that were raised by these findings. Secondly, methodological considerations and limitations of the experimental design will be outlined. And finally the implications of the findings of this study on future research will be discussed.

8.2 The effect of an established parasite infection on protein metabolism

The first aim of this study was to determine the effect of a parasite infection on protein metabolism in the lambs fed fresh forages, Lucerne and Sulla.

8.2.1 Experiment One; Lucerne

Faecal egg production peaked at day 26 post infection at approximately 6 000 egg per gram of wet faeces and the establishment rate of *Trichostrongylus colubriformis* in the small intestine of lambs fed fresh Lucerne was 60% (Chapter Two). There was no effect of parasitic infection on feed intake, liveweight gain or AA apparent absorption from the small intestine (Chapter Two).

In the MDV, the established infection had no effect on total AA flux (Chapter Two) and while parasites decreased fractional protein synthesis rate (FSR) in whole ileal tissue (Chapter Three) there was no effect on the FSR of the duodenal tissues. Despite the FSR being largely similar between the control and parasitised lambs, total irreversible loss rate (ILR) of valine across the MDV was increased by 45% in the infected lambs (Chapter Four), suggesting that valine utilised for protein synthesis, valine oxidation or both increased. This was largely due to an increase in the contribution of valine ILR from arterial sources. Despite an increase in valine ILR by the MDV tissues there was no effect on net valine appearance in the mesenteric drainage across the tissue bed. This suggests that the release of valine from protein degradation in the MDV may have also increased resulting in no net change in valine utilisation within the tissue bed.

Despite increased plasma flow in the PDV, there was once again no effect on net AA flux (Chapter Two), with a few notable exceptions including valine (Chapter Four). The PDV includes the MDV, mesenteric lymph nodes, large intestine and spleen (amongst others) and the FSR of these tissues was unaffected by infection (Chapter Three). Therefore, the increased valine ILR observed in the infected lambs fed fresh Lucerne, was most likely due to the alteration in the valine ILR in the MDV.

The portal vein is the major blood supply to the liver and together with the increased plasma flow through the hepatic artery, this resulted in an increased plasma flow across both the liver and TSP. While parasite infection did not affect the FSR of the liver (Chapter Three), it increased valine ILR across this tissue. This change in ILR may be due to either an increase in the synthesis of secreted proteins, and/or increase in the oxidation of AA which was matched by an increase in protein degradation in the liver. Despite the increase in ILR of valine into the TSP tissues, there was no net affect on the availability of valine to the rest of the tissues as indicated by similar net release of valine across the TSP.

The hind limb preparation includes contributions from muscle, skin and fat. The presence of parasites decreased FSR in the skin and increased muscle FSR albeit the latter estimate was unreliable (Chapter Three). The ILR of valine, trans-membrane transport of valine and degradation of protein within the posterior hind limbs were unaffected by parasitic infection (Chapter Four), which agree with the similar net flux of AA across the hind limbs presented in Chapter Two.

These results suggest that there is no re-partitioning of AA from the posterior hind limbs to the gastrointestinal (GIT) and liver during an established infection when lambs are fed fresh Lucerne. The increase in the arterial contribution to total valine ILR in the MDV and PDV tissues suggests that there is a diversion of AA from sources other than the tissues of the posterior hind-limbs or more likely due to the increase in plasma flow to the MDV and PDV tissues. Protein turnover is likely to be increased in the TSP tissues as a consequence of higher protein synthesis (and/or AA oxidation) and degradation

during parasitic infection as the net appearance of valine was unaffected by the parasitic infection. This suggests that an established parasitic infection may trigger a localised alteration in blood flow, AA metabolism and/or protein turnover in the affected tissue without significantly changing the metabolism of AA and proteins in tissues peripheral to the TSP tissues and without impacting negatively on the growth of the parasitised lambs.

8.2.2 Experiment Two; Sulla

Faecal egg production peaked at day 26 post infection at 1 800 eggs per gram of wet faeces and establishment rate of *T. colubriformis* in the small intestine of lambs fed fresh Sulla was 48% (Chapter Five). The presence of parasitic infection in the small intestine of the lambs decreased feed intake. While the change in liveweight was unaffected over the entire experimental period (48 d), over the last 20 d there was a significant reduction in liveweight gain in the infected lambs (Chapter Five). Intake, abomasal flow and apparent absorption of AA in the small intestine were reduced in the infected lambs (Chapter Five).

While plasma flow across the MDV was similar between the control and parasitised lambs, less AA were released from the MDV (Chapter Five). The reduction in AA released by the MDV may be due to the reduction in apparent AA absorption and/or more AA were utilised for protein synthesis or oxidation. Alterations in the protein turnover of this tissue was supported by the increased FSR in scraped duodenal and ileal tissues, although whole tissue was unaffected (Chapter Six). While the luminal contribution to total ILR in the MDV was decreased by infection, there was no effect of the infection on total ILR in the MDV (calculated from arterial and luminal sources; Chapter Seven). Together with the FSR data this suggests that there may have been a decrease in the oxidation of AA in the MDV, resulting in no net change in ILR. However, it is acknowledged that the continuous infusion technique will overestimate the FSR in the MDV due to the high rates of turnover and the exclusion of endogenous protein secretion in the FSR estimate.

Net AA flux across the PDV was once again lower in the infected lambs compared to the uninfected controls (Chapter Five). This was mostly likely due to the decreased release of AA from the MDV. The FSR in the mesenteric lymph nodes of the infected lambs was higher than that of the uninfected lambs, however the large intestine and the spleen FSR estimates were unaffected by parasitic infection (Chapter Six). Both valine and cysteine ILR estimates for the PDV were similar between the control and parasitised lambs, suggesting that there was no negative impact of the infection on the AA requirements of the PDV.

Plasma flow across the liver and TSP were unaffected by parasitic infection. With the exception of the branched-chain AA (BCAA), AA utilisation was similar in the liver between the control and parasite lambs. In the liver, FSR was doubled during infection (Chapter Six), however both valine and cysteine ILR were similar (Chapter Seven). This suggests that there may have been a reduction in valine oxidation in the liver or a decrease in the synthesis of export proteins by the liver, resulting in no net change in valine ILR. The increase in AA utilisation by the TSP was most likely due to the decreased contribution by the PDV because AA utilisation was similar in the liver (Chapter Five) as ILR in the TSP was not changed with the presence of adult *T. colubriformis*.

There was no effect of infection on net AA flux across the hind limbs, despite a decrease in the release of AA by the TSP in the infected animals (Chapter Five). Infected lambs had larger estimates of FSR in the muscle, however parasitic infection had no effect on skin FSR estimates (Chapter Six). This correlates with lower net uptake of valine and valine kinetics such as inward and outward transport, ILR and valine released from protein degradation in the hind-limb tissues, which may explain the reduction in liveweight and nitrogen retention that was observed in parasitised lambs. However, the transmembrane model did indicate some differences between the treatments which was dependant on the isotope of valine used. For example, based on the infusion of ^{13}C-valine into the abomasum there was no significant effect of parasite infection on

transmembrane valine kinetics. However, this was not the case when valine kinetics were calculated from ^{3}H-valine infused intravenously.

In conclusion, the first pass metabolism of AA was affected by the parasitic infection and this was likely to be attributable to lowered feed intake, apparent AA absorption and appearance of AA in the portal drainage in these animals. The results suggest that there was no increase in partitioning of AA from the hind limbs to the GIT or liver during an established parasite infection but the changes in valine kinetics in the whole body and tissue beds were mainly a consequence of the reduction in feed intake during the established parasitic infection.

Figure 8.1 Summary of amino acid (AA) metabolism (irreversible loss rate (ILR) or fractional protein synthesis rate (FSR)) in the lamb fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* compared to parasite-free controls.

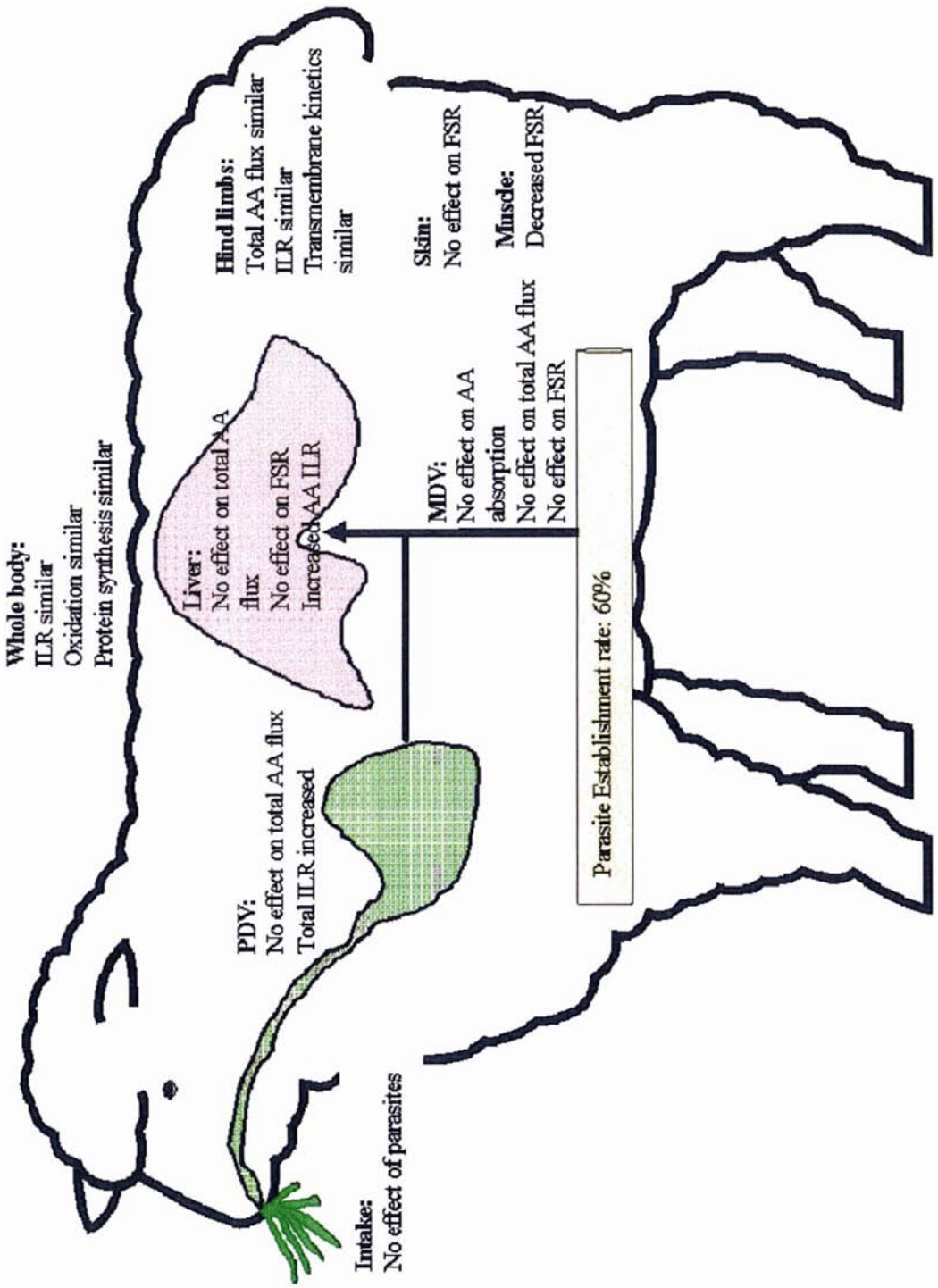
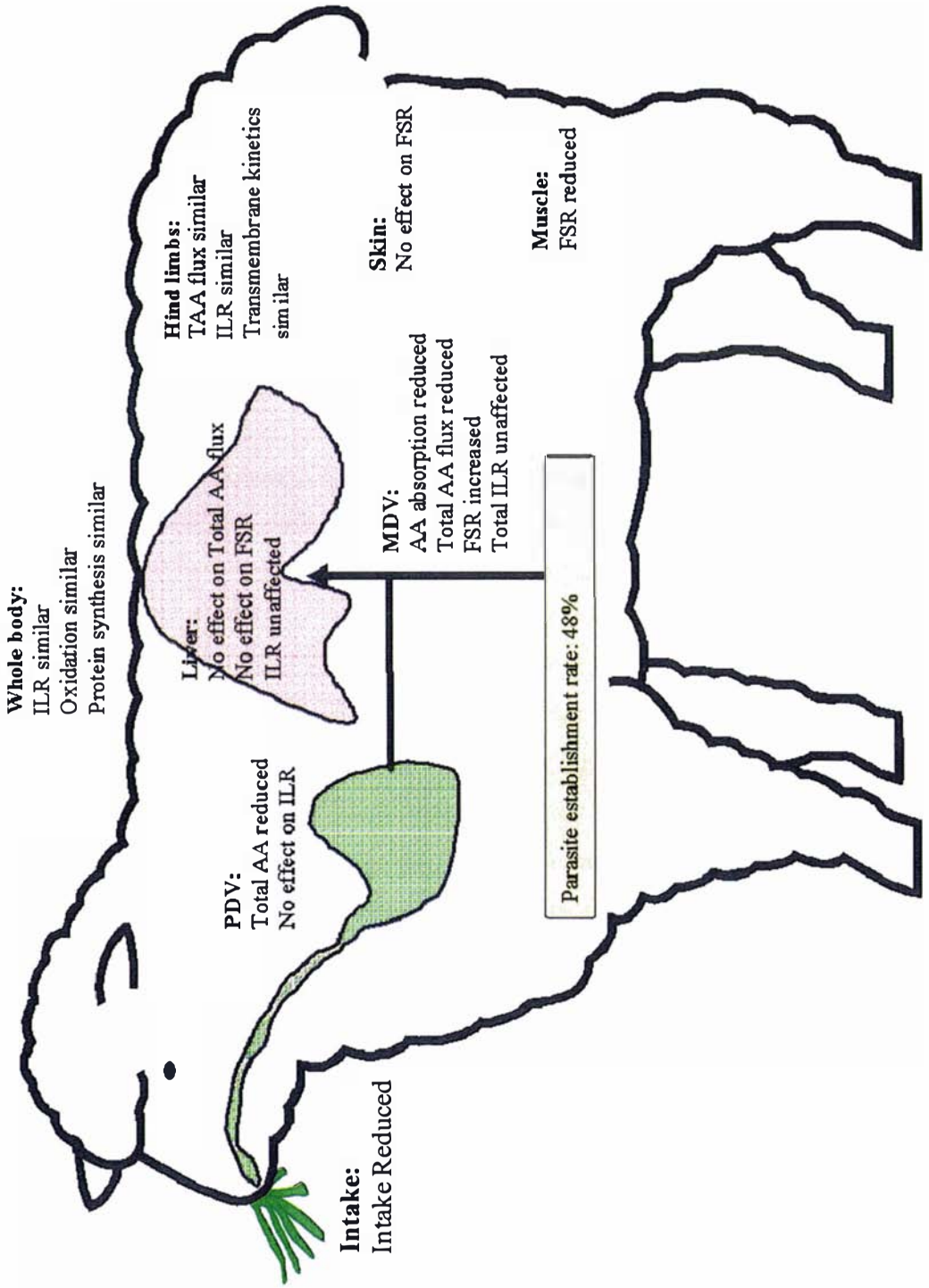


Figure 8.2 Summary of amino acid (AA) metabolism (irreversible loss rate (ILR) or fractional protein synthesis rate (FSR)) in the lamb fed fresh Sulla (*Hedysarum coronarium*) during a *Trichostrongylus colubriformis* infection compared to parasite-free controls.



8.3 Condensed tannins; what is their role during parasite infections?

The second aim of this study was to determine the effects of feeding CT on protein metabolism in the infected animal. A direct statistical comparison between the two forages is not possible. Furthermore, any comparisons between the infected lambs in this study are further confounded by differences in feed intake between the infected lambs fed Lucerne (790 g dry matter (DM) d^{-1}) and Sulla (690 g DM d^{-1}). However, the intake in the control lambs of Experiment One and Two were similar, therefore the effects of CT on protein metabolism in the uninfected lambs will be discussed.

8.3.1 The effects of condensed tannin on protein metabolism in the uninfected lamb

By comparing the means of the metabolic parameters obtained in the control animals fed Lucerne to that of control lambs fed Sulla, the effects of feeding CT on protein metabolism may be elucidated.

It was hypothesised that the presence of CT in the diet would result in an increase in the availability of dietary AA to the lambs, and therefore decrease the mobilisation of AA from tissues such as the skeletal muscle. In this experiment, the CT concentration in the Sulla was 2.6%. This level is traditionally viewed as lower than the range that has negative impacts on feed intake (CT concentration >8% dry matter (DM)), and comparing the intakes of control lambs fed both Lucerne and Sulla suggest that there was no adverse affect of the CT on intake levels.

In Chapters Two and Five, the apparent absorption and net flux of AA across the different tissue beds were measured. While the Sulla-fed lambs showed a higher proportion of AA digested in the small intestine than Lucerne-fed lambs (68 *vs.* 60%, respectively), this was not sufficient to avoid a decline in the apparent absorption of AA from the small intestine. Therefore, feeding Sulla did not provide additional AA from the small intestine in the control lambs.

Feeding forages that contain CT have increased liveweight and wool production (e.g., Burke *et al.*, 2002; Wang *et al.*, 1996). However, there is no published data available on the effects of CT on protein turnover in the productive tissues. In the current study, the estimate of skeletal muscle FSR in the Sulla-fed lambs were similar to those of the Lucerne-fed lambs. The skin estimates of FSR (10 to 12 % d^{-1}) were also fairly consistent between the Lucerne and Sulla-fed lambs. Therefore, feeding CT to lambs in this study did not improve production by increasing the amount of protein synthesised in the muscle and skin. However, in the current study intake was restricted to approximately maintenance requirements (800g DM d^{-1}) and therefore a larger difference between the two studies may become apparent at higher intakes.

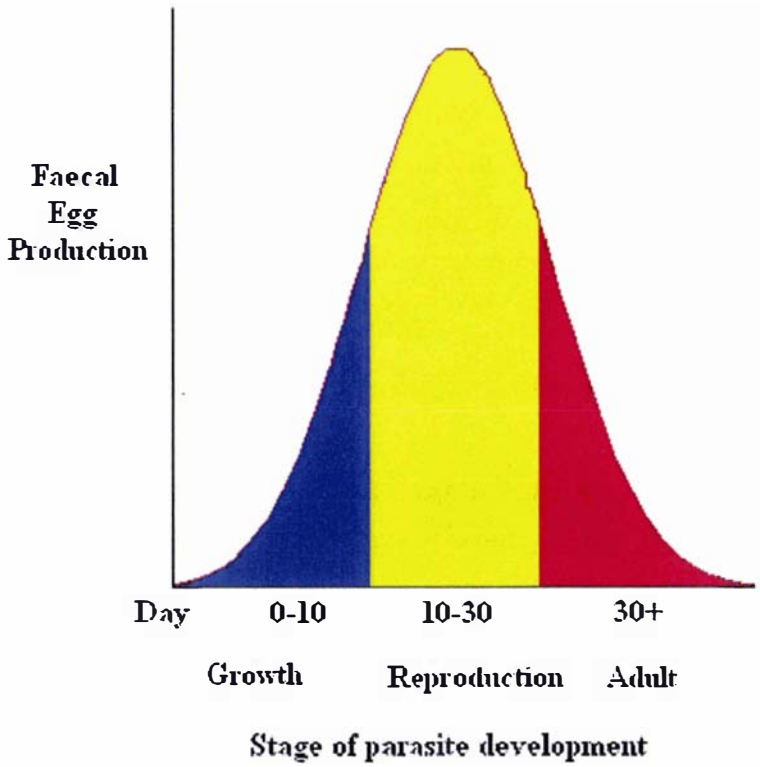
One explanation for how CT improve production may be due to an increase in the utilisation of dietary AA by the small intestine. In the control lambs fed Sulla the luminal contribution of valine ILR to total ILR in the MDV and PDV increased by *c.* 30% and 16%, respectively when compared to the estimates obtained in the control animals fed Lucerne. This suggests that the increase in dietary AA available to the tissues of the MDV and PDV may result in less protein degradation in the peripheral tissues to support the large AA requirement of the GIT. Overall the contribution of the MDV to the PDV valine ILR increased by *c.* 20% in the control lambs fed Sulla. This might be attributable to the endogenous loss of valine in the GIT being twice that of the control lambs fed Sulla.

8.3.2 When are the most significant periods of amino acid cost during parasitic infection?

The conclusions drawn from this study indicate that adult *T. colubriformis* populations in the small intestine of lambs impose little nutritional cost on the host 48 d post-infection. It is possible that any effects of the parasite burden occurred during the earlier stages of the trial and the data highlight a need to identify which stage(s) of intestinal parasitism need to be targeted in metabolic studies.

The three periods that are most likely to impact on the animals' metabolism are the initial larvae challenge, peak egg production and the presence of the established infection (Figure 8.3). The results presented in this thesis have shown that when food intake is not reduced there is little effect of the adult parasite on the host's metabolism. However, the effects of the initial stimulation of the host's immune response to the presence of parasite in the small intestine (i.e., the 'growth' period; Figure 8.3) need to be determined as this phase has the potential to be a major drain on the AA stores of the host. There is little information available on the nutritional demands of the parasite during its peak reproductive phase (approximately 3 weeks post infection). It is likely that any increase in nutrient requirements by the parasite for its own reproduction will have a negative impact on the host due to an increased utilisation of nutrients (either absorbed by the host, or sourced from the blood or mucosal tissue of the host).

Figure 8.3 A generalised representation of peak egg production and days post infection for sheep infected with a single dose of *Trichostrongylus colubriformis*.



8.4 Methodological considerations

8.4.1 Experimental design

In this study there were two major hypotheses: 1) That an established parasite infection would result in altered AA metabolism. 2) That lambs fed CT during an established parasite infection grow better than lambs fed forages that do not contain CT.

The first hypothesis was tested by comparing the control and infected lambs fed either Lucerne (Experiment One) or Sulla (Experiment Two). The observations from this hypothesis have been discussed in Section 8.2.

The second hypothesis was to be elucidated by comparing the infected lambs fed Lucerne and Sulla. The accepted practice for determining the effects of CT in ruminants is to orally dose several times a day or continuously infuse to the rumen, with polyethylene glycol (PEG) - a compound that preferentially binds with CT thus rendering them inactive (Kumar & Singh, 1984). Traditionally, the effects of CT in forages are determined by comparing PEG-dosed animals (i.e., no active CT) with animals not receiving PEG (and therefore have active CT). However, results published by Niezen *et al.* (1998) suggested that the administration of PEG had a negative impact on parasite establishment. Therefore, in order to elucidate the effects of CT on AA and protein metabolism during parasitic infection the experiment was split into two parts. In the first year Lucerne– a forage that has a high feeding value, but contains little or no CT was fed to 12 lambs (six uninfected control lambs and six infected lambs). In the second year Sulla – a forage similar in nutritive value to Lucerne with the exception that Sulla contains CT, was fed to another set of 12 lambs (six uninfected control lambs and six infected lambs). Identical infection and infusion protocols were used for both experiments and both were conducted at a similar time of the year (late summer). The lambs in both experiments were sourced from the same supplier and weaned at a similar liveweight in order to minimise prior exposure to parasites. However, this design means that we were unable to statistically analyse the effects of feeding CT on AA and protein

metabolism. This is due to the confounding impact that year and forage would have on the results.

The statistical model used in both experiments was a complete randomised block design with treatment and group used as a source of variation in the statistical model. Other alternative statistical models could have been employed. A factorial design would have been the ideal design. A factorial design in this case would involve two factors – feed (Lucerne *vs*. Sulla) and infection (control *vs*. parasite) for a total of 4 treatments with 6 observations per treatment (a total of 24 sheep). However, this design is not suitable as it would be impractical to maintain 24 multi-catheterised in one experiment. The second most suitable design would required the experiments to be conducted over a two year period, and involve feeding both Lucerne and Sulla in each year (i.e., 3 sheep per forage per treatment). However, the availability of the forages is a critical factor as well as forage quality and nutrient composition, which can be highly variable from year to year. Finally, the risk of losing a lamb during surgery is high. The numbers of animals undergoing surgery for each block needs to be superior to the required number for the experiment in order to make sure enough animals recover from the surgery. Therefore, by adopting the current approach investigating one forage per year, we have minimised the number of animals required to undergo surgical preparation.

8.4.1.1 Choice of significance level

In this thesis the results are declared to be significant if the probability is lower than 0.10, and tending to be significant when the probability is lower than 0.15. Leading researchers in the AA and protein metabolism area have previously used 'non normal' probability values. For example, Lapierre *et al*. (2000) considered their results to be significant if the probability is lower than 0.10, and defined a trend if the probability was between 0.10 and 0.20. This is due in part to the small numbers of degrees of freedom analysed statistically due to catheter failure or erroneous values within the data set.

8.4.2 Surgical preparation

The lambs in these experiments underwent catheterisation of multiple visceral and peripheral vessels. This is a highly invasive surgical preparation that has not been attempted in New Zealand previously. The surgery itself took around 4 h per animal to complete, with a maximum of 4 lambs prepared in any week. Therefore, surgery of the 12 lambs in each experiment was staggered over 3 weeks. Therefore, for statistical analysis of the results, lambs were blocked according to the week they underwent surgery, referred to as a group effect in the statistical model. The four lambs with the block were either kept as controls (2) or infected with parasites (2).

In year one (Lucerne experiment) catheter patency was approximately 99% with only one catheter failing over a 55 d period. In the second year (Sulla experiment) four catheters (2 in the mesenteric vein, and 1 in the portal vein and vena cava) failed due to fibrous sheaths forming over the end of the catheter reducing the patency to 95%.

8.4.3 Infection protocol

In these experiments, lambs were infected with 6 000 L3 *T. colubriformis* larvae per d for 6 d to provide a total dose of 36 000 larvae. This was essentially a 'single-dose' infection, as the larvae were split into 6 days to reduce the threat of rumen bypass and therefore maximize the establishment of larvae in the small intestine. However, trickle dose infections (i.e., constant exposure to infective larvae at least once a week) are more representative of what occurs on farm (MacRae, 1993). Much of the literature relating to parasitic infection in sheep is based on the trickle dose procedure (e.g., Sykes & Coop, 1976; Kimambo *et al.*, 1988a; 1988b; Athanasiadou *et al.*, 2000; Butter *et al.*, 2000; Yu *et al.*, 2000).

A single dose method of infection was used in the experiments for two reasons. Firstly, this is the first time an extensive surgical model combining the catheterisation of seven visceral and peripheral blood vessels and abomasal cannulae has been performed to investigate the effects of parasitic infection in lambs. It was unknown as to how successful the surgical preparation would be, and whether the lambs would be able to

deal with the continual onslaught of larvae as well as the demands caused by the presence of the adult parasite population after having undergone such a invasive procedure. Therefore, in order to maximise lamb survivability we used the single infection method. Secondly, there are few data available on the effects of an established adult worm burden on AA and protein metabolism. The use of a trickle dose means that the animal is dealing with the continual onslaught of new larvae as well as the existing adult populations. Therefore, any data relating to the alterations in metabolism in the trickle-infected animal may be due to the initial challenge by the parasite larvae, the developing adult worm, and finally the breeding adult worm. By utilising the single dose method we were able to study the adult population without any confounding effects of new larval challenges.

By utilising a single dose, measurement of the effect of an established adult infection on the AA and protein metabolism by the lamb with no complications of continual infection on the immune system were able to be assessed. However, by day 48 post infection little effect of the parasitic infection on AA and protein metabolism was observed in the Lucerne fed lambs. It is possible that any alterations in the lambs metabolism may have occurred earlier in the infection cycle. For example, alteration in AA partitioning within the lamb may occur during the initial infection period (days 0 to 10) due to the stimulation of the immune response. An increase in nutrient requirements by the parasite during its growth and reproductive cycle may also incur a metabolic cost on the host, with the period leading up to peak egg production (up to day 26 post infection) likely to alter the metabolism of the lamb. It is possible that between day 26 and 48 that the lambs metabolism has largely adjusted to the parasite burden and has returned to normal. In the Sulla-fed lambs, there were small alterations in AA and protein metabolism in the infected lambs.

8.4.4 Plasma flow

Technical considerations that need to be addressed from the present study include the measurement of blood flow across the splanchnic tissues using para-aminohippuric acid (PAH). Aside from an acetylation step in the liver, PAH is not metabolised by the

splanchnic tissues. Therefore, PAH was used to estimate the flow of plasma through the MDV, PDV, liver and TSP. The PAH can be deacylated by the addition of a boiling step in the analysis (Lobley *et al.*, 1995). Blood flow estimates are utilised to determine the net release or utilisation of AA and therefore an accurate measurement of this parameter is required. However, the estimates of plasma flow using PAH as a dye in the current studies (coefficient of variation (CV); 8-90%) and other studies (CV 20%; MacRae *et al.*, 1997; Greaney, 2001) were highly variable especially for the MDV plasma flow. This variation was attributed to either incomplete mixing of the dye in the blood vessel, movement of the catheter due to peristaltic gut contractions (MacRae *et al.*, 1997) or errors in the analytical procedure. Data in the literature relating to PAH analysis is conflicting (e.g., Smith *et al.*, 1945; Katz & Bergman, 1969) and therefore the use of PAH for estimating blood flow across the TSP is an important area which requires further clarification.

8.5 Conclusions and future research

From the results of this study three clear conclusions can be made. Firstly, the surgical preparation is a viable technique to measure the effects of parasite infections on AA metabolism within the lamb. Secondly, an established infection imposes no notable metabolic cost on the lamb when feed intake is not reduced. Thirdly, when feed intake is reduced, there is no mobilisation of protein from the hind limb, therefore other sources of AA and/or energy substrates must be utilised.

A major question resulting from these results is how did the lambs manage to cope with the presence of such a large parasite burden without any significant effects on their metabolism? In the future, when investigating these questions it is important to consider the effects of larval challenge *vs.* maintenance of worm burdens, previous exposure to the parasite, immune development and the metabolic activity of adult worms.

8.5.1 Future research

The effects of different stages of parasite infection on host metabolism are of importance as there is little information available on the nutrient requirements of firstly the immune

response generated by host in the presence of parasitic infection. Another stage of importance to the host in terms of the demands for nutrients by the parasite would be the period leading up to peak egg production (reproductive and growth requirements by the parasite). These requirements could be determined by utilising a 'single dose' with arterio-venous measurements taken at day 0-10 (initial infection) and around day 26 (peak egg production).

A trickle infection is more representative of what occurs on farm. Measuring AA and protein metabolism across the MDV, PDV, liver, TSP and hind limbs during a trickle infection may determine whether a shift in partitioning of AA from skeletal muscle to liver and GIT does actually occur. The effects of multiple parasite species on AA metabolism are also of importance due to the compounding effects mixed infections have on animal performance.

Intestinal parasite infections have resulted in altered food intake, however the mechanism(s) behind this effect are not clear. The reduction in feed intake of Sulla-fed lambs due to parasite infection but no effect in the Lucerne fed lambs despite a lower worm burden in the Sulla-fed lambs seems paradoxical. Whether the plant characteristics of Sulla affected feed intake due to palatability issues or whether they had a negative impact in the GIT is not known. As food intake is the driving force behind altered metabolism during parasitic infection these mechanism are of importance. The effects that intestinal parasites have on sections of the GIT other than where they inhabit are also of interest due to the alterations in the tissues of the PDV that could not be explained by the small intestine contributions.

8.6 References

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VIII Appendices

Appendix A

Table A.1 Parasite species and dosage rates in the literature cited in this thesis. Dosage rates are given as the number of parasites per day for the length of period (weeks or days). Unless stipulated in the table, the full reference can be found in the literature review.

Author	Parasite	Dose
Anthanasiadou *et al.*, 2000[1]	*T. colubriformis*[2]	2 0000 x 1
Anthanasiadou *et al.*, 2000[3]	*T. colubriformis*	3 000 5days/week for 10 weeks
Barger, 1972	*T. colubriformis*	12 000 x 1; 1 500 000 6 weeks later
Bown *et al.*, 1986	*T. colubriformis*	3 000/day for 12 weeks
Bown *et al.*, 1991[4]	*T. colubriformis; O. circumcincta*[5]	3 000 TC & 3000 OC/day for 18 weeks
Bown *et al.*, 1991[6]	*T. colubriformis*	3 000/day for 12 weeks
Butter *et al.*, 2000	*T. colubriformis*	3 000-6 000 5 day/week for 70 days
Coop & Angus, 1975	*T. colubriformis*	4 000 for 5 day/week for 10 weeks
Coop & Angus, 1976	*T. colubriformis*	2 500/day for 13 weeks
Coop *et al.*, 1977	*O. circumcincta*	1 000-5 000/day for 8 weeks
Coop *et al.*, 1979	*T. vitrinis*	2 500/day for 90 d
Coop *et al.*, 1982	*O. circumcincta*	0, 1 000, 3 000, or 5 000 per day for 84 d
Gregory *et al.*, 1985	*T. colubriformis*	2 500/day for 12 weeks
Jones & Symons, 1982	*T. colubriformis*	18 000/ week
Jones, 1982	*T. vitrinis*	25 000 x 5/ week for 14 weeks
Kimambo & Macrae, 1988	*T. colubriformis*	2 500/day for 34 weeks
Kimambo *et al.*, 1988	*T. colubriformis*	2 500/day for 25 weeks
Leyva *et al.*, 1982	*O. circumcincta*	4 000/day for 6 weeks
Neizen *et al.*, 1995	Natural infection	
Parkins *et al.*, 1990	*O. ostertagi; C. oncophora*[7]	OO: 2 000/day for 6 weeks; CO: 10 000/day for 6 weeks
Poppi *et al.*, 1986	*T. colubriformis*	2 500/day for 14 weeks
Roseby & Leng, 1974	*T. colubriformis*	30 000 x 1

Table A.2 Parasite species and dosage rates in the literature cited in this thesis. Dosage rates are given as the number of parasites per day (d) for the length of period (weeks or days). Unless stipulated in the table, the full reference can be found in the literature review. *Cont.*

Author	Parasite	Dose
Roseby, 1973	*T. colubriformis*	30 000 x 1
Roseby, 1977	*T. colubriformis*	30 000 x 1
Steel & Symons, 1980	*T. colubriformis*	300-30 000/week for 24 weeks
Steel *et al.*, 1982	*T. colubriformis; O. circumcincta*	Concurrent & mono species
Stephenson *et al.*, 1980	*Ascaris suum*	200 x 1
Sykes & Coop, 1976	*T. colubriformis*	2 500/day for 13 weeks
Sykes & Coop, 1977	*O. circumcincta*	4 000/day for 14 weeks
Symons & Hennessy, 1981	*T. colubriformis*	50 000 x 1
Symons & Jones, 1970	*N. brasiliensis*[8]*; T. colubriformis*	NB: 750 x 1; TC: 4 000 x 1
Symons & Jones, 1971	*N. dubis; T. colubriformis*	ND: 350; TC: 7 500
Symons & Jones, 1975	*T. colubriformis*	60 000 x 1
Symons & Jones, 1983	*T. colubriformis*	8 000 x 1
Symons *et al.*, 1974	*T. colubriformis*	7 000 x 1
Thomas & Ali, 1983	*H. contortus*[9]	2 500/week for 12 weeks
Yu *et al.*, 2000	*T. colubriformis*	2 500/day for 20 weeks

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5. *Ostertagia*
6. Bown MD, Poppi DP & Sykes AR (1991) Nitrogen transactions along the digestive tract of lambs concurrently infected with *Trichostrongylus colubriformis* and *Ostertagia circumcincta*. *British Journal of Nutrition* **66**, 237-249.
7. *Cooperia*
8. *Nematospiroides*
9. *Haemonchus*

APPENDIX B

B.1 Surgical procedures

B.1.1 Surgical procedures for vascular catheterisation and abomasal cannulation.

Lambs were fasted for 24 h prior to surgery, but allowed access to water during the fasting period. Anaesthesia was induced with Nembutal (0.5 mL kg body weight^{-1}; Chapters Two, Three and Four or a mixture of Diazapam (1 mg mL^{-1}) and ketamine (4 mg mL^{-1}: Chapters Five, Six and Seven and maintained with isoflurane (1.5%) and oxygen (98.5%) delivered via an endotracheal tube. An area approximately 50 cm^2 in size was shorn on both sides of the lamb following establishment of anaesthesia. The catheters were first inserted in the abdominal aorta and vena cava, secondly in the hepatic blood vessels, thirdly in the mesenteric blood vessels (Figure B.1) and finally a cannula was inserted in the abomasum.

B.1.2 Abdominal aorta and vena cava

Catheters were placed in the abdominal aorta and vena cava according to the surgical procedures described by Ortigues *et al.* (1994). The sheep were positioned in right lateral recumbency. An area between hip joint and the last rib and the site of exteriorisation on the back were scrubbed with surgical soap, rinsed and swabbed to remove any loose wool or skin, and rinsed again with a 70% hibitane solution (v/v in ethanol). The left side was draped with sterile surgical sheeting, and a 15 cm incision was made through skin and muscles parallel to the spine. Bleeding from skin and muscle tissues was prevented by cauterisation, suture or using forceps. Both sides of the incision were covered with sterilised gauze soaked in warm saline (37°C).

Blunt dissection was used to locate the abdominal aorta and vena cava between the renal and iliac vessels. The peritoneum was left intact and therefore the intestines were not directly exposed during this procedure. Ribbon was passed around the dissected vessel upstream and downstream to the intended site of insertion of the catheters. The ribbons were used for restricting blood flow during catheter insertion. Sterile gauze soaked with

warm saline was placed over the tissues surrounding the intended site of insertion to minimise the risk of damage to the tissues and to prevent tissue dehydration. A purse string (Ethibond 4-0, Ethicon, National Veterinary Supplies Ltd, Palmerston North, New Zealand) was sutured into the vessel. Blood flow was completely restricted by tightening the ribbons just prior to making an incision in the middle of the purse string using iris scissors, and the catheter inserted through the vessel wall, whilst accumulating blood (if any) was aspirated from the cavity. The purse string was tied off leaving 5 cm of the catheter inside the blood vessel with the tip located approximately 3 cm from the entry to the right and left iliac vessels. The blood flow restriction was then removed, with this procedure taking less than five minutes (*c.* 2-3 minutes) which is important to avoid post-surgical paralysis of the hind limbs. Patency of the catheter was checked, and the catheter secured by suturing the cuff glued to the catheter to the wall of the blood vessel. The vena cava catheterisation was performed first and the procedure repeated for the abdominal aorta.

All catheters were exteriorised through the muscle and skin layers at the top of the mid-side incision. Each catheter was identified by a specified colour of tape and a blunt needle (18 gauge) inserted in the end of each catheter. A loop of each catheter was left inside the sheep in order to avoid possible stress on the insertion site. Antibiotic powder was applied to the tissues before closing the incision and exteriorisation site. The catheters were coiled in a pouch made of PVC plastic with a velcro opening, attached to the back of the sheep.

B.1.3 Splanchnic blood vessel catheterisation

The sheep were then repositioned in a left lateral recumbency. An area between the hip and last rib down to the midline was scrubbed with 70% hibitane solution (in ethanol v/v), rinsed and swabbed to remove any loose wool or skin, and rinsed again with a 70% hibitane solution. The right side was draped with sterile surgical sheeting, and a 20 cm incision was made through the skin and muscles perpendicular but 5 cm caudal to the last rib. Bleeding was controlled by cauterisation, suture or forceps. The peritoneum was raised with forceps to prevent cutting the viscera and then opened the length of the

incision. Both sides of the incision were covered with sterilised towels soaked with warm saline.

B.1.3.1 Catheterisation of the visceral blood vessels

Catheterisation of the visceral blood vessels were performed as described by Huntington *et al.* (1989). The liver was palpitated cranial to the gall bladder to feel the indentations corresponding to the portal or hepatic blood vessels. With a scalpel blade, an incision was made on the top of the located blood vessel. If blood came out from this incision, the catheter was inserted through the opening of the blood vessel. Once the catheter was positioned in the blood vessel, patency of the catheter was verified by withdrawing blood. If successful the catheter was sutured in place using the cuff attached to the catheter, leaving approximately 7 cm of catheter within the vessel. This process was repeated on another vein located in the same lobe. When the second catheter was successfully inserted, oxygen concentration in the blood withdrawn from each vessel was checked using a blood gas analyser (ABL3, Radiometer Pacific Limited, Copenhagen) in order to determine which vessel was the portal and hepatic vein; portal blood has a higher oxygen concentration than hepatic blood. An additional verification was performed indirectly by filling the catheters with heparinised saline and holding the catheters at the same level. The liquid in both catheters flowed against gravity until sufficient pressure was encountered. The difference between the heights of each meniscus was determined by visual inspection; the hepatic vein has a lower blood pressure than the portal vein and therefore, will have the lower meniscus. If the catheters were not placed in the correct vessel, one of the catheters was removed and the process described above repeated until the placement of the catheters in the correct vessels was achieved. This rarely required having to insert more than three catheters to achieve a portal and hepatic catheterisation. The portal and hepatic catheters were sutured to the liver by using the cuffs glued to each of the catheters. Additionally, an atraumatic half-circle needle was used to form an overhanging knot loosely in the parenchyma, the catheters were placed within the knot and then secured using a square knot.

The small intestine was then exteriorised and displayed on the surgical drape. Towels soaked in warm saline were used to keep the intestine moist and warm. The mesenteric blood supply is more apparent on one side of the small intestine, and this side was used for performing mesenteric vein and artery catheterisation. The jejunal vein was catheterised and the tip of the catheter advanced so that it lay in the cranial mesenteric vein, immediately distal to the junction between the mesenteric and splenic veins. Ligatures (Ethibond Excel 3-0, Ethicon, National Veterinary Supplies Ltd, Palmerston North, New Zealand) were tied loosely distal and central to the intended site of incision. The distal suture was tied off and a small incision was made in the blood vessel. Gentle pressure was used to minimise bleeding until the sampling catheter was inserted and advanced into the cranial mesenteric vein. The tip of the catheter (10-12 cm from incision site) was palpitated within the cranial mesenteric vein just distal to the junction with the splenic vein in order to ensure the catheter did not lie in another branch of the arcade or in the portal vein. The central ligature was then tied off just in front of the catheter cuff after tip and catheter placement was confirmed. This catheter was identified as the sampling catheter. Another jejunal vein was located distal to the sampling catheter and the procedure described above was repeated for the insertion of the infusion catheter. The tip of the infusion catheter was at least a 10 cm distal to the tip of the sampling catheter. The catheters were anchored using nonresporbable suture (Braun Supramid size 3) attached to the cuff, and care was taken not to puncture the intestine or any blood vessels during the procedure.

The procedure for catheterisation of the mesenteric artery was similar to that for the mesenteric vein. After inserting approximately 20 cm of catheter into the vessel, the visceral tissue was palpitated to confirm the direction of the catheter in the mesenteric artery towards the caudal aorta rather than towards the distal portions of the mesenteric arterial system.

When all the catheters were correctly positioned, the intestines were rinsed with warm sterile saline to remove blood clots. A loop of each catheter was left inside the abdominal cavity in order to minimise the risk of gut strangulation.

B.1.4 Cannulation of the abomasum

A cannula was also inserted into the abomasum. Briefly, once all the splanchnic and mesenteric catheters were in place the animal was placed in a left lateral recumbency. The pylorus and abomasum were brought through the incision for the insertion of the cannula. The organs were maintained warm and moist by keeping the tissues covered with sterile towels that were replaced regularly.

A double purse string (Vicryl 00; National Veterinary Supplies Ltd, Palmerston North, New Zealand) was sutured into the abomasum and a 3cm incision made in the centre of the purse string. The cannula was inserted through the incision taking care to avoid contamination of the abdominal cavity with abomasal digesta. The purse string was tightened and tied off. A perforated plastic washer (4.5 cm diameter) was placed over the barrel of the cannula to speed up tissue adhesion. The abomasum was returned to the abdominal cavity and a brass stopper and wire was screwed into the cannula to prevent leakage of abomasum contents. A small (10-15 mm diameter) section of skin was removed about 2-3 cm caudal to the last rib and blunt dissection was used to access the abdominal cavity enabling the cannula to be drawn through the incision.

After the surgery, the brass stopper was removed and replaced with the cannula stopper. A washer was placed over the cannula and the barrel was held in place with rubber elastrator bands (used to dock lambs). Antibiotic powder was applied to the wounds to prevent infection and the lamb was returned to its metabolism crate. Once the lambs had recovered from the anaesthesia they were returned to a temperature controlled room (18°C) and offered a small amount of water and Lucerne pellets and chaffed hay.

The day following surgery the lambs were offered full feed and water rations. Rectal temperature was monitored and an intramuscular injection of 1% procaine penicillin (Bomacillin, Bomac Laboratories Ltd., Auckland, New Zealand) was given for 4 d post-surgery. A visual inspection of the exteriorisation site was done daily, with regular applications of antibiotic powder given. Catheters were checked for patency every 14 d

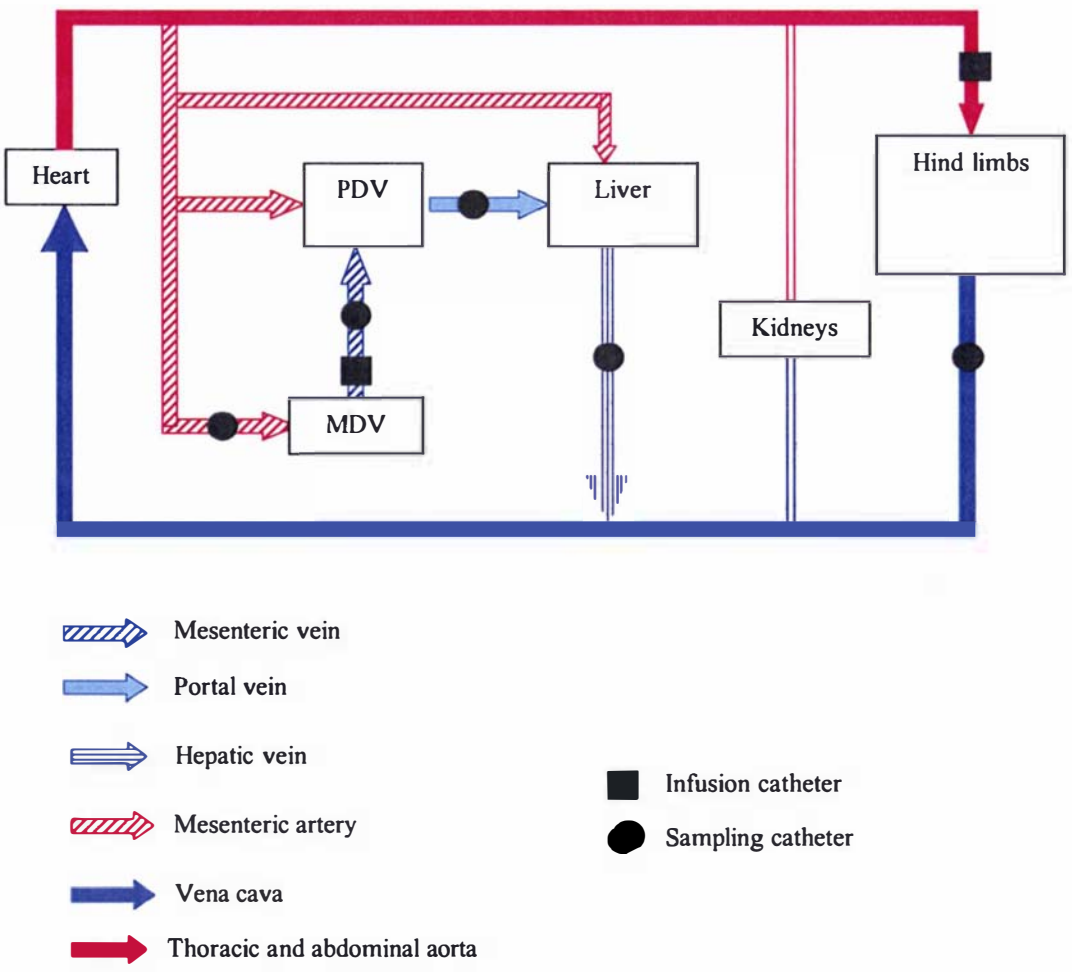
(see section B.2.2). Feed intakes and visual inspection of faeces were monitored daily through out the experimental period.

B.1.5 References

Huntington GB, Reynolds CK & Stroud BH (1989) Techniques for measuring blood flow in splanchnic tissues of cattle. *Journal of Dairy Science* **72**, 1583-1595.

Ortigues I, Durand D & Lefaivre J (1994) Use of para-amino hippuric acid to measure blood flows through portal-drained-viscera, liver and hindquarters in sheep. *Journal of Agricultural Science, Cambridge* **122**, 299-308.

Figure B.1 Picture of cathether placement for infusions and blood sampling



B.2. Catheter construction and verification of patentcy

B.2.1 Catheter construction

Catheters were constructed from Tygon microbore tubing as described below:

1) Cut 6m lengths of Tygon microbore tubing (ID 0.040"; OD 0.070"; Wall 0.015"; Scientific Supplies Ltd, Wellington, New Zealand) and fold in the middle. Knot both ends. This will make two catheters.
2) Put catheters in a 2% Triton mix (see Section B.2.1.1) in a suitable container and leave on agitator overnight.
3) Rinse the catheters as thoroughly as possible with deionised water. Note – not all the bubbles will be removed.
4) Add 1% Bomacillin solution (see Section B.2.1.2) to the container and leave the catheters soaking overnight whilst agitating.
5) Remove catheters from Bomacillin solution and hang to dry (usually 1 d is sufficient). Make sure the drying area is one that is not disturbed too often.
6) Make cuffs (see Section B.2.1.3 for details).
7) Cut the knot at the ends of the catheters and mark 30 cm from the catheter end that will be inserted into the blood vessel.
8) Insert cuffs using a drop of Bomacillin as a lubricant (Figure B.2).
9) Leave overnight to dry.
10) Add a drop of glue in-between the cuff and the catheter.
11) Leave overnight to dry.
12) Cut the catheter in half (i.e., so each catheter is about 3 m long and has a cuff attached).
13) Insert drawing up needle (18 gauge) to the "no-cuff" end.
14) Place a number of catheters into a measuring cylinder and add approximately 3 mL of Tridodecylmethyammonium chloride heparin (TDMAC; Polysciences Inc, Warrington, PA, US).
15) Using a 20 mL syringe draw the TDMAC heparin into the catheter in order to coat the inside of the catheter.

16) Leave the TDMAC heparin complex to drain out of the catheter by gravity and then use a syringe to "blow" the remaining TDMAC heparin complex out of the catheter with a empty 20 mL syringe.
17) Leave to dry.
18) Sterilise.

B.2.1.1 2% Triton

The 2% Triton (X-100; BDH Chemicals) was made as described below:

1) Tare 2 L bottle.
2) Add 40 g of Triton (using pipette tip that has been cut off).
3) Make up to 2000 g with deionised water.
4) Leave to mix in a tub of hot water.

B.2.1.2 1% Bomacillin

A 1% Bomacillian mixture was made using 1% procaine penicillin (Bomacillin, Bomac Laboratories Ltd., Auckland, New Zealand) as described below:

1) Tare 2 L bottle.
2) Add 20 g of Bomacillin.
3) Make up to 2000 g with deionised water.
4) Mix.

B.2.1.3 Making cuffs

Cuffs were used to attach the catheter to the wall of the blood vessel as described below (Figure B.2):

1) Cut approximately 8 mm of peristalic pump tubing (Green/Green Tygon tubing; 2.0 mL min^{-1}; 0.073" ID; A.I. Scientific Ltd., Auckland, New Zealand).
2) Thread the suture (Braun supramid suture; size 3 metric; National Veterinary Supplies, Palmerston North, New Zealand) through the tubing and back out the same side (see diagram) using a size 16 curved needle.
3) Cut a 15 cm suture.

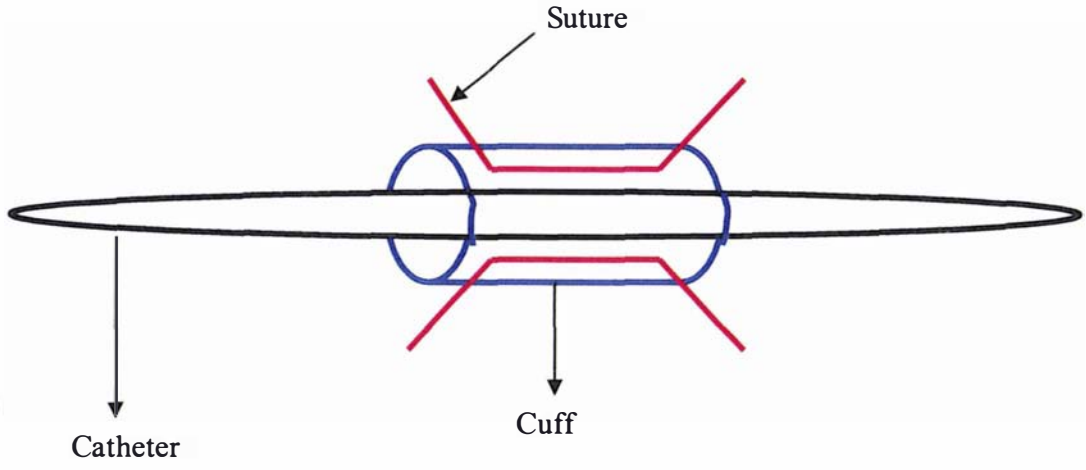
4) Repeat for the opposite side.

B.2.1.4 Heparinised saline

Heparinised saline was made according to the following steps:

1) Check batch of heparin for concentration of heparin (in iu mg^{-1}). Use this figure to calculate how much heparin is required to make the heparinised saline (i.e., 200 iu mL^{-1}; 50 iu mL^{-1} etc.).
2) Tare beaker.
3) Add the required amount of heparin.
4) Add 3 mL of Bomacillin.
5) Add sterile saline to 200 g (will give a final volume of 200 mL).
6) Add to stirrer plate until the heparin is totally dissolved.
7) Filter into a sterile bottle using a 35 mL syringe (use a small needle to let out air pressure inside the bottle).
8) Label with concentration and date of preparation.

Figure B.2 Cuff design and placement through the catheter.



B.2.2 Verification of the catheter patency for an arterial and venous vessels

This procedure applies to Tygon® catheter coated with the TDMAC heparin complex. Generally, these catheters can be closed by knotting the end twice (be sure that the knots are very tight). The extheriozation site should be checked regularly.

Instruments and solutions needed:

- Forceps covered with plastic tubing
- Heparin 50 iu mL^{-1} (always check if the solution is contaminated with bacteria)
- Heparin 200 iu mL^{-1} (always check if the solution is contaminated with bacteria)
- 2-3 mL syringes
- Swabs
- Container to warm up the heparinised saline
- Scissors
- Coloured tapes (to identify the type of catheter after checking patency)

Verification should be indicated in the experimental book with this minimal information:

- Animal number
- Date
- Type of vessel
- Able to infuse freely
- Able to withdraw freely (arterial only) or with a syringe

1) Frequency of verification is every fortnight unless there is blood visible in the catheter exiting on the back.
2) It is recommended to place the bottle (or bag) of heparanised saline into warm water until the solution reaches body temperature before infusing the heparin solution in the vessel.

3) Fill some syringes with normal strength heparin (50 iu mL^{-1}) for checking the catheter and some syringes with strong heparin (200 iu mL^{-1}) for leaving in the catheter.
4) Generally, 1.5 mL of solution is required for flushing the whole length of the catheter.
5) Clamp the catheter with a forceps just in front of the knots (be sure that you used forceps covered with plastic tubing otherwise you may damage the catheter).
6) Just cut the extremities of the catheters (between the forceps and first knot).
7) Place an 18 gauge cut off needle in the end of the cut of the catheter.
8) Remove the forceps and see if the blood is coming by itself (usually happen only with arterial). If so, just let the old blood (dark; generally soft clots) come out completely on a swab until the colour of the blood is a bright red.
9) If the blood is not coming by itself (arterial and venous catheters), flush slowly 0.3 ml of heparinised saline (normal strength = 50 iu mL^{-1}) and then check if the blood is coming by itself (usually arterial) or after withdrawing with a syringe (venous catheter). If so, proceed as 11). **Never infuse air in any catheter especially in an arterial vessel.**
10) If not, flush 1 mL of heparinised saline and try to withdraw gently with the 2-3 mL syringe.
11) If this is not working, check if the heparin solution in the catheter is moving down against gravity when the extremity of the catheter is move upward. Record the results in the book.
12) Clamp the catheter with the forceps to stem the flow of blood.
13) Place a syringe filled with strong heparin onto the catheter and remove the forceps.
14) Flush the catheter with 3 mL of the strong heparin (2000 iu mL^{-1}).
15) Clamp the catheter (leave enough free catheter to tie the knots) while pushing the last mL of heparinised saline and tie the catheter with two knots very tightly.

B.3 Chromatography for determination of amino acid concentrations in plasma and tissue free pool samples.

Derivatised plasma and tissue free pool samples (50 μL) were injected onto a Picotag C_{18} reverse phase column in an oven set to 46°C, with a 90 min run time between each injection. The elution system consisted of 2 mobile phases, buffer A (70 mM sodium acetate.3 H_2O adjusted to pH 6.5 using glacial acetic acid, containing 1.8% acetonitrile and 2.5 μL of EDTA) and buffer B (15% aqueous methanol, containing 45% acetonitrile). Buffer B was run in a gradient starting with an increase from 0% at 13.5 min to 0.5% at 13.51 min, followed by an increase with a number 3 curve to 2% at 24 min, then by a linear increase to 6% at 30 min, then a -2 curve to 23.5% at 50 min, and finally a linear increase to 36% at 62 min. Buffer B remained at 36% until 70 min when the gradient ended in a wash step of 100% B to remove residual sample from the column. The flow rate was set to 1.0 mL min^{-1}. Separated AA were detected by a UV detector set at 254 nm.

B.4 Amino acid fluxes through the digestive tract

Table B.1 Amino acid intake (g d^{-1}) in lambs fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite free controls. Results are presented as LSmeans and associated pooled standard deviation (SD).

		Control n=6	Parasite n=5	Pooled SD	P
Non-essential	Alanine	10.8	10.3	0.6	0.30
	Aspartate	25.4	24.3	1.5	0.30
	Glutamate	20.2	19.4	1.1	0.29
	Glycine	9.2	8.8	0.5	0.30
	Proline	9.3	8.9	0.5	0.29
	Serine	11.2	10.7	0.6	0.29
	Tyrosine	6.8	6.5	0.4	0.30
Essential	Arginine	8.6	8.2	0.5	0.30
	Histidine	3.9	3.8	0.2	0.30
	Isoleucine	6.4	6.2	0.4	0.29
	Leucine	14.3	13.6	0.8	0.30
	Lysine	11.2	10.7	0.6	0.30
	Methionine	2.5	2.3	0.1	0.28
	Phenylalanine	8.7	8.3	0.5	0.30
	Threonine	7.7	7.3	0.4	0.30
	Valine	7.7	7.3	0.4	0.30

Table B.2 The flux of amino acids through the abomasum (g d^{-1}) of lambs fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite free controls. Results are presented as LSmeans and associated pooled standard deviation (SD).

		Control n=6	Parasite n=5	Pooled SD	P
Non-essential	Alanine	6.8	7.3	0.3	0.06
	Aspartate	11.9	12.7	0.5	0.03
	Glutamate	12.4	13.4	0.6	0.03
	Glycine	6.1	6.5	0.2	0.02
	Proline	4.7	4.8	0.3	0.73
	Serine	5.9	6.5	0.2	0.01
	Tyrosine	4.9	5.2	0.3	0.08
Essential	Arginine	4.8	5.7	0.7	0.10
	Histidine	2.2	2.3	0.1	0.05
	Isoleucine	5.9	6.0	0.2	0.44
	Leucine	8.2	9.0	0.4	0.02
	Lysine	7.2	7.5	0.3	0.15
	Methionine	1.8	1.8	0.1	0.31
	Phenylalanine	5.2	5.7	0.2	0.02
	Threonine	5.9	6.2	0.3	0.09
	Valine	5.0	5.2	0.3	0.35

Table B.3 The flux of amino acids through the ileum (g d^{-1}) of lambs fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite free controls. Results are presented as LSmeans and associated pooled standard deviation (SD).

		Control n=6	Parasite n=5	Pooled SD	P
Non-essential	Alanine	2.6	2.7	0.6	0.81
	Aspartate	4.3	4.4	0.8	0.82
	Glutamate	4.9	5.0	1.0	0.92
	Glycine	2.7	2.8	0.5	0.79
	Proline	2.0	2.1	0.3	0.57
	Serine	2.7	2.8	0.5	0.71
	Tyrosine	1.7	1.8	0.4	0.77
Essential	Arginine	1.3	1.4	0.3	0.90
	Histidine	0.9	0.9	0.2	0.69
	Isoleucine	3.2	3.3	0.7	0.91
	Leucine	2.9	3.1	0.6	0.76
	Lysine	2.3	2.3	0.5	0.87
	Methionine	0.6	0.6	0.1	0.84
	Phenylalanine	1.5	1.6	0.7	0.86
	Threonine	2.4	2.6	0.6	0.68
	Valine	1.8	2.0	0.4	0.72

Table B.4 Apparent absorption of amino acids (g d^{-1}) in the small intestine of lambs fed Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite free controls. Results are presented as LSmeans and associated pooled standard deviation (SD).

		Control n=6	Parasite n=5	Pooled SD	P
Non-essential	Alanine	4.1	4.3	0.7	0.68
	Aspartate	7.6	8.1	1.0	0.53
	Glutamate	7.5	8.2	1.1	0.43
	Glycine	3.4	3.6	0.5	0.56
	Proline	2.7	2.5	0.3	0.38
	Serine	3.3	3.5	0.5	0.50
	Tyrosine	3.2	3.3	0.5	0.73
Essential	Arginine	3.4	3.6	0.4	0.37
	Histidine	1.3	1.4	0.2	0.76
	Isoleucine	2.7	2.8	0.6	0.89
	Leucine	5.3	5.7	0.6	0.45
	Lysine	4.9	5.1	0.6	0.70
	Methionine	1.2	1.2	0.2	0.72
	Phenylalanine	3.6	3.9	0.6	0.54
	Threonine	3.5	3.5	0.5	0.99
	Valine	3.2	3.2	0.6	0.99

Table B.5 Digestibility (as a proportion of feed intake) of amino acids in the small intestine of lambs fed Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite free controls. Results are presented as LSmeans and associated pooled standard deviation (SD).

		Control n=6	Parasite n=5	Pooled SD	P
Non-essential	Alanine	0.61	0.60	0.09	0.99
	Aspartate	0.63	0.64	0.07	0.88
	Glutamate	0.60	0.61	0.08	0.81
	Glycine	0.55	0.55	0.08	0.99
	Proline	0.57	0.53	0.05	0.36
	Serine	0.55	0.55	0.08	0.94
	Tyrosine	0.64	0.64	0.08	0.97
Essential	Arginine	0.71	0.72	0.07	0.87
	Histidine	0.60	0.59	0.07	0.88
	Isoleucine	0.46	0.46	0.13	0.99
	Leucine	0.64	0.65	0.07	0.94
	Lysine	0.67	0.67	0.07	0.93
	Methionine	0.65	0.67	0.09	0.78
	Phenylalanine	0.70	0.71	0.14	0.97
	Threonine	0.58	0.56	0.09	0.76
	Valine	0.62	0.61	0.09	0.80

B.5 Plasma amino acid data

B.5.1 Plasma amino acid concentration

Table B.6 The concentration of amino acids in the mesenteric vein ($\mu mol\ L^{-1}$) of lambs fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite free controls. Results are presented as LSmeans and associated pooled standard deviation (SD).

		Control n=6	Parasite n=5	Pooled SD	P
Non-essential	Alanine	119	88	44	0.33
	Asparagine	62	39	29	0.28
	Aspartate	11	8	3	0.23
	Carnithine*	22	24	5	0.63
	Citrulline*	153	133	46	0.54
	Glutamine	261	263	55	0.97
	Glutamate	21	21	5	0.83
	Glycine	268	280	80	0.84
	Hydoxy proline	15	15	2	0.64
	Ornithine*	59	71	23	0.46
	Proline	102	81	34	0.42
	Serine	71	54	34	0.48
	Taurine*	35	58	32	0.39
Essential	Arginine	142	134	49	0.81
	Histidine	42	41	11	0.90
	Isoleucine	89	72	25	0.35
	Leucine	146	110	42	0.26
	Lysine	106	114	39	0.79
	Methionine	48	38	15	0.35
	Phenylalanine	55	38	18	0.21
	Threonine	86	76	47	0.76
	Tyrosine	67	49	22	0.29
	Valine	267	245	52	0.56

*Not included in Total AA

Table B.7 The concentration of amino acids in the portal vein (μmol L^{-1}) of lambs fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite free controls. Results are presented as LSmeans and associated pooled standard deviation (SD).

		Control n=6	Parasite n=5	Pooled SD	P
Non-essential	Alanine	91	111	17	0.15
	Asparagine	42	43	11	0.87
	Aspartate	8	10	3	0.54
	Carnithine*	21	22	6	0.75
	Citrulline*	141	133	35	0.75
	Glutamine	271	267	49	0.91
	Glutamate	22	22	7	0.97
	Glycine	236	291	58	0.21
	Hydoxy proline	16	16	2	0.87
	Ornithine*	58	69	23	0.48
	Proline	78	83	17	0.64
	Serine	48	61	11	0.11
	Taurine*	35	58	36	0.36
Essential	Arginine	137	161	42	0.42
	Histidine	37	43	7	0.28
	Isoleucine	67	75	5	0.07
	Leucine	109	119	12	0.30
	Lysine	84	123	24	0.06
	Methionine	39	41	7	0.64
	Phenylalanine	41	42	5	0.67
	Threonine	66	73	22	0.64
	Tyrosine	51	53	10	0.76
	Valine	234	253	25	0.29

*Not included in Total AA

Table B.8 The concentration of amino acids in the hepatic vein (μmol L^{-1}) of lambs fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite free controls. Results are presented as LSmeans and associated pooled standard deviation (SD).

		Control n=6	Parasite n=4	Pooled SD	P
Non-essential	Alanine	72	88	20	0.29
	Asparagine	35	39	14	0.72
	Aspartate	8	9	4	0.68
	Carnithine*	22	24	7	0.59
	Citrulline*	140	144	42	0.91
	Glutamine	250	251	49	0.97
	Glutamate	28	31	10	0.69
	Glycine	211	277	62	0.19
	Hydoxy proline	14	15	2	0.45
	Ornithine*	59	69	24	0.59
	Proline	71	81	19	0.50
	Serine	38	54	15	0.21
	Taurine*	33	57	36	0.38
Essential	Arginine	120	133	44	0.69
	Histidine	34	40	8	0.42
	Isoleucine	63	77	10	0.11
	Leucine	103	118	15	0.24
	Lysine	78	115	22	0.06
	Methionine	32	39	6	0.16
	Phenylalanine	33	37	7	0.37
	Threonine	62	74	29	0.59
	Tyrosine	45	50	12	0.55
	Valine	228	257	27	0.19

*Not included in Total AA

Table B.9 The concentration of amino acids in the mesenteric artery (μmol L^{-1}) of lambs fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite free controls. Results are presented as LSmeans and associated pooled standard deviation (SD).

		Control n=6	Parasite n=5	Pooled SD	P
Non-essential	Alanine	74	83	16	0.40
	Asparagine	33	37	8	0.47
	Aspartate	7	8	3	0.35
	Carnithine*	23	22	7	0.76
	Citrulline*	74	78	15	0.72
	Glutamine	259	269	32	0.64
	Glutamate	55	61	14	0.55
	Glycine	220	263	49	0.23
	Hydoxy proline	15	15	1	0.45
	Ornithine*	55	63	22	0.52
	Proline	69	80	14	0.28
	Serine	37	49	9	0.10
	Taurine*	32	56	30	0.28
Essential	Arginine	112	120	32	0.73
	Histidine	30	30	18	0.98
	Isoleucine	58	68	4	0.02
	Leucine	98	111	12	0.16
	Lysine	70	97	19	0.09
	Methionine	34	36	4	0.50
	Phenylalanine	33	36	4	0.30
	Threonine	62	73	22	0.46
	Tyrosine	44	47	7	0.53
	Valine	223	252	25	0.14

*Not included in Total AA

Table B.12 Flux of amino acids across the portal-drained viscera (μmol min^{-1}) in lambs fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD). Positive values represent a net utilisation by the tissue, while a negative value represents a net release.

		Control n=6	Parasite n=5	Pooled SD	P
Non-essential	Alanine	-15.5	-28.2	15.3	0.27
	Asparagine	-11.7	-8.5	8.7	0.60
	Aspartate	-2.6	0.1	5.4	0.50
	Carnithine*	2.1	2.8	4.6	0.83
	Citrulline*	-84.2	-69.1	62.8	0.74
	Glutamine	-17.8	-3.2	43.2	0.64
	Glutamate	41.8	35.4	22.9	0.69
	Glycine	-22.7	-34.2	30.4	0.60
	Hydoxy proline	-1.8	-0.9	3.1	0.69
	Ornithine*	-4.1	-6.7	15.5	0.81
	Proline	-12.4	-6.9	12.2	0.53
	Serine	-14.7	-17.3	7.8	0.64
	Taurine*	-3.0	-4.9	7.8	0.73
Essential	Arginine	-21.0	-40.2	23.8	0.28
	Histidine	-9.8	-11.5	14.8	0.87
	Isoleucine	-11.3	-9.8	8.9	0.82
	Leucine	-15.1	-10.2	11.5	0.56
	Lysine	-18.7	-35.6	19.3	0.24
	Methionine	-6.5	-7.0	6.7	0.90
	Phenylalanine	-9.8	-7.7	6.5	0.64
	Threonine	-6.9	-3.9	8.3	0.62
	Tyrosine	-9.7	-8.2	7.3	0.78
	Valine	-9.7	-8.0	11.4	0.84

*Not included in Total AA

Table B.13 Flux of amino acids across the liver (μmol min^{-1}) in lambs fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD). Positive values represent a net utilisation by the tissue, while a negative value represents a net release.

		Control n=6	Parasite n=4	Pooled SD	P
Non-essential	Alanine	19.4	39.4	11.4	0.10
	Asparagine	8.2	7.6	4.5	0.86
	Aspartate	1.0	4.1	1.0	0.02
	Carnithine*	-1.4	-3.0	2.8	0.50
	Citrulline*	-0.8	-12.3	9.6	0.21
	Glutamine	28.0	16.1	27.6	0.63
	Glutamate	-3.2	0.7	10.5	0.68
	Glycine	32.6	30.8	20.8	0.92
	Hydoxy proline	2.3	1.6	2.0	0.70
	Ornithine*	-2.7	-5.3	6.2	0.63
	Proline	9.1	6.2	6.1	0.58
	Serine	12.0	15.6	7.5	0.56
	Taurine*	2.4	4.6	3.4	0.47
Essential	Arginine	8.4	31.4	21.1	0.25
	Histidine	2.8	-1.7	7.5	0.50
	Isoleucine	4.8	1.0	5.5	0.45
	Leucine	7.5	2.2	8.4	0.48
	Lysine	7.0	10.5	19.5	0.84
	Methionine	8.3	1.7	4.5	0.14
	Phenylalanine	9.8	8.9	2.4	0.66
	Threonine	4.9	3.4	8.2	0.84
	Tyrosine	8.2	5.4	4.3	0.47
	Valine	2.2	0.8	4.2	0.71

*Not included in Total AA

Table B.14 Flux of amino acids across the total splanchnic tissues (μmol min^{-1}) in lambs fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD). Positive values represent a net utilisation by the tissue, while a negative value represents a net release.

		Control n=6	Parasite n=4	Pooled SD	P
Non-essential	Alanine	3.9	11.2	9.7	0.20
	Asparagine	-3.5	-0.9	8.6	0.49
	Aspartate	-1.6	4.2	4.9	0.61
	Carnithine*	0.7	-0.2	7.7	0.37
	Citrulline*	-85.0	-81.4	74.9	0.69
	Glutamine	10.2	12.9	39.8	0.68
	Glutamate	38.6	36.1	4.2	0.15
	Glycine	9.9	-3.4	36.6	0.36
	Hydoxy proline	0.5	0.7	2.5	0.87
	Ornithine*	-6.8	-12	37.1	0.61
	Proline	-3.3	-0.7	10.3	0.73
	Serine	-2.7	-1.7	0.9	0.12
	Taurine*	-0.6	-0.3	5.5	0.22
Essential	Arginine	-12.6	-8.8	23.7	0.29
	Histidine	-7	-13.2	19.8	0.28
	Isoleucine	-6.5	-8.8	3.7	0.13
	Leucine	-7.6	-8	2.4	0.15
	Lysine	-11.7	-25.1	11.4	0.11
	Methionine	1.8	-5.3	6.4	0.08
	Phenylalanine	0.1	1.2	8.4	0.38
	Threonine	-2	-0.5	14.2	0.53
	Tyrosine	-1.5	-2.8	7.5	0.27
	Valine	-7.5	-7.2	9.7	0.43

*Not included in Total AA

Table B.15 Flux of amino acids across the hind limbs (μmol min^{-1}) in lambs fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD). Positive values represent a net utilisation by the tissue, while a negative value represents a net release.

		Control n=6	Parasite n=5	Pooled SD	P
Non-essential	Alanine	-3.0	-2.6	2.6	0.84
	Asparagine	0.9	1.9	1.6	0.38
	Aspartate	0.9	1.5	0.9	0.40
	Carnithine*	1.1	-0.9	2.3	0.26
	Citrulline*	-30.6	-30.7	15.8	0.99
	Glutamine	-27.9	-24.5	14.7	0.75
	Glutamate	19.9	24.6	4.0	0.13
	Glycine	-6.8	-9.6	17.5	0.82
	Hydoxy proline	-0.7	0.2	1.0	0.22
	Ornithine*	0.4	-2.0	9.2	0.72
	Proline	-1.4	0.8	2.2	0.29
	Serine	3.0	3.3	1.3	0.79
	Taurine*	-0.4	0.3	3.1	0.78
Essential	Arginine	-9.9	-11.3	11.5	0.86
	Histidine	-2.9	-6.6	8.3	0.54
	Isoleucine	1.5	1.6	1.3	0.93
	Leucine	2.5	3.3	1.7	0.52
	Lysine	-2.9	-7.0	9.7	0.55
	Methionine	-0.8	-1.0	2.0	0.84
	Phenylalanine	-0.3	0.3	0.6	0.18
	Threonine	1.2	1.8	4.6	0.86
	Tyrosine	-2.7	0.0	1.1	0.75
	Valine	5.1	7.2	8.1	0.71

*Not included in Total AA

APPENDIX C

C.1 Tissue extraction method

Weigh 1g of tissue into a pre-weighed tube, and record weight. Add 5 volumes of extraction Buffer (**1**), and record weight. Homogenise, and then centrifuge (4°C, Sorvall SM24, 15000 rpm, 30 min). This will produce a supernatant (free pool) and a pellet (protein-bound). This is shown diagrammatically in Figure C.1.

C.1.1 Free Pool AA

Into a pre-weighed tube (round bottom) weigh 2mL of supernatant. Add 1mL SDS/EDTA (**4**) and re-weigh, then add 0.2 mL DDT (**6**) and re-weigh. Add 0.1 mL Norleucine (**8**) and re-weigh. Mix with vortex and leave at room temperature for 15 min, then add 1mL of 30% TCA (**9**) and re-weigh. Centrifuge (4°C, 4000 rpm, 15 min) and filter resulting supernatant into a 4 mL vial.

C.1.2 Protein-bound AA

Wash pellet in extraction buffer (**1**) by adding 5 mL and mixing. Centrifuge (4°C, Sorvall SM24, 28000 g, 30 min) and discard resulting supernatant. Weigh pellet into pre-weighed 4 mL vial and freeze-dry.

C.1.3 Solutions:

1) TISSUE EXTRACTION BUFFER; 20 mM Tris pH 7.8; 2.5 mM EDTA; 0.3% SDS

Tris	1.211 *g*
EDTA	0.23 g
SDS	0.75 g
PMSF (**3**)	5 ml
H_2O	7.5 ml

Make to 200 mL with DI water. Adjust pH to 7.8 with HCl and adjust final volume to 250 mL with DI water.

2) 0.1 M DTT in 20 mM Tris pH 7.8

DTT	0.77 g

Dissolve in a final volume of 50 mL of 20 mM Tris pH 7.8

DTT is an anti-oxidant (especially for the sulphur AA)

3) Phenylmethylsulfonylfluoride (PMSF)
PMSF 500 mg
Dissolve in 50 mL isopropanol (propan-2-ol). Store in the freezer.

PMSF is TOXIC; it inactivates most enzymes

4) SDS/EDTA 0.75%/9 mM
SDS 1.88 g
EDTA 0.84 g
Dissolve in a total of 250 mL DI water.

EDTA is a complexing agent and soaks up metal ions.
SDS is a detergent and unravels the secondary structure of proteins.

5) 20 mM Tris pH 7.8
Tris 1.211 g
Dissolve in 200 mL of DI water. Adjust pH to 7.8 with concentrated HCl and adjust final volume to 250 mL.

6) 50 mM DTT in 20 mM Tris pH 7.8
DTT 0.39 g
Dissolve in a final volume of 50 ml of 20 mM Tris pH 7.8.

7) Phenol 0.1%
Liquid Phenol 0.1 mL
In 99.9 ml DI water.

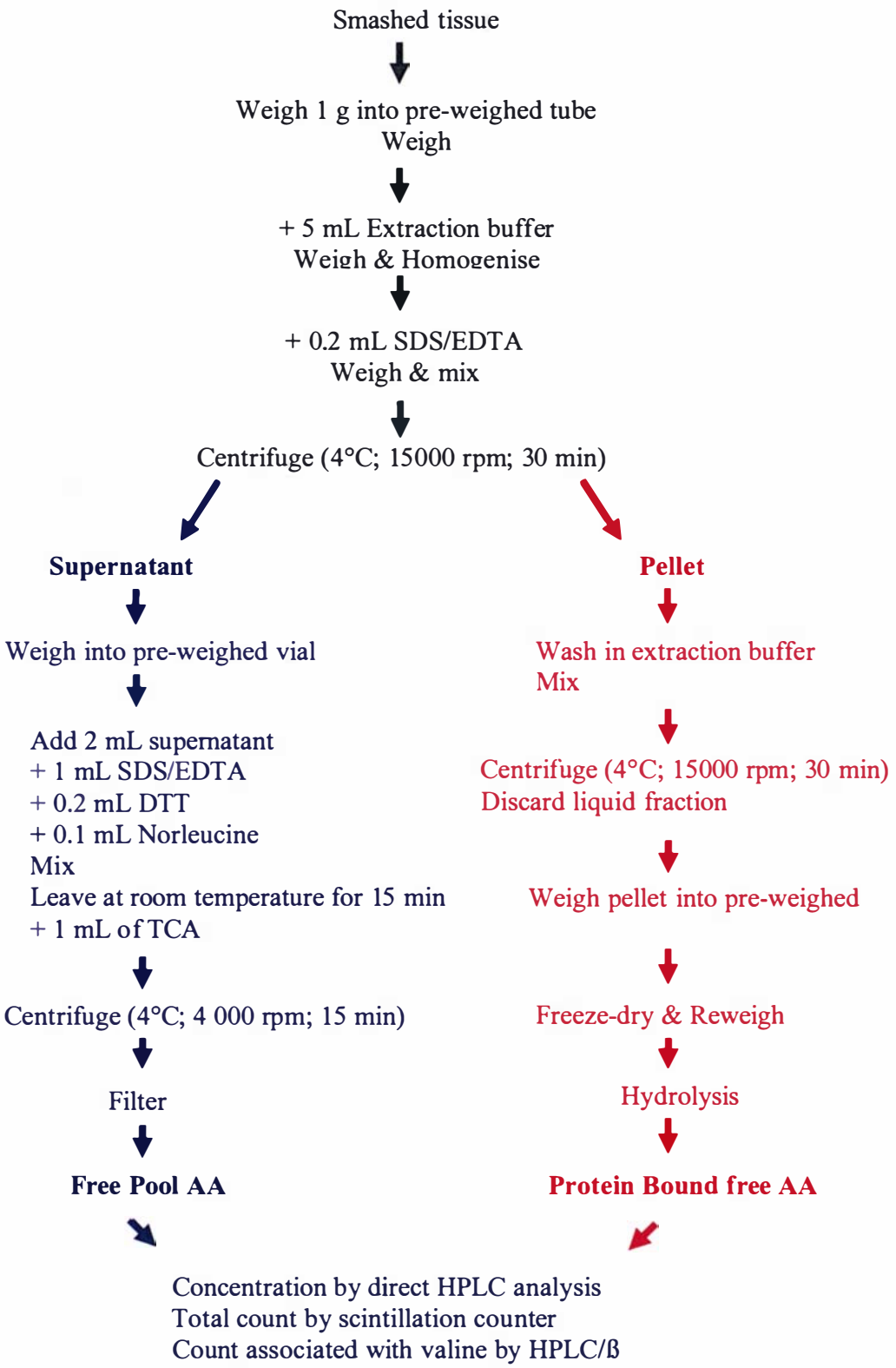
Phenol is used as an antibacterial agent and is TOXIC (nerve damage).

8) 3 mM Norleucine
Norleucine 19.68 mg
0.1 % Phenol 50 mL
Record weights.

9) 30% TCA
TCA 30 g
DI water 70 g

TCA precipitates protein.

Figure C.1 Tissue extraction



C.2 Estimation of protein fractional synthesis rates

The FSR of constitutive proteins is usually calculated using the equation from Waterlow *et al.*, (1978; Equation C.1)

Equation C.1

$$\frac{\text{SRA valine}_{(\text{protein bound})}}{\text{SRA valine}_{(\text{precursor pool})}} = \left(\frac{\lambda_i}{\lambda_i - \text{FSR}}\right) * \left(\frac{1 - e^{-\text{FSR}t}}{1 - e^{-\lambda i^t}}\right) - \left(\frac{\text{FSR}}{\lambda_i - \text{FSR}}\right)$$

where λ_i is the rate-constant describing the rise to plateau of SRA of free valine in the precursor pool. Usually λ_i is estimated from intermittent plasma samples taken at frequent intervals during the rise to plateau (e.g., 0, 5, 10, 15, 20, 25, 30, 50 min and hourly afterwards) until the completion of the infusion (Baracos *et al.*, 1991).

C.2.1 References

Baracos VE, Brun-Bellut J & Marie M (1991) Tissue protein synthesis in lactating and dry goats. *British Journal of Nutrition* **66**, 451-465.

Waterlow JC, Garlick PJ & Millward DJ (1978) Measurement of the rate of incorporation of labelled amino acids into tissue proteins. In *Protein turnover in mammalian tissues and in the whole body*, pp. 339-370 [JC Waterlow, PJ Garlick and DJ Millward, editors]. Amsterdam: North-Holland Biomedical press.

C.3 Amino acid concentrations

C.3.1 Free pool amino acid concentration

Table C.1 Amino acid concentrations (μmol L^{-1}) in the intracellular pool of whole duodenum tissue in lambs fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=5	Parasite n=4	Pooled SD	P
Non essential	Carnithine*	27.7	22.5	4.6	0.20
	Citrulline*	76.5	45.1	15.2	0.05
	Ornithine*	8.7	9.3	2.2	0.73
	Taurine*	548.3	337.1	199.6	0.23
	Alanine	222.2	145.3	55.5	0.13
	Asparagine	60.3	50.0	12.9	0.35
	Glutamine	261.3	225.6	53.2	0.43
	Glycine	570.5	517.0	146.1	0.66
	Hydroxyl-proline	29.8	26.5	10.0	0.69
	Proline	83.9	50.5	37.8	0.31
	Serine	148.6	112.6	33.1	0.22
Essential	Arginine	182.3	141.9	50.5	0.35
	Histidine	24.6	18.0	4.7	0.12
	Isoleucine	33.4	27.0	8.2	0.36
	Leucine	94.7	62.3	28.8	0.21
	Lysine	226.1	156.9	69.1	0.25
	Methionine	42.7	27.5	13.1	0.19
	Phenylalanine	52.7	41.0	16.1	0.39
	Threonine	88.7	62.6	23.9	0.22
	Tyrosine	51.6	41.2	17.8	0.48
	Valine	88.5	63.9	14.8	0.09

*not included in totals

Table C.2 Amino acid concentrations (μmol L^{-1}) in the intracellular pool of whole ileal tissue in lambs fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=5	Parasite n=4	Pooled SD	P
Non essential	Carnithine*	2847.8	2106.8	841.9	0.31
	Citrulline*	97.4	58.5	27.3	0.13
	Ornithine*	21.4	19.0	11.3	0.80
	Taurine*	1433.5	1006.7	507.9	0.33
	Alanine	415.3	310.6	45.1	0.03
	Asparagine	26.3	23.7	9.7	0.74
	Glutamine	1204.3	1210.0	356.8	0.98
	Glycine	292.3	203.2	50.1	0.07
	Hydroxyl-proline	15.3	15.2	5.3	0.97
	Proline	57.9	48.6	5.6	0.08
	Serine	66.7	59.9	7.3	0.28
Essential	Arginine	96.2	76.0	22.4	0.30
	Histidine	35.4	20.2	7.3	0.04
	Isoleucine	24.7	23.5	5.6	0.78
	Leucine	43.0	28.2	8.5	0.08
	Lysine	41.1	31.8	10.0	0.29
	Methionine	12.2	9.6	3.9	0.41
	Phenylalanine	14.4	10.3	2.9	0.13
	Threonine	61.8	48.1	7.7	0.07
	Tyrosine	21.1	16.9	3.4	0.17
	Valine	72.9	60.1	12.3	0.24

*not included in totals

Table C.3 Amino acid concentrations (μmol L^{-1}) in the intracellular pool of the mesenteric lymph nodes in lambs fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=5	Pooled SD	P
Non essential	Carnithine*	34.1	19.6	10.0	0.22
	Citrulline*	94.8	140.4	43.4	0.29
	Ornithine*	52.7	10.5	35.9	0.11
	Taurine*	580.8	740.0	191.4	0.39
	Alanine	97.5	110.9	59.7	0.81
	Asparagine	47.0	57.3	25.6	0.67
	Glutamine	253.6	326.2	71.5	0.31
	Glycine	520.8	641.2	113.3	0.29
	Hydroxyl-proline	16.6	21.1	6.5	0.47
	Proline	48.0	72.4	21.7	0.26
	Serine	56.3	74.6	35.9	0.59
Essential	Arginine	65.1	85.9	23.4	0.36
	Histidine	56.1	7.3	36.1	0.11
	Isoleucine	38.0	28.4	7.7	0.23
	Leucine	55.3	92.5	27.2	0.19
	Lysine	399.9	54.4	171.3	0.08
	Methionine	24.5	26.0	4.3	0.71
	Phenylalanine	19.6	35.6	9.3	0.12
	Threonine	58.5	27.0	11.9	0.07
	Tyrosine	39.2	37.2	8.0	0.79
	Valine	57.0	96.1	21.6	0.11

*Not included in totals.

C.3.2 Protein bound amino acid concentrations

Table C.4 Amino acid concentrations (μmol L^{-1}) in the protein bound fraction of scraped duodenal tissue in lambs fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=4	Pooled SD	P
Non essential	Alanine	48.7	52.8	9.5	0.57
	Aspartate	51.1	59.2	6.3	0.13
	Cysteine	6.0	7.2	0.8	0.12
	Glutamate	82.6	96.5	9.9	0.10
	Glycine	91.8	92.2	20.4	0.98
	Serine	27.5	31.8	3.0	0.10
Essential	Arginine	50.3	54.8	7.1	0.41
	Histidine	12.1	13.9	4.3	0.57
	Isoleucine	17.1	21.3	3.5	0.14
	Leucine	39.8	41.3	11.6	0.86
	Lysine	36.3	43.1	4.9	0.11
	Methionine	11.4	13.7	1.3	0.09
	Phenylalanine	19.9	23.6	2.3	0.07
	Threonine	23.2	28.7	2.6	0.03
	Valine	21.5	26.3	3.7	0.12

Table C.5 Amino acid concentrations (μmol L^{-1}) in the protein bound fraction of muscle in lambs fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=5	Pooled SD	P
Non essential	Alanine	54.60	56.47	5.94	0.66
	Aspartate	89.59	83.24	7.05	0.23
	Cysteine	10.51	9.62	0.62	0.12
	Glutamate	163.36	147.91	11.53	0.10
	Glycine	37.67	47.53	10.15	0.21
	Serine	39.97	38.15	2.43	0.31
Essential	Arginine	61.94	60.64	3.31	0.58
	Histidine	20.11	17.15	5.46	0.45
	Isoleucine	36.14	32.22	3.49	0.15
	Leucine	75.04	63.91	17.73	0.39
	Lysine	81.24	71.94	6.30	0.06
	Methionine	27.04	24.60	5.39	0.07
	Phenylalanine	35.28	33.44	2.22	0.27
	Threonine	44.82	41.40	3.16	0.16
	Valine	36.52	34.12	3.33	0.33

C.4 Literature comparisons

Table C.6 Fractional protein synthesis rates (% d^{-1}) reported in the literature for uninfected animal species obtained using different infusion procedures, including the precursor pool used (free pool: FP or plasma P), length of infusion (h) and amino acid used as tracer. Full details of the references can be found in Chapters Three and Six.

Precursor Pool	Species	Infusion	Duodenum		Ileum		Liver	Muscle	Skin	Spleen
			SM[1]	Whole	SM	Whole				
Free pool										
Chapter Three	**Lamb**	**C[2]; 8h; valine**	**9**	**19**	**8**	**49**	**37**	**>1**	**12**	**10**
Chapter Six	**Lamb**	**C; 8h; valine**	8	87	16	66	21	>1	8	27
Davis *et al.*, 1981	Lamb	C; 8h; leucine	.	.	.	.	15-54	3-5	12-35	.
Schaefer *et al.*, 1986	Sheep	C; 8h; leucine	.	.	.	.	21	2	.	.
Garlick *et al.*, 1980	Rat	F[3]; phenylalanine	.	.	.	.	83	16-21	.	68
Oddy & Lindsay, 1986	Lamb	C; 6h; leucine	.	.	.	.	.	4-6	.	.
Connell *et al.*, 1997	Lamb	C; 12h; phenylalanine	.	.	.	.	16	.	.	.
Baracos *et al.*, 1992	Goat	C; 8h; phenylalanine	.	107	.	.	27	4	11	38
Buttery *et al.*, 1977	Lamb	C; 7h; lysine	.	.	.	.	24	1-3	.	4
Wykes *et al.*, 1996	Pig	C; 8h; leucine	.	.	.	.	40	3-5	25	.
Lobley *et al.*, 1980	Cattle	C; 8h; tyrosine	.	.	.	.	32	2	6	.
Lobley *et al.*, 1994	Lamb	F; valine	42	.	31	.	22	.	.	.
Garlick *et al.*, 1973	Rat	C; 6h; tyrosine	.	.	.	.	50	7	.	.
Plasma										
Chapter Three	**Lamb**	**C; 8h; valine**	**9**	**54**	**5**	**18**	**17**	**>1**	**5**	**31**
Chapter Six	**Lamb**	**C; 8h; valine**	3	24	2	12	11	>1	4	5
Schaefer *et al.*, 1987	Sheep	C; 8h; leucine	.	.	.	.	12	1	.	.
Connell *et al.*, 1997	Lamb	C; 12h; phenylalanine	.	.	.	.	13	.	.	.
Baracos *et al.*, 1992	Goat	C; 8h; phenylalanine	.	21	.	.	13	2	3	9
Lobley *et al.*, 1980	Cattle	C; 8h; tyrosine	.	.	.	.	10	2	3	.

1. SM: smooth muscle, representing scraped tissue.
2. C: constant infusion.
3. F: flooding dose.

APPENDIX D

D.1 Transmembrane valine kinetics across the hind limbs

Figure D.1 ^{3}H-Valine kinetics (F; mmol d^{-1}) in the muscle of lambs fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* (P; n=5) or kept as parasite free controls (C; n=6). Results are presented as LSmeans and their associated pooled standard deviation (SD).

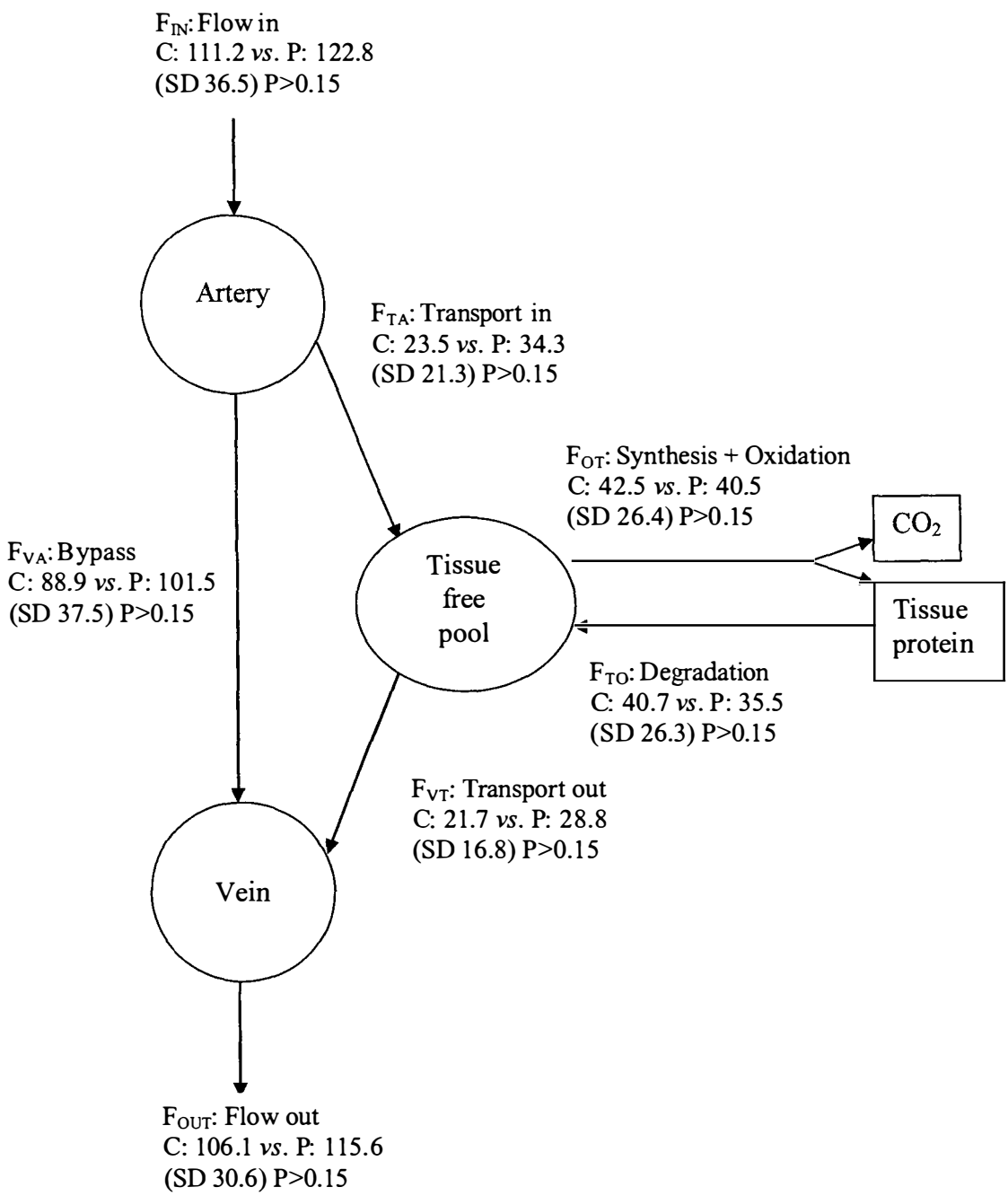
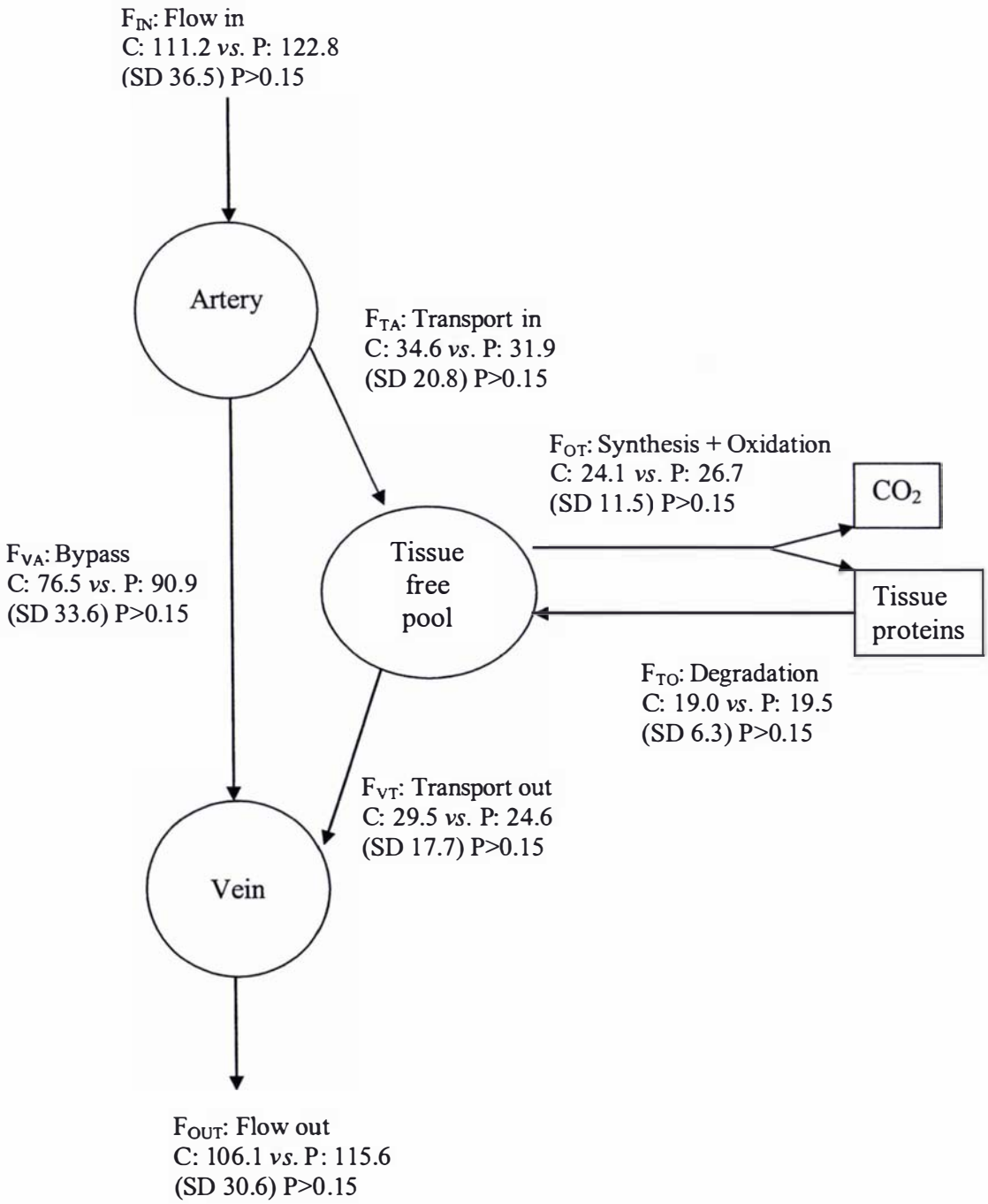


Figure D.2 ^{13}C-Valine kinetics (F; mmol d^{-1}) in the muscle of lambs fed fresh Lucerne (*Medicago sativa*) and infected with *Trichostrongylus colubriformis* (P; n=5) or kept as parasite free controls (C; n=6). Results are presented as LSmeans and their associated pooled standard deviation (SD).



APPENDIX E

E.1 Amino acid fluxes through the digestive tract

Table E.1 Amino acid intake (g d^{-1}) in lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with *Trichostrongylus colubriformis* or kept as parasite free controls. Results are presented as LSmeans and their pooled associated standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
Non essential	Alanine	6.7	5.4	1.0	0.05
	Aspartate	22.9	18.6	3.2	0.05
	Glutamate	12.9	10.4	1.8	0.05
	Glycine	5.6	4.5	0.8	0.05
	Proline	5.1	4.2	0.7	0.05
	Serine	6.2	5.0	0.9	0.05
	Tyrosine	3.8	3.1	0.5	0.05
Essential	Arginine	5.6	4.5	0.8	0.05
	Cysteine	1.9	1.5	0.3	0.04
	Histidine	2.5	2.0	0.4	0.05
	Isoleucine	4.1	3.3	0.6	0.05
	Leucine	8.9	7.2	1.3	0.05
	Lysine	7.2	5.8	1.0	0.05
	Methionine	2.2	1.8	0.3	0.04
	Phenylalanine	5.5	4.4	0.8	0.05
	Threonine	5.0	4.1	0.7	0.05
	Valine	4.8	3.9	0.7	0.05

Table E.2 The flux of amino acids through the abomasum (g d^{-1}) of lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with *Trichostrongylus colubriformis* or kept as parasite free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
Non essential	Alanine	7.8	6.1	1.3	0.05
	Aspartate	13.4	10.4	2.3	0.05
	Glutamate	15.0	11.9	2.6	0.07
	Glycine	6.6	5.2	1.0	0.05
	Proline	5.2	4.1	0.9	0.07
	Serine	6.3	5.1	1.1	0.09
	Tyrosine	5.9	4.3	1.1	0.03
Essential	Arginine	5.8	4.5	1.0	0.05
	Cysteine	2.1	1.8	0.5	0.30
	Histidine	2.7	2.1	0.4	0.03
	Isoleucine	5.4	4.1	0.9	0.02
	Leucine	10.3	7.7	1.7	0.03
	Lysine	8.6	6.7	1.4	0.05
	Methionine	2.3	1.8	0.4	0.08
	Phenylalanine	6.3	4.6	1.0	0.02
	Threonine	6.7	5.3	1.2	0.07
	Valine	5.9	5.0	1.9	0.46

Table E.3 The flux of amino acids through the ileum (g d^{-1}) of lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with *Trichostrongylus colubriformis* or kept as parasite free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
Non essential	Alanine	2.5	2.5	1.5	0.93
	Aspartate	4.0	4.5	2.6	0.73
	Glutamate	4.8	5.8	3.5	0.63
	Glycine	2.4	2.6	1.4	0.82
	Proline	2.1	2.8	1.5	0.47
	Serine	2.6	3.4	1.9	0.52
	Tyrosine	1.6	2.0	1.2	0.57
Essential	Arginine	1.5	2.3	1.4	0.36
	Cysteine	1.3	1.7	1.0	0.55
	Histidine	0.9	0.9	0.5	0.77
	Isoleucine	1.6	2.0	1.1	0.58
	Leucine	3.3	2.5	2.6	0.45
	Lysine	1.8	2.3	1.3	0.58
	Methionine	0.6	0.7	0.4	0.75
	Phenylalanine	1.6	2.1	1.2	0.56
	Threonine	2.6	3.6	2.1	0.50
	Valine	1.8	2.6	1.4	0.37

Table E.4 Apparent absorption of amino acids (g d^{-1}) in the small intestine of lambs fed Sulla (*Hedysarum coronarium*) and infected with *Trichostrongylus colubriformis* or kept as parasite free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
Non essential	Alanine	5.3	4.4	1.1	0.26
	Aspartate	9.4	7.6	1.9	0.22
	Glutamate	10.2	8.3	2.0	0.22
	Glycine	4.2	3.4	0.9	0.29
	Proline	3.1	2.3	0.8	0.20
	Serine	3.7	3.1	1.1	0.41
	Tyrosine	4.3	2.9	1.3	0.17
Essential	Arginine	4.4	3.2	0.7	0.07
	Cysteine	0.8	0.6	0.5	0.68
	Histidine	1.9	1.4	0.5	0.16
	Isoleucine	3.8	2.7	0.5	0.02
	Leucine	7.1	4.9	1.7	0.12
	Lysine	6.7	8.4	1.0	0.11
	Methionine	1.7	1.1	0.2	0.001
	Phenylalanine	4.7	3.1	1.2	0.10
	Threonine	4.1	3.4	1.0	0.40
	Valine	4.1	2.6	0.5	0.01

Table E.5 Digestibility (as a proportion of feed intake) of amino acids in the small intestine of lambs fed Sulla (*Hedysarum coronarium*) and infected with or without *Trichostrongylus colubriformis*. Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
Non essential	Alanine	0.69	0.72	0.15	0.74
	Aspartate	0.71	0.75	0.13	0.67
	Glutamate	0.68	0.72	0.14	0.71
	Glycine	0.64	0.68	0.16	0.71
	Proline	0.60	0.60	0.18	0.98
	Serine	0.59	0.64	0.18	0.71
	Tyrosine	0.72	0.74	0.14	0.86
Essential	Arginine	0.74	0.75	0.12	0.95
	Cysteine	0.38	0.45	0.29	0.76
	Histidine	0.70	0.70	0.14	0.98
	Isoleucine	0.70	0.70	0.13	0.96
	Leucine	0.68	0.68	0.15	0.98
	Lysine	0.79	0.81	0.10	0.84
	Methionine	0.73	0.72	0.14	0.91
	Phenylalanine	0.73	0.73	0.14	0.98
	Threonine	0.60	0.67	0.16	0.58
	Valine	0.70	0.65	0.12	0.58

E.2 Plasma amino acid data

E.2.1 Concentrations

Table E.6 The concentration of amino acids in the mesenteric vein (μmol L^{-1}) of lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with *Trichostrongylus colubriformis* or kept as parasite free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
Non essential	Alanine	257	206	44	0.11
	Asparagine	92	76	29	0.42
	Aspartate	19	22	11	0.66
	Citrulline*	32	40	7	0.11
	Glutamine	271	249	28	0.25
	Glutamate	97	105	39	0.76
	Glycine	403	493	106	0.21
	Hydoxy proline	19	23	8	0.45
	Ornithine*	26	28	5	0.44
	Proline	135	131	37	0.89
	Serine	96	107	22	0.46
	Taurine*	23	23	6	0.83
Essential	Arginine	126	110	33	0.46
	Cysteine	37	30	6	0.10
	Histidine	67	47	12	0.03
	Isoleucine	124	107	48	0.58
	Leucine	166	151	49	0.63
	Lysine	106	109	44	0.92
	Methionine	36	26	12	0.25
	Phenylalanine	72	61	20	0.38
	Threonine	102	86	34	0.46
	Tyrosine	87	77	25	0.51
	Valine	289	280	83	0.87

* Not included in totals

Table E.7 The concentration of amino acids in the portal vein (μmol L^{-1}) of lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
Non essential	Alanine	177	143	18	0.02
	Asparagine	51	45	11	0.35
	Aspartate	12	15	5	0.22
	Citrulline*	31	34	8	0.50
	Glutamine	256	254	27	0.88
	Glutamate	75	87	25	0.46
	Glycine	323	501	135	0.07
	Hydoxy proline	20	21	8	0.79
	Ornithine*	25	29	5	0.22
	Proline	93	94	17	0.94
	Serine	56	72	22	0.28
	Taurine*	19	23	5	0.27
Essential	Arginine	104	90	22	0.31
	Cysteine	31	28	6	0.37
	Histidine	55	38	8	0.01
	Isoleucine	85	77	24	0.58
	Leucine	112	115	27	0.89
	Lysine	72	92	34	0.35
	Methionine	22	17	5	0.16
	Phenylalanine	50	42	11	0.24
	Threonine	61	53	17	0.49
	Tyrosine	63	51	16	0.24
	Valine	241	243	56	0.95

* Not included in totals

Table E.8 The concentration of amino acids in the hepatic vein (μmol L^{-1}) of lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
Non essential	Alanine	144	123	16	0.04
	Asparagine	41	38	11	0.66
	Aspartate	10	14	5	0.19
	Citrulline*	28	35	9	0.22
	Glutamine	263	259	40	0.84
	Glutamate	86	94	24	0.57
	Glycine	315	471	127	0.06
	Hydoxy proline	18	21	7	0.54
	Ornithine*	24	30	6	0.12
	Proline	82	86	16	0.65
	Serine	46	68	21	0.11
	Taurine*	20	22	6	0.52
Essential	Arginine	93	83	22	0.46
	Cysteine	32	26	6	0.12
	Histidine	48	36	8	0.04
	Isoleucine	72	73	28	0.95
	Leucine	99	109	30	0.56
	Lysine	63	86	31	0.23
	Methionine	17	15	5	0.46
	Phenylalanine	39	35	11	0.59
	Threonine	51	53	19	0.83
	Tyrosine	51	45	17	0.54
	Valine	217	237	67	0.62

*Not included in Totals

Table E.9 The concentration of amino acids in the mesenteric artery (μmol L^{-1}) of lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
Non essential	Alanine	142	128	18	0.23
	Asparagine	38	39	10	0.86
	Aspartate	8	12	3	0.06
	Citrulline*	26	35	9	0.13
	Glutamine	283	292	40	0.71
	Glutamate	67	79	17	0.27
	Glycine	315	490	138	0.06
	Hydoxy proline	19	21	8	0.73
	Ornithine*	22	29	5	0.03
	Proline	77	88	17	0.28
	Serine	43	67	18	0.05
	Taurine*	20	22	6	0.47
Essential	Arginine	91	86	21	0.73
	Cysteine	31	26	6	0.20
	Histidine	47	38	9	0.10
	Isoleucine	64	72	26	0.60
	Leucine	87	108	28	0.24
	Lysine	57	87	29	0.11
	Methionine	16	16	5	0.99
	Phenylalanine	37	37	10	0.91
	Threonine	48	54	18	0.61
	Tyrosine	49	47	16	0.80
	Valine	203	235	64	0.41

*Not included in Totals

Table E.10 The concentration of amino acids in the vena cava (μmol L^{-1}) of lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
Non essential	Alanine	165	150	17	0.21
	Asparagine	36	38	9	0.75
	Aspartate	9	10	4	0.53
	Citrulline*	26	32	7	0.25
	Glutamine	319	317	45	0.93
	Glutamate	57	61	18	0.75
	Glycine	292	492	125	0.04
	Hydoxy proline	20	21	9	0.83
	Ornithine*	19	26	5	0.06
	Proline	79	88	14	0.33
	Serine	37	63	18	0.05
	Taurine*	20	22	6	0.68
Essential	Arginine	96	91	19	0.71
	Cysteine	33	26	6	0.09
	Histidine	47	39	10	0.26
	Isoleucine	59	71	22	0.41
	Leucine	82	107	26	0.17
	Lysine	62	84	27	0.22
	Methionine	17	17	6	0.84
	Phenylalanine	38	39	8	0.84
	Threonine	48	56	15	0.46
	Tyrosine	50	49	14	0.94
	Valine	200	232	63	0.43

*Not included in Totals

E.2.2 Amino acid net fluxes

Table E.11 Net flux of amino acids across the mesenteric-drained viscera (μmol min^{-1}) in lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Positive values represent a net utilisation by the tissue, while a negative value represents a net release. Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
Non essential	Alanine	-61.7	-55.2	44.0	0.83
	Asparagine	-28.9	-26.9	21.1	0.89
	Aspartate	-5.4	-7.3	4.4	0.54
	Citrulline*	-3.1	-2.4	2.5	0.69
	Glutamine	9.6	29.6	18.1	0.14
	Glutamate	-17.7	-22.9	8.2	0.37
	Glycine	-45.9	-41.5	27.6	0.82
	Hydoxy proline	-0.2	-0.4	0.5	0.66
	Ornithine*	-2.2	-1.2	1.3	0.31
	Proline	-30.7	-27.7	19.4	0.83
	Serine	-28.6	-26.7	16.8	0.87
	Taurine*	-1.5	-1.0	1.0	0.52
Essential	Arginine	-19.6	-16.6	15.6	0.78
	Cysteine	-3.2	-3.3	1.8	0.93
	Histidine	-11.2	-9.1	8.3	0.71
	Isoleucine	-32.1	-27.2	23.3	0.76
	Leucine	-42.9	-35.5	31.0	0.73
	Lysine	-27.7	-23.9	21.5	0.80
	Methionine	-10.5	-8.5	8.1	0.72
	Phenylalanine	-19.2	-16.4	14.3	0.78
	Threonine	-31.0	-10.3	14.4	0.15
	Tyrosine	-21.0	-18.1	14.2	0.77
	Valine	-47.7	-37.2	32.5	0.64

* Not included in Totals

Table E.12 Net flux of amino acids across the portal-drained viscera (μmol min^{-1}) in lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Positive values represent a net utilisation by the tissue, while a negative value represents a net release. Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
Non essential	Alanine	-47.6	-22.0	16.6	0.03
	Asparagine	-16.7	-8.6	5.8	0.06
	Aspartate	-4.3	-3.9	3.2	0.86
	Citrulline*	-3.7	0.7	2.8	0.04
	Glutamine	26.9	55.1	16.6	0.02
	Glutamate	-7.7	-11.6	16.6	0.72
	Glycine	-39.4	-17.0	22.9	0.16
	Hydoxy proline	0.3	-1.0	1.0	0.07
	Ornithine*	-3.7	-0.2	2.4	0.05
	Proline	-19.2	-10.9	5.8	0.05
	Serine	-15.6	-6.1	5.5	0.03
	Taurine*	-2.1	-0.6	1.8	0.22
Essential	Arginine	-11.3	-2.1	6.6	0.06
	Cysteine	-2.3	-1.9	1.7	0.65
	Histidine	-9.0	-1.1	3.5	0.01
	Isoleucine	-19.7	-7.2	7.7	0.03
	Leucine	-27.0	-10.7	10.4	0.04
	Lysine	-17.8	-9.1	10.4	0.22
	Methionine	-7.1	-1.3	2.9	0.01
	Phenylalanine	-14.9	-7.4	5.7	0.07
	Threonine	-15.6	3.4	4.3	0.00
	Tyrosine	-12.3	-5.8	6.3	0.14
	Valine	-26.3	-11.5	12.8	0.10

* Not included i n Totals

Table E.13 Net flux of amino acids across the liver (μmol min^{-1}) in lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Positive values represent a net utilisation by the tissue, while a negative value represents a net release. Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
Non essential	Alanine	40.9	29.7	19.1	0.37
	Asparagine	8.7	9.0	6.4	0.95
	Aspartate	0.2	2.0	4.4	0.51
	Citrulline*	0.1	-1.4	4.0	0.56
	Glutamine	13.4	-2.2	27.5	0.38
	Glutamate	-34.2	-12.5	19.2	0.11
	Glycine	43.1	42.1	19.4	0.94
	Hydoxy proline	0.9	0.4	2.7	0.77
	Ornithine*	-1.5	-1.9	3.0	0.82
	Proline	6.7	9.9	6.1	0.41
	Serine	9.7	5.1	12.6	0.57
	Taurine*	2.0	0.6	1.7	0.24
Essential	Arginine	5.7	8.9	7.2	0.51
	Cysteine	0.9	2.0	1.8	0.32
	Histidine	7.5	4.3	1.2	0.00
	Isoleucine	0.9	4.5	3.7	0.16
	Leucine	1.8	7.2	5.6	0.16
	Lysine	4.8	8.2	8.7	0.54
	Methionine	3.8	2.2	1.4	0.11
	Phenylalanine	11.1	9.4	5.1	0.60
	Threonine	9.5	-2.1	4.9	0.02
	Tyrosine	7.3	8.1	4.0	0.74
	Valine	-6.2	7.4	9.4	0.05

* Not included in Totals

Table E.14 Net flux of amino acids across the total splanchnic tissues (μmol min^{-1}) in lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Positive values represent a net utilisation by the tissue, while a negative value represents a net release. Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
Non essential	Alanine	-3.6	7.6	11.7	0.14
	Asparagine	-6.4	0.4	3.9	0.02
	Aspartate	-3.5	-1.9	4.4	0.55
	Citrulline*	3.1	-0.7	3.2	0.25
	Glutamine	43.4	52.9	23.3	0.50
	Glutamate	-36.2	-34.0	20.8	0.34
	Glycine	13.1	25.1	28.1	0.48
	Hydoxy proline	1.0	-0.6	3.1	0.41
	Ornithine*	-4.4	-3.2	3.1	0.53
	Proline	-9.7	2.6	8.1	0.03
	Serine	-5.4	-1.0	10.0	0.47
	Taurine*	-0.3	0.1	1.8	0.71
Essential	Arginine	-4.3	8.4	9.6	0.07
	Cysteine	-1.5	0.2	1.1	0.03
	Histidine	-1.3	3.2	3.1	0.04
	Isoleucine	-15.1	-2.7	8.9	0.04
	Leucine	-20.4	-3.4	11.5	0.03
	Lysine	-11.2	-0.9	9.8	0.10
	Methionine	-2.7	0.9	2.4	0.03
	Phenylalanine	-2.7	2.0	2.9	0.02
	Threonine	-5.1	1.5	3.8	0.04
	Tyrosine	-4.2	2.4	4.7	0.04
	Valine	-26.4	-8.4	17.9	0.12

* Not included in Totals

Table E.15 Net flux of amino acids across the hind limbs (μmol min^{-1}) of lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Positive values represent a net utilisation by the tissue, while a negative value represents a net release. Results are presented as LSmeans and their associated pooled standard deviation (SD).

		Control n=6	Parasite n=6	Pooled SD	P
Non essential	Alanine	-13.3	-10.5	6.0	0.47
	Asparagine	1.6	0.7	2.8	0.61
	Aspartate	-0.5	1.1	1.0	0.06
	Citrulline*	-0.1	1.6	1.6	0.13
	Glutamine	-17.4	-11.9	10.9	0.44
	Glutamate	7.2	9.3	5.2	0.53
	Glycine	-10.2	-1.6	12.6	0.31
	Hydoxy proline	-0.5	-0.2	0.6	0.52
	Ornithine*	0.7	1.5	0.3	0.37
	Proline	-1.2	1.0	5.5	0.54
	Serine	1.5	2.1	3.7	0.81
	Taurine*	0.0	-0.3	0.9	0.59
Essential	Arginine	-1.4	-1.3	4.6	0.97
	Cysteine	0.1	0.3	0.3	0.37
	Histidine	-0.8	-0.2	2.5	0.72
	Isoleucine	2.5	1.0	5.3	0.67
	Leucine	2.4	1.2	6.8	0.77
	Lysine	1.7	1.3	4.8	0.90
	Methionine	0.2	-0.5	2.0	0.62
	Phenylalanine	-0.4	-0.8	2.2	0.80
	Threonine	0.7	-0.6	3.1	0.57
	Tyrosine	-0.7	-0.7	2.4	0.98
	Valine	1.9	2.4	8.7	0.92

* Not included in Totals

APPENDIX F

F.1 Transmembrane valine kinetics across the hind limbs

Figure F.1 ^{3}H-Valine kinetics (F; mmol d^{-1}) in the muscle of lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with *Trichostrongylus colubriformis* (P; n=6) or kept as parasite free controls (C; n=6). Results are presented as LSmeans and their associated pooled standard deviation (SD).