

**PROTEIN NUTRITION OF LIVESTOCK GRAZING HIGH QUALITY
PASTURE**

A thesis

submitted in partial fulfilment

of the requirement for the Degree of

Doctor of Philosophy

at

Lincoln University

by

Othusitse Ricky Madibela

Lincoln University, Canterbury, New Zealand

2010

Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy

PROTEIN NUTRITION OF LIVESTOCK GRAZING HIGH QUALITY PASTURE

ABSTRACT

This thesis describes a series of four experiments designed to evaluate the role of the supply of protein in livestock grazing high quality pasture during mating and during pregnancy. The first two studies investigated the effects of high crude protein content of spring or autumn re-growth pasture on the reproductive performance of dairy cows and of ewes at mating. The last two studies investigated how the dietary supply of protein, body condition and their interactions contribute to the breakdown of immunity during the peri-parturient period in ewes and investigated underlying endocrine mechanisms.

In the first study (Chapter 3) cows were blood sampled via the tail vein during the breeding period in spring. Plasma was then analysed for urea concentration. Cows with high plasma urea (HPU) or low plasma urea (LPU) were defined as those with plasma urea concentrations of \geq or $<$ 44.9 mg/dl respectively. Lactating cows (n = 200) were also categorized into high milk producers (HMP) or low milk producers (LPM) relative to an average daily yield of 26.6 l/d. Pasture clipping showed an average pasture CP (crude protein) content of 223 g/kg DM. Concentrations of plasma urea ranged from 26.6 to 64.4 mg/dl. No correlation was observed between plasma urea concentration and either reproductive indicators or milk parameters. Mean blood

urea concentration of HPU cows was 50.8 compared to 38.5 mg/dl in LPU cows. There was a trend for more animals ($P = 0.09$) in the HPU group than in the LPU group not to return to oestrus. Cumulative pregnancy rate in HPU and LPU was similar except at week 6 after the start of mating when more ($P < 0.01$) HPU than LPU cows were pregnant. Calving to conception interval, calving interval and interval between conception and first service were similar ($P > 0.05$) between HPU and LPU cows. Gestation length, calving rate, milk yield and milk components were also similar ($P > 0.05$) between LPU and HPU cows. There was no difference ($P > 0.05$) in plasma urea concentrations between HMP and LMP milk producers. However, calving to conception interval, interval between calving and first service and calving interval were longer ($P < 0.001$), submission rate higher ($P < 0.001$) and NRR (Non-return rate) higher ($P < 0.05$) in LMP than HMP. The number of services, the interval between first and second service, gestation length and CR (calving rate) were similar ($P > 0.05$) between HMP and LMP cows. HMP had lower ($P < 0.001$) milk protein and fat concentrations than LMP cows. This information indicates that, despite the fact that plasma urea was consistently higher than levels in the literature which have been associated with reduced fertility in dairy cows; no impairment of reproductive performance was observed.

In the second experiment (Chapter 4) mature and dry Coopworth ewes were blocked by weight, body condition and previous prolificacy (high, HP vs low twinning frequency, LP) into two groups and thereafter randomly allocated to diet which were designed to provide either 1) high protein (163 g/kg DM, ryegrass/red clover pasture, HPP) or low protein (119 g/kg DM, hay and barley grain, HB) supply at joining. These were designed to provide high and low plasma urea concentration. Over a

period of 17 days, ewes recorded as mated were examined by laparoscopy, at which time there was no difference in blood urea concentration (58.6 vs 56.1 mg/dl) between HPP and HB groups. Fifty days after the start of joining the number of foetuses present was counted using ultrasonography. As a consequence of lack of difference in the plasma urea concentration, irrespective of treatment group, individual animals were categorized into high (HU) and low plasma urea (LU) status based on whether plasma urea concentration was higher or lower than the sample mean of 51.5 mg urea/dl. Lambs which weighed greater than the mean plus one standard deviation for their litter size were classified as oversize. Ovulation rate and conception rate were similar ($P > 0.05$) between HPP and BH and between HU and LU ewes. Ewes with previous high reproductive performance (HP) as would be expected had higher ovulation rate ($P < 0.001$) and conception rate ($P < 0.01$) than LP ewes. Embryo losses was not ($P = 0.06$) different between HB and HPP ewes. Urea category (HU vs LU) did not ($P > 0.05$) influence embryo mortality. Foetal loss, neonatal loss, total reproductive loss and mean lamb birth weight was were not affected by diet, nor urea category ($P > 0.05$). Single ovulations had tended ($P = 0.08$) to contribute to higher embryo loss compared to multiple ovulations, and, single foetuses suffered higher ($P < 0.001$) losses compared to multiples. While the study did not achieve large differences in plasma urea concentrations between diets, the levels of plasma urea operating were high yet reproductive wastage rates were similar to those recorded in the literature. Together with similar apparent lack of effect on a high plasma urea environment, the data suggest either that previous findings from controlled studies have a more complex aetiology or that pastoral animals can adapt to high tissue ammonia/urea status.

The third trial (Chapter 5) was designed to provide information on the supply of amino acids to the abomasum from protein supplementation which have previously been found to overcome dietary scarcity associated with limitation of peri-parturient increase in FEC.

Twin-suckling ewes were fitted with rumen and abomasal cannulae and grazed a ryegrass/clover sward (C) or the same sward but with a 500 g/h/d protein supplement (S). The trial was designed as a cross-over with two 14 day adaptation periods followed by two five-day digesta-sampling periods. All ewes were treated with anthelmintic 14 days after lambing. Weekly analysis of blood glucose was carried on whole blood and analysis of amino acids in plasma. The flows of amino acids (AA) and dry matter (DM) at the abomasum were measured during both sampling periods using intra-ruminally infused markers. Live weight and faecal egg count (FEC) were recorded weekly. Diurnal variation in AA flow at the abomasum peaked between 12:00 and 15:00 h and was greatest in S ewes. Flows of AA, including DAPA, were increased by supplementation by 16%, while sulphur amino acids (SAA) were the most enhanced (by 21%) and flows of leucine, lysine, glutamine and aspartate were increased by about 20%. There were significant time effects in rumen and abomasal pH ($P < 0.01$; in both cases in both periods) reflecting increase in pH after 09.00 h. During Period II, rumen pH in digesta of C ewes was significantly higher ($P < 0.001$) than that of S ewes (6.7 ± 0.05 vs 6.4 ± 0.05 for C and S ewes, respectively). Plasma AA concentrations ($P < 0.01$) were lower in S ewes 21 days after parturition, but similar ($P > 0.05$) to those of C ewes at other times. Forty-three days after lambing (after cross over), the order was reversed as plasma methionine and cysteine concentrations of C ewes became low ($P < 0.05$). These changes in plasma AA were accompanied by changes in body condition score between day 23 and 70 post-partum

whereby C ewes lost more body condition than S ewes. There was evidence for a lower FEC in S ewes, being 46 vs. 670 epg, respectively for S and C groups ($P = 0.08$) 21 days after anthelmintic treatment. There were higher ($P < 0.05$) blood glucose levels in C compared to S ewes at day +35 relative to lambing which was reversed and significantly higher ($P < 0.01$) for S ewes by day +56 from lambing (after treatments were reversed). There was no significant effect of treatment on live weight and lamb performance. There are limited data in amino acid supply on lactating ewes on pasture and the present study contributes additional information on the supply of amino acids at the abomasum. The prediction that flow rates that sulphur amino acids may have been enhanced to the greatest degree could be significant since sulphur amino acids are needed for the synthesis of glutathione for immune response. It can be calculated that supplementation to supply the quantities of S-amino acid at pasture would be needed, since it would not be possible for sheep to increase pasture intake to achieve similar S-amino acid flow. Increase in bypass amino acids in S ewes at certain times in the day probably suggests influence by protein supplementation at certain times of the grazing cycle. Reduced plasma free amino acids at day +21 relative to lambing, may indicate sparing of body protein breakdown by protein supplementation. However, the difference in blood glucose on day 35 and day 56 may indicate re-adjustment of hormonal settings, responsible for nutrient partitioning.

The last study (Chapter 6) used ewes during the peri-parturient period on pasture. Eighty pregnant ewes were allocated into four groups balanced for anticipated number of lambs. Group 1 had a high body condition score (BCS) of 4.0 which was maintained throughout pregnancy by pasture allowance (HM; $n = 20$). Group 2 ($n =$

40) had medium body condition (BCS 3.0) and were split into two subgroups; one was offered pasture to allow gain of condition (MH; n = 20) and the second allowed to lose condition by offering a low grazing allowance (ML; n = 20). Group 3 were thin ewes (BCS 2.4) and pasture allowance was designed to maintain this condition (LM; n = 20). These feeding regimes were maintained for 3 weeks from week -8 of pregnancy. During week -5 to -4 all ewes were acclimatized to a protein supplement (60 g/d). A glucose tolerance test (GTT) was conducted during week -4 after which half of the ewes in each group were offered a protein supplement at the rate of 500 g/d, creating -S and -NS groups. During wk -2, a second GTT was carried out. Animals were treated with an anthelmintic 3 wks before lambing, and were then challenged with a dose of 10 000 *Teladorsagia circumcincta* larvae on weeks -2 and -1 relative to lambing. Weekly recording of FEC, live weight and body condition was carried out. Lambs were weighed within 24 h of birth and again at 44 and 65 d of age. Computed tomography body scanning was carried out on ewes at weeks -8, -3 and +8 relative to lambing. There were no differences ($P > 0.05$) in lamb performance due to body condition or protein supplementation. FEC of all groups was low (≈ 9 peg) and there was no ($P > 0.05$) significant difference between ewes of different body condition or due to effects of protein supplementation. Ewes bearing/bearing multiple lambs had the highest FEC at day -32 and +12 relative to lambing, which was significant ($P < 0.05$) on the latter date. There were no significant effects of supplementation on parasite status.

There were differences in basal plasma glucose concentration between groups ($P < 0.001$), being highest in HM/S and least in ML/NS ewes and was generally higher ($P < 0.001$) during GTT 2 than GTT 1. Ewes carrying a single foetus had higher (P

<0.001) basal glucose than those carrying multiple lambs (2.2 vs. 1.7 mmol/L, respectively). Other plasma glucose response indexes were similar ($P < 0.05$) between groups. There were differences in insulin responsiveness between groups ($P < 0.001$), being highest in MH/S and least in ML/S ewes. Insulin responsiveness tended ($P = 0.06$) to be lower during GTT 1 than GTT 2, but was higher ($P < 0.01$) in ewes carrying singles than multiples. There was tendency for higher though non-significant, basal insulin concentrations in HM ewes. Insulin trends over time after glucose infusion suggest greater insulin response at GTT 1. Basal insulin was not correlated with CT muscle weight. Despite differences in body muscle mass at the start of the trial and differences induced by nutrition during late pregnancy, positive gains in muscle mass occurred during early pregnancy and muscle mass was similar in all groups by day 56 of lactation. Animals with greatest fat content at parturition (HM) mobilised the greatest amount and those with least fat (LM) deposited fat during lactation. Further experimentation may consider the use of the hyperinsulinemic-euglycemic clamp approach to more precisely estimate whether hormonal re-setting through insulin resistance may be involved in relaxation of immunity during the peri-parturient period.

Keywords; Fertility; urea; embryo; dairy cows; ewe; pasture; peri-parturient; protein; cytokines; immunity; insulin; glucose.

DEDICATION

This work is dedicated to the memory of my father, ES Madibela, he believed in education and also loved livestock. To my late great grandmother, Sejane “Mabicha” Kgaodi, she used to say hardwork never killed anyone, it was a valuable lesson.

To my mother, Gobotsamang Dorcus Kgaodi, for helping me with home-work during those early days of my education.

To my late sister Daphne

A wise man will hear and will increase learning; and a man of understanding shall
attain unto wise counsels – Proverbs 1:5

PREFACE

This story was overheard inside the gastrointestinal gut of one sheep somewhere in New Zealand.

Internal parasite X: It is now that time of the year when the farmer will soon come drenching his sheep and I hate that time because it mean the end of some of us.

Parasite Y: But there is an easy way of avoiding death

Parasite X: And how does one accomplish that?

Parasite Y: Just change your DNA

Parasite X: Do you mean by mutating I will survive the onslaught of anthelmintic?

Parasite Y: Yes

Somewhere in New Zealand on rye-red clover pastures, this story was also overheard

Sheep A: Do you know it is that time of the year when the farmer will be coming with that foul tasting drug, how I hate it.

Sheep B: It is not only the taste, that drug is no longer effective against worms

Ram C: So how can we tell the farmer to change the drug, may be I will just kick it off his hands

Sheep B: There is no need to be violent, just hide during the day of deworming

Sheep A: And how do I survive the worms?

Sheep B: I have discovered a secret; I jump over to the farm next door and help myself to some protein supplement.

Sheep A: How does the protein help with worms?

Sheep B: The protein help boost your immunity and your body can then fight the worms by itself.

Sheep A: Next time you go there call me

And that's how they survive internal parasites;..... stealing protein supplement from the neighbour.

ACKNOWLEDGEMENTS

My most heartfelt thanks go to The Almighty in Heaven for this precious gift of knowledge and for His guidance and grace.

I would like to acknowledge the guidance of my supervisor Professor Andrew Sykes, for giving me space for creative thinking and advising me without strangling my opinions. I would like to thank him for proof reading this manuscript.

I am thankful to associate supervisor Dr Alastair Nicol, for encouragements, listening and for his valuable input in this work.

Many thanks to my other associate supervisor Dr Jim Gibbs for helping with logistics, making sure I don't oversleep during ungodly sampling schedules, and for his valuable input.

I also would like to thank Dr José Laporte-Urbe for thought provoking discussions and for helping with sampling.

I am also indebted to Graig Trotter and Jessica Noel who helped me during sampling in wee hours of the night.

My gratitude goes to Chris Logan for providing logistics, technical advice and for carrying visual pasture assessment; Nigel Jay for his expertise in laparoscopy and CT scanning and Robin McAnulty and Cristin Lima for helping with parasitological work. Martin Ridgway and Rachel McCloy are recognised for their help with sampling and looking after the animals. Technical staff at Asheley Dene sheep farm is also acknowledged for looking after animals. Lincoln University Dairy staff is thanked for their assistance. All staff at Johnstone Memorial Laboratory is also acknowledged for their help.

I would like to thank Julie Furlong, Jenny Zhao, Hong Xie, Rosy Tung, Jeff Thackwell and Mayson Kay for their assistance in the laboratory.

My heart felt thanks go to my African friends at Lincoln; Omega, Dave, Mathew, Gillian, Samsideen, Zac, Yonas, Bernadina, Mariana Aragao Pereira, Danila and Innocent (Innocent, thanks for the supply of eggs; I needed some protein to boost my immunity). I also would like to thank Wilfred April for inspiring discussions during autumn of 2007.

My family in Botswana, thanks a lot, this degree is yours as much as it is mine. Your support is cherished thousand times. My sisters and brothers, thanks for the encouragements and believing in me. Special love goes to Lemo, Ausi Neo and Bofelo. I would like to thank my friends in Botswana, Prof Nelson Torto and Dr Madisa Mine, for their encouragements.

The Church of Forward in Faith Ministries in Christchurch is acknowledged for fellowship.

Kelemwork, thanks for the encouragements and motivation when I felt like I am losing grip on this thesis.

Botswana College of Agriculture is acknowledged for funding my studies.

TABLE OF CONTENTS

ABSTRACT	II
DEDICATION.....	IX
PREFACE.....	X
ACKNOWLEDGEMENTS.....	XI
TABLE OF CONTENTS.....	XII
LIST OF TABLES	XIV
LIST OF FIGURES	XVI
LIST OF PLATES.....	XIX
CHAPTER 1	1
1.0 Introduction	1
CHAPTER 2	5
2.0 Literature Review.....	5
2.1 Introduction	5
2.2 Effects of highly degradable protein on reproduction	6
2.2.1 <i>Nutrition</i>	6
2.2.2 <i>Effects urea and ammonia</i>	7
2.2.3 <i>Possible role of glutamine</i>	8
2.2.4 <i>Effects of urea/ammonia on circulating progesterone</i>	9
2.2.4 <i>Uterine environment</i>	9
2.2.4 <i>Evidence of effects of urea and ammonia from in vivo and field studies</i>	11
2.3 Protein nutrition in the parasitised ewes.....	12
2.3.1 <i>The gastrointestinal tract</i>	12
2.3.2 <i>The role of nutrition</i>	14
2.3.3 <i>Inadequacies of protein</i>	16
2.3.3 <i>Supplementation strategies</i>	16
2.3.4 <i>Mobilised body reserves</i>	18
2.4 The role of protein in immune function.....	19
2.4.1 <i>Amino acids</i>	20
2.5 Flow rate of nitrogen in GI of parasitized ewe	24
2.6 Is there a hormonal link to immune function?.....	25
CHAPTER 3	29
Highly degradable crude protein in spring during mating does not impair reproduction in dairy cows.....	29
3.1. Introduction	29

3.2. Material and methods	30
3.3. Results	35
3.4. Discussion.....	41
CHAPTER 4.....	50
Highly degradable crude protein from autumn pasture at mating neither caused embryo mortality nor resulted in oversize lambs	50
4.1. Introduction	50
4.2. Materials and methods.....	51
4.3. Results	54
4.4. Discussion.....	60
CHAPTER 5.....	62
Amino acid supply in twin-suckling ewes grazing spring pasture	62
5.1. Introduction	62
5.2 Material and methods	64
5.4. Results	81
5.5 Discussion.....	102
CHAPTER 6.....	114
Effects of body condition and protein supplementation on glucose and insulin after exogenous glucose infusion and on FEC during peri-parturient period in <i>Teladorsagia circumcincta</i> challenged ewes	114
6. 1 Introduction	114
6.2 Material and methods	116
6.3 Results	124
6.4 Discussion.....	140
CHAPTER 7.....	149
General Summary	149
References	156
Appendices	171
Publications and conferences during the course of the study.....	175

LIST OF TABLES

Table 3 1: Chemical composition (g/kg DM) and ME (MJ/kg DM) of ryegrass-clover pasture samples from different paddocks.....	36
Table 3 2: Comparison of some reproductive parameters between the Experimental and the Whole-Herd cows grazing high protein rye-clover pasture during spring-mating season	38
Table 3 3: Reproductive parameters and milk production of spring-mated dairy cows grazing high-protein pasture and grouped as either having high (HPU) and or low plasma urea (LPU) and as high or low milk producers.	39
Table 3 4: Correlations of milk parameters and plasma urea concentrations of spring-mated dairy cows grazing high protein pastured with reproductive indexes	40
Table 3 5: Descriptive characteristics and likelihood ratios for multiple categories of plasma urea of cows diagnosed as pregnant or open, 3 weeks after the start of mating.....	40
Table 4 1: Chemical composition of pasture, hay and barley (as % DM).....	56
Table 4 2: Mean ewe live weights, plasma urea concentrations and lamb birth weight of sheep of different plasma urea and prolificacy on autumn pasture during mating	58
Table 4 3: Re-analysis of mean birth weight of oversize lambs, adjusting for birth type (singles, twins and triplets).....	58
Table 4 4: Potential reproduction (OR) and reproductive wastage of sheep on two feed treatments with different plasma urea concentrations at mating on autumn pasture and effect of prolificacy	59
Table 5 1: Dry matter (g DM/kg fresh weight) and chemical composition (g/kg DM) of protein nuts and pasture.....	81
Table 5 2: Pasture and nutrient intake by twin-rearing ewes grazing spring pasture supplemented with protein nuts (Supplemented) or without a supplement (Control) during Period I and II ¹	84
Table 5 3: Amino acid intake (g/day) by twin-rearing ewes grazing spring pasture supplemented with protein nuts (Supplemented) or without a supplement (Control) during Period I and II ¹	85
Table 5 4: Plasma EAA, BCAA and SAA concentration (μ M) in twin-rearing ewes grazing spring pasture with or without a protein supplementation.	93
Table 5 5: Amino acid content of rumen bacteria (g/kg) of twin-rearing ewes grazing spring pasture	100

Table 5 6: Flow of DM, water, TAA, essential amino acids, cysteine, DAPA, and taurine through the abomasum in twin-rearing ewes grazing spring pasture with or without a protein supplementation.	101
Table 5 7: Plasma EAA, BCAA and SAA concentration (μM) in twin-rearing ewes grazing spring pasture with or without a protein supplementation at day 21 post lambing.	111
Table 6 1: Lamb birth weight and average daily gain/ewe at 44 and 65 days of age of lambsborn from ewes of different body reserves grazing spring pasture with or without a protein supplement.	127
Table 6 2: Correlation coefficients between reference body tissues levels and cavalieri body tissue levels.....	127
Table 6 3: Changes in CT-determined tissues composition of ewes in the treatment groups at different periods in the breeding cycle at the three measurement periods while grazing spring pasture with or without a protein supplement	128
Table 6 4: Basal glucose (mmol /L whole blood), glucose increment (mmol/L), peak glucose (mol/L whole blood), glucose AUC (mmol/L x min), turnover rate (k, % /min), half life ($t_{1/2}$, min), and CLR (min) of parasitized pregnant ewes of different body reserves grazing spring pasture with or without a protein supplement during GTT 1 and GTT 2..	138
Table 6 5: Plasma basal insulin (pmol/L), peak insulin (pmol/L), insulin increment (pmol/L), insulin turnover rate (%/min), insulin half life (min) and insulin AUC pmol/L xmin) after glucose infusion in parasitized pregnant ewes of different body reserves grazing spring pasture with or without a protein supplement at -26 and -12 day relative to lambing	139
Table 6 6: Correlations between body tissue mass and between body condition score, basal blood glucose and plasma insulin (day -26) with mass body tissue (kg) at day -54, -21 and +65 from lambing	139

LIST OF FIGURES

Figure 3 1: Distributions of plasma urea concentration of all the samples [A] taken during the 6 weeks of sampling, a subsample of cows sampled ± 10 days relative to AI [B] and cows with more than one calving (older cows) [C].	37
Figure 3 2: Cumulative pregnancy rate of HPU and LPU cows 3 to 24 weeks after start of breeding	41
Figure 4 1: Plasma urea concentration of ewes at laparoscopy	57
Figure 5 1: Live weight of twin-rearing ewes grazing spring pasture with or without a protein supplement (a) and Total daily growth (ADG; g/ewe) of their twin lambs and estimated dam milk yield (b)	86
Figure 5 2: Back-transformed FEC (a) and blood glucose (b) of twin-rearing ewes grazing spring pasture with (S) or without a protein supplement (C)	87
Figure 5 3: Plasma total amino acid (a) and total glucogenic amino acid (b) concentration in twin-rearing ewes grazing spring pasture with (S) or without a protein supplement (C)	88
Figure 5 4: Plasma total essential amino acid (a) and total branched-chain amino acid (b) concentration in twin-rearing ewes grazing spring pasture with (S) without a protein supplement (C)	89
Figure 5 5: Plasma methionine (a) and cysteine (b) concentration in twin-rearing ewes grazing spring pasture with (S) or without a protein supplement (C)	94
Figure 5 6: Plasma glutamine (a) and glutamic acid (b) concentration of twin-rearing ewes grazing spring pasture with or without a protein supplement	95
Figure 5 7: Diurnal variation in rumen and abomasal digesta pH of twin-rearing ewes grazing spring pasture with or without a protein supplement during Periods I and II	96
Figure 5 8: Diurnal variation in total AA (a), EAA (b), BCAA (c) and SAA (d) concentrations (g/kg) of bacterial isolate from the rumen of twin-rearing ewes grazing spring pasture with or without a protein supplement	97
Figure 5 9: Diurnal variation in abomasal total AA, EAA, BCAA, and SAA flows in parasitized twin-rearing ewes grazing spring pasture with or without a protein supplement	98
Figure 5 10: Diurnal variation in abomasal dry matter, glutamine, leucine and DAPA flows in parasitized twin-rearing ewes grazing spring pasture with or without a protein supplement	99

Figure 5 11: Diurnal variation in the flows of DM (a) and total AA (b) in twin-rearing ewes grazing spring pasture without a supplement (C).....	105
Figure 5 12: Diurnal variation in the flows of DM (a) and total AA (b) in twin-rearing ewes grazing spring pasture with or without a supplement.....	106
Figure 5 13: Diurnal variation in total nitrogen (TN) and bacterial nitrogen (BN) flow in twin-rearing ewes grazing spring pasture with or without a protein supplement and A and B representing by-pass nitrogen at those times	107
Figure 6 1: Experimental design; Ewes of different body condition grazing spring pasture with or without a protein supplement were challenged with <i>T. circumcincta</i> larvae. During mid-pregnancy there were fed to maintain body condition (HM and LM), gain condition (MH) or lose condition (ML). CT= computer tomography, GTT = glucose tolerance test, PA = protein acclimatization, AT = Anthelmintic treatment, LC = larvae challenge.....	118
Figure 6 2: Live weights (a, b) and body condition score (c, d) of parasitized pregnant- and suckling ewes of different body condition grazing spring pasture with or without a protein supplement. PS = prior supplementation.....	129
Figure 6 3: Percentage changes in CT estimated muscle (a) and fat (b) contents of the treatment groups measured between week -8 and week -3 weeks and between week -3 and week 8 relative to lambing.....	130
Figure 6 4: Effects of body condition (a), protein supplementation (b) and combination of body condition and supplementation for HM and MH (c) and for LM and ML (d) on FEC of ewes grazing spring pasture during pregnancy and suckling.....	131
Figure 6 5: Back-transformed FEC of ewes grazing spring pasture during pregnancy and suckling and either bearing multiple (M) or single lambs (S).....	132
Figure 6 6: Whole blood glucose profiles after glucose infusion in parasitized pregnant ewes of different body condition while grazing spring pasture with or without a protein supplement at day -26 (a; before supplementation commences) and at day -12 (b, c; after supplementation was started) relative to lambing	135
Figure 6 7: Whole blood glucose and insulin profiles after glucose infusion in parasitized pregnant ewes of different body and grazing spring pasture at day -26 (a, b) and day -12 (c, d) relative to lambing for a subsample of 12 ewes.....	136
Figure 6 8: Effects of protein supplementation on blood glucose and insulin profiles after glucose infusion in parasitized pregnant ewes of different body condition at day -12 relative to lambing and grazing spring pasture.....	137
Figure 6 9: Back-transformed FEC of parasitized ewes grazing spring pasture during pregnancy and suckling with H or L insulin Resp/kg fat (a), H or L glucose CLR/kg fat (b)	

and combination of insulin Resp/kg fat and glucose CLR/kg fat and (c) and AUCin/kg LW
(d) at day -12 relative to lambing 147

LIST OF PLATES

Plate 5.1: The cannula (C) was made from cannula barrel with round lug (Cb), 2 latex flange (Lf), PVC flange (Pf), retaining clip (Rc) and a plunger stopper with a paper clip (Ps).....	66
Plate 5.2: Sampling crush	68
Plate 5.3: Individual pen where ewes were fed protein supplements	69
Plate 5.4: Sheep carrying a backpack holding a peristaltic pump and two Viaflex bags at a paddock.....	71

CHAPTER 1

1.0 Introduction

Protein is an important nutrient for lactation, growth and development and for establishment and maintenance of immunity. When fed at higher than conventional recommendations of AFRC (1993) and NRC (1985) it can have both positive and negative effects on individual animals. On the one hand, during early pregnancy, nutrient demands of the early embryo are quantitatively minute, but are very specific in both their qualitative and temporal expression (Robinson et al., 1999b). Excessive dietary protein intake during early lactation has been associated with decreased fertility in dairy cows (Hammon et al., 2005; Rhoads et al., 2006). When dietary protein is highly degradable or excessive provision of protein or energy deficiency leading to amino acid deamination, ammonia is produced and is detoxified in the liver to urea (Canfield et al., 1990; Elrod and Butler, 1993) and is reflected in the high plasma urea in plasma or serum and reproductive fluids (Hammon et al., 2005). The underlying causes of reproductive inefficiency in ruminants with elevated plasma urea or ammonia are unclear (Hammon et al., 2005). However, increases in concentration of these chemicals have been associated with decreased conception rates (Butler et al., 1996) and reduced fertilization, altered embryo development, decreased embryo survival (Blanchard et al., 1990; Rhoads et al., 2006) and 'large offspring syndrome' (McEvoy et al., 1997). The syndrome is associated with increase in birthweight to various degrees, dystocia and often gestation is extended (Young et al., 1998). The increase in size is frequently much greater than that which can be generated from post mating alterations in the maternal nutrition (Robinson et al., 1999a). Other features associated with syndrome include increase prenatal losses, breathing difficulties, reluctance to suckle, sudden perinatal death (Young et al., 1998) and a shift in

the growth rate of key foetal organs relative to the overall growth rate of the foetus (Sinclair et al., 1997).

On the other hand, during late pregnancy or early lactation, the nutrient needs of grazing ruminants increase considerably. The foetus accumulates almost 80% of eventual mass at birth during the final third of pregnancy (Faichney and White, 1987) and nutrient demands for lactation are in excess of three times maintenance requirement. Nutritional challenges are especially great in late pregnancy or early lactation for ewes carrying or nursing multiple lambs, which are an addition to the challenges of gastrointestinal parasites, exposure to cold weather or availability of low-quality pasture. Sheep production in New Zealand (NZ) is based on pasture, using ryegrass-white clover forage systems with little supplementation (Sykes and Coop, 2001). In the light of evidence that feeding greater than conventional requirements of protein may be beneficial for immune function, there is uncertainty about the benefits of supplementation. Offering more protein than requirements for growth (Bown et al., 1991a; Kambara et al., 1993) or reproduction (Houdijk et al., 2001b) has enabled sheep to withstand negative effects of nematode infection through improving resilience and/or resistance.

Protein leakage into the GIT during nematode infection results in the animal synthesising more protein to replace these losses and represents a nutritional cost (Poppi et al., 1986). Many components of the immune system are proteinaceous in nature (Coop and Holmes, 1996) and the pathophysiology associated with establishment of immune function is considered to have a high demand for protein (Colditz, 2008). The work of Donaldson et al. (2001) showed that this extra protein could be more than 30% above the metabolisable protein recommendations for pregnant ewes according to AFRC (1993). However, it is likely that such responses involve a requirement for a specific amino acid rather than for

protein *per se* (Hoskin et al., 2002) and identification of the specific amino acids requires further elucidation (Donaldson et al., 1998). One such attempt suggested that supplying cysteine and glutamine might improve both productivity and immune function (Hoskin et al., 2002). Most of the work that has established that response to nematode infection is sensitive to protein supply was carried out with pen-fed animals and for comparison, it is important to quantify the normal supply of amino acids to infected ewes on pasture, for which there are few studies.

Other studies have shown that body protein reserves play an important role in overcoming the consequences of dietary metabolisable protein (MP) scarcity that results in rise in FEC during peri-parturient period (Houdijk et al., 2001a). Mandatory evolution-based processes that regulate partitioning of nutrients (dietary and mobilized) during the peri-parturient period may be involved in the breakdown of immunity and are perhaps linked by the endocrine environment that regulates both reproduction and lactation. During late-pregnancy, leptin concentrations are reduced (Bonnet et al., 2005) and there is an increase in glucose resistance (Pettersen et al., 1993). In addition there is possible interplay of insulin and leptin on each other's production (Ehrhardt et al., 2001), on gluco-regulation and on immunity (Ingvarsen and Boisclair, 2001). The possibility that these endocrine changes are drivers of the breakdown in immunity in the peri-parturient period in the ewe is a research-worthy area, especially since pro-inflammatory cytokines produced during response to infection are considered to have the capacity to induce a state of resistance to insulin, growth hormone and IGF-1 (Colditz, 2004).

This thesis presents a series of experiments which investigated the supply of protein to livestock grazing pasture during mating or late pregnancy and early lactation. It explores the theory that degradable protein in high-quality pasture may compromise fertility when

mating of cows occurs on high quality spring pasture and of ewes on autumn pasture growth. The studies also tested hypothesis that increased MP to parasitized ewes grazing pasture during the peri-partum period would help re-establish immune function. It also seeks to establish the possible role of endocrine changes normally associated with nutrient partitioning as a mechanism involved in relaxation of peri-parturient immunity in the ewe.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Introduction

Livestock farming in NZ is based on ryegrass-white clover pasture (Waghorn and Clark, 2004) and is largely independent of costly supplements (Sykes and Coop, 2001). Absence of supplementation means that pasture has to be of high quality to meet the requirements of animals for high levels of production and characteristically pasture contains high levels of crude protein, ranging from 15 to 30% (Chaves et al., 2002; Moller et al., 1993). In general this has advantages in ensuring high levels of production and the timing of parturition in spring ensures that high-quality pasture is available when the demands of late pregnancy and lactation are high. However, the possibility that of high levels of nitrogen from highly degradable protein have impact on embryo development and on fertility has been highlighted (Kenny et al., 2001; Laven et al., 2002; McEvoy et al., 1997; Robinson et al., 2006; Wittwer et al., 1999) in the Northern Hemisphere. This has potential implications for the NZ dairy industry since reliable re-breeding within 3 months of parturition in spring is important to maintain an annual calving interval. While this is not an issue in sheep in spring, given the shorter gestation period, mating is usually planned within a period of lax feeding of high-quality autumn regrowth pasture. There is no tangible evidence that detrimental effects of highly degradable nitrogen do occur under NZ pastoral conditions. Studies in NZ have, however, shown high blood urea concentrations in cows on spring pasture (Auldish and Prosser, 1998; Kolver and Macmillan, 1993, 1994; Moller et al., 1993) or high milk urea concentrations (Kolver and Macmillan, 1993).

Despite the competitive advantages of sheep production under pastoral systems in NZ outlined by Fisher (2004) and the high-quality of pasture, this system faces the challenges

of internal nematode parasite infections. Because of favourable conditions of warm temperature and soil moisture, (Armour, 1980; Gibbs, 1982) large numbers of infective stage larvae can be available during spring when susceptible neonates are born. On the other hand, there is evidence that even high-quality pasture may not meet the protein requirement for maintenance of immunity with consequent peri-parturient increase in faecal egg count (FEC). Late pregnancy and early lactation are periods of high nutrient requirement (Kahn et al., 1999). Both ME and MP requirements in twin bearing ewe are increased such that the ratio of requirement of MP:ME approaches 9.0g MP/MJ ME (Kahn, 2003) and may be further elevated during parasite challenge. It has been observed that the reproductive ewe experiences greater breakdown of immunity than non-pregnant animals during the peri-parturient period (Houdijk et al., 2005). Protein supplementation of ewes on high-quality pasture (Kahn et al., 2003a; Sykes, unpublished data) can reduce FEC. Such responses probably involve a requirement for specific amino acid rather than protein *per se* (Hoskin et al., 2002). For instance, glutamine (Hoskin et al., 2002; Rogero et al., 2008) and cysteine (Hoskin et al., 2002; Liu and Karlsson, 2004) have been suggested as important for immune responses, but these specific amino acids may not be supplied by pasture in sufficient amounts to stimulate immune function in high-producing sheep. The actual supply is, however poorly understood.

2.2 Effects of highly degradable protein on reproduction

2.2.1 Nutrition

The genetic determination of birth weight comes from a contribution from both the paternal and maternal genome, but nutrition of the female animal and its effect in the uterus microenvironment plays a major role. For the foetus to reach full genetic potential it must develop in an optimal intrauterine environment with adequate supply of oxygen and

nutrients, free from harmful effects of toxic agents and pathogens (Rees and Harding, 2004). Earlier studies (Faichney and White, 1987; Mellor, 1983) on birth weight, evaluated effects of nutrition during the last trimester of pregnancy. The foetus accumulates almost 80% of eventual mass at birth during the final third of pregnancy (Faichney and White, 1987). However, evidence shows that although the nutrient demands of the early embryo are quantitatively minute, they are very specific in both their qualitative and temporal expression (Robinson et al., 1999b). This is because nutrition during foetal growth and indeed during early- and mid-pregnancy could inflict a legacy of developmental changes that affect size, viability and health of the neonate and its post-natal performance (Robinson et al., 1999a). This has led to a theory of early life nutrient re-programming. This describes a phenomenon whereby a stimulus or insult at a critical, sensitive period of early life has permanent effects on structure, physiology and metabolism (Godfrey, 2002). How nutrition mediates this re-programming is a topical issue in both livestock and human foetal developmental research. Reports from animal experiments (Ashworth and Antipatis, 2001; Barnes, 2000; Bishonga et al., 1996; Dawuda et al., 2002; McEvoy et al., 1997) have identified feed metabolites or constituents or level of feeding (Adamiak et al., 2005) as contributing factors to a mechanism that results in variation in growth, health status of the embryo/foetus and the associated 'large-offspring syndrome'. These putative constituents have included urea and ammonia from diets high in protein.

2.2.2 Effects urea and ammonia

The idea that ammonia from high-protein diets can be detrimental to embryo development was prompted by the consistent observation of declining fertility in dairy herds offered high-protein diets. Early studies (Folman et al., 1981; Jordan and Swanson, 1979a) reported that feeding excessive levels of protein can have negative effects on conception rate. Folman et al. (1981) also observed that intake of a ration containing 20% crude

protein (CP) resulted in high plasma urea concentrations. High circulating urea concentrations were found to be associated with high plasma ammonia and uterine fluid urea concentrations by Jordan et al. (1983). Though total crude protein intake itself may not have had a direct impact on fertility, its association with a consistent decline in conception rates led to a productive line of enquiry as evidenced by a subsequent study by Ferguson and Chalupa (1989). These authors postulated that high concentrations of ammonia, as a by-product of nitrogen metabolism in the rumen may impair sperm, ova or early embryo survival. They concluded that fractionation of (CP) into degradable- (DP) and undegradable protein (UDP) appeared to better describe protein-induced reduction in fertility rather than CP intake *per se*. Subsequent studies observed reduced numbers of fertilized ova (Blanchard et al., 1990), reduced first conception rates and elevated plasma urea nitrogen (Canfield et al., 1990; Elrod and Butler, 1993) in association high DP intake. The mechanism by which these effects are mediated was thought to be through alteration of the uterine environment (Canfield et al., 1990; Jordan et al., 1983).

2.2.3 Possible role of glutamine

The change in uterine environment, as determined by the change in pH, reflects an altered uterine secretory activity (Elrod and Butler, 1993; Elrod et al., 1993). Elrod et al. (1993) found that both DP and UDP and not just the DP fraction were linked to these changes in the uterus. This idea advanced by Elrod et al. (1993) corroborates a hypothesis by Ferguson and Chalupa (1989) who proposed that reproduction in young cows is more sensitive to absorbed amino acid than excess ruminal ammonia. Elrod and Bulter (1993) reported that elevated urinary ammonia concentration at day 7 (luteal phase) coincided with an aberrant uterine pH and proposed that detrimental effects on fertility may be ascribed to excessive ammonia possibly derived from glutamine. Within tissue cells, glutamate can react with ammonia (enzyme; glutamine synthetase) to form glutamine. In

this way, energy-yielding molecules for the embryo entering the Krebs cycle through 2-oxo-glutarate would be reduced.

2.2.4 Effects of urea/ammonia on circulating progesterone

An earlier study reported that feeding high-protein diets reduced blood progesterone in dairy cows compared to those offered a low-protein diet (Jordan and Swanson, 1979b). Bishonga et al. (1996) also reported reduced circulating progesterone during the pre-ovulatory period in ewes fed high-urea diets; however, subsequent embryo mortality was independent of alterations in progesterone concentration. Contradictory studies by McEvoy et al. (1997) found that added dietary urea had no effects on pre-ovulatory progesterone concentrations. Mann et al. (2003) also found that in beef heifers, enhancement of embryo development was not related to changes in circulating progesterone levels. Therefore, if there is any direct detrimental effect on the oocyte before fertilization it seems unlikely to be mediated through progesterone effects. Collectively, these studies suggest that the adverse effects of high intakes of DP are more likely to be acting in the reproductive tract than at the hypothalamic and pituitary level.

2.2.4 Uterine environment

The fact that alteration of the uterine environment in early pregnancy may affect embryo development was earlier reported by Miller and Moore (1976). The greatest changes in the uterine environment occur during the mid-luteal phase, a critical period for early embryo development that ultimately determines long-term embryo survival (Rhoads et al., 2006). Because it provides the nutritional environment for the early embryo, the metabolite and ionic composition of oviductal fluid is clearly important (Kenny et al., 2002). An altered secretory activity of the uterus during the luteal phase resulted in decreases in

concentrations of P, Mg and K in cows fed a diet containing 23% CP compared to 12 % CP and these mineral changes were related to increased plasma urea and blood ammonia concentrations (Jordan et al., 1983). However, with the exception of calcium, Kenny et al. (2002) did not find any effect of elevated systemic ammonia or urea on mineral concentrations (K, Mg and Na) from oviductal fluid of cows at day 2 and 8 of the oestrus cycle when urea or ammonia was infused into the reproductive tract. Rhoads et al. (2006) recorded a decrease in uterine pH during urea infusion at day 7 after estrus. The importance of the uterine environment in embryo development is that a non-synchronous uterus is hostile to the developing embryo (Barnes, 2000). This may occur when the embryo develops in a dam fed a highly nitrogenous diet (Dawuda et al., 2002; Hammon et al., 2005; McEvoy et al., 1997). Indeed ammonia and urea concentrations in utero-oviductal fluid were found to be higher in ewes fed a high urea diet (Madibela et al., 1995; McEvoy et al., 1997) and were associated with reduced embryo survival. Urea and/or ammonia in the uterus may cause disruptive its function through alteration of the ionic composition of the uterine fluid and consequently the uterus pH (Elrod and Butler, 1993; Elrod et al., 1993). High-protein diets lowered uterine pH at day 7 (Elrod and Butler, 1993) and during the luteal phase (Elrod et al., 1993) in dairy cows. It seems that detrimental effects on the embryo can occur within the uterus at the pre-implantation period, from conception to 7 days after pregnancy (Young et al., 1999). Some studies have reported this critical time to be 17 days after ovulation (Dawuda et al., 2004), on the day of insemination (Dawuda et al., 2002), before day 7 of pregnancy (Rhoads et al., 2006) or as early as 3 days post-insemination (McEvoy et al., 1997). Hammon *et al.* (1997) found that when ammonia was introduced to the oocyte during maturation or during fertilization, there were no effects on cleavage rate, day 8-morula and blastocyst formation, though day 12 blastocyst formation was affected. These *in vitro* effects of ammonia may be associated with effects observed *in vivo*. Uterine fluid ammonia and urea nitrogen concentration at

day 7 were found to be higher in dairy cows with higher plasma urea nitrogen (≥ 20 mg/dl vs < 20 mg/dl) (Hammon et al., 2005). The challenge would be to narrow the window at which this occurs and to elucidate the mechanisms through which these changes affect the embryo.

2.2.4 Evidence of effects of urea and ammonia from *in vivo* and field studies

Even though *in vitro* studies have not pinpointed the mechanism involved in development of large offspring syndrome, these studies have practical significance where highly degradable protein is fed, resulting in elevated levels of blood urea and ammonia concentrations during the breeding period. Such a situation may be manifested when cows are turnout to spring grass especially in grass-based feeding systems like that of NZ or in the tropics where urea is used to supplement low-quality cereal forages. Several studies have reported elevated blood urea or ammonia concentrations in cows or sheep grazing spring pastures (Kenny et al., 2001; Laven et al., 2002; Wittwer et al., 1999) or when animals are fed rapidly degradable urea based diets (Bishonga et al., 1996; Dawuda et al., 2004; Dawuda et al., 2002; Madibela et al., 1995; McEvoy et al., 1997; Rhoads et al., 2006). In dairy studies, Dawuda et al. (2004), Dawuda et al. (2002) and Laven et al. (2004) did not find any compromise in embryo development and attributed this to the ability of cows to adapt to high urea levels. In addition, Kenny et al. (2002) did not observe any detrimental effect on embryo development in dairy heifers grazing nitrogen fertilized pastures, despite high blood ammonia. Rhoads et al. (2006) made similar observations with embryos from treated and control cows at time of embryo collection, but embryos from cattle consuming high-urea diets resulted in lower pregnancy rates. This may be pointing to the fact that embryos that are exposed to ammonia may be predisposed not solely to degeneration and death but occasionally instead to abnormal, non-lethal re-programming of development (McEvoy et al., 1997). This means embryo may develop into phenotypic

normal offspring but manifest large offspring syndrome or offspring with breathing difficulties, reluctance to suckle, sudden perinatal death (Young et al., 1998) and a shift in the growth rate of key foetal organs relative to the overall growth rate of the foetus (Sinclair et al., 1997). Contrary to Dawuda et al. (2004) and Laven et al. (2004), a study by Wittwer et al. (1999) observed an association between high milk urea and low fertility in dairy cows grazing spring pastures. However, embryo survival in cows which were twenty days pregnant was not affected by readily degradable nitrogen in heavily fertilized pasture indicating that probably the effect may have occurred before this period (Laven et al., 2002). These contradicting reports may also be due to the possibility that cows in herds with already high conception rates may be less affected by increasing blood urea nitrogen compared with cows in herds with low conception rate (Ferguson et al., 1993). It would be worth investigating whether similar detrimental effects on embryo in livestock grazing pasture with highly degradable protein occurs in livestock in NZ and is the subject of work described in Chapters 3 and 4 of this thesis.

2.3 Protein nutrition in the parasitised ewes

2.3.1 The gastrointestinal tract

To better understand the nutritional and metabolic changes, and immune responses that take place within the gastrointestinal (GIT) tract, it is worthwhile to look briefly at the dynamics of a normal GIT. In the sheep the GIT contributes a substantially (32 to 45%) to whole-body protein synthesis (MacRae et al., 1997a). According to MacRae *et al.* (1997a), van Goudoever et al. (2000) and Loblely et al. (2003), the high metabolic activity is necessary for the integrity of the GIT to meet the demands of digestion, absorption and to produce large amounts of immunoglobulins and mucins within the mucosa. A study by van Goudoever et al. (2000), using pigs, found that inadequate protein intake did not affect

total lysine use by the portal-drained viscera, implying a high obligatory need for lysine by the viscera. In addition, it appears that even in the well-fed state the GIT uses amino acids solely from circulating blood (MacRae et al., 1997a) whereas during restricted protein intake, the source of amino acid (i.e. lysine) is switched to mixed dietary and systemic sources (van Goudoever et al., 2000). However, there may be an endogenous cost (Lobley et al., 2003) due to this high amino acid sequestration by GIT tissue from the systemic circulation. For instance, as a consequence of high usage of some specific amino acids, the visceral growth and metabolism are well maintained whereas supply to peripheral tissues such as skeletal muscles and mammary gland is compromised (El-Kadi et al., 2006; MacRae et al., 1997a; van Goudoever et al., 2000). This background information in the metabolism of the GIT under normal conditions seeks to prime the reader to the consequences of changes to the GIT environment due to parasitism or increased nutrient demands due to a combination of parasitism and the nutrient demands for foetal growth or lactation.

The importance of the GIT is also derived from the fact that about 70% of the body's population of immune cells reside within the villus or Peyer's patches (Reeds and Burrin, 2000). In response to gastrointestinal nematode challenge, the gut mucosal mast cells population (MMC) increase in number and resistance to challenge is indicated by appearance of globule leucocytes which are considered the mature stage of or degranulated MMC (Israfi et al., 1996). Leucocytes produced in bone marrow populate the gut mucosa and have a requirement for glutamine (Colditz, 2002, 2004). Eosinophils from constitutive bone marrow and probably from the mucosal tissue may be considered an important first line of defence in determining if in-coming helminth larvae can become established (Behm and Ovington, 2000). Immunity is manifested by reduction in worm burden (Israfi et al., 1996) and during this time changes in the mucosa include increased infiltration of the

mucosa with mast cells (MMC), globule leucocytes (GL) and eosinophils, and plasma leakage (Colditz, 2003; Israf et al., 1996). Some acute phase proteins which are initiated by pro-inflammatory cytokines during infection are also synthesised in the gut (Colditz, 2002) and may partly account for the increased amino acid sequestration observed by Yu et al. (2000). Mucus from goblet mucus cells is believed to facilitate worm expulsion and contain high concentrations of threonine, serine and proline which may result in deficiencies for other processes if mucus production is increased (Knox et al., 2006). In addition to a possible change in the profile of nutrient utilisation by the skeletal tissues due to the acute phase response (APR), nutrient requirements for increased production of leucocytes, of acute phase proteins and immunoglobulins will be increased (Colditz, 2003). Thus, besides the physiological drain of protein into the GIT tract, the establishment of an immune response creates a nutritional penalty to the host animal (Greer et al., 2005; Poppi et al., 1986).

Young lambs without immune function slowly develop immunity upon exposure to intestinal nematodes. There is evidence that immunity is lost around parturition in ewes (Barger, 1993). There is considerable evidence that immune responses are protein/amino acid demanding (Donaldson et al., 2001; Houdijk et al., 2000; Kahn et al., 2003a) and that even high-quality pasture may not optimise the speed and extent of development of immunity. Preliminary studies (Huntley et al., 2004; Sykes et al., 2007) have shown that local inflammatory cells (MMC and GL) of the GIT tract are reduced during the peri-parturient period when protein supply is restricted.

2.3.2 The role of nutrition

Even though malnutrition and high susceptibility to parasitism often occur concurrently (Liu and Karlsson, 2004), the level of worm burden and productivity will depend on how

different predisposing factors affect the magnitude to which animals will exhibit resilience or resistance to the infection. Resilience is understood to refer to the ability of the animal to support an equivalent production to uninfected counterparts while faced with the pathogenic challenges of an infection while a resistant animal is the one which has the greater immune capability to control infection (establishment or maintenance of a nematode population). Among factors, such as cold stress and litter size (Xie et al., 2004), nutrition of the host seems to be a major factor affecting immune competency and the host-parasite interaction (Kahn, 2003). Reduced performance in terms of growth results from reduction in voluntary feed intake which is an initial consequence of worm infection (Coop and Holmes, 1996; Liu and Karlsson, 2004) and the nutritional cost imposed by development of immunity (Greer et al., 2005; Poppi et al., 1986). It has been demonstrated that increasing the plane of nutrition in general, evoked resilience in growing lambs (Valderrabano et al., 2002) and resistance to parasitism in the peri-parturient ewe (Valderrabano et al., 2006; Valderrabano and Uriarte, 2003), accompanied by increased concentration of circulating eosinophils. In particular, increased dietary metabolisable protein (MP) has been found to be more important than energy (Bown et al., 1991a; Donaldson et al., 2001) and that body protein status was more effective than energy status (body fat), (Houdijk et al., 2001a). This may be understandable because, besides depressed food intake due to cytokine activity, pro-inflammatory cytokines can induce metabolic changes that are associated with protein wasting by stimulating skeletal and protein catabolism and enhancing efflux of glutamine and other amino acids from the tissue as fuel to the immune system (Grimble, 1998). However, conventional diets, including high-quality pasture, may not be able to supply protein in sufficient amounts for the development of immune function.

2.3.3 Inadequacies of protein

Central to the aspect of the activation of immune responses is the modification of nutrient metabolism. For instance, the role of glutamine as a substrate for synthesis of nucleic acids in enterocytes and leucocytes during APR and the cysteine requirement for synthesis of immunoglobulins, mucins and glutathione are considered to be increased (Colditz, 2003). The peri-parturient period, during which host resistance is disrupted (Kahn, 2003), is characterised by large increases in requirement for nutrients, an imbalance between nutrient demand and supply as well as low partial priority of the immune function for MP compared to pregnancy and lactation (Coop and Kyriazakis, 1999). These factors predispose to protein deficiency in the breeding ewe. Indoor (Donaldson et al., 2001; Houdijk et al., 2000) and grazing studies (Kahn et al., 2003b; Kahn et al., 2003a; Sykes, unpublished data) have shown that breakdown of immunity during the peri-parturient period can be affected by protein supply. In particular, increasing the supply of MP by supplementation in grazing ewes is most effective in reducing the extent of relaxation in immunity during the peri-parturient period, a period of high demand for MP (Kahn et al., 2003a).

2.3.3 Supplementation strategies

Supplementation could result in either, directly supplying nutrients to meet the animal's immune needs or indirectly by restoring body-tissue reserves which the animal would later draw on to boost immune function. For instance, when protein supplements were offered during the pre-partum period (Kahn et al., 2003a), the resultant body protein pools appeared to be a source of labile amino acids for the immune response during the postpartum period and reduction in FEC has observed. In addition high protein diets offered to young animals for a short period (9 weeks) after weaning resulted in reduced FEC for 69 weeks at grazing and this reduction was accompanied by improved wool and

body growth (Datta et al., 1999). This strategy of offering high-protein diets for short periods may be a sensible way of using scarce resources since proteins are expensive. In a reproductive ewe, carry-over effects were observed during the peri-parturient period (Houdijk et al., 2001a; Houdijk et al., 2000) when a high-protein diet was offered during mid-pregnancy. Advantages to the immuno-responsiveness of the short-term feeding of high-protein diets were suggested by high antibody responses to *Haemonchus contortus* and *Trichostrongylus colubriformis* (Datta et al., 1999) and by a higher proportions abomasal globule leukocytes in *Teladorsagia circumcincta*-infected sheep (Houdijk et al., 2000).

During the dry season in the tropics, the quality and quantity of dry matter declines and supplementation is an adopted strategy to supply necessary nutrients for animal maintenance. Without such supplementation, small ruminants will be very susceptible to internal parasites. Under such dry and hot conditions, the number of internal parasites is usually low, however Torres-Acosta et al. (2004) still observed that several non-supplemented kids were shedding eggs during the dry period indicating that animals were infected. Therefore, if such supplementation provides biomass, in addition to helping animals exhibit a better resilience and/or resistance against internal parasites, the strategy would serve a good dual purpose. Supplementary feeding of Criollo kids during the dry season with a similar diet used by Aguilar-Caballero et al. (2002) consisting of sorghum and soybean, did not affect FEC and peripheral eosinophil counts but nevertheless improved resilience of the kids (Torres-Acosta et al., 2006). Results from Liu *et al.* (2005b) suggest that maintaining sheep in good body condition could help to prevent the establishment of parasite infection.

2.3.4 Mobilised body reserves

The period of transition between late-pregnancy and early lactation presents an enormous metabolic challenge to high-yielding ruminants such as dairy cows (Bell, 1995) or multiple-bearing ewes. In late pregnancy, most of the carbon and nitrogen required for foetal growth and metabolism is supplied by glucose and amino acids, with amino acids accounting for 30% of foetal oxidation (Faichney and White, 1987). Maternal adaptations, in response to these increased requirements, include substantial catabolism of muscle protein and fat tissues (Robinson, 1986; Robinson et al., 1978). Given that during parasitic infection cytokines create metabolic changes that result in mobilised nutrients to the immune function (Grimble, 1998), therefore, one would expect competition between reproduction and the immune system. In parasitized ewes during the peri-parturient period, breakdown in immunity has been attributed to a prioritization of nutrients towards reproduction rather than the immune system (Coop and Kyriazakis, 1999). Shedding of eggs during the peri-parturient period, including early lactation is a source of infection for lambs. To overcome this breakdown of immunity and hence reduce pasture contamination, it has been found that increasing nutrition (Houdijk et al., 2000) or reducing demand for protein of the ewe, by lamb removal is effective (Houdijk et al., 2006). Animals in good body condition before infection stand a better chance of overcoming the breakdown in immune function. Some studies have found that body reserves in terms of fat stores are effective while some studies observed effectiveness in body protein. For instance, pregnant ewes which were made to store more fat reserves in early pregnancy, by offering a high plane of nutrition before infection with *Haemonchus contortus* had lower FEC (Valderrabano et al., 2006; Valderrabano and Uriarte, 2003). Back-fat thickness declined after infection, indicating mobilised nutrients which were accompanied by a decrease in worm burden at lambing. This probably suggests the role of energy. Resilience to gastrointestinal nematode infection appears to be sensitive to both ME and MP intake

(Kahn, 2003). However, earlier studies (Bown et al., 1991a; Donaldson et al., 1998) demonstrated that it was MP and not energy which was important enhancing resistance in gastrointestinal nematode. This was later suggested by Houdijk et al. (2001a) who reported a 3-fold increase in FEC of lactating ewes when ewes were fed to reduce body protein during mid-pregnancy while FEC was not affected when ewes were fed to maintain body reserves during mid-pregnancy. Endogenous-derived MP may be serving a similar purpose to that of dietary MP. This immune response to protein supply may not be out of step with an ability of maternal tissues to mobilise or deposit amino acids in response to protein supply. Net flux of amino acids to and from skeletal muscle is sensitive to protein nutrition in late pregnancy and by manipulating protein and energy contents of the diet, it is possible to alter protein accretion independently of fat accretion (McNeill et al., 1997). These metabolic changes may be under hormonal control, a subject which will be dealt with in See Section 2.6.

2.4 The role of protein in immune function

One feature of gastrointestinal tract helminthosis is endogenous protein loss (Yu et al., 2000). The immune response, involving local inflammatory responses, epithelial cell secretions and antibody production are additional protein costs (Sykes and Coop, 2001). The extra protein would be used to support the pathophysiological responses in the intestinal tissue to parasite infection which results in increased protein metabolism (Yu et al., 2000). Bown et al. (1991a) demonstrated that increasing the supply of protein by abomasal casein infusion could counteract the reduction in nitrogen deposition and the use of metabolisable energy for growth in parasitised sheep. Fish meal as a source of undegraded protein increased the rate of worm expulsion, and this increase was correlated with increased levels of circulating eosinophils and concentrations of intestinal sheep mast cell proteases (van Houtert et al., 1995), improved weight gain, lower FEC and worm

burden in reproductive ewes (Donaldson et al., 1998). Undegraded protein delivers more amino acids directly to the abomasum having escaped fermentation in the rumen such as when casein was infused post-ruminally by Bown et al. (1991a). Besides the use of dietary protein *per se*, to improve both resilience and resistance, it has also been demonstrated that urea supplementation, which would be anticipated to increase microbial protein synthesis in the rumen, reduced FEC and total worm numbers in sheep (Knox and Steel, 1996). However, Scott et al. (1998) were unable to demonstrate effect of urea supplementation on FEC in sheep infected by *H. contortus*. According to Coop and Holmes (1996), supplementation with urea-molasses blocks (UMB) alone will not fully overcome adverse effects of parasitism whereas addition of extra protein to the UMB supplementation will increase the resilience of the host. Though the effects of protein has been demonstrated as shown above, identification of the precise component of the protein responsible for the effects such as specific amino acids requires further elucidation (Donaldson et al., 1998).

2.4.1 Amino acids

Under normal circumstances, amino acid metabolism within the GIT and its accessory organs make a greater contribution to total body amino acid turnover and can have an important bearing on the availability of amino acids for the support of other productive functions (Reeds and Burrin, 2000). The pathophysiological changes that occur during inflammation (in human subjects) exert a large metabolic demand on amino acid metabolism (Grimble and Grimble, 1998) and is also the case in animals infected with internal parasites. Thus, it is conceivable that by preferentially meeting the metabolic needs of the mucosa by first-pass use during protein-limiting conditions (including parasite infection), systemic availability of essential amino acids would be low (van Goudoever et al., 2000). In sheep infected by *T. colubriformis*, Yu et al. (2000) observed that leucine use was increased by 24 % across the gastrointestinal tract with the extra supply coming from

arterial sources with consequent reduction in net influx of leucine to other tissues. Reeds and Burrin (2000) reported marked variation in the degree to which different amino acids were utilised by the intestine in the piglet, indicating substantial modification of the mixture of amino acids available to the animal as a consequence of intestinal metabolism. Parasitic infection is likely therefore to not only cause re-distribution of amino acids between small intestine and peripheral tissues but also cause excessive amino acid catabolism resulting in net reduction in amino acid available for muscle tissue synthesis (Bermingham et al., 2006). This is because the composition of specific amino acids of the intestinal and liver protein is different from that of skeletal muscle protein in growing sheep, with higher proportion of cysteine and methionine in the gut and liver than in the carcass (MacRae et al., 1993). This may be attributed to the fact that 70% of immune cells reside in the intestine (Reeds and Burrin, 2000). However, according to Grimble (1998) the provision of amino acids from endogenous sources may be a better strategy because the amino acid profile more closely matches the demands of the inflammatory process than do those derived from an unreliable dietary nutrient intake. On the other hand, a fish meal-based diet (Donaldson et al., 1998; Donaldson et al., 2001) and xylose-treated soybean (Houdijk et al., 2003), have been found to be effective in peri-parturient ewes, and so has cotton seed meal in growing lambs (Datta et al., 1998). This could have been so, probably because these diets were able to supply amounts of amino acids sufficient to increase the supply of the most rate-limiting amino acid (s).

According to Grimble and Grimble (1998) many substances produced in enhanced amounts in response to cytokines in human subjects, are rich in specific amino acids. The unfavourable correlation between FEC and wool growth (Liu and Karlsson, 2004; Miller et al., 2000) suggests that nematode-associated immune responses compete for nutrients that are critical for wool growth (Liu and Karlsson, 2004). The important limiting amino acids in wool growth are the sulphur-containing amino acids; cysteine and methionine. In human

subjects, it has been postulated that following infection and trauma, there is enhanced requirement for sulphur- and other amino acids (Grimble, 1992). There are also large decreases in plasma glycine, serine and taurine concentrations, which may indicate enhanced utilization of glycine, serine and sulphur amino acids, and a shortfall of provision from endogenous sources (Grimble and Grimble, 1998). Similar elevation in requirement may be occurring in livestock infected with internal parasites. In rats infected with live *Escherichia coli*, methionine trans-sulphuration flux to cysteine in both plasma and liver was increased by 80% (Malmezat et al., 2000). Evidence of such needs comes from the composition of certain components of the immune system displaying a high content of sulphur-containing amino acids (Sykes and Coop, 2001) and this may form the bases for argument for competing demands for these amino acids as indicated by Liu and Karlsson (2004) and Liu et al. (2005a). Cysteine requirements for immunity-associated responses may be high, possibly due to the high concentration of cysteine in mucins, a component of mucus (Liu et al., 2003). Leucotrienes, which are involved in cell-signalling, are rich in cysteine (Coop and Sykes, 2002). The proliferation of the lymphocytes and the cells of the GI tract place a high demand for glutamine (Hoskin et al., 2002). Glutathione (GSH) consists of glycine, glutamic acid and cysteine while metallothioneins contain glycine, serine, cysteine and methionine. These latter two, GSH and metallothionein, are produced in large amounts in response to cytokines in humans (Grimble and Grimble, 1998) and GSH is important for several T-cell functions (Miller et al., 2000). A recent report (Liu et al., 2005b) has revealed a depletion of blood GSH in parasitized lambs given food at maintenance or 1.5 x maintenance and this was observed even in parasite-resistant genotypes of sheep. Since GSH is derived from glycine, glutamic acid and cysteine (Grimble and Grimble, 1998), the depletion of GSH during nematode infection occurred when nutrient supply was relatively low and amino acids for biosynthesis of GSH were reduced (Liu et al., 2005b). The biochemical changes which take place during

inflammation exert a large metabolic demand on amino acid metabolism (Grimble and Grimble, 1998). For instance, in the gut mucosa, large populations of leucocytes utilise much of the glutamine provided by the diet and since muscle has a high capacity to synthesise glutamine, the muscle serves as a reservoir which supplies the pro-inflammatory cytokines with this amino acid to fuel leucocyte proliferation (Colditz, 2002). This may suggest that providing either free glutamine or glutamine-rich protein diets would improve the outcome of parasite infection. However, Preiser et al. (2003) found that, in human subjects, mucosal glutamine concentration was increased after administration of an enteral feeding solution containing glutamine-rich proteins but not additional free glutamine. Similarly, increasing the availability of sulphur-containing amino acids may be expected to improve both wool production and immune competency (Liu and Karlsson, 2004). Hoskin et al. (2002) reported that cysteine and glutamine supplementation through the abomasum reduced peripheral eosinophilia and increased apparent nitrogen retention of parasitized sheep. Specific amino acids required by the immune system may be important, singly or in combination. For instance, in chickens challenged with sheep erythrocytes, the immune response was highly related to branched-chained amino acids, arginine plus lysine and less with sulphur-containing amino acids or aromatic amino acids (Konashi et al., 2000). Though additional protein or amino acids are able to help restore immune function or acquisition of immunity, in the ruminant animal the challenge is that rumen microbes alter the composition of dietary amino acids. There is no information on the supply of amino acids passing from the abomasum in lactating ewes on pasture, on the effect of infection or on the changes in amino acid supply due to protein supplements which have previously observed to enhance immune responses to nematode infection.

2.5 Flow rate of nitrogen in GI of parasitized ewe

There are few studies on nutrient flow through the abomasum in lactating ewes grazing pasture. Results from studies (Dove and Milne, 1994) suggested that the bulk of non-ammonia nitrogen (NAN) leaving the rumen was of microbial origin in ewes grazing pasture. However, earlier studies from this research group showed that protein supplementation to grazing lactating ewes increased abomasal NAN, microbial nitrogen (Dove et al., 1985) and dry matter flow compared to controls (Dove et al., 1988). In addition, NAN flow to the duodenum in cows grazing pasture was increased when cows were supplemented with corn (Berzaghi et al., 1996). A high compared to a low CP (14.5 vs. 11.0%) supplement offered to lactating cows also increased amino acid flow to the duodenum (Klusmeyer et al., 1990). A diurnal variation in nutrient flow through the abomasum was observed (Dove et al., 1988). In lactating dairy cows Robinson et al. (2002) also found diurnal patterns in amino acid flow in duodenal digesta depending on the time of feeding slowly degraded protein, either 08:00 or 00:30 h. The variability was greater in cows fed during the day than those fed during the night (Gill and Robinson, 1995). Though feed intake would increase digesta flow (Ali and Hennessy, 1995) or total amino acid flow (Scholljegerdes et al., 2004) limitations to increased production on pasture-based systems due to low pasture dry matter intake is recognised (Kolver, 2003). Reduced flows of cysteine and methionine at the abomasum in sheep grazing pasture have been observed (McMeniman et al., 1986).

When an amino acid mixture was infused directly into the rumen of sheep, fractions of lysine, threonine and methionine were incorporated directly into microbial protein (Cottle and Velle, 1989). Interestingly enough, pregnancy alters the amount of protein (Gonzalez et al., 1985) and total amino acids (Coffey et al., 1989) presented to the sheep abomasum,

probably by increasing the rate of passage (Gonzalez et al., 1985). It would be interesting to record these changes in parasitized ewes during the peri-parturient period.

Earlier work with parasitized GIT tract-cannulated lambs (Poppi et al., 1986) found that parasite infection increased the flow of non-ammonia nitrogen and NH₃-N at the terminal ileum even though absorption from the small intestine endogenous secretions such as plasma proteins appears to be unaffected (Bown et al., 1991b). A 4g N/d increase in flow to the terminal ileum was recorded by Kimambo et al. (1988) during week 6 and 12 of dosing with *Trichostrongylus colubriformis* in growing lambs. Roseby (1977) also observed that water, dry matter and nitrogen content were lower in the rumen and higher in the abomasum, small intestine and caecum-proximal colon of infected sheep. A recent study (Bermingham et al., 2008) has shown that flow of amino acids through the abomasum was increased in *Trichostrongylus colubriformis*-infected lambs but no difference was observed at the ileum. For animals supplemented with undegraded protein, it is expected that this difference would be sustained at the ileum with extra protein partitioned to the immune system. In the lactating ewe, it is only after the needs for milk production are achieved that extra protein is made available to the immune system (Houdijk et al., 2003) consistent with the fact that immune response are more sensitive than lactation to protein supply (Houdijk et al., 2000). Such changes in nutrient partitioning to different organs, life functions and end-products are under the control of hormones (Friggens and Newbold, 2007).

2.6 Is there a hormonal link to immune function?

The possibility that hormonal involvement in the immune function in nematode-infected animals has been raised in literature. This emanated from effects that could not be accounted for by protein supplementation or body condition especially in the peri-parturient ewe (Houdijk et al., 2005; Kahn, 2003) and prompted the idea that other factors

may be involved. Classical biology teaches us that nutrient metabolism and partitioning are regulated by hormones and it would not be unreasonable to assume an involvement of hormones in the breakdown of immunity during the peri-parturient period. A recent review by Kelley et al. (2007) recognises hormone-hormone and hormone-immune system interactions that co-stimulate animal cells in a disease-free or stressed state. This balance is upset during infection and autoimmune diseases. Research from human medicine and rodent models for type 2 diabetes has identified hormonal impairment due to hyper-production of cytokines. In particular tumor necrosis factor alpha (TNF- α) has been found to interfere with insulin action by decreasing tyrosine phosphorylation of both the insulin receptors and insulin receptor substrate 1 (Hotamisligil, 1999; Hotamisligil et al., 1994), thus interfering with signalling of insulin. Other molecules which have been implicated are leptin (Hotamisligil, 1999), IGF-1 (Blanchard et al., 2004), while interleukin 1 (IL-1), IL-6 and interferon are reported to affect glucose homeostasis in various tissues (Hotamisligil et al., 1993). Besides acting on insulin receptors, these cytokines also affect the hypothalamic pituitary axis (Johnson et al., 1997) suggesting an integrated neuroimmune system. *In vitro* studies showed that when fat cells are exposed to TNF- α they become insulin resistant (Pallares-Trujillo et al., 2000) and TNF- α protein expression has been found to be high in adipose tissue (Lavigne et al., 2001). Coincidentally, it is the adipose tissue which is involved in insulin resistance (Blouet et al., 2006). However, there may be differences as to which of the tissues, muscle or fat tissues, has a prominent role in insulin resistance in ruminants as compared to humans. Pregnancy causes a decrease in glucose uptake in both muscle and adipose tissue in sheep (Petterson et al., 1993). Taken together, the inhibitory actions of cytokines on hormones, (i.e. IGF-1, growth hormone, (GH), and insulin) during infection in relation to their normal interaction with hormones, suggest that such events are likely to be major causes of GH resistance and reduction in growth of children and animals during inflammatory states (Kelley et al., 2007).

The implication of leptin in insulin resistance (Hotamisligil, 1999) is interesting. Since leptin is produced by adipose tissue and its level in plasma indicates energy status (Delavaud et al., 2007) it would appear that conditions that promote partitioning of nutrients during the peri-parturient period would create insulin resistance and that leptin may be a participant. Its link to the immune system through up-regulating secretion of pro-inflammatory cytokines such as TNF- α , IL-6 and IL-12 in macrophages (Matarese et al., 2007) means that cytokine-mediated insulin resistance may be initiated by a hyperleptinemic status of the animal. In a review article, Altmann and Von Borell (2007) concluded that plasma leptin reflects overall fatness, which includes visceral fat depots (there are higher correlations between plasma leptin and visceral fat than between plasma leptin and carcass fat (Altmann et al., 2005)). Therefore, the use of leptin as a selection tool in breeding programs appears limited due the fact that visceral fat is less important for the meat industry. However, the site of leptin production may be important for immune function when viewed against the recent findings of de Oliveira et al. (2009) that the action of insulin is down-regulated during intestinal inflammatory response in mesenteric adipose tissue. Leptin is also produced by the gastric mucosa (Pico et al., 2003). The increase in leptin concentration during pregnancy (Ehrhardt et al., 2001) appears to be counter-intuitive to its normal role as a satiety factor (Hill, 2004) and the fact that it is only females that are susceptible to autoimmunity and their relative hyperleptinemic nature may be a link to the rise in FEC during the peri-parturient period in the ewe. However, Bonnet et al. (2005) reported a decrease in leptin in goats, 130 days before kidding, while a study by Muhlhausler et al. (2002) indicated that as maternal leptin was reduced in control compared to well-fed ewes at late pregnancy, foetal leptin concentration was unaffected. When viewed against the homeorhetic theory (Friggens and Newbold, 2007), foetal-derived leptin may be influencing maternal metabolism to change the dynamics of glucose

utilization towards preferential use by the foetus rather than to re-build maternal stores through the actions of insulin, particularly where maternal nutrition is limiting and maternal leptin is reduced (Hill, 2004). While it has been demonstrated that insulin regulates plasma leptin in dairy cows by stimulating adipose tissue receptors during late pregnancy (Leury et al., 2003), insulin-leptin setting in the ewe during pregnancy may be different. Ehrhardt et al., (2001) reported an increase in plasma leptin during pregnancy, which did not require adiposity or energy balance and was also unrelated to insulin action on glucose. The link between body tissue mobilisation and breakdown of immunity in nematode-infected ewes (Houdijk et al., 2001a) may also be the factor participating in hormonal resistance during infection, particularly insulin resistance.

The underlying mechanisms resulting in overcoming the breakdown of immunity during the peri-parturient period through increased dietary and mobilised MP have not been investigated. Specific amino acids have been implicated, but which amino acids and how much of these amino acids still remains unclear. Hormonal involvement and its interaction with the pro-inflammatory response in marshalling nutrient partitioning during the peri-parturient period seems an attractive hypothesis for the low partial priority for MP for immune function relative to pregnancy and lactation. The work reported in Chapter 5 and 6 of this thesis attempts to contribute new insights in this area.

CHAPTER 3

HIGHLY DEGRADABLE CRUDE PROTEIN IN SPRING DURING MATING DOES NOT IMPAIR REPRODUCTION IN DAIRY COWS

3.1. Introduction

Ammonia and urea have been found to affect embryo development both *in vitro* (Thompson, 2000) and *in vivo* in sheep (McEvoy et al., 1997). High concentrations of ammonia were measured from reproductive fluids of dairy cattle with high plasma urea (Hammon et al., 2005). Cows with high plasma urea concentration had low uterine pH during the luteal phase (Elrod and Butler, 1993; Elrod et al., 1993) and reduced fertility (Elrod and Butler, 1993). Embryos exposed to ammonia may be predisposed not just to degeneration and death but occasionally instead may appear physically normal (McEvoy et al., 1997) with abnormalities become evident later during development. It appears that damage to the integrity of the embryo occurs around the pre-implantation period, between conception and 7 days of pregnancy (Rhoads et al., 2006). Some studies have reported this critical time to be 17 days after ovulation (Dawuda et al., 2004), on the day of insemination (Dawuda et al., 2002) or as early as day 3 post-insemination (McEvoy et al., 1997).

When mating of cows occurs on spring pasture it may be expected that animals would be affected by ammonia in a similar way to cows when fed total mixed ration containing high amounts of highly degraded protein (Kenny et al., 2001; Laven et al., 2002; McEvoy et al., 1997; Robinson et al., 2006; Wittwer et al., 1999). This period represents the first flush of new pasture growth that can contain very high levels of quickly degradable protein (Dawuda et al., 2004). A few studies on pasture-based feeding have observed high plasma ammonia concentrations in dairy cows (Kenny et al., 2001; Laven et al., 2002). However,

these studies were inconclusive while those of Wittwer et al. (1999) associated high milk urea concentrations with reduced fertility in grazing cows. Pasture in NZ contain high crude protein concentrations (Chaves et al. 2002; Moller et al., 1993). Studies conducted in NZ have shown that, indeed, cows grazing spring pasture have high blood urea concentrations (Auldism and Prosser, 1998; Kolver and Macmillan, 1993, 1994; Moller et al., 1993) or high milk urea concentrations (Kolver and Macmillan, 1993). Urea values of 5.1-8.2 mmol/l reported in these studies are similar to or higher than the minimum plasma urea concentration of 5.1 mmol/l reported by McEvoy *et al.* (1997) to be detrimental to embryo development in sheep. However, only one study by Moller et al. (1993) found an association between blood urea concentration and fertility, and higher urea concentrations were evident in herds with large numbers of anoestrous cows.

The present study was set up to determine if dairy cows grazing spring pasture during mating are exposed to high blood urea concentration and therefore at risk. The second objective was to determine whether high blood urea concentration was a constant feature in individual animals and assess any association with cow reproductive performance.

3.2. Material and methods

3.2.1 Animal Management

Dairy cows used in this study were part of the Lincoln University commercial herd which had close to 650 milking cows. The management of the herd during the experiment period followed the routine commercial management. Cows rotationally grazed 22 paddocks of irrigated ryegrass/clover pastures to provide for an intake of 17 DM kg/cow/d. Pasture biomass was determined by visual assessment every Tuesday of the week after initial measurements using a rising plate meter. An experienced operator (Farm Manager) visually assessed herbage production after been calibrated to herbage mass by the rising

plate meter. Walking across the paddock the operator selected an area that represented the average dry matter and quality of the paddock and by comparing herbage height, density, growth stage, dry matter concentration and canopy structure with previous experience, the operator estimated production to within 1 cm of the soil surface. Breeding was done using AI but in the later part of the breeding programme, bulls were introduced to mate cows that did not conceive within the 9-week breeding period. Sensitivity of heat detection was increased by placing tail paint on the tail-head of each cow.

3.2.2 Blood sampling and urea analysis

Blood samples were obtained through the tail vein from a random sample of about 200 cows once *per* week during the six-week breeding period giving 1189 samples from a total of 528 cows. Blood (5ml) was collected from the tail vein into EDTA coated tubes (15% tripotassium EDTA; Becton Dickinson Vacutainer Systems, Plymouth, UK) at 1500 h during milking t. Blood tubes were immediately placed on ice and taken to the laboratory, where plasma was harvested by spinning at 3000 rpm for 10 min at 4°C (Mistral 3000, England). Plasma was placed into two 5 ml plug-top test tubes (Biolab, Christchurch, New Zealand) and these were labeled and stored at -20°C pending urea analysis. During the day of the analysis the samples were placed on a bench and left to thaw and a subsample aliquot transferred into sample cups (# 1027 115; Roche Diagnostics, GmbH, D-68298, Mannheim, Germany). Plasma urea concentration was determined on a Cobas Mira Plus autoanalyser (Roche Diagnostics, Switzerland) using kit #11489364 (Roche Diagnostics, GmbH, D-68298, Mannheim, Germany). The method is based on hydrolyzing urea with urease and the resulting ammonia converted to L-glutamate. The accompanied oxidation of NADH to NAD⁺ is then measured by kinetic UV at absorbance of 340nm. The sensitivity of the urea assay was 5.0 mg/dl.

3.2.3 Pasture sampling

The morning prior to the time of blood sampling (afternoon milking), herbage was hand-plucked from the grazing horizon (about 5cm above ground level) along a zigzag transect through the paddock. Three plucks were obtained from approximately 5-6 sites at each paddock break to estimate the nutritive value of the grazed portion of the canopy. The samples were thoroughly mixed. The samples were freeze-dried, ground and then analysed for composition using near infrared spectroscopy (NIR Systems Model 5000, Foss, Hillerod, Denmark). Pasture spectra and reference data derived local samples were used to calibrate and cross validate equations (Software; Win ISI III Version 1.50) for predicting the various parameters.

3.2.4 Reproduction data collection

Lincoln University Farm (LUDF MINDA) data was used to retrieve dates when cows were inseminated, calved, previous calving records and outcome of pregnancy diagnosis (PD). Mating started on 19/10/2005 and ended on 21/12/2005. Blood sampling started on 03/11/2005 and ended on 07/12/2005. Because blood sampling dates did not coincide with artificial insemination (AI) dates for some animals, only those whose sampling dates fell within ± 10 days of AI date were included in the analysis ($n = 211$; Experimental group). Ultrasonography data after 21, 42, 63 84, 105 133 and 168 days relative to start of mating were used to determine pregnancy rates. In some cases PD data was missing and hence sample number was reduced. Ideally an efficient mating policy should be targeting three cycles, however, there were some cows that went on to a fourth cycle and bulls were put in from 23/12/2005 to 3/02/2006 to mate these cows. Successive calving dates were used to calculate calving interval (CI) and calving rates (CR). Calving to re-conception interval (CCI) was calculated as days between previous calving in 2005 and current conception.

Submission rate (SR) was the proportion of cows detected in oestrus and inseminated within the first 3 weeks of the start of breeding season. Non-return rate (NRR) was defined as the proportion of cows that were not submitted again for mating after the second oestrus. It was observed that some cows did not calve at the expected calving dates, indicating that the recorded AI date was not the ones that resulted in pregnancy. These animals had 300 or more days gestation and were assumed to have been served by the bull (n= 17). These animals were not included when analyzing for calving to re-conception interval and gestation period. During the sampling period one milk test was done 11 days (30/10/2005) after the start of the breeding period and milk data was retrieved from LUDF MINDA records.

3.2.5 Data handling and statistical analysis

Data were captured and processed with Microsoft Excel 2002. Before creating different datasets, individual cow number was verified against its unique identity number and its presence at each activity. Data handling also included identification of anomalies such as duplicate records, incomplete records as well as creating specific milk production and reproductive variables. Urea status and milk production categories were taken to be independent variables and were used to create biological groups. For instance, cows with high plasma urea (HPU) or low plasma urea (LPU) were defined as cows with plasma urea concentrations of ≥ 44.9 mg/dl or < 44.9 mg/dl respectively. Plasma urea concentration was further divided into 4 categories of increments of 4.9 mg/dl and this was used for a likelihood ratio test. Lactating cows (n = 200) were also categorized into high milk producers (HMP) or low milk producers (LPM) relative to an average daily yield of 26.6 l/d.

Seven pregnancy rate variables; pregnancy rate after 3 weeks (PR3), cumulative pregnancy rate after 6 weeks (CPR6), after 9 weeks (CPR9), after 12 weeks (CPR12), after 15 weeks (CPR15) after 19 weeks (CPR19) and after 24 weeks of mating (PR24) were created using ultrasonography data and were tested against urea status (HPU and LPU) using Chi-square (proportions) analysis (SAS, 1999). Expected values were calculated as follows;

$$P = \text{Total number in HPU or LPU} / \text{total number of observations} \quad [1]$$

$$EV = \text{Total number of animal that become pregnant} \times P \quad [2]$$

Where; P is the proportion of animals with HPU or LPU
and EV is expected values

Submission rate (SR), non-return rate (NRR) and CR were similarly tested against urea status and milk production (HMP and LMP). The effects of plasma urea status and milk category on interval variables; calving to first service (ICFS), calving to conception (CCI), gestation, CI and first to second service (IFSS) were analysed by a general linear models (GLM) procedures (SAS, 1999). Effects of plasma urea status and milk category on plasma urea concentrations, number of services, milk yield, milk fat, milk protein and milk solids and were analysed by GLM as above. Previous calving date was used as a co-variate during statistical analysis for milk yield and milk components. Interactions between urea status and milk category was also investigated. Correlations between milk data and reproductive indexes were estimated. A regression relationship was established between milk yield with CCI and ICFS. To test whether high-plasma urea concentration was a common feature of individual cows grazing high-quality pasture, repeatability and reproducibility of urea concentration was obtained from restricted maximum likelihood (REML) estimation of variation components (Dal Zotto et al., 2008) using SAS (2002-2003) linear model.

Four category of urea status (incremental ranges of 4.9 mg/dl) were used to calculate likelihood ratio (LR) according to (Ferguson et al., 1993). Briefly, for each plasma category the number of cases was divided by the total pregnant or non-pregnant case, respectively. Then the likelihood ratio was attained by dividing percentage of cows pregnant by percentage of non-pregnant cows. A likelihood ratio of >1 implies an increased likelihood of pregnancy and that of <1 implies an inverse association. This test was chosen because over ranges of underlying pregnancy rates (PR) that may be observed in cows, likelihood ratios can be calculated to express changes in PR as plasma urea (PU) increases or decreases (Ferguson et al., 1993). Only information for the first service in each cow was used to avoid confounding effects of repeated measures and repeat breeding (Butler et al., 1996).

This experiment was carried out under the authority of Lincoln University Animal Ethics Committee (AEC) # 112.

3.3. Results

Chemical composition of the pasture clippings as cows rotated around the grazing paddocks from week 1 to 5 is shown in Table 3.1. Pasture clipping show an average CP (crude protein) of 223 g/kg DM. The ratio crude protein:water soluble carbohydrate (CP:WSC) was lower than 2.0. The average dry matter digestibility of pasture clippings was 798 g/kg DM. Distribution curves of plasma urea concentrations for all the samplings and subsequent subsets are shown in Figure 3.1. All the distributions had a similar average plasma urea concentration and standard deviation, (mean \pm sd); A = 46.2 ± 9.51 , B = 44.9 ± 7.74 and C = 45.7 ± 7.67 mg/dl.

Table 3 1: Chemical composition (g/kg DM) and ME (MJ/kg DM) of ryegrass-clover pasture samples from different paddocks

Paddock	OM*	CP	WSC	NDF	ADF	DMD	CP:WSC	ME
1	877	217	163	407	229	807	1.3	11.7
2	880	230	121	431	236	793	1.9	11.6
3	914	170	196	468	256	735	0.9	11.2
4	889	225	184	386	213	807	1.2	11.9
5	900	273	150	366	192	848	1.8	12.6
Average	892	223	162.8	411.6	225	798	1.4	11.8

* OM, Organic Matter; CP, Crude Protein; WSC, Water Soluble Carbohydrates; NDF, Neutral Detergent Fibre; ADF, Acid Detergent Fibre; DMD, Dry matter Digestibility; ME, Metabolisable Energy

Reproductive performance of the whole herd and the experimental herd is shown in Table 3.2. Reproductive performance of the sample cows was similar to that of the whole herd (Table 3.2) indicating that the sample was representative of the herd.

Reproductive parameters of the experimental herd after cows were designated as high plasma urea (HPU) or low plasma urea (LPU) are shown in Table 3.3.

Cows with high plasma urea concentration (HPU) had blood urea concentration of 50.8 compared to 38.5 mg/dl of LPU cows. There was a tendency for more ($P = 0.09$) animals in the HPU group than LPU not to return to oestrus. Cumulative pregnancy rate (Figure 3.2) shows that HPU and LPU had similar performance except at week 6 after the start of mating when more ($P < 0.01$) HPU were pregnant than LPU cows. Calving to conception interval, CI and ICFS were similar ($P > 0.05$) between HPU and LPU cows (Table 3.3). Gestation, CR, milk yield and milk components were also similar ($P > 0.05$) between the LPU and HPU cows. There was no difference ($P > 0.05$) in plasma urea between high (HMP) and low milk producers (LMP).

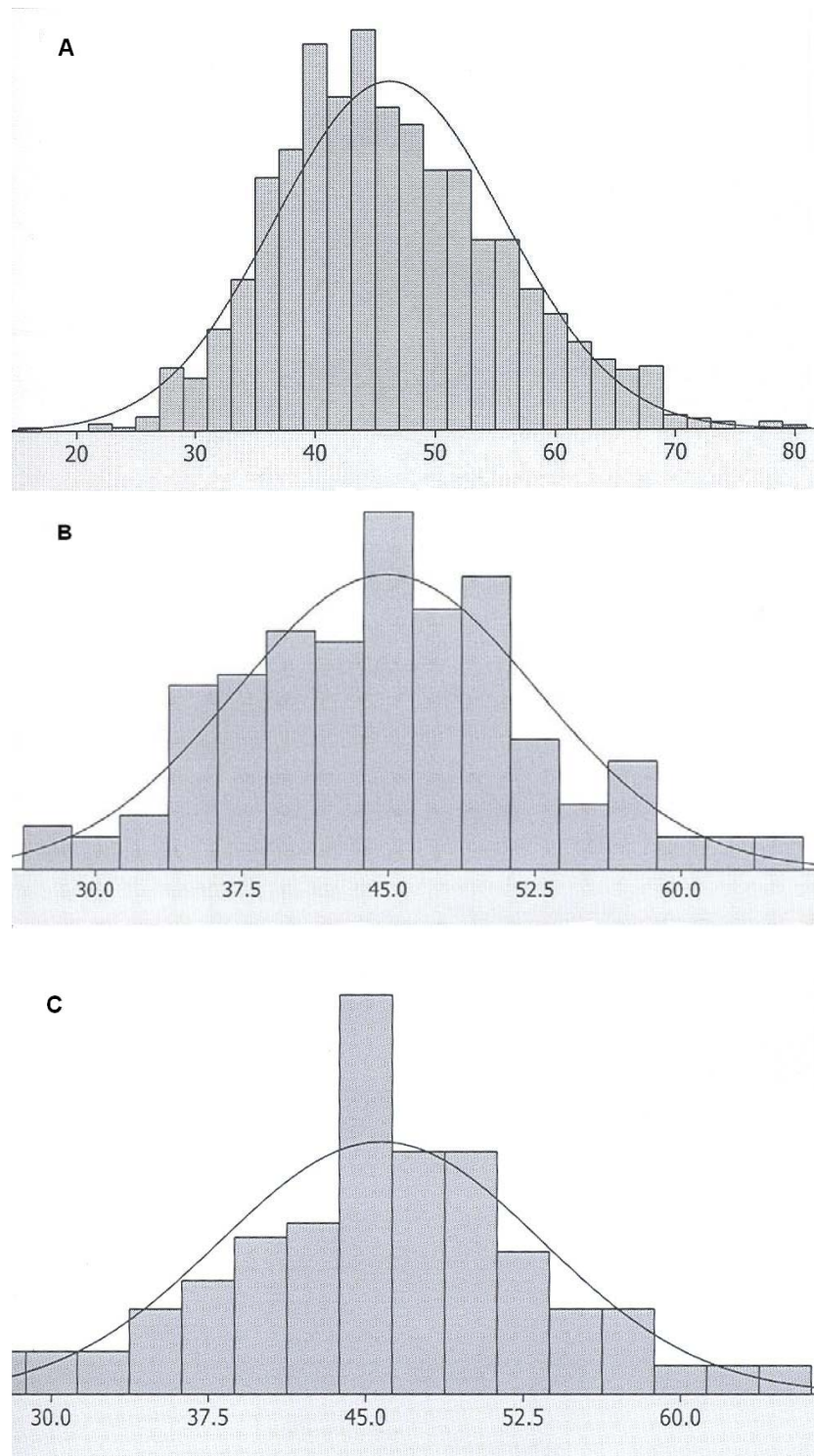


Figure 3 1: Distributions of plasma urea concentration of all the samples [A] taken during the 6 weeks of sampling, a subsample of cows sampled ± 10 days relative to AI [B] and cows with more than one calving (older cows) [C].

Table 3 2: Comparison of some reproductive parameters between the Experimental and the Whole-Herd cows grazing high protein rye-clover pasture during spring-mating season

Parameter	Experimental cows		Whole Herd	
	Sample size	Value	Sample size	Value
Submission rate (%) ¹	211	81.0	627	85.2
PR at 3 wks (%)	174	56.9	540	51.4
CPR6 (%) ²	208	71.6	636	71.9
CPR9 (%)	201	76.6	623	76.3
CPR12 (%)	206	82.5	639	85
CPR15 (%)	209	89.5	646	89.9
CPR19 (%)	193	92.8	609	92.1
CPR24 (%)	168	99.4	520	99.6
Calving rate (%)	136	100.0	-	-

¹Submission rate = Cows detected in oestrus and inseminated within the first 3 weeks of start of breeding season; PR = Pregnancy rate; ²CPR = Cumulative pregnancy rate

Calving to conception interval, ICFS and CI ($P < 0.001$) were longer while SI ($P < 0.001$) and NRR ($P < 0.05$) were higher in LMP than HMP cows. The number of services, IFSS, gestation interval and CR were not different ($P > 0.05$) between HMP and LMP cows (Table 3.3). Milk fat and protein were high ($P < 0.001$) in LMP than HMP cows.

No correlation was found between plasma urea and reproductive indexes or between plasma urea and milk production, neither was any association observed. Milk production data was highly and negatively correlated to CCI, ICFS and CI (Table 3.4). Though the regression analysis of milk yield with CCI and ICFS results were significant ($P < 0.001$; Equation 2 and 3) milk yield only explain 9 and 21% of the variance, respectively.

$$\text{CCI} = 139.4 - 1.61\text{MY} \quad (R^2 = 0.09) \quad [2]$$

$$\text{ICFS} = 118.7 - 1.51\text{MY} \quad (R^2 = 0.21) \quad [3]$$

where MY = milk yield.

Table 3 3: Reproductive parameters and milk production of spring-mated dairy cows grazing high-protein pasture and grouped as either having high (HPU) and or low plasma urea (LPU) and as high or low milk producers.

Parameter	Urea				Milk			
	n	HPU	LPU	SL ¹⁰	n	HMP	LMP	SL
Reproductive parameters								
PU (mg/dl) ¹	211	50.8±1.1	38.5±1.1	***	200	45.6±0.6	44.8±0.5	NS
SR (%) ²	211	85.0	77.5	NS	134	71.9	94.3	***
Services ³	211	1.5±0.1	1.6±0.1	NS	136	1.6±0.05	1.7±0.05	NS
NRR (%) ⁴	211	53.0	41.4	0.09	136	52.3	64.8	*
CCI (d) ⁵	137	86.1±1.8	87.8±1.9	NS	136	79.8±2.8	92.7±2.6	***
IFSS (d) ⁶	92	23.9±1.3	22.6±1.1	NS	55	20.8±1.6	22.2±1.8	NS
Gestation (d)	136	282.9±3.	278.5±3.3	NS	136	281.0±1.7	283.3±1.7	NS
		2						
CI (d) ⁷	137	368.4±2.	370.0±2.2	NS	136	360.8±3.1	376.0±3.0	***
		0						
ICFS (d) ⁸	137	78.0±1.0	79.2±1.1	NS	136	69.9±2.4	85.6±2.2	***
CR (%) ⁹	136	100	100	NS	136	100	100	NS
Milk Production								
Milk yield (l/d)	200	27.1±0.4	26.7±0.4	NS	136	31.6±0.5	21.2±0.4	***
Fat (%)	200	4.5±0.08	4.4±0.08	NS	136	4.3±0.1	4.7±0.08	***
Protein (%)	200	3.5±0.03	3.5±0.03	NS	136	3.4±0.03	3.6±0.02	***
Solids (kg)	200	2.1±0.04	2.1±0.03	NS	136	2.4±0.04	1.8±0.03	***

¹PU = Plasma urea; ²SR = Submission rate; ³No of services = includes natural breeding; ⁴NRR = Non-return rate; ⁵CCI = Calving to Conception Interval; ⁶IFSS = Interval between first and second service; ⁷CI = Calving Interval; ⁸ICFS = Interval between calving and first service; ⁹CR = Calving rate; ¹⁰SL = Significant level

Correlation coefficient between plasma urea concentration and reproductive indicators and between plasma urea and milk parameters were small and not significant (Table 3.4). CCI, ICFS and CI were negatively ($P < 0.001$) correlated with milk yield and milk solids (Table 3.4).

Table 3 4: Correlations of milk parameters and plasma urea concentrations of spring-mated dairy cows grazing high protein pastured with reproductive indexes

Parameter	CCI	ICFS	CI	IFSS	Plasma urea
Plasma urea	0.08 ^{NS}	0.08 ^{NS}	0.08 ^{NS}	0.04 ^{NS}	
Milk yield (l/cow)	-0.31***	-0.46***	-0.36***	-0.02 ^{NS}	0.04 ^{NS}
Milk fat (%)	0.05 ^{NS}	0.07 ^{NS}	0.09 ^{NS}	-0.03 ^{NS}	-0.02 ^{NS}
Milk protein (%)	0.12 ^{NS}	0.23**	0.17*	-0.15 ^{NS}	0.05 ^{NS}
Milk solids (kg/d)	-0.29***	-0.45***	-0.34***	-0.04 ^{NS}	0.004 ^{NS}

Significance level: NS = P > 0.05; * = P < 0.01; ** = P < 0.01; *** = P < 0.001

Repeatability coefficient (r) was 0.21 and reproducibility coefficient (R) was 0.55.

Concentrations of plasma urea ranged from 26.6 to 64.4 mg/dl. The pregnancy rate probability pattern did not follow any logical trend. It started at 55.3% for cows with plasma urea of ≤ 39.9 mg/dl, was 48.9% for those cows within a class of 40-44.9 mg/dl, but was 64.4% for those with a class of 45-49.9 mg/dl and 59.1% for those cows with plasma urea class equal or more than 50 mg/dl (Table 3.5). Table 3.5 also shows that as plasma urea increased between cows, the likelihood of pregnancy also increased, but at plasma category 40-44.9% the likelihood ratio was at its lowest at 0.73.

Table 3 5: Descriptive characteristics and likelihood ratios for multiple categories of plasma urea of cows diagnosed as pregnant or open, 3 weeks after the start of mating

PU category ¹	(n)	Plasma urea mg/dl ²	Pregnancy rate %	Percentage of non-pregnant and pregnant cows in PU category ³		Likelihood ratio ⁴
				Non-pregnant	Pregnant	
≤ 39.9	38	36.4 \pm 0.98 ^c	55.3	22.8	21.2	0.93
40-44.9	47	44.1 \pm 0.96 ^b	48.9	32.0	23.2	0.73
45-49.9	45	46.3 \pm 0.87 ^b	64.4	21.3	29.3	1.37
≥ 50	44	53.4 \pm 0.97 ^a	59.1	24.0	26.3	1.09

¹PU = Plasma urea; ²Different superscripts within a column are different at (P < 0.05); ³Percentage represents the number of cases in each PU category divided by the total pregnant or non-pregnant cases.

⁴Percentage of cows pregnant divided by the percentage of cows not pregnant

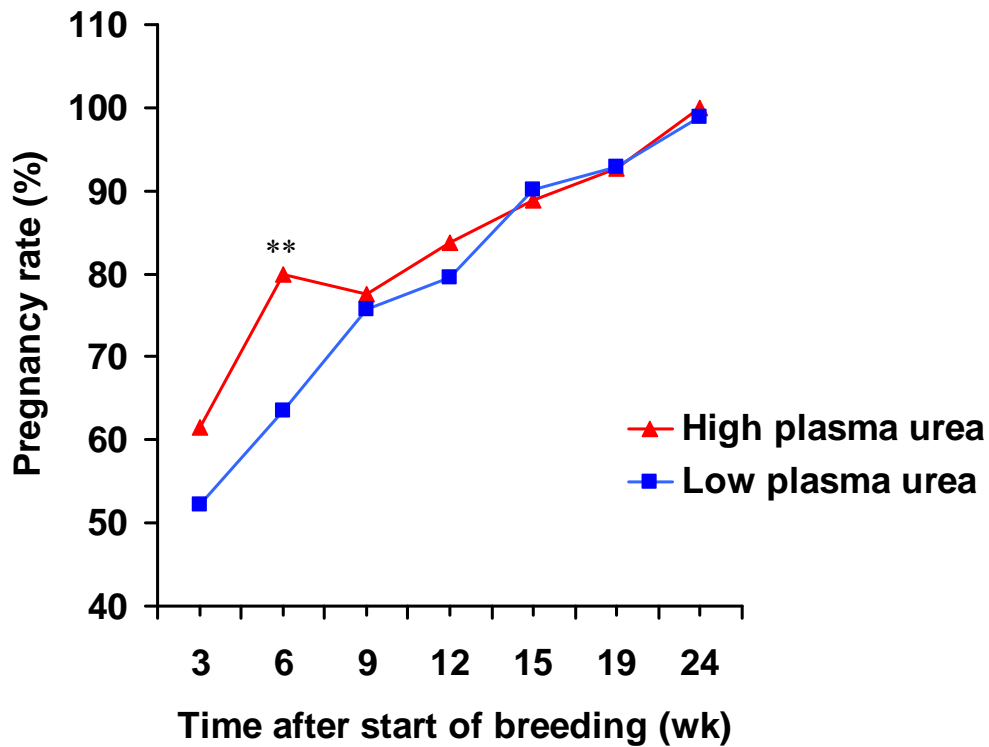


Figure 3 2: Cumulative pregnancy rate of HPU and LPU cows 3 to 24 weeks after start of breeding

3.4. Discussion

Results of the present study seem to indicate that high circulating plasma urea is not associated with reduced reproductive performance in this herd. However, repeatability of urea concentration measure was low. This may be expected since the number of urea concentration measurements per individual were low therefore decreasing accuracy associated with each measurement. Also if there was selective grazing but some cows, which represent environmental influence on repeatability, it may be expected that repeatability would be low, but then, because the interval between observation was short (7 days), this could lead to high repeatability since cows would have been in the same physiological state. Reproducibility as a measure of variation over the 6 week sampling

period was average. Despite high plasma urea concentrations, reproductive performance of the animals in the current study was similar to that of those reviewed by McDougall (2006) for dairy cows under pasture-based systems in Australia and New Zealand. Buckley et al. (2003) found a submission rate of 81% under Irish pasture-feeding systems, however pregnancy rates of 3 weeks and 6 weeks of mating were lower than those found in the present study. Lack of difference in submission rates between HPU and LPU contradicts observations made by Westwood et al. (2002) who found that cows with higher concentrations of urea were less likely to show oestrus. There was no difference in number of services between HPU and LPU groups, consistent with a recent study by Ordonez et al. (2007) where the number of services *per* pregnancy of cows grazing fertilised pasture and those on unfertilised pasture were similar (1.3 vs 1.2). In contrast, reproductive performance in dairy cows in USA showed an increase in service *per* conception from 1.8 to 3 (Washburn et al., 2002) which was attributable to genetic improvement and modern management practices that were accompanied by increased milk production. In the present study there was no difference between HPU and LPU in all reproductive parameters except for NRR which tended to high (53.0 vs 41.4%; $P = 0.09$), and PR at 6 weeks of mating ($P < 0.05$) when HPU cows showed a superior performance (80.0 vs 63.89% in PR) compared to LPU. In general, the lack of an effect was contrary to what was expected because based on the literature a decline in dairy cow fertility has been association with higher plasma urea concentrations.

Regardless of high plasma urea concentrations in the present study, there was no evidence of impairment in reproductive performance. For instance, HPU cows had an average plasma urea concentration of 50.8 mg/dl, much higher than the value suggested by Ferguson et al. (1993). In addition other studies have indicated that lower plasma urea concentrations than those value observed for HPU (50.8 mg/dl) and similar to those of

LPU cows (38.5 mg/dl), were associated with decreased conception rate (Canfield et al., 1990), decreased pregnancy rate (Butler et al., 1996; Rhoads et al., 2006) and lowered uterine pH at day 7 of oestrus (Elrod et al., 1993). The current study however corroborates that of Ordonez et al. (2007) and Kenny et al. (2001) which contradict the above mentioned studies. The difference between the studies of Canfield et al. (1990) and Bulter et al. (1996), and those of Ordonez et al. (2007), Kenny et al. (2001) and the current study is that the high blood urea concentration in the latter studies occurred after cows consumed high protein pasture while in the former, blood urea concentration was generated by feeding a highly degradable total mixed rations (TMR). Later Kenny et al. (2002) caused an elevation of plasma urea concentration in dairy cows by venous infusion of urea to achieve a concentration of 86.9 mg/dl in oviductal fluid but did not observe any change in oviductal composition except for calcium concentration, which decreased. After adding 250 g *per* cow/day of urea to an isoenergetic and isoproteinous TMR diet of dairy cows in early lactation, Dawuda et al. (2004) and Laven *et al.* (2004) did not find any impairment of reproduction, in the function of the corpus luteum, in follicular development or in embryo growth. According to Dawuda et al. 2004; 2002 the deleterious effects of urea nitrogen on the embryo occur at the time of insemination. In the present study, despite the HPU cows having comparable plasma urea concentration to that observed by Dawuda et al. (2002) (50.8 vs 54.2mg/dl) around insemination, no effects were observed. The suggestion that cows can adjust to the harmful effects of urea (Dawuda et al., 2004; Dawuda et al., 2002; Laven et al., 2004) has recently been reiterated by Ordonez et al. (2007). This may suggest that both HPU and LPU cows were affected to the same extent (Ordonez et al., 2007; Young et al., 1998). This stems from a background of hypothesis that, culture system-derived ammonia (Sinclair et al., 1999; Young et al., 1998) or ruminally derived ammonia (McEvoy et al., 1997) may not just lead to degeneration and death, but to a normally looking offspring with more subtle developmental disorders which might

impinge on postnatal growth and longevity (McEvoy et al., 1997; Sinclair et al., 1999; Young et al., 1998). If dairy cows consuming high protein pasture-based diets, such as in the present study, are able to tolerate high blood urea concentrations, it would be interesting to know how this adaptation is manifested.

Attwood et al. (1998) observed that forage-grazing sheep, deer and dairy cows harbour - ammonia hyper-producing bacteria due to the presence of high protein and low soluble carbohydrate concentrations in NZ pasture. The semi-continuous nature of grazing may have produced conditions that allow cows to adapt to high blood urea concentration. Part of this adaptation may have been partly achieved in the rumen itself, as has been alluded to in the case of sheep by Abdoun et al. (2003). Using isolated ruminal epithelium of sheep, these authors found that diet altered the function of the rumen epithelium, modulating the effect of ammonia on Na^+ transport. Having grazed high-protein pastures for a long time, it may be expected that the rumen and tissues of dairy cows are adapted to high rumen ammonia. Abdoun et al. (2003) found that Na^+ transport was enhanced in concentrate- and urea-fed animals, but not in maize-starch-fed sheep, suggesting minor effects of energy intake on adaptation and hints of an effect of ammonia. Probably such an adaptative mechanism by the rumen epithelium may also be a feature in maintaining homeorhesis by the embryo within a high ammonia or urea uterine milieu. Ammonia and urea from utero-oviductal fluid were found to be higher for ewes fed a high urea diet (Madibela et al., 1995; McEvoy et al., 1997). The presence of urea or/and ammonia in the uterus may disrupt functions of the uterus through alteration of the ionic composition of the uterine and consequently its pH (Elrod and Butler, 1993; Elrod et al., 1993). Lane and Bavister (1998) reported that the 2-cell hamster embryo uses the Na^+/H^+ antiport to regulate pH. It could be speculated that probably the ruminant embryo exposed to high concentrations of ammonia may be using the same mechanism in the same way as is the case with rumen

epithelium. The integrity of osmo-regularity by ammonia and/or urea-challenged embryo may be maintained by improved metabolism of amino acids such as glycine which have been found by van Winkle Lon et al. (1990) to protect the pre-implantation mouse embryo by acting as an intercellular osmolite. Besides adaptation by dairy cows to high ammonia from high-protein pastures, Tamminga (2006) recently suggested that polyunsaturated fatty acids (PUFA) in fresh grass may be beneficial to the rumen environment by counteracting the negative effects of high rumen degradable protein.

Adaptation to high circulating urea concentrations mentioned above may have been further strengthened by farmers' selection through culling animals that do not conceive during the mating season. Royal et al. (2000) reported that decline in pregnancy rate was associated with the lengthening of the average calving interval in dairy cows in the UK. Cows with a long calving interval and those calving late, fail to make use of available food early in the season in a seasonal production system like those in NZ (Haile-Mariam et al., 2003).

Another important goal in pasture-based feeding, besides efficient use of pasture, is to manage the herd reproductive performance to achieve a compact calving interval of 365 days (Harris and Kolver, 2001; McDougall, 2006; Verkerk, 2003) and this can be realized by culling cows that fail to conceive within 12-14 weeks of breeding season (McDougall, 2006). Basically, this means that farming under pasture-based feeding systems selects for high reproductive performance (Harris and Kolver, 2001) as well as gains in milk productivity. It can be inferred from the aforementioned argument that, if high ammonia and/or urea as a result of consuming pasture with highly degradable protein does not affect reproductive performance then farmers must be indirectly selecting for cows with high fertility amidst conditions of high blood urea.

The results of Ferguson et al. (1993) give weight to this line of argument. Inferring from their data, these authors observed that as serum urea nitrogen (SUN) increased, the likelihood of conception would decrease but cows in herds with a high conception rate (CR) will be less affected by increasing SUN compared with low CR herds, an observation affirmed by the present study. The present herd is known for a high conception rate but cows also consume high-protein spring pasture during mating and the results from the present study suggest that as plasma urea increased, the likelihood for pregnancy also increased albeit with a very small percentage. The conclusions of Ferguson et al. (1993) give impetus to the suggestion that selection for high reproductive performance under a high crude protein environment may be the reason for lack of an effect. This relationship could explain why there is contradiction in the literature about the effects of urea on reproduction since cows with low fertility would be adversely affected while those with high reproductive performance would be minimally affected. Alternatively, the high culling pressure imposed by farmers may be creating an illusion of high performance because under-performing cows are continually been replaced by heifers.

It has been proposed that partitioning of ingested energy and mobilization of body reserves by high-producing dairy cows contributes to reduced fertility (MacMillan et al., 1996). The present results show that HMP had lower SR and NRR than LMP cows consistent with other reports (Berry et al., 2003) on association of milk yield with pregnancy rate to first service. However, this difference was not reflected in CCI, CI and ICFS which were shorter for HMP cows than LMP. Lucy (2001) reports that days-open and interval to first service decrease for herds stratified from lowest to highest production, because in high-producing cows, a higher level of reproductive management such as oestrus detection compensates for a slight decline in reproductive efficiency. In the present study, cows categorized either as HMP or LMP were managed as one herd and there was no differential

reproductive management between the two groups of cows. According to Lucy (2001) adoption of North American genetics into NZ herds to boost milk yield would eventually result in low reproductive performance due to the greater reproductive demands placed on dairy cattle in NZ. A report by McDougall (2006) observed that reproductive performance varies among production systems, with pasture based systems of Australia and NZ having better reproduction than has been reported from USA and Europe. Plasma urea concentration in HMP and LMP cows was similar, further emphasizing the hypothesis that cows may be adapted to high plasma urea.

Cows selected for high milk production as represented by North American herds may reflect a change in endocrinology of the dairy cows over the years (Bousquet et al., 2004) making them vulnerable to high ammonia levels. Dairy cows in NZ with North American ancestry may perform less well than their NZ counterparts in terms of reproduction due to this genetic variability, a concern reiterated by Verkerk (2003). Studying feeding systems x genetic effects on reproduction in USA herds, Kearney et al. (2004) did not find any genotype x nutrition interaction on reproductive performance, suggesting that these traits may be under genetic influence and would still be manifested under NZ conditions if these genotypes are imported to NZ. Smith et al. (2001) ascribed the large variation in reproductive response to high-protein diets to variation in how different proportions of overseas genotype are present in NZ herds and how these traits influence adaptation to high-protein diets. Perhaps, this explains the results observed by Moller et al. (1993) who reported an association between urea concentration with fertility, where higher urea levels was evident in herds with high levels of anoestrus.

Selective culling for milk yield has the potential to create an inverse relationship between high yield and fertility (Royal et al., 2000) and the link in that relationship has been suggested as negative energy balance (NEB) (MacMillan et al., 1996; Tamminga, 2006).

High-producing cows consume large amounts of energy to cope with the demands of production and reproduction simultaneously (Kearney et al., 2004), but the fact that cows with North American genetics partition more energy toward lactation at the expense of reproduction (Lucy, 2001), means reproduction is compromised. For instance, in Holstein herds, as fat-corrected milk (FCM) increases, days-open tends to increase (Washburn et al., 2002; Westwood et al., 2002). In addition, the negative energy balance may be inherently associated with increase of milk yield as a consequence of mobilizing body reserves to meet the demand of milk production during early lactation. This period coincides with the time when the cow is expected to return to breeding activities. In Australia, Westwood et al. (2002) found that cows that lose less than 51 kg of body weight 6 weeks after the start of lactation were 3.7 times more likely to conceive at first service compared with herd mates that lost double that body weight. In NZ, Kolver et al. (2000) found that though North American Holstein heifers had high live weight and produced more milk, they only gained 5% of post-calving live weight by the end of their first lactation as compared to NZ heifers that gained 14% of post-calving live weight. Because NZ dairy cows are smaller in size and lose less body condition during early lactation, they may not encounter problems of negative energy balances to a similar magnitude as North American cows.

Conclusion and implications

Dairy cows grazing high-protein pasture diets have high circulating urea concentrations. Plasma urea concentration was consistently higher than 43 mg/dl through the sampling time. However, no evidence of impairment of reproductive performance was observed. It is concluded that a high reproductive performance index within a high crude protein environments may be the reason for lack of an effect. It is suggested that those animals observed to be susceptible to the detrimental effects of high circulating blood urea concentrations may be animals in overseas environment which have been selected for high

milk yield with little selection pressure for good reproductive performance. Alternatively, the high culling pressure inherent in the seasonal supply systems in NZ may be creating an illusion of high performance because under-performing cows are continually being replaced by more fertile reproductive heifers. High milk production had a negative effect on a few reproductive indexes probably indicating the effects of negative energy balance.

CHAPTER 4

HIGHLY DEGRADABLE CRUDE PROTEIN FROM AUTUMN PASTURE AT MATING NEITHER CAUSED EMBRYO MORTALITY NOR RESULTED IN OVERSIZE LAMBS

4.1. Introduction

Embryo mortality and oversize offspring may be a consequence of genetic aberration but according to Meyer et al. (1994) it is unclear what percentage of embryo losses is of genetic origin. However, high levels of ammonia in body fluids, as a consequence of highly degradable crude protein in sheep diets have been implicated in compromising embryo development (Bishonga et al., 1996). Embryos exposed to ammonia may degenerate (McEvoy et al., 1997) or their metabolism and growth may be enhanced (Berardinelli et al., 2001; McEvoy et al., 1997). Hence, embryos may be predisposed not just to degeneration and death but occasionally instead may appear physically normal (McEvoy et al., 1997) but may result in oversize lambs. Kelly (1982) reported reproductive wastage of 24.9% in sheep in the South Island of NZ and partitioned it into failure to ovulate, fertilisation failure, embryo mortality and prenatal mortality. Pastures in NZ contain high levels of crude protein (Chaves et al., 2002; Moller et al., 1993) and it has not been shown if the wastage reported by Kelly (1982) could be partly due to ammonia and/or urea toxicity from highly degradable crude protein in autumn pasture. After evaluating relationships between reproductive traits and environmental cues, Kleemann and Walker (Kleemann and Walker, 2005b) concluded that nutritional cues play a major part in reproductive traits in sheep in South Australia and in particular advocated further research into possible peri-conceptional dietary factors controlling embryo loss emanating from

partial failure of twin ovulations. Therefore, the aim of the present study was to determine plasma urea concentrations in ewes grazing high-protein autumn pasture during mating and to determine their possible effects on ovulation rate and conception, embryo survival, and birth weight of their lambs by comparison with ewes mated under conditions designed to provide a lower plasma urea environment.

The study was carried at Ashley Dene Farm of Lincoln University and was conducted under the guidelines of Lincoln University Animal Ethics Committee (Authority # 124).

4.2. Materials and methods

4.2.1 Animal and feeding management

Four hundred and fifty-four mature, non-pregnant Coopworth ewes were adapted by feeding hay and barley grain for nine days. The grain was initially offered at 100 g/ewe/day and then increased stepwise by 50 g/ewe until they were consuming 250 g/ewe/day on day 5 and held at this rate until day 9 after which was again increased to 300 g/ewe/day on day 10. The ewes were blocked by weight, body condition and previous prolificacy (high, HP vs. low twinning frequency, LP) into two groups. The groups were then randomly allocated to diets designed to provide high (high-protein pasture, HPP; n = 227) or low (very short pasture supplemented with barley/hay diet, BH; n = 227) dietary protein. Mean body weight and body condition score (BCS) were 61.0 ± 0.5 kg and $BCS = 2.7 \pm 0.03$, and 60.9 ± 0.5 kg and $BCS = 2.7 \pm 0.03$ for HPP and BH, respectively. Pasture was a sward of ryegrass (*Lolium perenne*)/and red clover (*Trifolium pratense*) and HPP were allocated a weekly strip of pasture behind electric fences to supply an estimated 1 kg dry matter (DM)/day. BH ewes were given 300 g of barley grain and 700 g of hay in addition to grazing of very short pasture. Weekly DM allowance of HHP was monitored by pasture clipping before and after grazing using quadrat. A 0.2 m² quadrat was thrown at

stratified way within the strip to be grazed into areas of high-, medium- and low-growth patches and all the biomass inside the quadrat was cut to 2.5 cm ground level. DM (kg/ha) obtained was averaged and used to adjust allowance based on number of animals in the paddock. It was assumed that very short pasture in paddock where BH ewes were kept would not contribute any significant amount into DM intake hence pasture intake was not estimated. A representative sample was taken to the laboratory for analysis of chemical composition. Samples of pasture, grain and hay were dried, ground and then analysed for OM, CP, NDF, ADF, DMD and ME using near infrared spectroscopy (NIR).

4.2.2 Mating and ovarian examination

Four harnessed entire rams were introduced to each group on the same day the diets commenced. Ewes were mustered at 7 day intervals during a period of 17 days and marked ewes were drafted off, denied food and water for 24 h and subjected to laparoscopic examination of their ovaries. Ewes were sedated with an intramuscular injection of 1 ml acepromazine maleate (ACEZINE 10; Acepromazine Maleate 13.5 mg; Delvet Pty Ltd, NSW, Australia). Ovaries were examined according to Mckelvey et al. (1986). Briefly, following the clipping of wool from the abdominal wall anterior to the udder, the ewes were suspended head down in a laparoscopy cradle at an angle of 45° to the horizontal. One ml of local anaesthetic (2 % lignocaine hydrochloride; Lopaine, Ethical Agents Ltd, Auckland, New Zealand) was used around the abdominal site where the incision was to be made. Two stab incisions were made about 2 cm on either side of the midline about 10 cm anterior to the udder to permit entry of an endoscope and a pair of laparoscopy grasping forceps. After insufflation of the abdominal cavity with carbon dioxide, both ovaries were located and corpora lutea counted (\approx estimate of ovulation rate; OR).

Examined ewes were removed from the experimental mob and were put on routine maintenance grazing. At approximately 50 days after ram introduction, the ewes were scanned for pregnancy status (litter size). Two blood samples were collected through a jugular vein into Li-heparin coated tubes (Becton Dickinson Vacutainer Systems, Plymouth, UK) from each ewe; one during allocation of treatment diets and a second one at laparoscopy. Blood tubes were immediately placed on ice and taken to the laboratory where plasma was harvested by spinning at 3000 rpm for 10 min at 4°C. Plasma was obtained by pipetting off the supernatant using a 3 ml plastic transfer pipette (Samco Scientific Corporation, San Fernando, USA) into a 5ml plug top test tube (Biolab, Christchurch, New Zealand). Plasma samples were stored at -20°C pending analysis. Baseline and laparoscopy urea concentrations were determined from plasma on a Cobas Mira Plus autoanalyser (Roche Diagnostics, Switzerland.) using kit # 11489364 (Roche Diagnostics, GmbH, D-68298, Mannheim, Germany). Full recording of ewes at lambing (birth date, litter size, birth weight, and sex and lamb survival) was made. Birth date was checked against laparoscopy and expected conception date to eliminate animals which conceived to ovulations other than those observed. Embryo loss was estimated by subtracting the number of foetuses at ultrasonography examination from the number of corpora lutea recorded at laparoscopy. Foetus loss was estimated by difference between the number of lambs born and number of foetuses at ultrasonography examination. Pregnancy rate (PR) was estimated from the proportion of ewes not marked again by rams in the following week. Neonatal loss was defined as lambs born dead or dying within 24 h of both.

4.2.3 Statistical analysis

Birth date was checked against laparoscopy and expected conception date and 131 animals were found to have conceived to ovulations other than those observed and were excluded

from the analysis. The resultant sample size, live weights and BCS at joining were BH; n = 165, 60.5 ± 0.4 kg and 2.8 ± 0.03 and HPP; n = 158, 60.2 ± 0.4 and 2.8 ± 0.03 , respectively. Effects of diet treatment and prolificacy on ewe parameters, plasma urea and birth weight were assessed using a general linear model (GLM) procedures (SAS 1999). Ewes, irrespective of treatment group, were categorized into high urea (HU) and low urea (LU) when their plasma urea concentration was higher or lower than the sample mean of 51.5 mg urea/dL at laparoscopy and the differences between the two groups were examined as for diet treatments. An arbitrary definition was developed for oversize lambs in order to estimate proportions of embryos that may be affected between the groups. Lambs which weighed greater than the mean plus one standard deviation (SD) ($5.0 + 1.2$ kg) were classified as oversize. The lambs were also stratified by birth-type. Lambs which weighed greater than the mean plus 1 SD for their litter size ($5.6 + 1.1$, $4.8 + 1.2$ and $4.2 + 1.2$ kg for singles, twins and triplets respectively) were also classified as oversize. Analysis for proportions was carried out using Chi-square (SAS 1999). Reproductive wastage data were analysed using Proc Mixed procedure (SAS 1999); with ewe live weight as a random factor. Results are reported as means \pm se as appropriate.

4.3. Results

4.3.1 Diet chemical analysis and estimated CP and ME intake

Results of the chemical analysis of pasture, hay and barley grain are shown in Table 4.1. Based on an estimated intake of 1 kg DM of pasture for HPP and 1 kg DM of hay and barley for BH, ME and CP daily intakes were estimated to be 11.8 MJ ME/ewe DM and 163 g CP/ewe for HPP and 11.4 MJ ME/ewe and 119g CP/ewe for BH.

4.3.1 Live weights and urea concentration

The mean live weight, plasma urea concentrations and the proportions of oversize lambs are given in Tables 4.2 and 4.3. Mean live weights of ewes at laparoscopy and mean birth weight were not affected ($P > 0.05$ in all cases) by treatment, urea category or prolificacy (Table 4.2). The proportions of lambs categorised as oversize and their mean birth weight were similar ($P > 0.05$) between dietary treatments, between urea categories and between prolificacy groups. Even when lambs were discriminated by their birth-type (i.e. singles vs. twins vs. triplets) (Table 4.3) there was no difference (12.1 vs. 13.3%; $P > 0.05$) in the proportion of oversize lambs. Their weights were also similar (6.6 ± 0.2 vs. 6.7 ± 0.2 kg; $P > 0.05$) between BH and HPP and similar (6.6 ± 0.2 vs. 6.7 ± 0.2 kg; $P > 0.05$) between HU and LU, respectively. However, there were fewer ($P < 0.05$) twin lambs that were oversize than singles and triplets (9.3 vs. 17.8 vs. 18.2 %) and they weighed 6.8 ± 0.2 , 7.3 ± 0.2 and 5.9 ± 0.4 kg, respectively, for twins, singles and triplets. In addition there was no ($P > 0.05$) difference in the mean birth weight of oversize lambs in HP and LP ewes and their proportions were 10.9 and 16.5 % ($P = 0.086$) respectively, (Table 4.3). Mean initial live weight of ewes was significantly and positively correlated with number of eggs shed ($r = 0.31$; $P < 0.001$). The correlation coefficient was again examined for ewe weight at laparoscopy and it was still positive and highly significant though poorer ($r = 0.21$; $P < 0.001$). The dependence of no of eggs shed on live weight was different at weighing stages; being greater at the start of the trial than at laparoscopy ($R^2 = 0.095$; $P < 0.001$ vs. $R^2 = 0.045$; $P < 0.001$). The distribution of plasma urea concentration at laparoscopy is shown in Figure 4.1. It was slightly high at laparoscopy for HPP ewes (58.6 ± 0.1 vs. 56.1 ± 0.1 mg/dl; $P < 0.001$) and as expected was higher ($P < 0.001$) in HU than LU (65.4 ± 1.0 vs. 49.3 ± 0.4 , $P < 0.001$). However, there was no ($P > 0.05$) difference in plasma urea due to prolificacy grouping.

Table 4 1: Chemical composition of pasture, hay and barley (as % DM)

Component	Pasture	Hay	Barley grain
Organic matter (%)	80.7	89.5	95.6
Crude protein (%)	17.6	11.8	12.0
Soluble CHO (%)	16.0	13.2	-
Acid detergent fibre (%)	25.1	31.7	6.8
Neutral detergent fibre (%)	43.3	53.5	19.0
Digestible organic matter (%)	79.3	69.7	94.2
Metabolisable energy (MJ/kg DM)	11.8	10.4	13.6

4.3.2 Reproductive performance and wastage

Reproductive parameters are shown in Table 4.4. Mean ovulation rate was similar (1.6 ± 0.1 egg/ewe) for HPP and BH and between HU and LU ewes. However, as expected those ewes with high reproductive performance (HP) had an ovulation rate of 1.9 ± 0.1 compared with 1.3 ± 0.1 egg/ewe ($P < 0.001$) for those with low reproductive performance (LP). Diet treatment and urea category did not affect ($P > 0.05$) pregnancy rate.

Embryo loss tended ($P = 0.06$) to be greater in BH than HPP ewes. Urea category and prolificacy did not ($P > 0.05$) influence embryo loss. Foetal loss, neonatal loss and total loss were not affected by treatment, urea category or prolificacy ($P > 0.05$). Eggs shed as a singleton tended ($P = 0.08$) to contribute more to embryo loss compared with multiple ovulations (19.3 ± 4.6 vs. 8.1 ± 3.3). More lambs from single ovulations died immediately after birth though the difference was not statistically significant (6.2 ± 2.6 vs. 1.9 ± 2.3 ; $P > 0.05$; for singles and multiples, respectively). Neither corpus luteal category (eggs shed as singleton or multiples) nor foetal category (foetus carried as singleton or multiples) affected ($P > 0.05$) foetal loss.

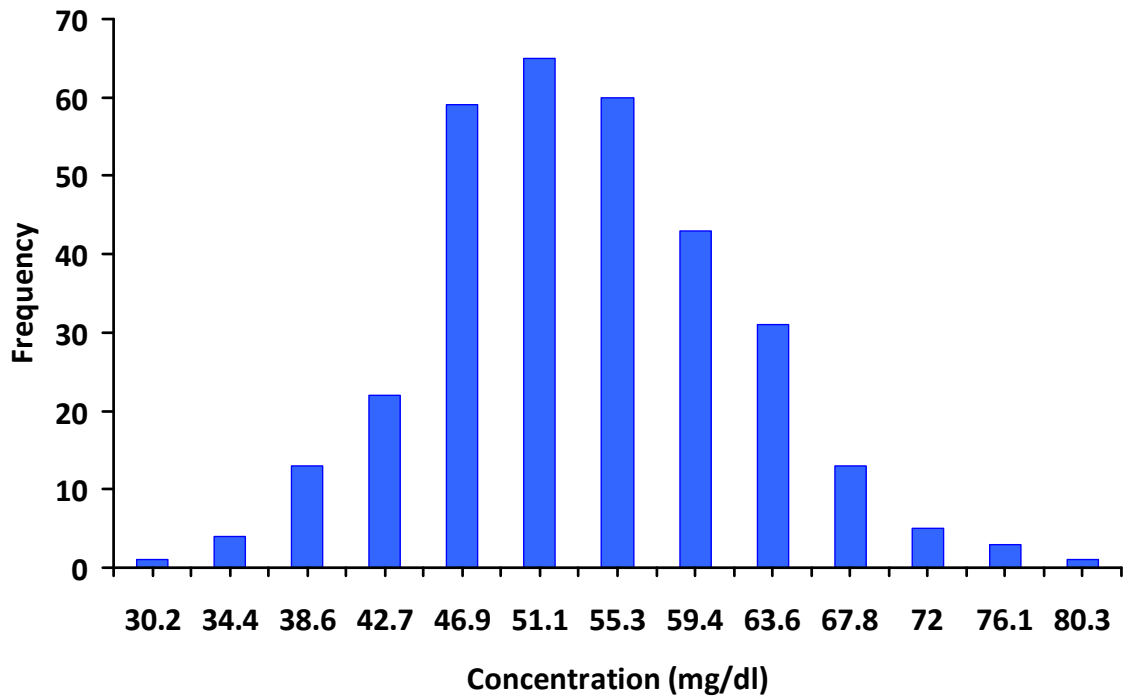


Figure 4 1: Plasma urea concentration of ewes at laparoscopy

There was no correlation between embryo loss and urea concentration at laparoscopy ($r = -0.04$; $P = 0.511$). That between embryo loss and OR was, however, significant ($r = 0.29$; $P < 0.001$). However, the regression of embryo loss on urea concentration at laparoscopy was low and not significant and was described by the following equation;

$$\text{Embryo loss (\%)} = 18.48 - 0.12\text{Urea} \quad (R^2 = 0.0015; P = 0.06)$$

According to the formula above, at plasma urea of 30.7 mg/dl found by McEvoy et al. (1997), to cause embryo death, loss would be 14.7%.

Foetal category affected ($P < 0.001$) total reproductive loss with foetuses carried as singles suffering higher losses compared to multiples (34.5 ± 4.5 vs. 13.6 ± 4.2). There was a negative and a highly significant correlation between foetal loss and lambing rate ($r = -0.30$; $P < 0.001$). Though total reproductive loss was not correlated to plasma urea concentration at laparoscopy ($r = 0.043$; $P > 0.05$), it was positively and significantly correlated to number of eggs shed ($r = 0.21$; $P < 0.001$).

Table 4 2: Mean ewe live weights, plasma urea concentrations and lamb birth weight of sheep of different plasma urea and prolificacy on autumn pasture during mating

	Treatment			Urea category			Prolificacy		
	HPP	HB	SL*	HU	LU	SL	HP	LP	SL
Urea (B) (mg/dl) ¹	49.6±0.9	49.3±0.9	NS	51.1±1.4	47.8±0.5	*	48.2±0.8	50.7±1.1	*
Live weight (kg)	59.4±0.6	60.4±0.6	NS	60.1±0.9	59.7±0.4	NS	59.5±0.5	60.3±0.7	NS
Urea (L) (mg/dl)	58.6±0.6	56.1±0.7	***	65.4±1.0	49.3±0.4	***	57.8±0.6	57.1±0.8	NS
Birth weights (kg)	4.9±0.1	4.9±0.1	NS	4.9±0.1	4.9±0.1	NS	4.9±0.1	4.9±0.1	NS
Oversize (%)	13.8	13.1	NS	13.3	13.2	NS	12.0	16.5	NS
Oversize weight (kg)	6.9±0.1	6.8±0.1	NS	6.9±0.1	6.9±0.1	NS	7.0±0.1	6.7±0.1	NS

* SL = Significance level, NS = P>0.05, * = P <0.05, ** = P<0.01, *** = P<0.001

¹B = baseline; L = at laparoscopy

Table 4 3: Re-analysis of mean birth weight of oversize lambs, adjusting for birth type (singles, twins and triplets)

	Treatment			Urea category			Prolificacy		
	HPP	HB	SL	HU	LU	SL	HP	LP	SL
Oversize (%)	13.3	12.1	NS	12.0	12.3	NS	10.9	16.5	0.086
Oversize weights (kg)	6.7±0.2	6.6±0.2	NS	6.6±0.2	6.7±0.2	NS	6.8±0.2	6.5±0.2	NS

* SL = Significance level, NS = P>0.05, * = P <0.05, ** = P<0.01, *** = P<0.001

Table 4 4: Potential reproduction (OR) and reproductive wastage of sheep on two feed treatments with different plasma urea concentrations at mating on autumn pasture and effect of prolificacy

	Treatment			Urea category			Prolificacy		
	HPP	HB	SL	HU	LU	SL	HP	LP	SL
OR (ova/ewe)*	1.6±0.1	1.6±0.1	NS	1.6±0.1	1.6±0.4	NS	1.9±0.1	1.3±0.1	***
PR (%)	92.1	92.1	NS	92.3	92.0	NS	95.8	85.7	**
LR (lamb/ewe)	1.5±0.1	1.4±0.1	NS	1.4±0.1	1.5±0.04	NS	1.6±0.1	1.3±0.1	***
Embryo loss (%)	10.9±2.7	16.5±2.8	0.06	13.3±4.1	14.1±1.8	NS	11.2±2.6	16.2±3.1	NS
Foetal loss (%)	6.4±1.7	6.1±1.7	NS	7.9±2.5	4.7±1.1	NS	5.3±1.5	7.3±1.9	NS
Neonatal death (%)	3.8±2.3	4.2±2.3	NS	4.0±3.3	4.0±1.7	NS	5.9±2.3	2.2±2.6	NS
Total loss (%)	22.5±4.0	25.5±4.0	NS	24.5±6.0	23.6±2.5	NS	23.4±3.8	24.6±4.6	NS

*OR = ovulation rate, PR = pregnancy rate, LR = Lambing rate (lamb born *per* ewe lambing).

4.4. Discussion

Diet type had no effect on ovulation rate, pregnancy rate, embryo mortality, foetal death or lamb viability. The anticipated difference in plasma urea concentration between diet types was not achieved and may reflect the possibility that although ewes were kept on short pasture less than 3 cm in length, degradable protein intake was greater than anticipated. However, there was no correlation between embryo loss and urea concentration and no evidence that the 30.7 mg urea/dL critical threshold observed by McEvoy et al. (1997) was associated with differences in embryo loss. Embryo loss observed in the present study is similar to previous studies (Kelly, 1982; Kleemann and Walker, 2005a) under normal grazing systems of NZ and Southern Australia. In the literature, degeneration of embryos in ewes fed diets containing high proportions of urea was attributed to ammonia which was postulated to be toxic to the embryo (McEvoy et al., 1997). In other studies, diets containing high levels of protein were found to up-regulate embryo metabolism (Madibela et al., 1995), which may result in early death. This is in complete contrast to the present data since if any thing, the trend was for embryo mortality in ewes on the high-protein pasture to be lower than that in ewes fed the hay/barley diet. Even when ewes were categorised as having high blood urea concentrations, embryo mortality, foetal death or neonatal viability were not different. There are two inferences from the lack of difference. Either both groups were affected but to a similar degree or high plasma urea concentrations were without effect, possibly as a consequence of adaptation. Recent studies with dairy cows similarly found no impairment of reproductive performance when serum urea was increased from 37 to 43 mg urea/dL (Dawuda et al., 2004), from 27 to 42 mg/dL (Ordonez et al., 2007) or from 38.5 to 50.8 mg urea/dL (Madibela et al., 2008) as a result of offering 14g/kg of urea/molasses as part of diet or due to urea fertiliser treatment of pasture. Plasma or serum urea concentrations in the present work and that of Ordonez et al. (2007) were 150 to 200 % greater than the 30 mg/dl reported by McEvoy et al. (1997) to be associated

with abnormal embryo development in sheep. Moreover, plasma urea concentrations in excess of 40-50 mg/dl are common in pastoral situations (Sykes, 1978) as are high reproductive rates (Davis et al., 1987). In the present work, 99.7% of ewes had plasma urea concentrations higher than 30.7 mg/dL, implying that, if indeed high plasma urea was detrimental to embryo development, then almost all the ewes would be expected to have been affected. This suggests either longer-term adaptation to high plasma urea concentrations in grazing sheep or a more complex relationship between diet and embryo development as discussed by Ordonez et al. (2007).

Conclusions

While the study did not achieve large differences in plasma urea concentrations between diets, the high levels of plasma urea measured were accompanied by normal reproductive wastage rates. Together with similar findings from studies in dairy cattle (also chapter 3), the data suggest either that findings from controlled studies have a more complex aetiology or that pastoral animals can adapt to tissue ammonia/urea status resulting from large amounts of highly degradable protein in their diets.

CHAPTER 5

AMINO ACID SUPPLY IN TWIN-SUCKLING EWES GRAZING

SPRING PASTURE

5.1. Introduction

Increasing levels of nutrition of livestock have led to enhanced expression of immunity and improved resilience against parasitic infection. Protein supply has been found to be particularly important. Bown et al. (1991a) demonstrated that nitrogen retention in infected sheep was increased fourfold by abomasal infusion of protein but only twofold by infusion of energy. Fish meal as a source of rumen by-pass protein increased the rate of worm expulsion in lambs (van Houtert et al., 1995) and reduced the peri-parturient increase in FEC and reduced worm burdens in reproductive ewes (Donaldson et al., 1998). Resilience of lambs (Bown et al., 1991a) or dairy goats (Chartier et al., 2000) or resistance in ewes (Donaldson et al., 1998; Donaldson et al., 2001; Houdijk et al., 2001a; Houdijk et al., 2000; Houdijk et al., 2005) to infection have been enhanced by supply of protein. However, such responses probably involve a requirement for specific amino acids rather than protein *per se* (Hoskin et al., 2002) and therefore the identification of specific amino acid profile requires further elucidation.

Under normal circumstances amino acid metabolism within the gastro intestinal tract (GIT) and its accessory organs makes a large contribution to amino acid turnover and can have an important bearing on the availability of amino acids for the support of other productive functions (Reeds and Burrin, 2000). It is in the GIT where *Trichostrongylus colubriformis*, a major sheep parasite in NZ, comes into contact with the epithelium of the small intestine (Bermingham et al., 2005). Thus it is conceivable that by preferentially meeting the

metabolic needs of the mucosa for immune and tissue repair responses by “first-pass” use during protein-limiting conditions (as imposed by parasite infection), systemic availability of essential amino acids would be low (van Goudoever et al., 2000). This was observed by Yu et al. (2000) who reported that in *T. colubriformis* infected sheep, leucine use by the gastrointestinal tract was increased by 24 %, though a large proportion of the extra amino acids appeared to come from arterial sources. Net influx of leucine to other tissues was reduced. Valine fractional synthesis rates doubled in mesenteric lymph nodes, duodenal smooth muscle and ileal smooth muscle of *T. colubriformis* infected compared to control lambs whereas rates in skeletal muscle, the skin and the liver remained unchanged (Bermingham et al., 2006).

The negative correlation between FEC and wool growth (Liu and Karlsson, 2004; Miller et al., 2000) suggests that nematode-associated immune responses compete for sulphur-containing amino acids that are critical to wool growth (Liu and Karlsson, 2004). It has been postulated that following infection and trauma, there is an enhanced requirement for sulphur-containing and related amino acids in human subjects (Grimble, 1992). The possible elevation in requirement was suggested by the composition of certain components of the immune system which display a high content of sulphur amino acids (Sykes and Coop, 2001). For instance, the cysteine requirements for immunity-associated responses could be expected to be high, due to the high concentration of cysteine in mucins, a component of mucus (Liu et al., 2003). The proliferation of lymphocytes and cells of the GIT places a high demand for glutamine (Hoskin et al., 2002). In fact, glutathione (GSH) consists of glycine, glutamic acid and cysteine while metallothioneins contain glycine, serine, cysteine and methionine. These two, GSH and metallothionein are produced in large amounts in response to cytokines responsible for immune and inflammatory reactions in humans (Grimble and Grimble, 1998) and GSH is important for several T-cell functions

(Miller et al., 2000). Therefore, the specific amino acids required for the immune system in parasite-infected animals need to be identified if feeding strategies are to be developed to sustainably enhance immunity in infected animals. However, since most proteins are altered by rumen microbes it is important to know how much and which amino acids reach post-ruminal regions of the gut and probably become available for immune function.

There is limited data on protein supply beyond the rumen in lactating ewes on pasture (Dove and Milne, 1994; Dove et al., 1985; Dove et al., 1988) and there are none which describe the effect on amino acid supply of protein supplementation regimes which enhance immunity (Sykes, unpublished data). Therefore, the aim of the present study was to determine the effect of supplementation with rumen-protected protein (fishmeal) on the amino acid supply to parasitized twin-rearing ewes on pasture. In the light of findings in previous studies (Dove et al., 1988) of diurnal variation in the flow of dry matter and non-ammonia nitrogen (NAN) through the abomasum, the present work was designed to confirm this possibility on amino acid flow. The trial also aimed to determine any changes in amino acid composition of microbial protein and its flow through the abomasum. The concentrations of amino acids in systemic circulation in ewes were also determined.

5.2 Material and methods

5.2.1 Animals and surgery

Twenty-four pregnant (scanned as carrying twins) Coopworth ewes were assembled at the Johnstone Memorial Laboratory. Two months before parturition twenty ewes were equipped with rumen and abomasal cannulae. The abomasal cannula was inserted according to Greer (2005). Briefly, a cannula with internal and external flanges was fitted after denying animals feed and water for 24 h before surgery. The cannulae were constructed using a 5 cm polyvinylchloride (PVC) barrel with a round lug at one end, two

oval latex flanges and a PVC flange. The cannula barrel passed through a hole in the centre of the flanges. One latex flange was held in place proximally by the lug of the cannula barrel and the second distally by a PVC flange and a tight retaining clip. The cannula was closed using removable stoppers made by shortening the syringe plunger (Terumo Syringe; Terumo Corporation, Philippines) and locked in place using a paper clip (Figure 5.1). During the operation, general anaesthesia was induced by 20 ml of mixture of 0.3-0.7 % diazepam (Pamlin) and 10-13 % Ketamine hydrochloride (Ketamine) both sourced from Parnell Laboratories New Zealand Limited, Auckland, New Zealand. An initial dose of 12 ml (0.2 ml *per* kg of live weight) was given intravenously with occasional “top up” when necessary. After exteriorising the abomasum, a purse-string suture was made around the area where the cannula was to be inserted, about 10 cm anterior to the pylorus, to allow for collection of digesta passing through the abomasum. The cannula was inserted through an incision made between the string suture which was then tightened and tied. The abomasum was returned into the abdominal cavity and the cannula exteriorised through a stab incision made between the two hindmost ribs and held in place by the flanges and a rubber retaining clip. Each animal was given 5 ml of 33-38% dihydrostreptomycin sulphate (Depomycin; Intervet Limited, Upper Hutt, New Zealand) and 16-24% penicillin g procaine (Intervet Limited, Upper Hutt, New Zealand) intramuscularly as a prophylactic antibiotic and 1.5 ml Flunixin Injection as an anti-inflammatory (5-9.5% Flunixin meglumine; Parnell Laboratories New Zealand Limited, Auckland, New Zealand). Animals were allowed to recover before sampling took place. After-surgery care included giving each animal 60 ml Ketol (Bomac Laboratories Limited, Auckland, New Zealand) and allowing animals to recuperate in hay-bedded pens for two days. During this time, ewes were offered chopped alfalfa hay and each ewe was given 5 ml Depomycin, 1 ml Flunixin and 60 ml Ketol daily and after two days they were maintained on ryegrass/clover pasture. A rumen cannula was inserted on the left side over the anterior dorsal abdomen on

the remaining fourteen ewes after 6 animals were removed after developing complications ranging from loss of the abomasal cannula to herniated fistula. The cannulation and fitting of the cannula was according to the methods of Hecker (1969). Briefly, after clipping the wool and disinfecting the site, an incision 5 cm long was made in the ventro-caudal direction through the skin from the last rib and from the end of the transverse process of the first lumbar vertebra. The underlying abdominal muscles and peritoneum were separated by blunt dissection to form an opening 5 cm long. A part of the rumen wall was withdrawn and held with forceps while a clamp was applied and the screws tightened. A suture was then placed through the skin, the rumen wall under the clamp, and the skin on the other side and was tied over the clamp. This suture held the skin near the middle of the clamp. After 6 to 8 days when the rumen fold sloughed off, the clamp was removed and a flexible rubber cannula was inserted. Wound hygiene was maintained throughout the experiment by clipping and washing the area around the fistulas with diluted savlon at least once a week.

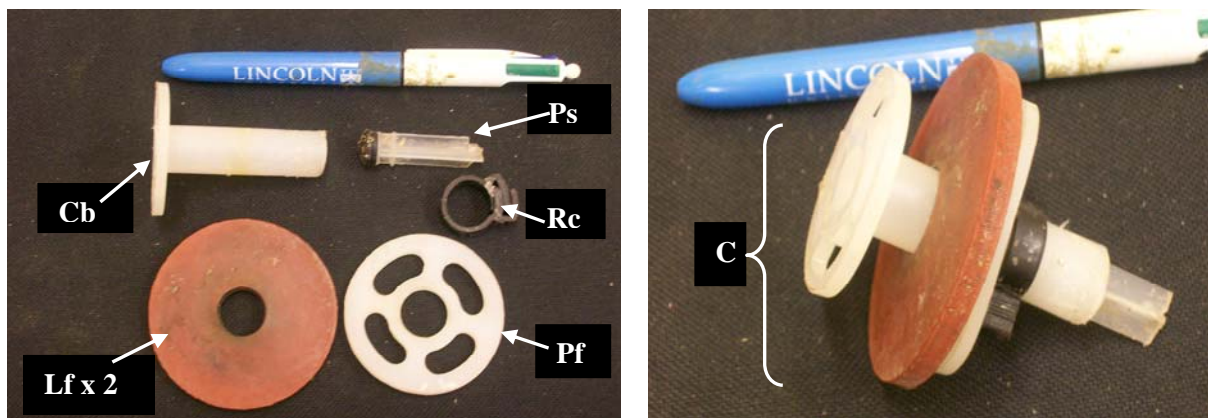


Plate 5.1: The cannula (C) was made from cannula barrel with round lug (Cb), 2 latex flange (Lf), PVC flange (Pf), retaining clip (Rc) and a plunger stopper with a paper clip (Ps)

5.2.2 Treatments and animal management

Three weeks before parturition the 14 ewes, weighing 65.1 ± 1.50 kg were stratified by weight into two groups in a crossover design. The two groups were then randomly allocated into one of two treatments; 1) ryegrass (*Lolium penenne*)/clover (*Trifolium repens* var Huia) + 500 g/d/ewe protein supplement (S; n = 7) and 2) ryegrass/clover alone (C; n = 7). Both groups grazed the same potentially nematode-contaminated pasture. The formulation and rate of supplementation was based on the prediction by Donaldson et al. (2001) of the MP supply consistent with maintenance of immune function (low FEC) during the peri-parturient period and experience with supplementation regimes which reduced FEC of ewes on pasture (Xie, 2003). The treatments were anticipated to provide 1.0 x metabolisable energy (ME) requirement in both groups, and 1.0 and 1.3 x metabolisable protein (MP) requirement in C and S ewes respectively, based on AFRC (1993) recommendations for twin-suckling ewes. During lactation a milk yield of 3.0 kg/d was assumed, which was typical of the breed of ewe used in the trial (Geenty and Sykes, 1986). Available pasture biomass was initially estimated by pasture rising plate meter (Filip Folding Pasture meter, Feilding, NZ) and excess pasture was mowed from 24 to 20 cm. During adaptation, ewes grazed paddocks adjacent to the experimental paddocks and pasture intake was estimated by pasture clipping (3 random sites *per* paddock, before and after a grazing period of 7 days). Thereafter, pasture availability was assessed visually on weekly basis by an experienced operator. An attempt was made to equalise ME intake of S and C ewes by allocating a smaller paddock to the S ewes but attaining higher MP intake through offering the supplement (see section 5.2.3 for Supplementation). Animals grazed as separate groups behind electric fences to force them to graze to the same height in an attempt to equalise larval and ME intakes.

Protein supplementation was introduced to the S ewes 2 weeks before lambing. After lambing, S ewes continued on the supplement for a further 42 days until they were eating 500 g/ewe/d after which sampling took place for 5 days. At day +50 relative to lambing, groups were reversed and the previously non-supplemented ewes allowed to get accustomed to the protein supplement for 14 days before being sampled for another 5 days. All animals were drenched with levamisole (7.5 ml *per* kg LW; Novartis, New Zealand Ltd) 14 days after lambing to get rid of nematode parasite from pasture and to be able to examine animal response to parasite challenge. During days 35 to 42, all ewes were trained to go through a sampling crush (Plate 5.2) and accustomed to feeding pens. Sampling was done by two people stationed at two different crushes to ensure that the whole process of sampling and supplement feeding took as little time as possible.



Plate 5.2: Sampling crush

5.2.3 Supplementation

The protein nuts (see Table 5.1 for chemical analysis) consisted of 40 g molasses/kg, 200 g fishmeal/kg, 200 soya/kg, 240 g barley/kg and 320 g bran/kg. Ewes were initially introduced to 50 g supplement/ewe/day at 09.00 h on a group basis within the paddock. At lambing, the supplement was gradually increased stepwise, by 100 g/ewe/d, until they were consuming 500 g/day on a group basis. During this period, all ewes were trained to go through pens and sampling crushes and accept harnesses and infusion equipment. From d +35 relative to lambing protein supplementation was provided on an individual basis in individual pens (Plate 5.3). One of the seven S ewes did not consume the supplement. In order to encourage acceptance during the adaptation period, 50 g of protein nuts was introduced through the rumen cannula. For the rest of the animals, if 20 min elapsed without complete consumption of allocated supplements the remaining feed was likewise placed inside the rumen at the end of the feeding interval during the adaptation period.



Plate 5.3: Individual pen where ewes were fed protein supplements

5.2.4. Cr-EDTA, Ytterbium acetate and alkane dosing

Five days before collection, a backpack (made of a sealable plastic container and attached to a saddle made of polythene foam) holding a multichannel peristaltic pump (CPP30, ChemLab, England) and two Viaflex bags (each containing 900 ml liquid Chromium-EDTA (Cr; 2.77 g/L) and ytterbium; 150 mg/L) was held on the back of each sheep. Cr-EDTA was prepared by dissolving 71 g of $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ in 1000ml of water and 100 g EDTA-disodium salt in 1500 ml of water. The EDTA solution was then added to the Cr solution and then boiled for 1 h in a covered beaker. After cooling, excess EDTA mixture was neutralised with 20 ml 1 M CaCl and the pH adjusted to 6.5 with about 60 ml of 10 M NaOH. A solution of ytterbium acetate was prepared by dissolving 3.04 g in one litre of nanopure water and diluted to 1 in 10 to get 150mg/l of pure ytterbium. The backpack was held by a means of straps after ewes had been previously trained to carry it (see Plate 5.4). The straps from the saddle went around the belly of the sheep to keep the backpack in place. The markers were continuously infused over 24 h and fresh bags were placed daily at 09.00 h. The battery operated pumps were previously calibrated to deliver 37.5 ml/ h of solution but actual volume infused was determined by measuring the volume of residual Cr-EDTA and Yb solution the following day. To estimate nutrient intake an alkane pellet (150 mg of C32; dotriacontane) was prepared by wrapping the alkane in tissue paper and was placed directly into the rumen mat via the rumen cannula every day at 09.00 h, three days prior to sampling, and terminated two days before the end of sampling.



Plate 5.4: Sheep carrying a backpack holding a peristaltic pump and two Viaflex bags at a paddock

5.2.5 Sampling

5.2.5.1 Blood, faeces and weights

Prior to introduction of the protein supplement, on d -17 relative to lambing, animals were bled from the jugular vein (10 ml) into Li-heparin coated tubes (Becton Dickinson Vacutainer Systems, Plymouth, UK) and then weekly thereafter at 09.00 h before supplementary feeding. The tubes were placed on ice and taken to the laboratory where plasma was harvested by spinning the blood at 3000 rpm for 10 min at 4°C. Blood samples were also taken on day -1, +1, +2 and +5 relative to day of abomasal and rumen digesta sampling (where day +1 was taken as first day of sampling). During the same times when blood was sampled, faecal samples (20 g) were taken from the rectum, placed in a jar and analysed while fresh for faecal egg count (FEC). During the five-day digesta-sampling period, daily faecal samples were also taken, frozen, and bulked for each animal. Live weights were recorded on a weekly basis. Body condition score was taken at 23, 52 and 70

days after lambing. Lambs were weighed within 12 h of birth and then at 9, 21, 28 and at 35 days of age and weighing was then discontinued because of the pressure of work. Lambs were not allowed to get access to their dam's supplementary feeds so that lamb live weight gain could be used to estimate milk yield.

5.2.5.2 Pasture and protein nuts

Herbage samples (200 g) were obtained by diagonal transect sampling, twice weekly after 12.30 h during the digesta sampling period and stored in the freezer pending analysis for chemical composition and amino acid profile. Daily pasture samples for alkane analysis were taken 2 d prior to digesta sampling and 2 d before the end of sampling, during both sampling periods. Protein nuts (50 g) were sub-sampled during daily feeding, bulked and stored until analysed for chemical composition and amino acids.

5.2.5.3 Rumen and abomasal digesta

Digesta was sampled to provide samples to give a pattern of 3 h intervals during a 24 h d by sampling at 12 or 15 h intervals over the 5 d. This was to reduce disturbance to the pattern of grazing while allowing estimation of diurnal variation in flow. Approximately 300 ml of rumen digesta was collected from the ventral sac under mild vacuum induced in a PVC tube attached to a 100ml syringe. The digesta was placed into a 500 ml plastic container and mixed using a glass rod before pH was measured (ISFET pH meter, KS701, Shindengen Electric Manufacturing Co. Ltd, Japan). About 100 ml rumen digesta was sub-sampled and transferred with 1:1 v/v of rumen buffer (0.9% NaCl, 0.1% Methyl cellulose and 0.01% Carboxyl-methyl cellulose) to a 250 ml plastic bottle. The suction pump was flushed with clean water between each sampling. The abomasal samples were obtained into 500 ml plastic containers by removing the plunger from the cannula barrel. The abomasal digesta was mixed using a glass rod before pH was recorded. Two subsamples (200 ml) were obtained and placed into two 250 ml plastic bottles. Both the rumen and

abomasal samples were placed inside a bin filled with ice and later stored in a freezer prior to analysis. The sampling container, the glass rods and the pH meter were cleaned between sampling.

5.2.6 Processing of rumen digesta for rumen microbes

To extract bacterial protein, rumen samples were processed according to modified methods of Ranilla and Carro (2003) and Cecava (1990). This involves a series of centrifugation and vortexing to isolate rumen microbes and the detailed processing methodology is given in Appendix 5.1. Before analysis for amino acid, samples were pooled together across subjects due to small quantities of samples.

5.2.7 Analysis

5.2.7.1 Chromium-EDTA

Chromium in abomasal digesta was analysed according to the methods of Murthy et al. (1971). The standard for chromium analysis was prepared by dissolving 76.9 mg of chromium (III) chloride 6-hydrate (MW 266.4) in 4 ml 3M HCL in a 100 ml volumetric flask. Final concentration of 150 mg /l chromium (150 ppm) was achieved by topping up with nanopure water. Eight standards (1, 2, 3, 4, 5, 7, 10, 15mg/l) of 7.5 ml were prepared using a blank abomasal digesta supernatant as a matrix diluent and used to draw the standard curve. Duplicate subsamples of digesta were centrifuged at 13000 rpm for 30 min at 4°C in a Beckman J2 MI centrifuge using a JA 14 rotor (Bekman Instruments, California, USA). The supernatant fraction was carefully pipetted off into a clean tube (a visible purple colour indicating the presence of Cr/EDTA). The supernatant was further diluted 1:10 with blank abomasal fluid in order to fit within the standard curve concentration. Analysis was carried out using normal burner, acetylene instrument grade gas and compressed air in Atomic Absorption Spectrophotometer (Model 5100 PC, Perkin-

Elmer Corporation, Norwalk, Connecticut, USA). The results were recorded as mg/l concentration of Cr.

5.2.7.2 Ytterbium

Ytterbium was analysed in the abomasal digesta according to a modification of the method of Siddons et al. (1985). Standards were prepared by dissolving 0.050 g of ytterbium acetate (MW 350.24) in nanopure water to volume in a 100 ml volumetric flask to achieve a final concentration of pure ytterbium (MW 173.04) 250 mg /l ytterbium (250 ppm). Five standards (5, 10, 15, 20, 25 mg/l) were made for preparation of a standard curve using blank abomasal fluid. The abomasal fluid was mixed well and used as whole (not centrifuged). All standards including blank digesta for the blank standards (20 ml) were taken through the entire method of analysis. An acid solution was made by adding 20 ml nitric acid and 1.9 g/l potassium chloride (1 mg potassium *per* ml) to approximately 800 ml nanopure water and making up to a litre. Twenty ml of abomasal digesta sample or standards in duplicates were placed into the pre-weighed 20 ml scintillation vials and reweighed. The samples/standards were then dried at 100 °C for approximately 48 h in a drying oven. The samples/standards were then reweighed after cooling in a desiccator to calculate DM. They were then ashed at 550 °C for eight hours. The weight of the ash was recorded and then extracted out with 20 ml acid solution (HNO₃/KCL) by shaking continuously for six hours using a shaker (Gerhardt, Bonn). The ash was then allowed to settle out of solution for approximately two days before analysis. Analysis was carried out using the nitrous oxide burner (blue), nitrous oxide, acetylene instrument grade gas and compressed air using Atomic Absorption Spectrophotometer (Perkin-Elmer, 5100 PC, Norwalk, Connecticut, USA). Results were recorded as mg ytterbium/l digesta.

5.2.7.3 Glucose

Glucose concentration was measured from whole blood of ewes immediately after collection. This was achieved by glucose kit (Accu-Chek[®], Advantage II; Roche Diagnostics GmbH, D-68298 Mannheim, Germany) by touching and holding a drop of blood to the edge of the test strip which contains glucose dehydrogenase in the presence of coenzyme PQQ. Glucose in blood is converted to gluconolactone and the reaction creates a small electric current that is read by the meter.

5.2.7.4 Amino acid analysis in abomasal digesta, ruminal bacteria, pasture and protein nuts samples (Hydrolysis method)

Eighteen commercial amino acids (AA) in an assorted solution with concentration of 2.5mM (Sigma, Aldrich, Australia), diaminopimelic acid (DAPA) and taurine (the latter two were prepared in-house to a solution of 2.5 mM from stock powder) were used to prepare standards for construction of standard curves. Freeze-dried abomasal digesta, the rumen bacterial pellet (from Section 5.2.6), pasture and protein nut samples were ground to pass through a 0.5 mm sieve (Tecator 1094 homogenizer, France) and about 75 mg weighed, in duplicate, into screw-top kimax tubes. Hydrolysis was by a modified method of Fountoulakis and Lahm (1998) and it is detailed in Appendix 5. 2. The Hewlett Packard 1100 Series HPLC system with Chemstation software (Agilent Technologies, Waldbronn, Germany) was used to determine amino acids according to the methods of Heems et al. (1998) and Carducci et al. (1996) utilising a 250 mm x 4.6 mm, 5µm Reverse Phase Prodigy column (Phenomenex, New Zealand) heated to 40°C.

5.2.7.5 Oxidation method for analysis of cysteine and methionine in abomasal digesta, ruminal bacteria, pasture and protein nuts samples

Cysteine and methionine were analysed according to modified methods of McNabb et al. (1993). Sulphur amino acids are destroyed to varying extents by HCL hydrolysis. Cysteine and methionine were therefore oxidised to cysteic acid and to methionine sulfone, respectively, followed by hydrolysis (Gehrke et al., 1987). The oxidation method is detailed on Appendix 5.3. The determination of cysteine and methionine was carried out by HPLC.

5.2.7.6 Plasma amino acids

Standards used for plasma free amino acids were prepared the same way as in section 5.2.7.4 but with the addition of glycine standards. Standards were diluted 1 in 2 with control plasma from pooled plasma samples. After thawing, 500 µl of plasma sample was pipetted into 1.5 ml tubes in duplicates. Tubes received 15 µl of internal standard (α -amino butyric acid), including the solvent blank. Samples were deproteinised by adding 50 µl of 0.2 M sulfosalicylic acid as modified from the methods of Qu et al. (2001). The mixture was vortexed for 1 min and incubated at room temperature for 20 min. Tubes were centrifuged at 13000 rpm for 5 min in a micro centrifuge (Biofuge Pico, Kendro Laboratory Products, Germany). The supernatant was placed in separate 1.5 tubes and 5 µl sodium hydroxide solution (4 M) was added to neutralise the sulfosalicylic acid. The contents were mixed well and filtered through a 33 mm 0.22 µm PES syringe filter (Biolab, New Zealand) into a HPLC vial containing a vial insert and cap. Fifty microliter (50µL) of control plasma was used to dilute the standard filtrate in a HPLC vial containing a vial insert at 1 to 2 dilution and contents mixed. Samples were analysed using HPLC utilising a 250 mm x 4.6 mm, 5µm Reverse Phase Prodigy column (Phenomenex, New Zealand) heated to 40°C.

5.2.7.7 *FEC*

Egg count in faecal samples (FEC) was measured by flotation in brine using a modification of the McMaster method (MAFF, 1979) and expressed as eggs per gram (peg) of fresh faeces.

5.2.7.8 *Chemical composition of pasture and protein nuts*

The freeze-dried pasture samples and the protein nuts were milled through a 1mm screen before crude protein (CP), organic matter (OM), crude fat (CF), acid detergent fibre (ADF), neutral detergent fibre (NDF), water soluble carbohydrate (WSC), digestible organic matter (DOM), digestible dry matter (DDM) and metabolisable energy (ME) were determined using near infrared spectroscopy (NIR Systems, Model 5000, Foss, Hillerød, Denmark).

5.2.7.9 *Alkane analysis*

Freeze dried pasture and daily faecal samples were milled through a 1mm screen before being analysed for alkane according to a modification of method of Dove et al. (1996). There may have been alkanes in the supplement, however the supplement was not included in the analysis of alkane. An internal marker (C34; 0.4 ml) was placed into 70 ml kimax tubes containing either 1 g dried faeces or 2 g dried pasture sample. Potassium hydroxide (KOH) in methanol was added to each tube (10 ml for faecal and 20 ml for pasture samples). The tubes were closed and left overnight and the following day were heated in an oven at 90 °C for 3.5 h with vortexing every hour. When the contents of the tubes were reduced, methanol was added. After 3.5 h, the tubes were placed in a preheated water bath at 60 °C. To each faeces sample, 5 ml of nanopure water and 5 ml heptane was added but the amounts of nanopure water and heptane was doubled for herbage samples. The tubes

were closed, shaken and then returned to the water bath to allow a biphasic layers to form. To extract the alkane, syringes filled with $\frac{3}{4}$ silica gel (0.063-0.200 mm) were used. The syringes were washed with heptane in a vacuum manifold before use. An equivalent number of tubes to sample tubes were labelled and aligned with taps in the vacuum manifold. Vacuum was turned on and top layer of the biphasic was sucked slowly through silica gel into the new tubes. The elutant went through a series of washes (3 times for faecal and 4 times for herbage samples) by shaking with 5 ml of heptane. The equipment was rinsed through with 10 ml of heptane. Any discolouration was eliminated by passing elutant through series of silica column syringes and rinsed with final flush of 10 ml heptane until the final collection was clear. Tubes containing clear elutant were placed in an oven at 90°C to evaporate the heptane. Alkane was recovered by addition of 0.7 ml heptane to each tube and then vortexed. The contents of the tubes were transferred to vials for gas chromatography (HP 6890, Hewlett-Packard Company, Wilmington, USA) analysis. The chromatography utilised a 30 m x 0.53 mm x 1.50 μ m column Zebron ZB-1 set to 245 °C.

5.3. Calculations and Statistical analysis

5.3.1 Pasture, nutrient and amino acid intake

Pasture intake was estimated by using faecal alkane data from 4 animals for each treatment group and this was over-sight during laboratory analysis of alkane in the faeces samples (during sampling all animals were sampled). However, mean DM intake of each group (i.e. S and C) was used during calculation of nutrient intake of individual animal in each group respectively. The plant odd-chain alkane *i* (C33) and the dosed even chain alkane *j* (C32) were chosen as external and internal marker respectively and these were fitted in the formula by Dove and Mayes (1991);

$$\text{Intake} = (F_i/F_j \times D_j) / ((H_i - (F_i/F_j \times H_j)) \quad [5.1]$$

F_i = Faecal concentration of C33; F_j = Faecal concentration of C32; D_j = daily dose of C32

H_i = Herbage concentration of C33; H_j = Herbage concentration of C32

Nutrient (ME, CP, MP) and amino acid intake was calculated from the product of mean DM intake (kg) estimated from alkane analysis and nutrient concentration (g/kg DM). MP intake was estimated according to AFRC (1993) and the following assumptions were made; 1) rumen outflow rate of effective rumen degradable protein of pasture and protein supplement to be 0.08/hour; 2) level of feeding was taken to be 3.0; 3) to estimate DUP the following formulas were used;

$$\text{DUP} = 0.9[(\text{UDP}) - 6.25(\text{ADIN})] \quad [5.2]$$

In which ADIN was estimated by a formula;

$$\text{ADIN} = \text{ADF}/62.5 \quad [5.3]$$

5.3.2 Faecal egg count average daily gain and milk yield

Faecal egg count (FEC) was log-transformed [$\log_{10}(\text{FEC}+1)$] before analysis but was reported as back-transformed values. Average daily gain (ADG) for lambs was calculated by subtracting birth weight from live weight and dividing by days to get cumulative ADG. Based on the combined daily growths of twin lambs, milk yield was estimated from the assumption that 1 g weight gain was supported by 6 g milk (Peart, 1968).

5.3.3 Digesta flow rates

Marker concentrations were expressed as proportion of marker infused each day. True digesta was calculated according to Faichney (1980). Ytterbium measured zero in the filtrate fraction, but a value of 0.001 was instead used in the formula (Appendix 5.4). Dry matter flow was calculated by multiplying true digesta flow by DM of abomasal digesta. Thereafter, flow of each amino acid was calculated by substituting their concentration

(g/kg DM) in abomasal digesta for that of Cr-EDTA from the solid-phase and then multiply this by true digesta flow (Faichney, 1980).

All time-series data were subjected to restricted maximum likelihood (REML) to estimate repeated measures by the use of statement Repeated within Proc Mixed procedure of SAS (2002-2003) to estimate variances and covariance (Holland, 2006) for crossover and missing data. The compound symmetry (CS) structure proved to be the appropriate within-subject variance covariance structure for these data according to Akaike's information criterion (Wolfinger and Chang, 1998). The exception was plasma lysine for which autoregression of order one (AR (1)) structure was used because CS structure resulted in infinite likelihood. The model for FEC, blood glucose, body weight, lamb ADG, ewe body condition score and plasma free amino acids included effects of ewe, treatment, time and treatment x time interaction. Lambing occurred within a week so lambing date as a covariate had no effect ($P > 0.05$) on FEC, blood glucose, lamb ADG or plasma amino acids and was thus omitted from the model.

To tease out the nature of trends in plasma amino acids over time, the effect of time was broken down into polynomial contrasts. Time was dropped from the class statement, making it a continuous variable and then it was included three times on the model statement to construct non-orthogonal polynomial items (Wolfinger and Chang, 1998). The model for rumen and abomasal digesta data included effects of ewe, period, treatment, time and treatment x time interaction. Ewe x treatment x period was used to specify variation between animals as a random effect except for DM flow rate in which only ewe was used. During analyses of differences between C and S ewes for rumen bacterial N flow, ewe effect was dropped from the model because samples were pooled together and minimum significant level was assumed at $P < 0.10$. Period effects were dropped from the model

used for statistical analysis of amino acid concentration in the bacterial pellet because it had no ($P > 0.10$) effect. Additional analysis of variance at each time point was carried out when treatment and time interacted. Differences in pasture DM, ME, CP, MP and amino acid intake between C and S ewes were tested by ANOVA using GLM procedure of SAS (2002-2003). Results are reported as least squares means ($P < 0.05$, except for the rumen pellet, analysis of which was $P < 0.10$). For FEC, statistical inference was based on log-transformed data. Where there were differences due to treatment, pair wise comparisons of means were tested by differences of least squares means.

5.4. Results

5.4.1 Diet chemical composition and required ME and MP

The chemical composition of the protein nuts and pasture is shown in Table 5.1. Fibre components of pasture increased during Period II while levels of CP, WSC, DDM and ME decreased. Protein nuts had high CP, ME but low fibre components.

Table 5 1: Dry matter (g DM/kg fresh weight) and chemical composition (g/kg DM) of protein nuts and pasture

Parameters	Protein nuts	Pasture			
		Period I (C)	Period I (S)	Period II (C)	Period II (S)
Dry matter	861	923	926	944	934
Organic matter	916	902	904	915	917
Crude protein	338	155	151	128	114
Crude fat	42	-	-	-	-
Acid detergent fibre	71	231	235	297	300
Neutral detergent fibre	295	423	434	524	536
Water soluble CHO	-	216	219	174	188
DOM*	889	-	-	-	-
Digestible DM	-	789	786	689	680
ME (MJ/kg DM)	13.5	11.8	11.8	10.6	10.4

*Digestibility of organic matter

The concentration of C32 in faeces samples were similar ($P > 0.05$) for C and S ewes (0.25 vs. 0.27 mg/g for C and S ewes, respectively). However, the concentration of C33 was higher ($P < 0.001$) in C than S ewes (0.41 vs. 0.35 mg/g). During Period I C32 concentration in faeces samples was lower ($P > 0.05$) than during Period II, but C33 concentration was significantly higher in Period I than Period II (0.42 vs. 0.34 mg/g). The concentration of C32 was similar ($P > 0.05$) in pasture from different paddocks grazed by C and S ewes (8 vs. 6 ppm) but C33 was higher ($P < 0.01$) in pasture grazed by C than S ewes (110 vs. 100 ppm). During Period I and II C32 in pasture was similar (6 vs. 8 ppm; $P > 0.05$) but C33 was higher ($P < 0.001$) in Period I than II (110 vs. 97 ppm).

5.4.2 Pasture, nutrient and amino acid intake

Pasture dry matter and nutrient intakes are shown on Table 5.2. Pasture intake tended ($P = 0.06$) to be greater in C than S ewes but total dry matter intake and ME intake were similar ($P > 0.05$) between the C and the S animals. Crude protein and especially estimated MP intake were increased ($P < 0.05$ and $P < 0.001$, respectively) by supplementation. Intake of pasture, dry matter ($P < 0.05$), ME, crude protein ($P < 0.001$) and MP ($P < 0.01$), were higher during Period I than Period II. There was no treatment x period interactions ($P > 0.05$).

Calculated total amino acid intake is shown in Table 5.3. Intakes of total amino acids (TAA), essential amino acids (EAA) ($P < 0.01$), SAA ($P < 0.001$) and branch-chained amino acids (BCAA) ($P < 0.05$) were higher for S ewes except for valine, which was similar ($P > 0.05$) between groups. Intakes of TAA, EAA, BCAA ($P < 0.01$) and SAA ($P < 0.05$) were greater during Period I than II except for arginine and histidine which were similar ($P > 0.05$) between the two periods. There was no treatment x period interactions ($P > 0.05$).

5.4.2 Ewe weight, body condition score, FEC, blood glucose, milk yield and lamb weight

Trends in live weight, lamb average daily gain (ADG), FEC and blood glucose are shown in Figures 5.1 to 5.2. A highly significant treatment x time interaction was observed for live weight ($P < 0.001$). Body condition score was higher ($P < 0.05$) in the C (2.8 ± 0.11) than in S ewes (2.3 ± 0.10) at 23 days after lambing. At 52 and 70 days after lambing, both C and S sheep had similar ($P > 0.05$) body condition (2.6 ± 0.16 vs. 2.3 ± 0.15 and 2.3 ± 0.16 vs. 2.1 ± 0.18 for C and S ewes, respectively). Lamb ADG was slightly but not significantly higher ($P > 0.05$) in C than S ewes (Figure 5.1b). Milk yield of C and S ewes was similar (3659.7 ± 331.95 vs. 3359.1 ± 307.33 g/day, respectively; $P > 0.05$) and there was no treatment x time interaction ($P > 0.05$).

No ($P > 0.05$) treatment effect was observed for FEC (33 ± 0.8 vs. 35 ± 0.7 peg) for C and S ewes, respectively. Twenty-one days after anthelmintic treatment, FEC of C rose rapidly compared to S animals (670 vs. 46, respectively; $P = 0.08$) (Figure 5.2a) and was reduced after treatments were reversed. There was a highly significant ($P < 0.001$) time effect on FEC.

No treatment effect was observed for blood glucose (2.21 ± 0.06 vs. 2.17 ± 0.05 mmol/L for C and S ewes, respectively). However, there was a tendency ($P = 0.07$) for a treatment x time interaction due to high ($P < 0.05$) glucose levels in C ewes at day +35 relative to lambing and due to a significantly higher ($P < 0.01$) blood glucose concentration in S ewes at day +56 relative to lambing (Figure 5.2b).

Table 5 2: Pasture and nutrient intake by twin-rearing ewes grazing spring pasture supplemented with protein nuts (Supplemented) or without a supplement (Control) during Period I and II¹.

Parameter	Treatment		Period		Main effects	
	Control	Supplemented	I	II	Treatment	Period
Pasture intake (g DM/d)	2629±139.95	2245±135.20	2659±135.20	2216±139.95	0.06	*
Total DM intake (g/d)	2629±139.95	2676±135.20	2874±135.20	2431±139.95	NS	*
ME intake (MJ ME/d)	29.6±1.51	30.9±1.50	34.3±1.46	26.2±1.51	NS	***
CP intake (g/d)	375.8±18.10	442.8±17.48	478.4±17.48	340.2±18.10	*	***
MP supply (g/d) ²	309.3±13.30	434.4±12.85	399.6±12.85	344.0±13.30	***	**

* = P < 0.05; ** = P < 0.01; *** = P < 0.001 and NS = P > 0.05

¹Based on mean DM intake of 4 ewes from each group

²Calculated from AFRC (1993)

Table 5 3: Amino acid intake (g/day) by twin-rearing ewes grazing spring pasture supplemented with protein nuts (Supplemented) or without a supplement (Control) during Period I and II¹.

Parameter	Treatment		Period		SEM	Main effects	
	Control	Supplemented	I	II		Treatment	Period
Methionine	3.4	11.7	8.0	7.1	0.21	***	*
Leucine	28.5	35.3	39.0	24.8	1.26	*	**
Lysine	21.3	25.8	26.4	20.7	0.99	*	*
Isoleucine	13.6	16.8	18.5	11.9	0.62	*	**
Phenylalanine	14.4	18.4	18.9	13.9	0.68	*	**
Valine	17.2	20.4	22.9	14.7	0.87	NS	**
Arginine	16.8	26.5	22.4	20.9	0.81	**	NS
Threonine	17.1	20.5	23.7	13.9	0.79	*	***
Histidine	6.9	10.1	9.1	7.9	0.46	**	NS
Total Amino Acid	305.7	400.9	424.6	282.1	13.45	**	**
Total EAA	139.1	185.5	188.7	135.8	6.46	**	**
Total BCAA	59.3	72.5	80.3	51.4	2.73	*	**
Total SAA	8.2	27.4	19.1	16.4	0.52	***	*
Cysteine	4.8	15.7	11.1	9.3	0.32	***	*
Taurine	2.3	1.8	2.9	1.1	0.12	*	***

* = P < 0.05; ** = P < 0.01; *** = P < 0.001 and NS = P > 0.05

¹Based on mean DM intake of 4 ewes from each group

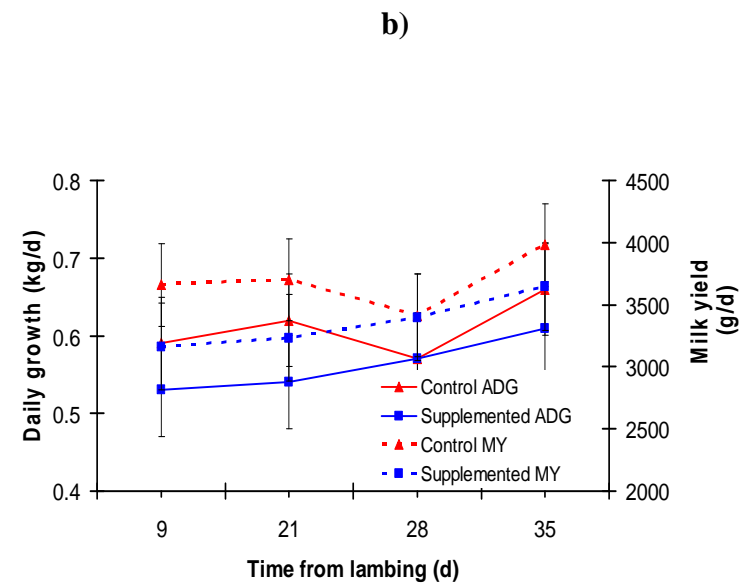
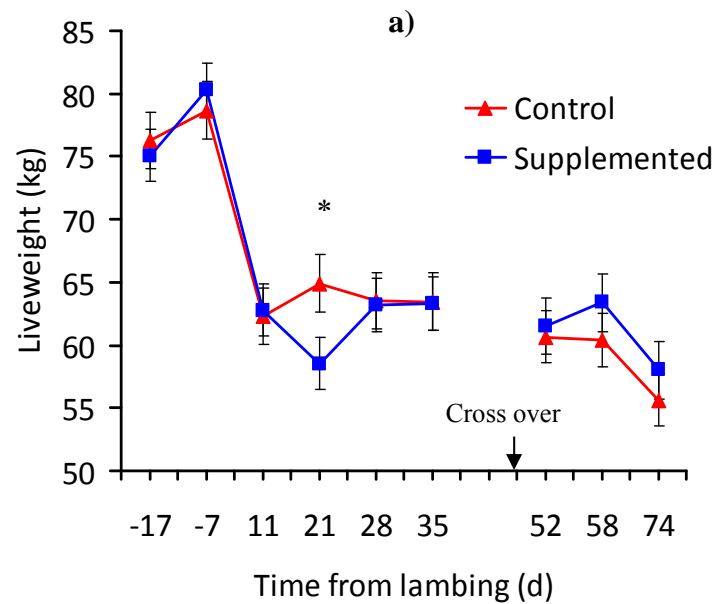


Figure 5 1 Live weight of twin-rearing ewes grazing spring pasture with or without a protein supplement (a) and Total daily growth (ADG; g/ewe) of their twin lambs and estimated dam milk yield (b)

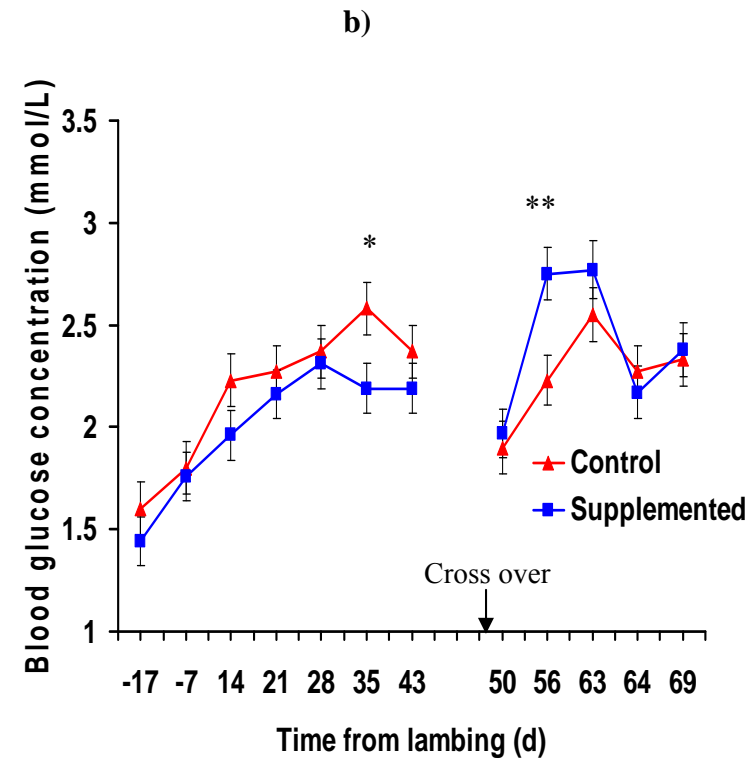
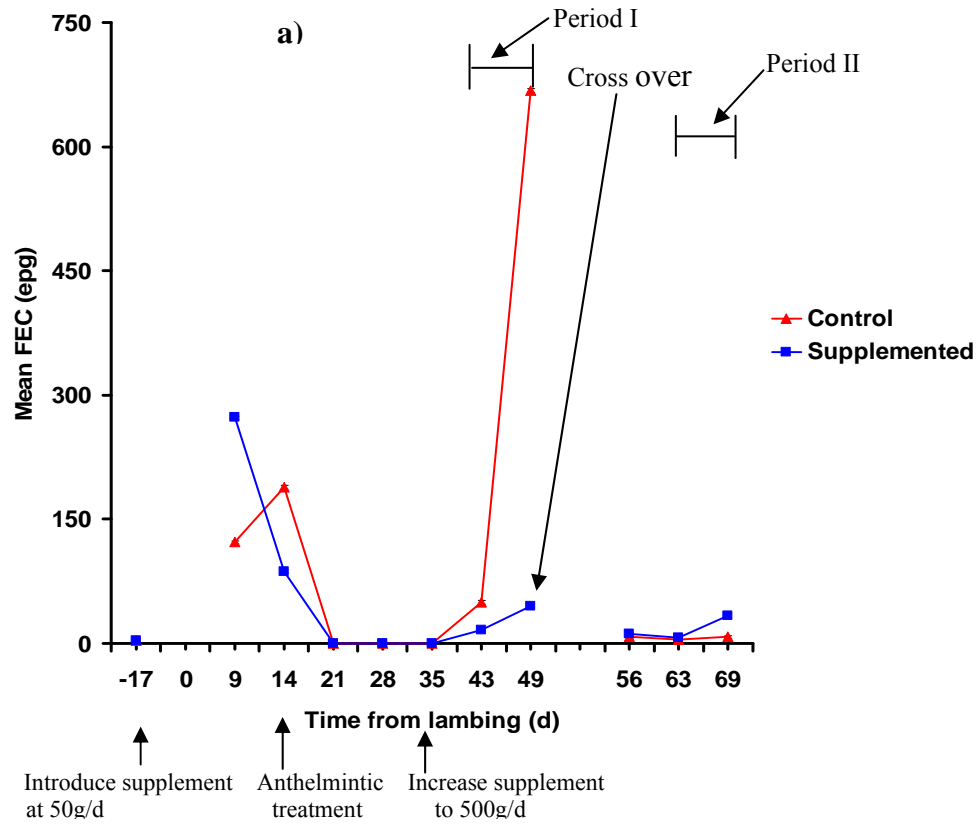


Figure 5 2: Back-transformed FEC (a) and blood glucose (b) of twin-rearing ewes grazing spring pasture with (S) or without a protein supplement (C)

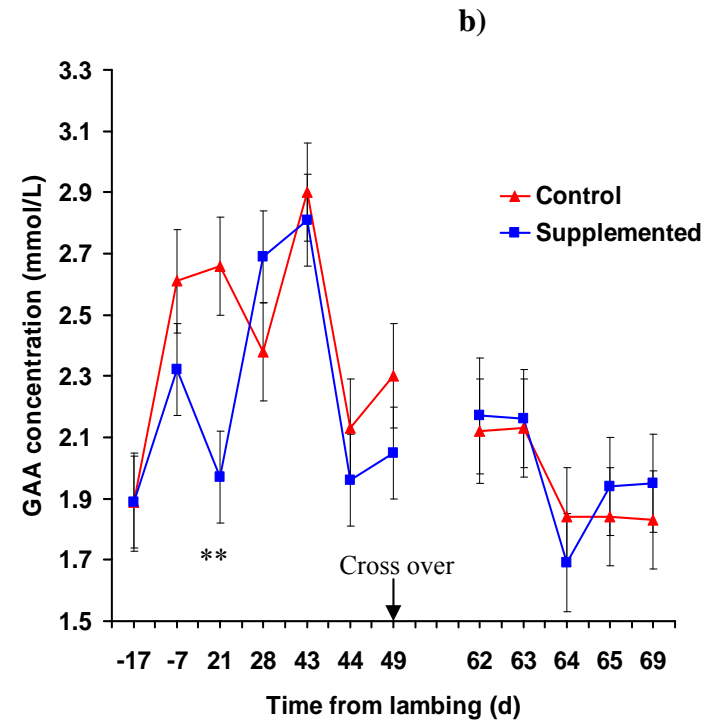
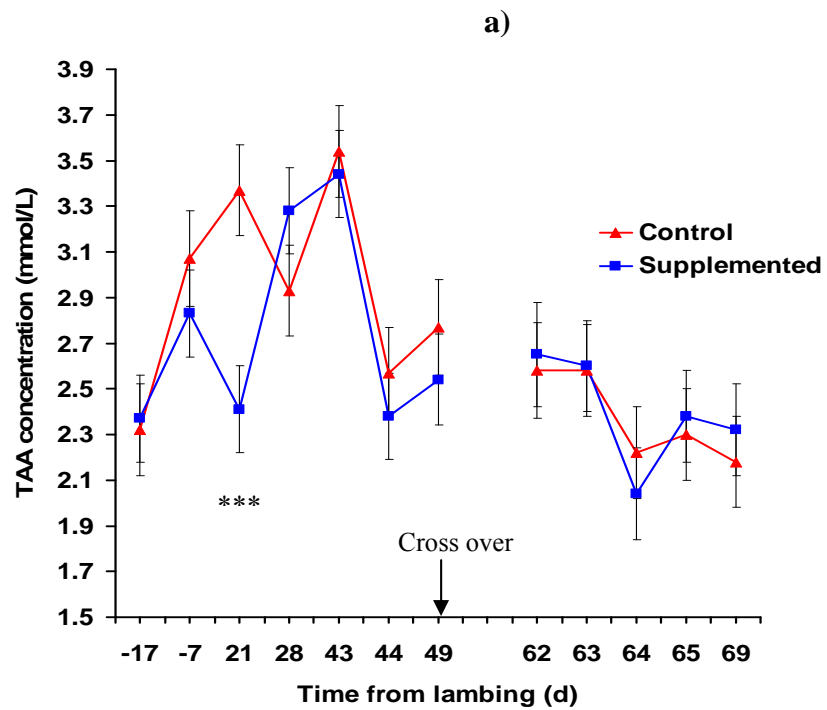


Figure 5 3: Plasma total amino acid (a) and total glucogenic amino acid (b) concentration in twin-rearing ewes grazing spring pasture with (S) or without a protein supplement (C)

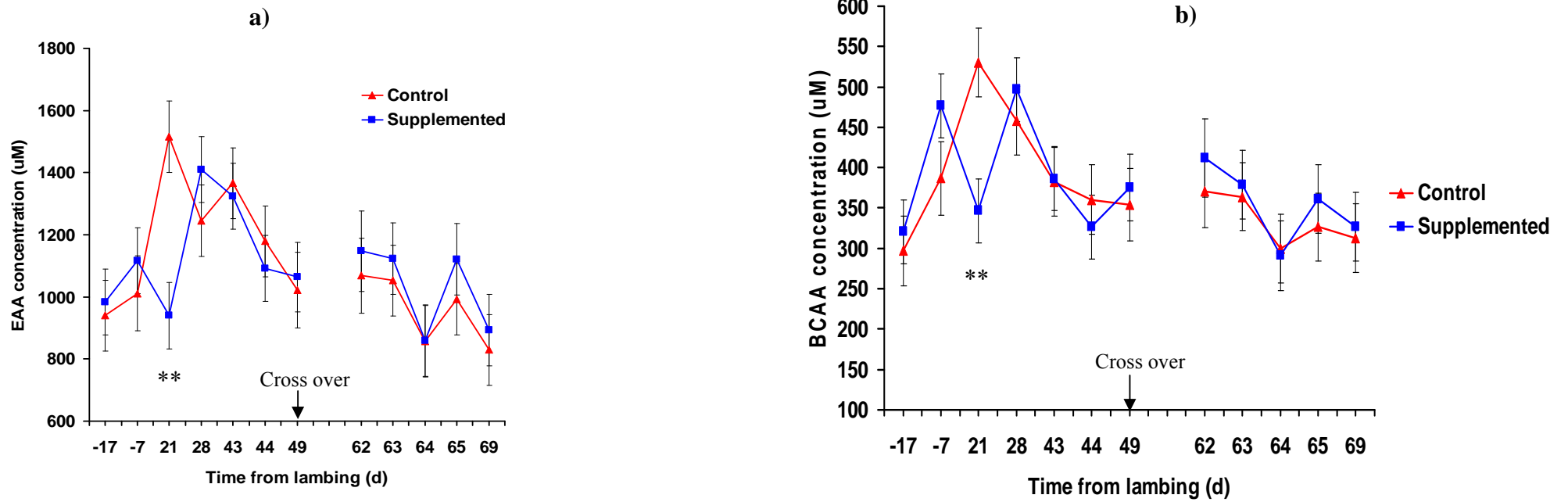


Figure 5 4: Plasma total essential amino acid (a) and total branched-chain amino acid (b) concentration in twin-rearing ewes grazing spring pasture with (S) without a protein supplement (C)

5.4.3 Plasma amino acids

Mean plasma amino acid concentrations and trends over time are presented in Tables 5.4 and Figures 5.5 to 5.6. There was a highly significant ($P < 0.001$) effect of time on plasma amino acid concentrations. Polynomial contrasts (Table 5.4) showed most amino acids had significant quadratic components ($P < 0.05$ or greater) indicative of a rise in concentration during early lactation and subsequent fall, glutamic acid, aspartate and proline being the exceptions. Time-related changes had highly significant linear and cubic effects ($P < 0.001$, in both cases). Plasma concentrations of total AA, EAA ($P < 0.001$), BCAA and total glucogenic amino acid (GAA; those amino acids with potential to yield glucose via phosphoenolpyruvate (D'Mello, 2003)) were lower ($P < 0.01$) in S ewes at day 21 after lambing but similar ($P > 0.05$) to those of C ewes at other times. Plasma concentrations of certain individual amino acids – viz, threonine, valine, isoleucine, leucine, histidine, lysine, methionine, serine ($P < 0.01$), arginine, glutamine, cysteine, glycine ($P < 0.05$), phenylalanine, glutamic acid, tryptophan and tyrosine ($P < 0.001$) of S ewes were also lower at day 21 after lambing. These trends for selected individual AA are shown in Figures 5.5 and 5.6. An exception was taurine for which plasma concentrations were high in S ewes on day 21 ($P < 0.05$) post lambing (Table 5.5). A treatment x time interaction was observed for most EAA, BCAA and SAA indicating that the different groups responded differently over time and hence different concentration patterns, except for threonine, phenylalanine, lysine, glutamine, taurine and aspartate for which groups had similar patterns (Table 5.4). There was no overall ($P > 0.05$) treatment effect on plasma essential and branched-chain amino acid concentration except for serine ($P < 0.01$) and cysteine ($P < 0.05$). Tryptophan ($P = 0.09$) and tyrosine ($P = 0.07$) tended to be reduced in S ewes. Supplemented animals had high plasma concentrations of cysteine and taurine ($P < 0.05$) in both cases. Total plasma SAA levels in S sheep were marginally higher but not significantly different ($P > 0.05$) from those of C ewes. Slightly higher, but statistically

insignificant ($P > 0.05$), concentrations of glutamine, glycine and alanine were recorded in plasma of C than in S animals.

5.4.4 Rumen and abomasal pH

Trends in rumen and abomasal pH during Periods I and II are shown in Figure 5.7. During Period I there was no ($P > 0.05$) treatment effect on rumen pH or abomasal pH. There was, however, a tendency for a treatment x time interaction in rumen pH ($P = 0.07$) but none ($P > 0.05$) in abomasal pH. A time effect was observed in both the rumen pH ($P < 0.01$) and the abomasal pH ($P < 0.001$) during Period I with pH increasing after 9.00 h. During Period II, rumen pH in digesta of C ewes was significantly higher ($P < 0.001$) than that of S ewes (6.71 ± 0.054 vs. 6.42 ± 0.053 for C and S ewes, respectively). There was no ($P > 0.05$) treatment x time interaction in rumen pH while there was tendency ($P = 0.06$) for one in abomasal pH. Time effect was highly significant ($P < 0.001$) in rumen pH and significant ($P < 0.01$) in abomasal pH (Figure 5.7) with rumen pH decreasing at 21.00 h and abomasal pH of C ewes increasing at 15.00 h in Period II.

5.4.5 Rumen bacterial AA concentration, abomasal DM flow and abomasal AA flow

Preliminary analysis did not show any effect of Period on rumen bacterial amino acid concentration, hence data from Period I and II were combined. Concentrations of rumen bacterial AA are shown in Table 5.6 and Figure 5.8 with missing data for 24.00 h sampling. Trends in flow of DM and amino acids are shown in Table 5.6 and Figures 5.9 and 5.10.

Rumen bacterial amino acid concentrations and TAA were slightly but insignificantly ($P = 0.72$ to $P = 0.23$) higher in S than C ewes (Table 5.5 and Figure 5.8). There was highly significant time effect whereby amino acid concentrations were higher between 06.00 and 12.00 h and then decreased between 15.00 and 18.00 h (Figure 5.8).

Supplementation increased flows of DM, total AA, EAA, SAA, BCAA, individual AA and DAPA ($P < 0.001$) through the abomasum (Table 5.6). Supplementation also resulted in highly significant ($P < 0.001$) treatment x time interaction in DM, total AA, EAA, SAA, BCAA, individual AA and DAPA flows (Table 5.6 and Figures 5.9 and 5.10). Flow rates were higher during Period I than Period II (Table 5.6). These increases in flows occurred in supplemented ewes within 3 h of offering the supplement at 9.00 h and lasted up to 21.00 h (Figures 5.9 and 5.10) except for dry matter in which the increase lasted only up to 15.00 h.

Table 5 4: Plasma EAA, BCAA and SAA concentration (μM) in twin-rearing ewes grazing spring pasture with or without protein supplementation.

Parameter	Treatments		Main effects				Interaction effects
	Control	Supplemented	Treatment	Linear ²	Quadratic	Cubic	Treatment x Time
Threonine	323±25.90	315±24.81	NS	NS	***	NS	NS
Valine	157±11.15	158±10.68	NS	NS	***	*	*
Isoleucine	103±5.68	105±5.43	NS	***	***	*	*
Leucine	110±6.63	111±6.26	NS	NS	*	***	*
Phenylalanine	59.0±2.67	54.4±2.57	0.05	*	***	***	NS
Histidine	52.4±2.50	52.4±2.39	NS	*	**	NS	***
Lysine	113±7.48	113±7.18	NS	**	***	***	*
Arginine	158±9.53	170±9.13	NS	NS	***	**	*
Methionine	22.2±1.52	21.9±1.45	NS	NS	***	*	*
Total EAA (mmol/L)	1.09±0.06	1.09±0.07	NS	NS	***	NS	**
Total SAA	92.6±4.49	100.1±4.32	NS	***	***	**	**
Total BCAA	370.3±23.03	374.9±22.05	NS	*	***	**	*
Total GAA (mmol/L)¹	2.22±0.09	2.13±0.09	NS	*	***	NS	*
Glutamine	281.9±11.94	268.4±11.48	NS	***	***	*	NS
Glutamic acid	127.0±5.97	125.8±5.71	NS	***	NS	***	***
Taurine	78.9±4.64	90.2±4.45	*	***	***	**	NS
Cysteine	70.4±3.47	78.2±3.35	*	***	***	*	***
Glycine	614.1±31.03	566.0±29.85	NS	NS	*	**	***
Aspartate	4.6±0.53	4.9±0.51	NS	*	NS	NS	NS
Tryptophan	33.6±1.74	31.14±1.68	0.09	**	***	***	***
Tyrosine	67.9±4.10	61.0±3.96	0.07	NS	***	*	**
Proline	120.0±6.22	125.2±6.00	NS	NS	NS	*	0.07
Serine	77.5±5.00	63.9±4.82	**	***	***	**	*
Alanine	215.7±8.69	196.6±8.29	NS	***	***	*	**
Total AA (mmol/L)	2.70±0.11	2.60±0.10	NS	**	***	NS	*

¹Glucogenic amino acids minus asparagine

²Time effects partitioned by polynomial contrasts into linear, quadratic and cubic effects

* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$ and NS = $P > 0.05$

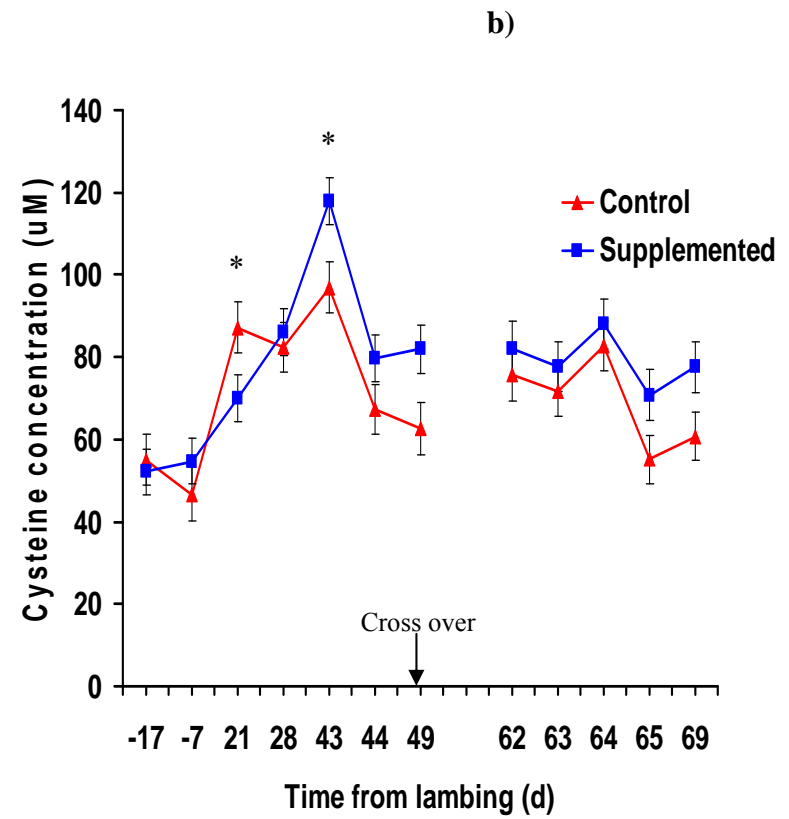
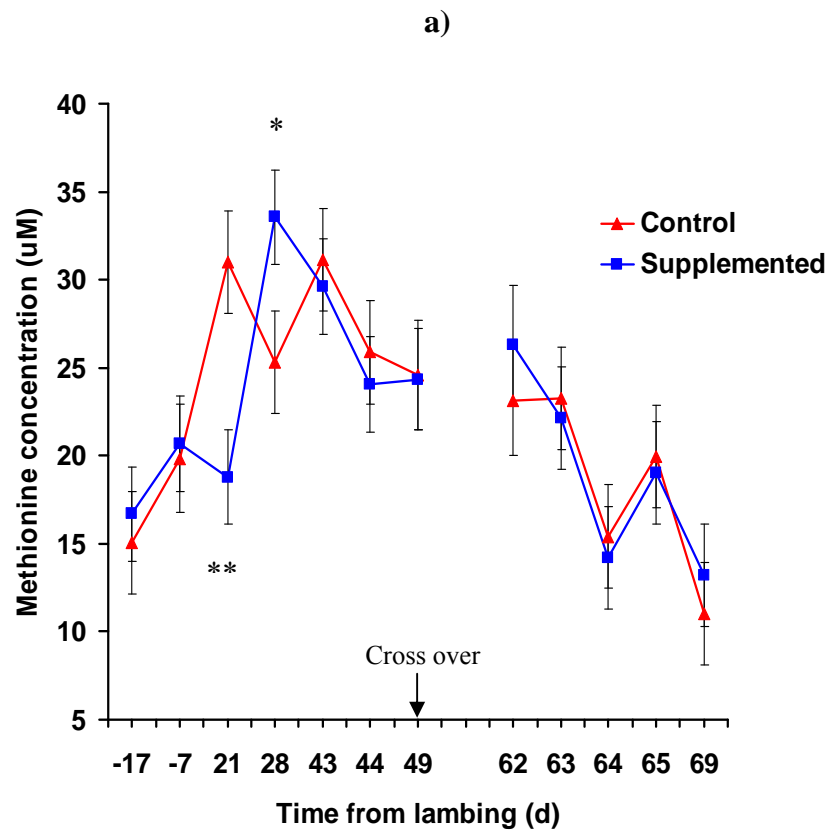


Figure 5 5: Plasma methionine (a) and cysteine (b) concentration in twin-rearing ewes grazing spring pasture with (S) or without protein supplement (C).

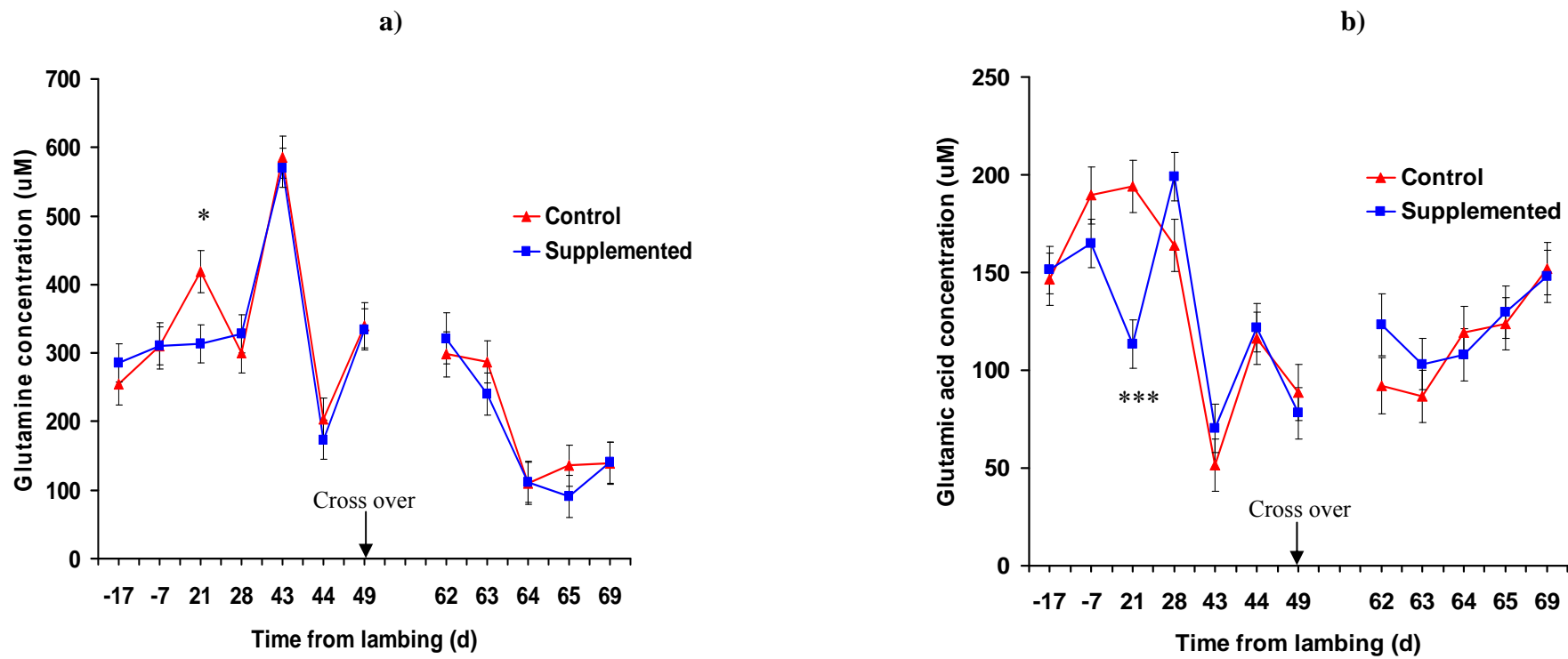


Figure 5 6: Plasma glutamine (a) and glutamic acid (b) concentration of twin-rearing ewes grazing spring pasture with or without protein supplement

pH

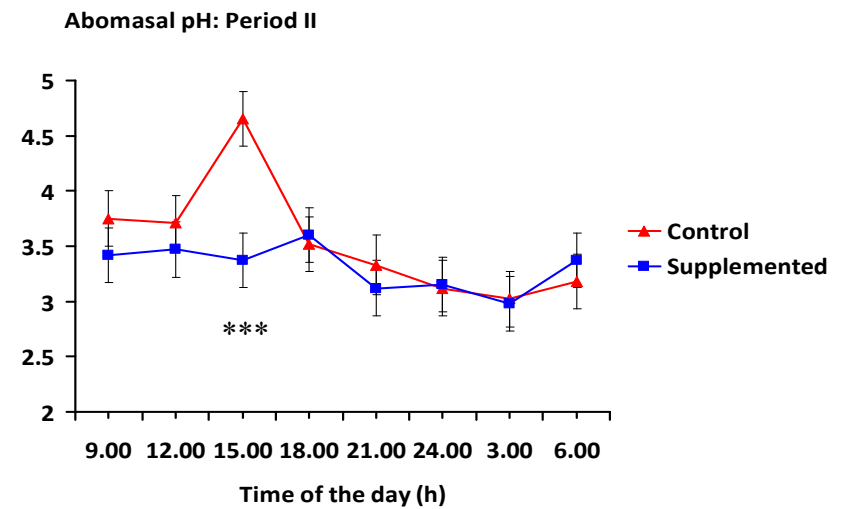
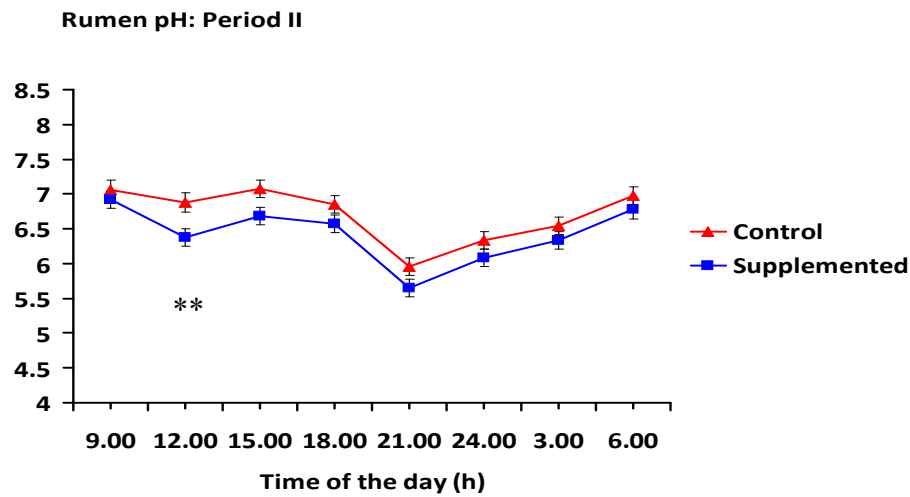
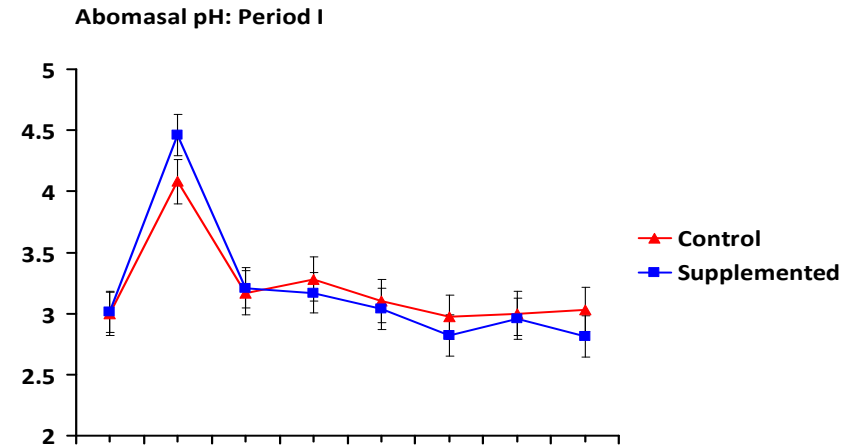
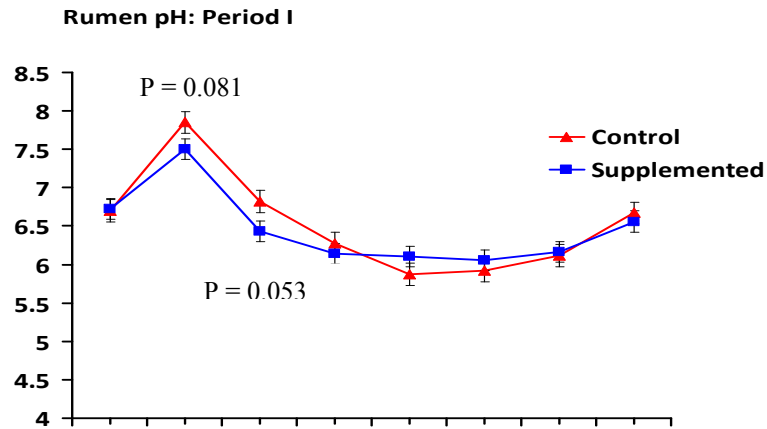


Figure 5 7: Diurnal variation in rumen and abomasal digesta pH of twin-rearing ewes grazing spring pasture with or without protein supplement during Periods I and II

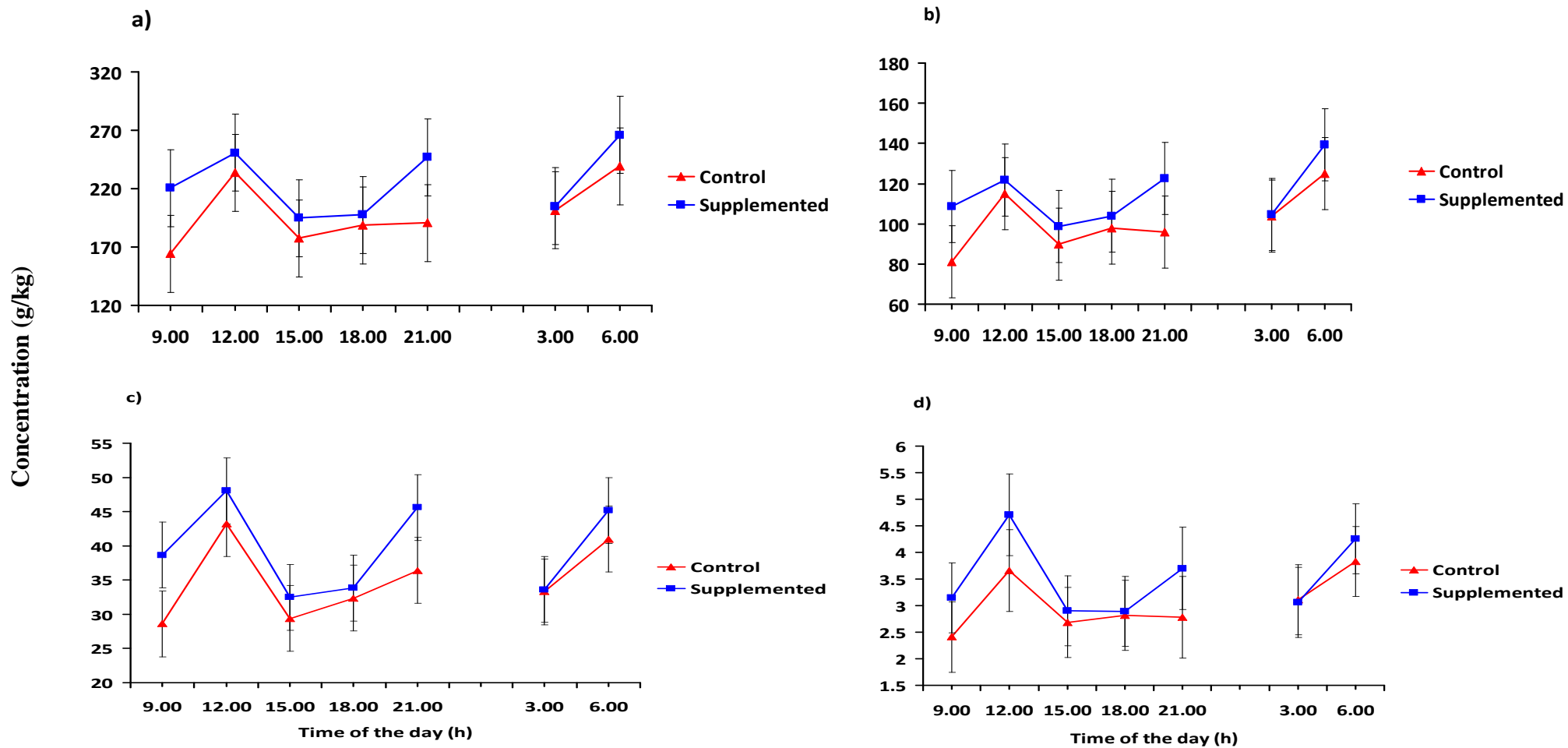


Figure 5 8: Diurnal variation in total AA (a), EAA (b), BCAA (c) and SAA (d) concentrations (g/kg pellet) of bacterial isolate from the rumen of twin-rearing ewes grazing spring pasture with or without protein supplement

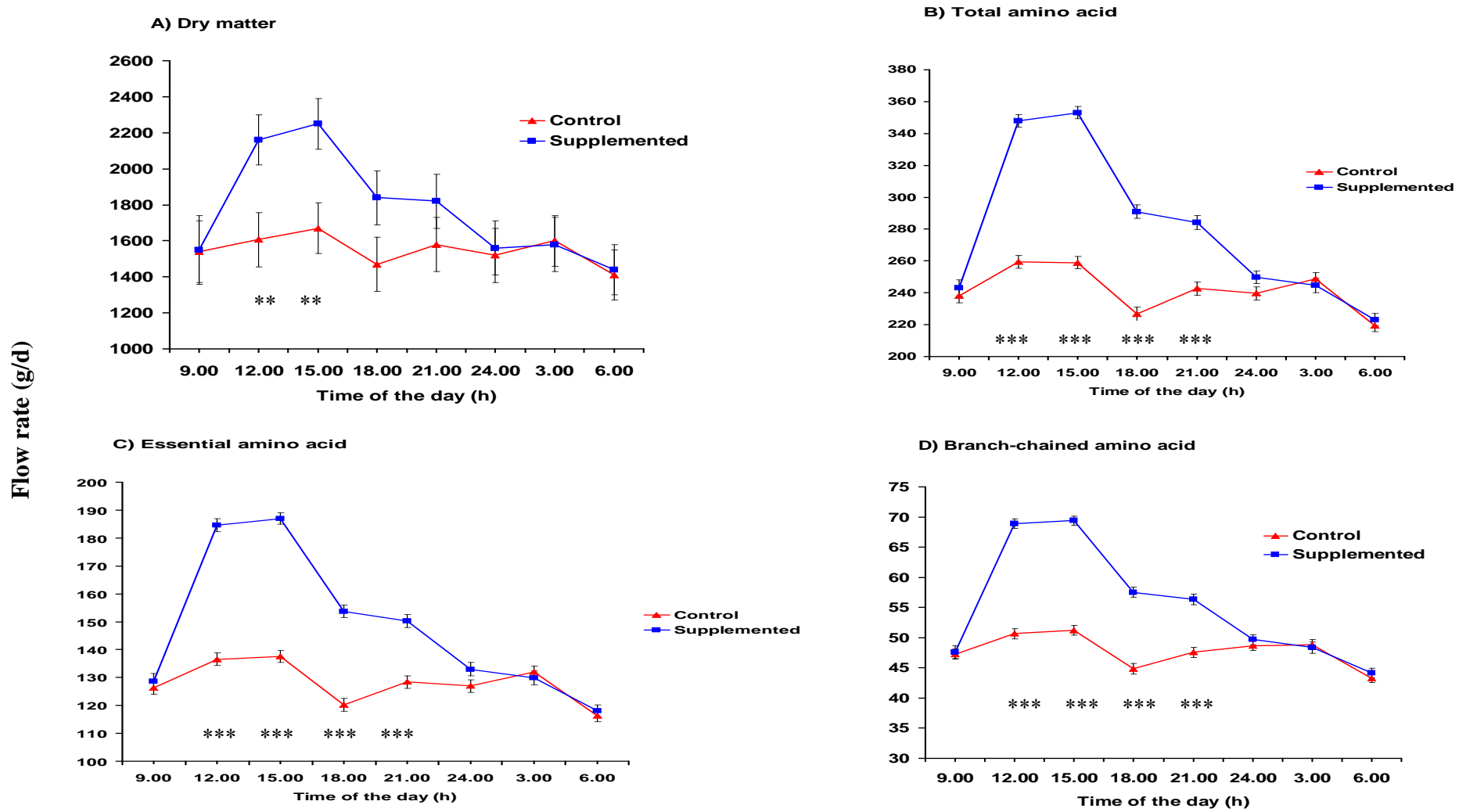


Figure 5 9: Diurnal variation in abomasal DM, total AA, EAA and BCAA flows in parasitized twin-rearing ewes grazing spring pasture with or without protein supplement

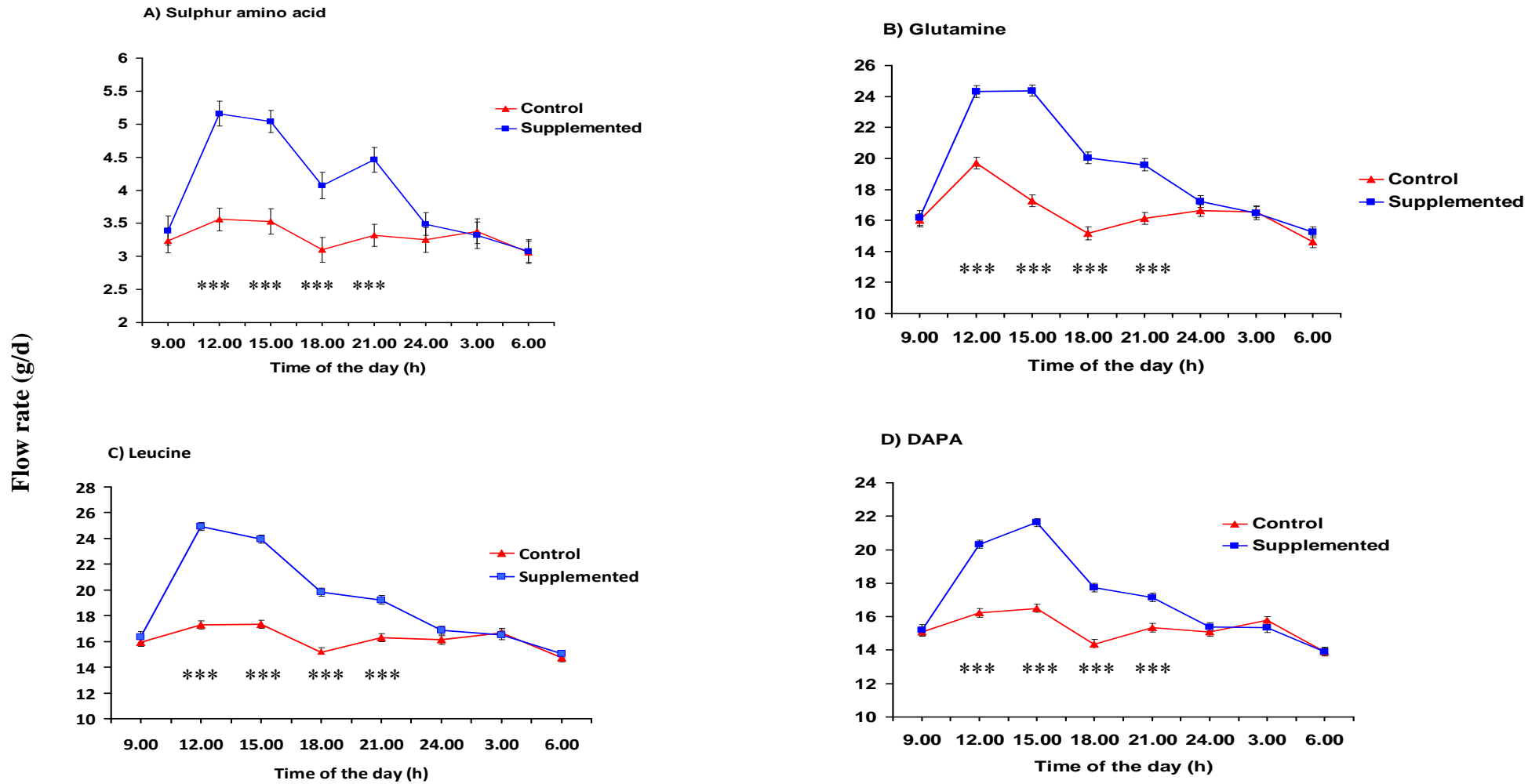


Figure 5 10: Diurnal variation in abomasal SAA, glutamine, leucine and DAPA flows in parasitized twin-rearing ewes grazing spring pasture with or without protein supplement

Table 5 5: Amino acid content of rumen bacteria (g/kg of pellet) of twin-rearing ewes grazing spring pasture with or without protein supplement

Parameter	Control	Supplemented	SEM	Main effects ¹		
				P value	Time	Trt x Time
Threonine	9.6	10.9	1.54	0.61	***	NS
Valine	9.7	11.0	1.07	0.47	**	NS
Isoleucine	9.4	10.7	0.67	0.31	**	NS
Leucine	15.9	17.9	2.35	0.59	**	NS
Phenylalanine	9.2	10.2	0.73	0.42	***	NS
Histidine	4.1	4.7	0.22	0.23	*	NS
Lysine	17.0	18.6	1.95	0.63	***	NS
Arginine	9.7	11.2	1.56	0.57	NS	NS
Methionine	1.9	2.3	0.32	0.63	**	NS
Total EAA	101.3	114.3	15.95	0.62	*	NS
Total SAA	3.0	3.5	0.58	0.67	**	NS
Total BCAA	34.9	39.6	4.06	0.50	***	NS
Total AA	199.3	225.9	29.23	0.59	***	NS
DAPA	1.8	2.1	0.15	0.24	*	NS
Taurine	1.3	1.6	0.50	0.75	*	NS
Cysteine	1.1	1.3	0.26	0.73	*	NS

¹Due to small quantities of bacterial pellet during amino acids chemical analysis significant level was set at P<0.10. (i.e. P<0.10 = *; P<0.05 = **; P<0.01 = ***).

Table 5 6: Flow of DM, water, TAA, essential amino acids, cysteine, DAPA, and taurine through the abomasum in twin-rearing ewes grazing spring pasture with or without protein supplement.

Parameter	Treatment		Period		SEM	Effects	
	Control	Supplemented	I	II		Period	Trt x Time
Dry matter (kg/d)	1.56	1.78	1.83	1.51	0.08	***	***
Water (l/d)	51.0	47.9	57.3	41.6	2.80	**	*
Total AA (g/d)	241.7	279.6	283.2	238.1	1.60	***	***
Threonine	15.9	18.3	18.6	15.6	0.08	***	***
Valine	15.8	18.1	18.4	15.5	0.09	***	***
Isoleucine	15.8	18.2	18.4	15.5	0.09	***	***
Leucine	16.2	19.0	19.1	16.1	0.11	***	***
Phenylalanine	15.8	18.2	18.5	15.5	0.09	***	***
Histidine	15.4	17.6	17.8	15.2	0.15	***	***
Lysine	16.2	19.0	19.1	16.1	0.11	***	***
Arginine	15.9	18.5	18.6	15.7	0.10	***	***
Methionine	1.7	2.1	2.0	1.8	0.05	**	***
Total EAA	128.0	148.1	149.8	126.4	0.80	***	***
Total SAA	3.3	4.0	3.9	3.4	0.08	***	***
Total BCAA	47.8	55.4	56.0	47.2	0.30	***	***
Glutamine	16.5	19.3	19.3	16.5	0.16	***	***
DAPA	15.3	17.1	17.5	14.9	0.09	***	***
Taurine	15.3	17.1	17.5	14.9	0.09	***	***
Cysteine	1.6	1.9	1.9	1.6	0.03	***	***

5.5 Discussion

Intensive studies such as this can not use the large number of animals needed for parasitological studies and as such, less weight can be attributed to the parasitological data. However, the conditions used achieved the enhancement of MP supply suggested by Donaldson et al. (2001) and Houdijk et al. (2005) that lead to improved ability to limit the peri-parturient limitation of immunity. In addition, the present work mirrors the studies of Xie (2003) in which sheep grazing similar pastures and which were offered the same quantities of the same supplement did show a highly significant reduction in FEC from 99 to 2 eggs during the first 6 weeks of lactation. The strengths of the present work are therefore in providing an indication of likely changes in amino acid supply occurring in the work of Xie (2003). Except for data on flows of non-ammonia nitrogen and amino acid in grazing lactating ewes (Dove et al., 1988; Dove et al., 1985; McMeniman et al., 1986), indoor-fed pregnant and lactating sheep (Gonzalez et al., 1985) and of amino acid in soy bean-supplemented indoor pregnant ewes (Coffey et al., 1989), to the best knowledge of the author, the current work is the first to quantify amino acid supply in parasitised twin-suckling ewes on pasture with or without a fishmeal-based supplement.

Generally, supplementation increased the flow of amino acid through the abomasum by 16% on a daily basis. This increase was greater between 12.00 to 21.00 h when the difference in flow was for total AA (+36%), EAA (+36%), BCAA (+36%), SAA (+45%), DAPA (+31%), glutamine (+30%), aspartic acid (+66), cysteine (+43%) and taurine (+31%). An increase in the flow of escape protein relative to bacterial protein (Robinson et al., 2002) would account for such an abrupt increase of flow in DM and amino acids between 12.00 and 21.00 h in S ewes.

The 16% increase in amino acid flow from the rumen of S ewes suggests that supplementation provided both dietary AA and microbial protein. Enhancement of microbial protein was inferred from DAPA data. DAPA is a constituent of the cell wall of many species of bacteria. The fluxes of DAPA were also increased in S ewes from 12.00 to 21.00 h. DAPA was also used to calculate total bacterial nitrogen flowing at the abomasum by dividing the N:DAPA ratio of bacterial isolate by the N:DAPA ratio of abomasal digesta and multiplying this quotient by total abomasal N flow rate (Volden, 1999). Nitrogen in the bacterial isolate and in the abomasal digesta was derived from total amino acid (TAA) concentration by dividing TAA by 6.25. Mean total nitrogen (TN) and bacterial nitrogen (MN) flow of S and C ewes was 45.5 ± 3.59 vs. 38.2 ± 3.57 g/d and 32.2 ± 1.96 vs. 31.6 ± 1.79 g/d for S and C ewes, respectively. Dove and Milne (Dove and Milne, 1994) estimated flow of microbial nitrogen in ewes grazing spring/summer pasture to be 37.2 g/d, which is slightly greater than flows observed in the C ewes in the present study. These differences between S and C ewes in bypass protein (TN – MN) flow suggest that supplementation doubled bypass nitrogen in S ewes. Flow of bacterial N in S ewes at 15.00 h was high (39.5 ± 4.85 vs. 26.3 ± 4.86 g/d; $P < 0.1$; for S and C ewes, respectively; Figure 5.13), indicating a shift in microbial supply at this time and suggesting a possible enhancement of microbial protein synthesis in supplemented ewes. Reduction in rumen pH as a consequence of supplementation has been observed previously (Bargo et al., 2002) and when rumen pH is below 6.0, the rumen microbial population may shift from more acid-sensitive to acid-tolerant genera (Dehority, 2003). However, in the present study rumen pH values for both S and C were above 6 except at 21.00 h in both cases. It is likely that supplementation may have changed the balance of the microbial population because supplements containing undegraded protein also supply organic matter that provides energy for additional microbial protein synthesis (O'Reagain and McMeniman, 2002). Cottle and Velle (1989) postulated that fractions of administered lysine, threonine and

methionine could be incorporated directly into microbial protein. If this was the case in the present study, then the slight increase in some amino acids in bacterial pellet of S ewes (Table 5.5) may imply that AA incorporation directly into microbial protein may have been enhanced. From Figure 5.13, rumen bypass nitrogen flow can be extrapolated from each sampling point for each treatment group by subtracting bacterial nitrogen from total nitrogen flow; e.g. A and B for C and S ewes, respectively at 15.00 h and indicated an enhanced by-pass nitrogen by the supplement particularly in the afternoon.

Flows were not consistent throughout the day. Diurnal variation in flow of DM and amino acid (Figure 5.11) in C group was lower than that in a study by Dove et al. (1988) in which DM and NAN flow ranged from 15% below to 30% above mean daily flow. Diurnal variation in flow of DM and amino acids for C ewes in the present study ranged from 5% below and 10% above mean daily flow (Figure 5.11). However, of particular interest was the pattern of the flows of AA in the present study in which the peak occurred from 12.00 to 15.00 h and another at mid-night to 3.00 h while in the study by Dove et al. (1988) the peak was reached at mid-night. Thomson et al. (1985) noted that in grazing lambs, the rumen reached its maximum level of fill at the end of the afternoon grazing, with additional grazing period during the night, perhaps explaining the existence of peak flow of DM and NAN at mid-night. However, in the present study the early peak between 12.00 and 15.00 h may be indicating high fractional outflow rate of digesta perhaps due to the rumen reaching its maximum fill much earlier. A shorter half-life of fluid and particulate matter in the rumen of sheep with high dry matter intakes result in faster flow rate of digesta through the abomasum (Ali and Hennessy, 1995) as a result of propulsive activity of the rumen (Dove et al., 1988). In ewes given the supplement, diurnal fluctuation was prominent at 12.00h, ranging from 15% below and 30% above the mean daily flow, and the mid-night peak observed in the control ewes was not evident (Figure 5.12). It also

seems that supplementation with protected protein may have altered the pattern of bacterial nitrogen flow through the abomasum. Protein nuts were offered at 9.00 h, and as shown in Figure 5.13, bypass nitrogen peaked between 12.00h and 15.00 h, but it was not until 15.00 h that a peak in bacterial nitrogen flow was observed, 6 h later. A delay of 6-7 h between consumption and a change in AA profile of duodenal digesta was observed by Robinson et al. (2002) when dairy cows were given a protein supplement containing fishmeal as was the case in present study.

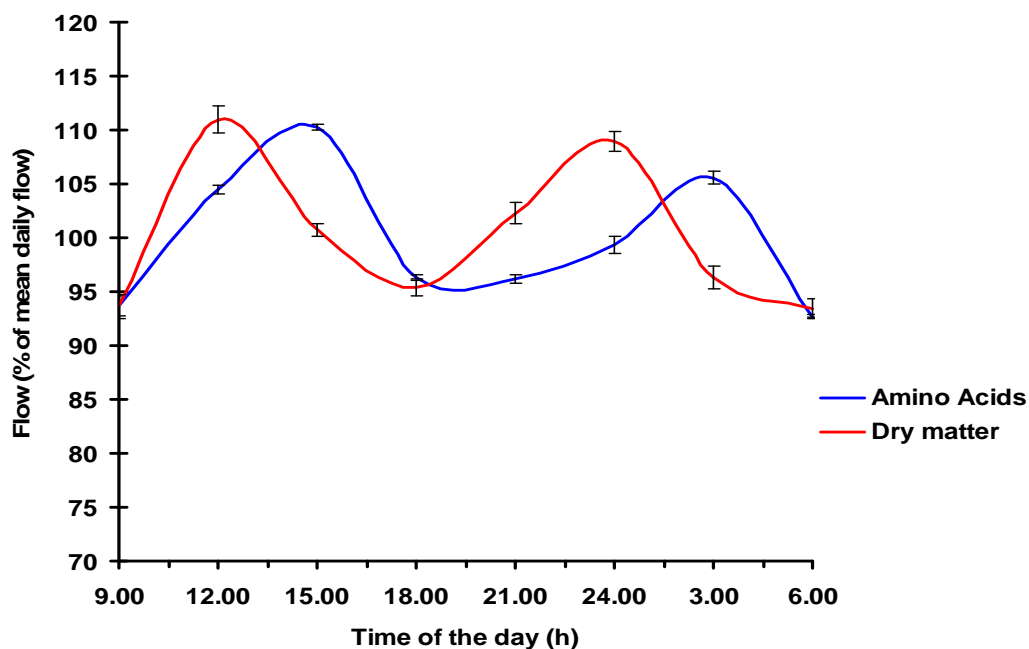


Figure 5 11: Diurnal variation in the flows of DM and total AA in twin-rearing ewes grazing spring pasture without a supplement (C)

To be able to compare this data with other studies, amino acid flows were expressed as *per* unit dry matter intake (DMI). Though pasture intake was estimated using only four ewes from each treatment group, these appeared to provide realistic values for intake based on calculated energy requirement of ewes for milk yield and according to AFRC (1993) nutrient requirements. In addition, the present pasture intake of 2.6 kg is comparable to intake of 36-38 g DM /kg LW of sheep on pasture observed by Cruickshank (1986).

Comparison with dairy cattle data showed that total amino acid flow of C ewes (91.9 g/d/kg DMI) were similar to those of lactating cows grazing ryegrass/white clover (93.0 g/d/kg DMI) or only ryegrass (97.6 g/d/kg DMI) offered *ad libitum* by Kolver et al. (1999). In addition, flows of branch-chain amino acids (valine, isoleucine and leucine), threonine and lysine were comparable to those of dairy cows in the afore-mentioned study (6.0 vs. 5.0; 6.0 vs. 4.0; 6.2 vs. 7.0; 6.0 vs. 5.2 and 6.2 vs. 5.3, for valine, isoleucine, leucine, threonine and lysine, respectively).

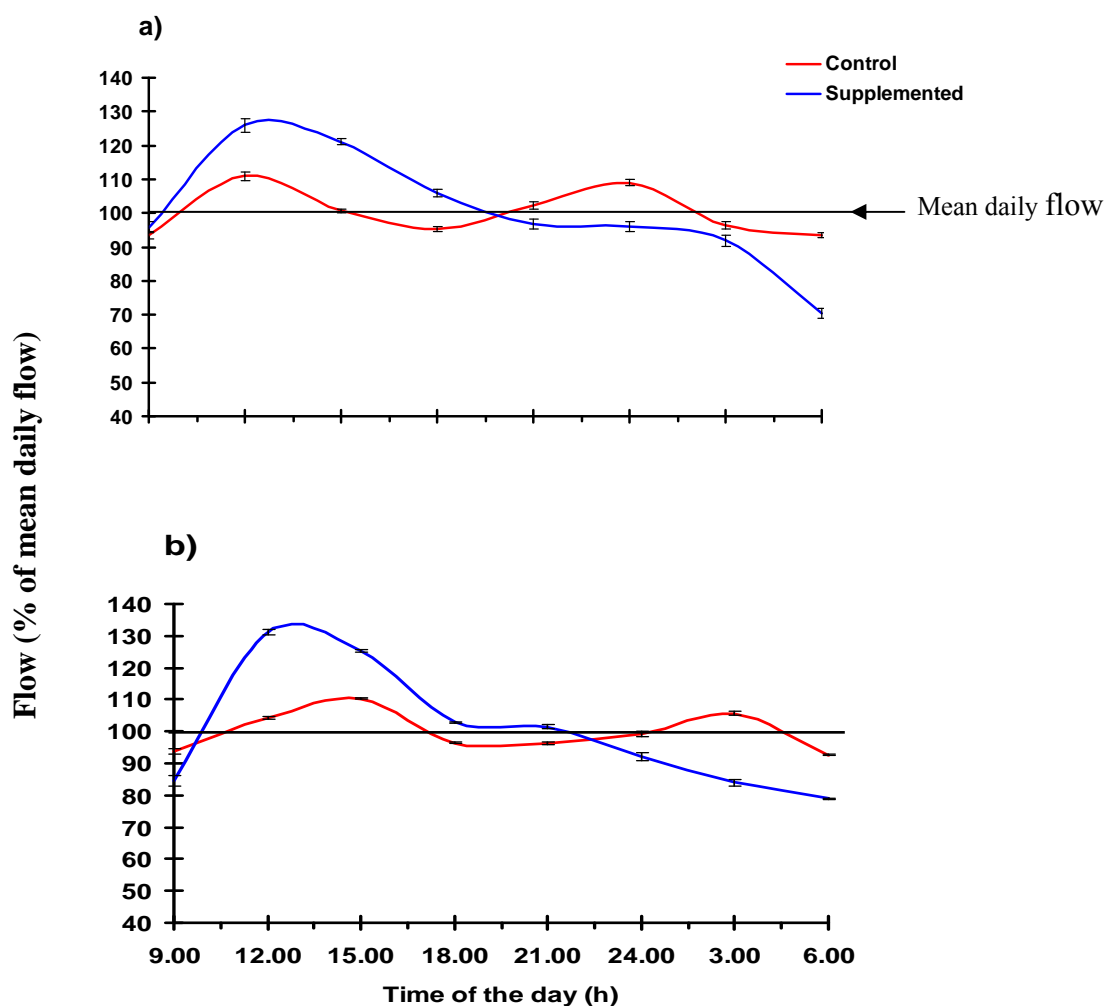


Figure 5 12: Diurnal variation in the flows of DM (a) and total AA (b) in twin-rearing ewes grazing spring pasture with or without supplement

Twin-suckling ewes in early lactation have high nutrient requirements, with feeding levels comparable to high-yielding dairy cows (AFRC, 1993) and it is therefore not surprising

that scale to intake, amino acid flow in the present study was comparable to that of dairy cows. Though there has been debate as to whether or not data from sheep and cattle could be used interchangeably for research, it would appear from the literature that sheep could be used as a model for cattle in some but not all situations (Sankey, 2005). The similarity of amino acid flows through the abomasum of sheep and cattle could be such that data may be used interchangeably, especially when animals are given high-quality pasture.

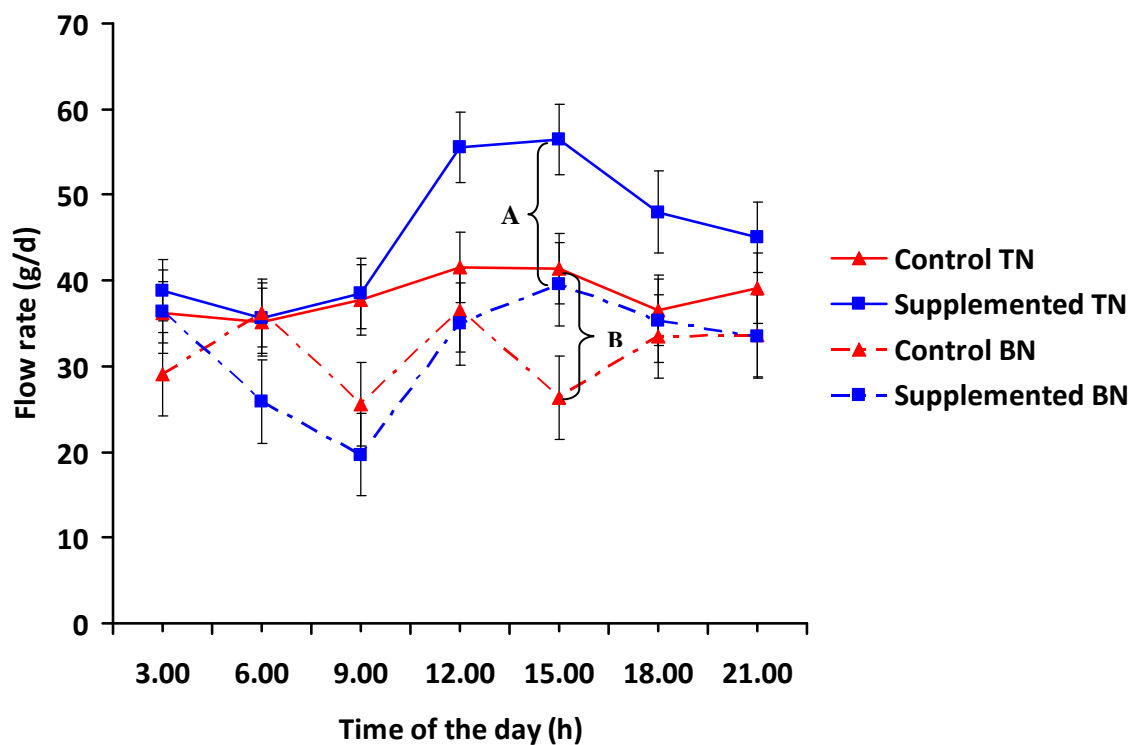


Figure 5 13: Diurnal variation in total nitrogen (TN) and bacterial nitrogen (BN) flow in twin-rearing ewes grazing spring pasture with or without a protein supplement and A and B representing by-pass nitrogen at 15.00h

Sulphur amino acids could be potentially limiting for immune function, judging from their low concentrations in abomasal digesta of C compared with S ewes and the 19-23% increase in flow due to supplementation. The potential for an effect in limiting FEC is especially the case since cysteine and methionine are important in the synthesis of glutathione, a component of anti-oxidant defences (Grimble et al., 1992). However, this could not be determined directly, but the hypothesis that sulphur AA are the most likely

limiting AA is consistent with the prediction of methionine to be limiting for milk production from pasture diets (Kolver, 2003; Kolver et al., 1999). Branch-chained amino acids (BCAA), the flows of which were increased by 16% in the present study have previously been associated with amino acid – hormone interactions. These interactions are understood to result from the fact that BCAA are not metabolised by the liver and their presence in the peripheral circulation may act as nutrient signals to hormonal activity (Lobley, 1998). The AA flows observed in the present study were measured from a similar supplementation study by Xie (2003) which reported a reduction in FEC in grazing ewes and it may be suggesting a maximum amount of MP that needs to be offered. However, the prospect that the improved immune response shown to MP is to a particular rate-limiting amino acid has not been adequately tested (Sykes, 2008). On that basis, feeding graded amounts of rumen-protected protein to provide scaled levels of amino acids will be needed to achieve this goal. The present work simply defines appropriate rates of supplementation which could provide such a response.

If, hypothetically, one may consider the observed augmented flow rates of AA to represent the needs of the immune response, one may need to ask; to what extent would pasture intake need to increase to match these without a need for supplementation? On this basis, it was calculated that DM intake would have to increase to 3.2 kgDM/d for most AA, 3.8 kgDM/d for phenylalanine, arginine, histidine and glutamine but to 9.0 kgDM/d for the sulphur AA. This is well above the generally accepted predicted maximum intake of 2.6 kg DM/d (4% of body weight). Therefore, it seems likely that supplementation would be needed.

There is a scarcity of information on the concentrations of plasma free AA in ewes grazing pasture. The present free AA concentrations are comparable to data by Sano et al. (2004)

where 2 year old wethers were given 2.15 x maintenance CP intake. A recent field study in United Kingdom with parasite-free ewes of different genotypes (Allison et al., 2008) reported a plasma free BCAA value of 566.1 μM which is similar to the 530.4 μM observed in the present study. Similarly, alanine, (261.6 vs. 275.6 μM), phenylalanine (72.2 vs. 72.1 μM), glycine (433.5 vs. 481.5 μM), isoleucine (128.4 vs. 144.8 μM), leucine (173.1 vs. 155.5 μM), methionine (34.5 vs. 31.0 μM), serine (80.0 vs. 109.3 μM) and tyrosine (92.4 vs. 104.7 μM) relative concentrations were also comparable those at 21 days after lambing in C ewes. Plasma AA concentrations were low during pregnancy and increased around week 3 after lambing either in a linear (isoleucine, lysine, SAA, total AA, glutamine, glutamic acid, taurine, cysteine, tryptophan, serine and alanine) or in quadratic or cubic fashion (Table 5.4). A study by Masters et al. (1993) reported decline in plasma AA in unsupplemented ewes during the last 3 weeks of pregnancy but later increased during the first 3 weeks of lactation. Though plasma AA concentrations in the present study were not affected by treatment, with the exception of cysteine and taurine which were increased in S ewes, plasma AA concentrations were lower 21 days post-partum in the supplemented ewes (see Table 5.7 below). After injecting methionine into the abomasum of ewes Stewart et al. (1993) observed a reduction in plasma EAA concentration on day 7 and 21 of lactation. Recently, Pitta et al. (2009) found that 3-methyl histidine in plasma was reduced by supplementing grazing ewes with willow stem cuttings and the authors attributed this to reduced degradation of body protein. Without supplementation, concentrations of arginine, lysine and threonine increased during the first 3 weeks of lactation (Masters et al., 1993) but skeletal muscle degradation tended to be low with increased dietary CP intake (Sano et al., 2004). Thus, the degradation of skeletal muscle could have been the reason for the phenomenon observed on day 21 post-partum in the present study, though it could also have been an artefact since during this time ewes were being offered only 100 g/ewe/d of the supplement. However, changes in body

condition score between day 23 and 70 post-partum suggests that mobilisation of body reserves was greater for C than S ewes, with C having lost body condition than S ewes at end of the study. This reinforces the notion that C ewes mobilised body reserves to buffer the shortfall of AA to support milk production while S ewes utilised part of the dietary nutrients to support lactation.

Observation of enzymes for transsulfuration in the skin of sheep led to a suggestion that wool-production responses to methionine supplementation may be due to increased supply of cysteine from transsulfuration of methionine (Liu et al., 2000). Based on that, it is tempting to suggest that in the present study, increased plasma methionine in C ewes 3 weeks after lambing may have increased the flux of substrates through the transsulfuration pathway and increased cysteine synthesis. High plasma cysteine concentration in S ewes, 3 weeks later may suggest that transsulfuration had occurred. This hypothesis is consistent with data of Malmezat et al. (2000) in which fractional turnover of plasma methionine was found to be 3 times higher than that of cysteine.

The up and down trend of glutamine and glutamate between day 28 and 49 postpartum may be reflecting the interchangeable capacity of these AA in the inter-cellular matrix and their utility in mucosal metabolism. Pathways of mucosal intermediary metabolism that utilise the carbon skeleton of glutamine can apparently utilise glutamate equally well (Reeds and Burrin, 2001). However, metabolism of glutamine extracts 25-33% of arterial glutamine in a single pass from the mesenteric artery of rodents (Wu, 1998) while in well-fed piglets, the mucosal GSH-glutamate derived comes largely from direct metabolism of enteral glutamate (Reeds et al., 1997). The low level of glutamate in the present study may indicate a preferential oxidation of enterally delivered glutamate (Wu, 1998) and a reduced

enrichment of the extra-intestinal tissues. In the diseased state, post-absorptive levels of glutamate may be depressed even further by the need to synthesis glutathione.

Table 5 7: Plasma EAA, BCAA and SAA concentration (μM) in twin-rearing ewes grazing spring pasture with or without protein supplement at day 21 post lambing.

Parameter	21 days			
	C	S	% Difference	SL ¹
Threonine	479.3±42.73	321.0±40.82	-33.0	**
Valine	230.1±20.07	150.3±18.58	-34.7	**
Isoleucine	144.8±11.19	95.9±10.36	-33.8	**
Leucine	155.5±12.56	100.4±11.63	-35.4	**
Phenylalanine	72.1±5.72	40.3±5.30	-44.1	***
Histidine	59.0±4.92	36.6±4.56	-38.0	**
Lysine	187.5±15.97	127.1±14.79	-32.2	**
Arginine	155.9±17.65	105.8±17.12	-32.1	*
Methionine	31.0±2.92	18.8±2.70	-39.4	**
Total EAA (mmol/L)	1.5±0.11	0.94±0.11	-37.3	***
Total SAA	118.2±7.95	88.9±7.36	-24.8	**
Total BCAA	530.4±42.66	346.5±39.49	-34.7	**
Total GAA (mmol/L)²	2.7±0.16	2.0±0.15	-25.9	**
Glutamine	418.5±30.54	313.4±28.27	-25.1	*
Glutamic acid	194.1±13.35	113.2 ±12.36	-41.7	***
Taurine	48.4±9.96	75.3±9.13	55.6	*
Cysteine	87.2±6.12	70.1±5.66	-19.6	*
Glycine	481.5±58.00	660.8±56.31	37.2	*
Aspartate	5.3±1.35	3.0±1.25	-43.4	NS
Tryptophan	41.5±3.40	19.6±3.14	-52.8	***
Tyrosine	104.7±7.86	56.6±7.28	-45.9	***
Proline	133.4±15.48	98.6±14.34	-26.1	NS
Serine	109.3±9.28	75.4±8.59	-31.0	**
Alanine	275.6±17.57	164.6±16.27	-40.3	***
Total AA (mmol/L)	3.4±0.20	2.4±0.19	-29.4	***

¹SL = Significance level; ²Glucogenic amino acids minus asparagine

* = P < 0.05; ** = P < 0.01; *** = P < 0.001 and NS = P > 0.05

Arterial amino acid utilisation by the gut was also observed in parasitized sheep by Yu et al. (2000) who reported a total 24% increase in leucine sequestration across the GIT and that most of the leucine came from arterial sources. In the present study, plasma leucine was depressed in supplemented ewes at week 3 after lambing and it would be interesting to find how much supplementation affected the ratio of leucine drawn from arterial compared to gastric absorption. Based on the conditions of the current study, it would be difficult to

apportion how much and which amino acids were used for pregnancy/lactation and which for immune function. However, it possible that protein supplementation in S ewes may have reduced the rate of catabolism to allow dietary AAs to be used for lactation without completely eliminating the need from uptake of arterial AA.

The number of animals needed to establish variance in parasitological measurements would need to be higher than the number of ewes used in the present study. However, body weight has been reported to be allocated low priority of scarce nutrients compared to reproduction (Houdijk et al., 2000), nevertheless it seems in the present study, protein supplementation did not result in weight gain, but instead reduced the rate of muscle degradation. The fact that daily gain of twin lambs from S ewes was similar to that of controls indicates that milk yield was not affected. This suggests a greater propensity of parasitized ewes to partition nutrients towards lactation and thus validates a hypothesis by Coop and Kyriazakis (1999) and Houdijk et al. (2000) that maternal body weight and immunity have a relatively lower priority for the allocation of scarce nutrients than reproduction. This priority of scarce MP allocation to milk production over immunity functions may be gradual rather than absolute (Houdijk et al., 2003).

During pregnancy and lactation, females develop insulin resistance, reflecting a decreased ability of adipose and skeletal tissues to utilise glucose (Ehrhardt et al., 2001). Sano and Terashima (2001) found that tissue responsiveness to insulin was influenced by cold exposure and that effects of cold stress were modified by level of dietary protein intake. In the present study, could it be possible that the surge in AA after feeding influences this tissue-endocrine axis rather than simply providing a fuel?

Conclusions

There is limited data on the amino acid supply to lactating ewes on pasture and the present study contributes additional information on supply of amino acids beyond the rumen.

Prediction from flow rates that sulphur amino acids may be enhanced to the greatest degree by the supplementation could suggest that these may be responsible for the observed responses to fish meal supplements as sulphur amino acids are needed for the synthesis of glutathione. BCAA which were also enhanced by 16% may act as a signal to tissues and hormonal activity about nutrient availability. These results suggest the need for direct supplementation ewes at pasture since it would not be possible to attain high enough pasture intakes to achieve similar amino acid flow observed. Diurnal variation was greatest in the supplemented ewes but the pattern was altered and different from that of ewes without a supplement. The amino acids flows observed were measured from a similar supplementation which previously reported a reduction in FEC in grazing ewes and probably indicate maximum amount of MP needed for improved immune responses.

CHAPTER 6

EFFECTS OF BODY CONDITION AND PROTEIN SUPPLEMENTATION ON GLUCOSE AND INSULIN AFTER EXOGENOUS GLUCOSE INFUSION AND ON FEC DURING PERI- PARTURIENT PERIOD IN *TELADORSAGIA CIRCUMCINCTA*- CHALLENGED EWES

6. 1 Introduction

After contrasting their data on the immune response of ewes in the peri-parturient period to protein nutrition and to body reserves with that of Donaldson et al. (1998), Houdijk et al. (2000) suggested the existence of an interaction between specific body reserves, protein nutrition and the expression of immunity to parasites. A nutrient-partitioning framework proposed by Coop and Kyriazakis (1999) as a nutritional basis for the peri-parturient breakdown in immunity argues that body nutrients would be used to support reproduction rather than immune function, when MP is scarce. Under normal conditions, during the breeding cycle, the maternal body undergoes unavoidable endocrine and compositional changes designed to provide nutrients for lactation (Robinson, 1986) and the channelling of nutrients to reproduction at the expense of immune function may simply be following an evolutionary 'wiring' set to enhance reproductive success in the female.

Mobilization of body reserves is regulated by hormones and happens due to homeorhetic mechanisms under genetic drives (Friggens and Newbold, 2007). If body nutrients are mobilised by peri-parturient ewes to overcome limitations of dietary ME or MP supply caused by the combined demands of reproduction and those of the immune system, as

suggested by the work of Houdijk et al. (2001a), it would then be expected that an association between hormonal action and immune function could exist. In peri-parturient ewes, hormonal effects may be specifically associated with preparation for lactation, but an unequivocal role for prolactin or any other hormone has not yet been identified (Barger, 1993). However, it has recently been reported by Kelley et al. (2007) that since the brain can orchestrate responses from leukocytes through autonomic nervous and endocrine systems, their interactions occurring at the hypothalamic-pituitary axis would result in pituitary hormones mediating the effects of the central nervous system on immune responses. For instance, growth hormone (GH), insulin-like growth factor 1 alpha (IGF-1 α) (Kelley et al., 2007), insulin (Campos and Baumann, 1992) and leptin (Ingvarsen and Boisclair, 2001) have been linked with immune function. Therefore, it is logical that pregnancy or lactation associated with hormonal changes may lead to compromised immunity since according to Robinson (1986) pregnancy/lactation and to Coop and Kyriazakis (1999) immune function results in body compositional changes to provide nutrients for either reproduction or immunity. In this regard, insulin resistance has been reported to be associated with diseases and infections, but most significantly with obesity and type 2 diabetes (Hotamisligil, 1999). The cause of this resistance has been reported to be due to molecular cytokines that inhibit hormonal action. For instance, pathological situations associated with high tumour necrosis factor alpha (TNF $-\alpha$) show a state of insulin resistance (Hotamisligil et al., 1993; Pallares-Trujillo et al., 2000) while suppressors of cytokine signalling (SOCS) attenuate insulin signalling by binding to the insulin receptors and reducing their ability to phosphorylate insulin receptor substrate (IRS) (de Oliveira et al., 2009)

During the peri-parturient period, female sheep experience insulin resistance (Pettersson et al., 1993) and leptin declines during early lactation (Ehrhardt et al., 2001). Leptin and

insulin serve as adiposity signals (Baskin et al., 1999; Demas and Sakaria, 2005) about current body energy status. It may be plausible at this point to ask; “is the loss of immune response to a nematode infection due to hormone-induced changes in metabolism during late pregnancy such as insulin resistance? And do body composition and dietary protein supply influence these changes?” There is no documentation of whether such changes in insulin sensitivity could be related to the peri-parturient breakdown of immunity in livestock and whether very high levels of supply of amino acid influence these. Nevertheless, studies in humans (Piatti et al., 1994), rats (Blouet et al., 2006) and in sheep (Sano and Terashima, 2001) have suggested that high protein intakes may enhance tissue sensitivity to insulin (insulin sensitivity). Therefore, the aims of this trial were to determine whether body condition influenced immune responses and whether this response is reinforced by protein supplements in nematode infected ewes during the peri-parturient period. In addition, the study aimed to determine whether variation in glucose tolerance as an index of insulin sensitivity was associated with the peri-parturient breakdown in immunity to nematode and the extent to which this was influenced by body condition and by protein supply.

6.2 Material and methods

6.2.1 Experimental design

Eighty ewes previously scanned as either twin- or single-bearing and destined to lamb around 24th August 2007 were selected from a large flock. Four groups, balanced for anticipated number of lambs, were selected. Group 1 (n = 20) were ewes with high initial body condition score (BCS) (mean \pm SE; 3.9 ± 0.09) in which BCS was maintained throughout pregnancy by manipulating pasture allowance (HM). Groups 2 and 3 were ewes of medium BCS (3.3 ± 0.07) and were randomly allocated into two subgroups, one of which was offered pasture to allow gain of condition (MH; n = 20) and the second offered

a low grazing allowance to induce loss of body condition (ML; n = 20). Group 4 were thin ewes with BCS of 2.4 ± 0.09 which was maintained by routine pasture allowance (LM; n = 20). These feeding regimes were maintained for 20 days (from day -54 to -35 relative to parturition). Body condition was monitored throughout this time to ensure that ML lost and MH ewes gained condition. HM and LM ewes were run as one mob separately from ML and MH ewes. From day -35 to -26 relative to anticipated date of parturition all ewes were introduced to a protein supplement (60 g/d) while at pasture to allow acclimatization. A glucose tolerance test (GTT) was conducted on all sheep during day -26 relative to lambing, after which the protein supplement was increased to 500 g/d, to half of the ewes in each group and supplement withdrawn from the remainder creating supplemented (-S) and non-supplemented (-NS) subgroups. During day -12 relative to parturition, a second GTT was carried out. All animals were treated with anthelmintic on day -21 and were challenged with 10 000 *Teladorsagia circumcincta* larvae on days -14 and -7. Protein supplementation was continued until day +69 relative to lambing. The layout of the trial is given in Figure 1. Faecal samples were taken, live weight and body condition recorded at the start of the trial and thereafter weekly. Lambs were weighed within 24 h of birth and again at 44 and 65 d after birth. Computer tomographic scanning was carried out on ewes at days -54, -21 and +56 relative to lambing.

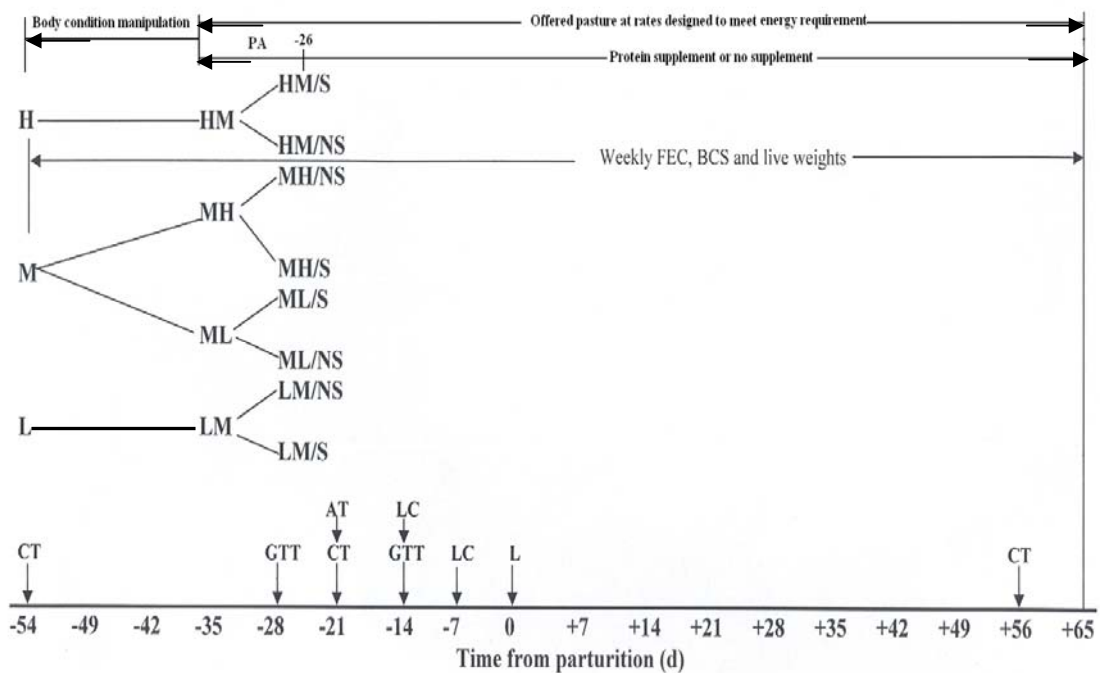


Figure 6 1: Experimental design; Ewes of different body condition grazing spring pasture with or without a protein supplement were challenged with *T. circumcincta* larvae. During late-pregnancy there were fed to maintain body condition (HM and LM), gain condition (MH) or lose condition (ML). CT= computer tomography, GTT = glucose tolerance test, PA = protein acclimatization, AT = Anthelmintic treatment, LC = larvae challenge

6.2.2 Supplementation

Ewes grazed an established rye grass (*Lolium penenne*) – clover (*Trifolium repens* var Huia) sward. The protein supplement was fishmeal-based and identical in composition to that described in Chapter 5. From day -26, S groups of each treatment (i. e. HM/S, LM/S, MH/S and ML/S) were run as one mob while the NS group (i. e. HM/NS, LM/NS, MH/NS and ML/NS) run as another single mob on two separate paddocks. Pasture availability was estimated by visual height assessment by experience personnel to assure biomass availability of approximately 2200 kg DM/ha, to provide an energy intake comparable to requirement.

6.2.3 Glucose tolerance test and blood sampling

Animals were brought indoors and housed for 20 h before GTT which took place twice, on days -28 and -14 before anticipated lambing date. The methods of Kaneko (1989) were used. Sterile glucose solution (50% w/v Baxter New Zealand, Auckland, NZ) was administered over 1 minute at the rate of 0.44g/kg LW (Regnault et al., 2004). To calculate glucose dosage, live weight at 6 (GTT 1) and 3 days (GTT 2) prior to commencement of GTT were used. Glucose was delivered through the jugular vein using a 100 ml syringe. The zero time was taken as the time when total glucose load had been given. During GTT, blood (about 5 ml) was collected from the contralateral vein into Li-heparin-containing tubes (Becton Dickinson Vacutainer Systems, Plymouth, UK). Glucose analysis was done within 5 min of blood collection on samples collected at 0, 5, 10, 20, 40, 80 and 160 min after glucose infusion. Plasma was harvested by spinning the blood at 3000 g for 10 min at 4°C and the supernatant pipetted by a 3 ml bulb pipette (SAMCO Scientific group, Mexico) into two 5 ml plug top test tubes (Biolab, Christchurch, NZ) for each animal. Samples were labelled and stored at -20°C pending analysis.

6.2.4 Parasitology

The animals were drenched with Scanda (80.0 g/L levamisole HCL and 45.3g/L ofendazole; Shering-Plough Animal Health Ltd, Wellington, New Zealand) at a rate of 1 ml *per* 10 kg live weight on day -21 relative to lambing. Sheep grazed natural pasture known be infected by nematode parasites and infection was supplemented by challenge with third-stage infective larvae of *T. circumcincta*, administered by dosing with a filter paper pellet onto which a solution containing 10 000 larvae had been pipetted. FEC were carried out on fresh faeces as described in Chapter 5.

6.2.6 Computer tomography (CT)

Changes in volume of fat, muscle and bone during the course of the experiment were estimated *in vivo* using X-ray computed tomography (Prospeed, General Electric Medical Systems; Wilwaukee, WI USA). Scanning procedure was similar to that of Young et al. (1996). Animals were fasted for a minimum of 12 h prior to scanning (except scanning during day -21, relative to lambing) and were sedated with 1.5 ml of acepromazine maleate (ACEZINE 10; acepromazine maleate 13.5 mg; Delvet Pty Ltd, NSW, Australia) given intramuscularly, at least 30 min before scanning. Animals were restrained in a cradle while lying on their back as outline by Nsoso (1995). Cross-sectional scans were taken on each animal at the thoracic vertebrae 7 (TV7), lumbar vertebrae 1 (LV1), lumbar vertebrae 4 (LV4) and the ischium (ISC) and from each scan image, areas and image densities were obtained for fat, muscle and bone components of the carcass. In addition 10 (during first scanning), 8 (during second scanning) and 6 ewes (during the third scanning) were randomly selected to represent big, medium and small ewes for the estimation of total carcass tissue weights using the 'calvaleri principle' developed by Gundersen et al. (1988). The procedure was carried out according to the modified methods of (Young et al., 1996). In brief, instead of being precisely positioned in terms of the scanner table bed position, the first scan was chosen at random and subsequent slides made at equal spacing of 55 mm through the animal to provide an estimate of volume of 3-dimensional images. The shape and the orientation of the animal were not important (Nsoso, 1995). Determination of the area of muscle, fat and bone on each scan and the measurement of linear dimensions were done using the Sheep Tomogram Analysis Routines software (Jones et al., 2004). Individual gutted images were 'electronically' dissected into total fat, muscle and bone areas. Thereafter slices for each tissue for each animal were summed and multiplied by the distance between slices (55 mm) to obtain tissue volumes. Volumes were converted to

weight by multiplying by standard density values for carcass fat, muscle and bone of 0.925, 1.031 and 1.549 kg/dm³, respectively (Nsoso, 1995).

6.2.7 Analysis

6.2.7.1 Glucose and insulin

Glucose concentration was determined within 5 min of sampling on whole blood as outlined in Chapter 5. Insulin in plasma was analysed at Endolab (Canterbury Health Laboratories, Christchurch, New Zealand) on a limited number (12) of twin-bearing ewes after reviewing the variation in glucose tolerance data, because of cost. Selected samples represented ewes with high, medium and low live weight. Samples were mixed with an equal volume of 25% polyethylene glycol 6000 and centrifuged to remove antibodies. The supernatants were analysed for insulin using the automated Elecsys 2010 analyser (Roche, Mannheim, Germany). The inter- and intra-assay CVs were 2.0% and 3.1% respectively.

6.2.8 Calculations

6.2.8.1 Average daily gain (ADG)

ADG for lambs was calculated as in Chapter 5 at 44 and 65 d of age.

6.2.8.2 Turnover rate, increment, half-life of blood glucose and insulin

Fractional turnover rate or disappearance rate (k) was calculated as;

$$k (\% \text{min}) = ((\ln A_{\text{peak}} - \ln A_{80}) / (80 - T_{\text{peak}})) * 100 \quad [1] \quad (\text{Kaneko, 1989})$$

where A_{peak} is the peak level of either plasma glucose or insulin, A_{80} is the plasma glucose or insulin level 80 min after glucose infusion and T_{peak} (min) is the time at which the peak was observed.

Increment of either glucose or insulin was defined as the difference between the peak level and basal level. Half-life ($T_{1/2}$), the time required for either of glucose or insulin concentration to fall by one half was calculated as;

$$T_{1/2} \text{ (min)} = (\ln 2/k) * 100 \quad [2] \quad (\text{Kaneko, 1989})$$

Area under the curve (AUC) for glucose and insulin was calculated according to Billo (2007) using a trapezoid rule during the 160 min (AUC) infusion period starting at 5 min post-infusion.

Glucose clearance rate (CLR, min) was calculated according to Regnault et al. (2004) as follows;

$$\text{CLR (min)} = \text{glucose dose (mmol/L)} / \text{AUC mmol/L x min} \quad [3]$$

where AUC = area under the curve.

6.2.9 Statistical analysis

Two ewes from ML/NS and one from MH/NS died before completing the trial and their data were excluded. Differences in lamb performance (birth weights, average daily gains/ewe) and ewe tissue weights (muscle, bone and fat) were analysed by analysis of variance (ANOVA) using the general linear model (GLM) procedures of SAS (2002-2003). Sex, date of birth and rearing-rank were used to adjust lamb performance; where no significant effects were observed these were dropped from the model. The term birth type (Btype) was used to mean number of foetuses carried by ewes or born (single vs. multiples). Btype and together with rearing-rank, were used as covariates for CT tissue weights when appropriate. Blood glucose and insulin responses (AUC, k, $T_{1/2}$, CLR, increment, peak level and basal level) were analysed using Proc Mixed procedures of GLM SAS (2002-2003) where ewe was used as a random factor. Basal glucose was used as a

covariate where appropriate and interaction of treatment x period was tested but where no effect was observed, it was excluded from the model. Data from ewes carrying twins were used to analyse for insulin, therefore birth type was dropped from the statistical model. Correlations among CT body tissues and between body tissues and BCS were evaluated. In addition, correlations between tissue reference and “cavalieri” values (from a sample of 10 randomly chosen ewes) were computed to verify if averages of three section-scanning were a good representation of whole body tissue levels. Basal glucose and basal insulin concentration were also evaluated for their correlation with CT muscle and fat.

All time-series data were subjected to restricted maximum likelihood (REML) by the use of statement Repeated within Proc Mixed procedure of SAS (2002-2003) to estimate variances and covariance (Holland, 2006). Ante-dependent order one (FEC) and autoregression order one (dam performance and blood metabolite data) structures proved to be appropriate within-subject variance covariance structure according to Akaike’s information criterion (Wolfinger and Chang, 1998). The model for FEC, live weight, body condition score, blood glucose and blood insulin included the effects of treatment and treatment x time. However, ewe, treatment birth-type and rearing-rank were used as random factors to specify between-animal variation in the FEC and animal performance model. Ewe, birth type and live weight were used as random factors for plasma glucose concentration while birth type was excluded for the insulin and glucose subsample data. All twelve animals in the insulin data subset were carrying twins. Minimum significance level was set at $P < 0.1$ for significant difference for results of the insulin subset samples.

This experiment was carried out according to Lincoln University Animal Ethics Committee (AEC) authority # 201.

6.3 Results

6.3.1 Live weights, body condition score, average daily growth

More ewes gave birth to multiple lambs than to singles (8 triplet; 51 twin and 18 single-bearing ewes). During the suckling period for some ewes, one of the multiple lambs died resulting in a different ranking at days 44 and 65 of age (3 triplet; 45 twin and 26 single- at 44 d of age and 3 triplet; 40 twin and 31 single- rearing ewes at 65 d of age). The data on ewe live weight, body condition score and lamb average daily weight gain are shown in Figures 6.2 and Table 6.1. The aim of reducing or increasing live weight and body condition score in ML and MH animals, respectively, was only accomplished for weight as indicated in both Figures 6.2(a) and (c), and Figures 6.2(b) and (d) from day -54 to day -32, relative to lambing. Fifteen days before lambing HM ewes were 21, 8.5 and 13.4 kg heavier ($P < 0.001$) than LM, MH and ML ewes, respectively, while LM ewes were 12.5 ($P < 0.001$) and 7.6 kg ($P < 0.01$) lighter than MH and ML ewes, respectively. MH ewes were 5 kg heavier ($P < 0.05$) than ML ewes. Fifteen days before lambing HM had a higher (4.0 ± 0.11 ; $P < 0.001$) BCS than LM, MH and ML ewes (2.5 ± 0.11 , 3.4 ± 0.11 and 3.1 ± 0.12 for LM, MH and ML ewes, respectively). During this time, LM ewes had poorer ($P < 0.001$) BCS than MH and ML. MH and ML ewes however, had similar ($P > 0.05$) body condition 15 days prior to lambing. There was no ($P > 0.05$) difference in lamb birth weight due to supplementation of their dams nor was there any difference in ADG/ewe at 44 and at 65 days of age. However, there were trends indicating heavier lambs born from supplemented ewes at day +65 relative to lambing (Table 6.1).

6.3.2 Body composition

The computer tomographic estimates of body composition are presented in Tables 6.2, 6.3 and Figure 6.3. Reference muscle and fat were significantly ($P < 0.001$) and highly correlated to “cavalieri” muscle and fat (Table 6.2). Fifty-four days before lambing HM

ewes had the greatest ($P < 0.001$) body muscle and body fat (Table 6.3), though bone weights were similar ($P > 0.05$) (Table 6.3). Changes in CT muscles indicated that ML ewes had lost most muscle, followed by HM ewes between days -54 and -21 relative to parturition (Figure 6.3a). There was no change in muscle of MH ewes while LM/S gained 0.2 and LM/NS lost 0.3 kg of muscle. At this time, CT fat weights were significantly lower ($P < 0.001$) for LM/S and LM/NS ewes (than other groups) but ML/NS had similar ($P > 0.05$) amounts to LM/NS ewes. LM ewes lost the most fat (2.9 kg), while ML lost 2.7 kg of fat (Figure 6.3b) between days -54 and -21 relative to lambing. CT fat weight of MH ewes increased by 0.1 kg while that of HM ewes decreased by 0.4 kg between day -54 and -21 relative to lambing. Interestingly between days -21 and +56 relative to lambing, HM/NS ewes had lost the most fat (3.4 kg), followed by HM/S (2.9 kg) and MH/NS (2.2 kg). In contrast, during this period LM/S had shown increase in fat (1.2 kg) while ML/S lost 1.6 kg of fat. There was a high and significant correlation (Table 6.6) between muscle and fat weights ($r = 0.69$, $r = 60$ and $r = 49$; $P < 0.001$) on day -54, -21 and +65, respectively, and between body condition score and fat weight ($r = 0.77$ and $r = 0.83$; $P < 0.001$) on day -54 and +65, respectively.

6.3.2 Faecal egg count

Faecal egg counts are shown in Figures 6.4 and 6.5. There was no ($P > 0.05$) significant difference in FEC between ewes of different body condition (Figure 6.4a). However, starting from day -38 to day -32 relative to lambing, ewes kept in low body condition (LM) or moved from medium to low body condition (ML) tended to have higher FEC but this was not significant. There were no consistent trends in FEC after lambing and no effect of protein supplementation (Figure 6.4b). Animals in group LM, whether supplemented or not tended (not significant) to have highest FEC but similarly, so did the supplemented group in greatest body condition (HM/S), in this case significantly so on days 12 ($P = 0.05$) and 17 ($P < 0.05$) after lambing (Figure 6.4c and d). Ewes bearing/rearing multiple lambs had

higher FEC counts at day -32 and +12 relative to lambing than ewes bearing/rearing single lambs, which was significant ($P < 0.05$) on the latter date (Figure 6.5).

Table 6 1: Lamb birth weight and average daily gain/ewe at 44 and 65 days of age of lambs born from ewes of different body reserves grazing spring pasture with or without a protein supplement.

Treatment	At birth			44 d old			65 d old					
	Wt (kg)	N*			ADG/ewe (g/d)	N			ADG/ewe (g/d)	N		
		Tri	Tw	S		Tri	Tw	S		Tri	Tw	S
HM/NS	5.4 ± 0.20	1	7	2	535 ± 53.2	0	6	3	487 ± 30.6	0	6	3
HM/S	5.4 ± 0.19	2	7	1	478 ± 50.5	2	5	3	542 ± 28.4	2	3	5
LM/NS	5.6 ± 0.21	0	7	3	464 ± 56.5	0	7	2	423 ± 32.3	0	6	3
LM/S	5.3 ± 0.19	0	6	4	501 ± 50.5	0	6	4	497 ± 28.5	0	6	4
MH/NS	5.5 ± 0.19	1	7	1	544 ± 53.2	0	6	3	488 ± 30.0	0	5	4
MH/S	5.6 ± 0.20	1	5	4	486 ± 53.2	1	4	4	547 ± 30.0	1	3	5
ML/NS	5.1 ± 0.20	2	5	1	516 ± 56.5	0	6	2	456 ± 32.3	0	6	2
ML/S	5.7 ± 0.19	1	7	2	430 ± 50.5	0	5	5	503 ± 28.4	0	5	5
S L	NS				NS				NS			

*Number of triplets, twin and single lambs

Table 6 2: Correlation coefficients between reference body tissues levels and Cavalieri body tissue levels

Cavalieri	Muscle			Fat			Bone		
Reference	-54	-21	+65	-54	-21	+65	-54	-21	+65
Muscle	0.89***	0.98***	0.93**						
Fat				0.99***	0.99***	0.97**			
Bone							0.37 ^{NS}	0.17 ^{NS}	0.75 ^{0.09}

Table 6 3: Changes in CT-determined tissue composition of ewes in the treatment groups at different periods in the breeding cycle at the three measurement periods while grazing spring pasture with or without a protein supplement

Treatments combination									Treatment effects
BCS	HM		LM		MH		ML		
Suppl	HM/S	HM/NS	LM/S	LM/NS	MH/S	MH/NS	ML/S	ML/NS	SL
Muscle (kg)									
-54 days	12.0 ± 0.28 ^a		9.1 ± 0.28 ^c		10.3 ± 0.28 ^b		11.0 ± 0.29 ^b		***
-21 day	11.2±0.36 ^a	10.8±0.40 ^{ab}	9.3±0.36 ^c	8.8±0.40 ^c	10.3±0.40 ^{ab}	10.3±0.38 ^{ab}	9.4±0.38 ^c	9.8±0.46 ^{bc}	***
+56 days	11.2± 0.39	11.0±0.39	10.0±0.35	10.1 ±0.39	10.1±0.39	10.8 ± 0.37	10.7 ± 0.39	11.1 ± 0.31	NS
Fat (kg)									
-54 days	9.4 ± 0.49 ^a		6.7 ± 0.53 ^b		6.9 ± 0.38 ^b		8.6 ± 0.38 ^a		***
-21 day	9.0±0.51 ^a	9.0±0.58 ^a	3.4±0.48 ^d	4.3±0.54 ^{cd}	7.0±0.53 ^b	7.0±0.53 ^b	6.0±0.52 ^b	5.8±0.62 ^{bc}	***
+56 days	6.1 ± 0.39 ^a	5.6 ± 0.44 ^{ab}	4.6 ± 0.40 ^{bc}	4.6 ±0.43 ^{bc}	6.0 ± 0.43 ^a	4.8 ± 0.41 ^{bc}	4.2 ± 0.49 ^c	4.2 ± 0.49 ^c	**
Bone (kg)									
-54 days	3.1 ± 0.18		2.6 ± 0.17		2.8 ± 0.18		3.0 ± 0.18		NS
-21 day	2.8±0.10 ^{ab}	2.9±0.11 ^a	2.3±0.10 ^c	2.3±0.11 ^c	2.4±0.10 ^c	2.4±0.10 ^c	2.4±0.10 ^c	2.5±0.12 ^{cb}	***
+56 days	2.7 ± 0.12	2.7 ± 0.13	2.5 ± 0.12	2.5 ± 0.13	2.5 ± 0.13	2.7 ± 0.12	2.6 ± 0.12	2.6 ± 0.14	NS

^aMeans across rows with different superscript are different at P < 0.05

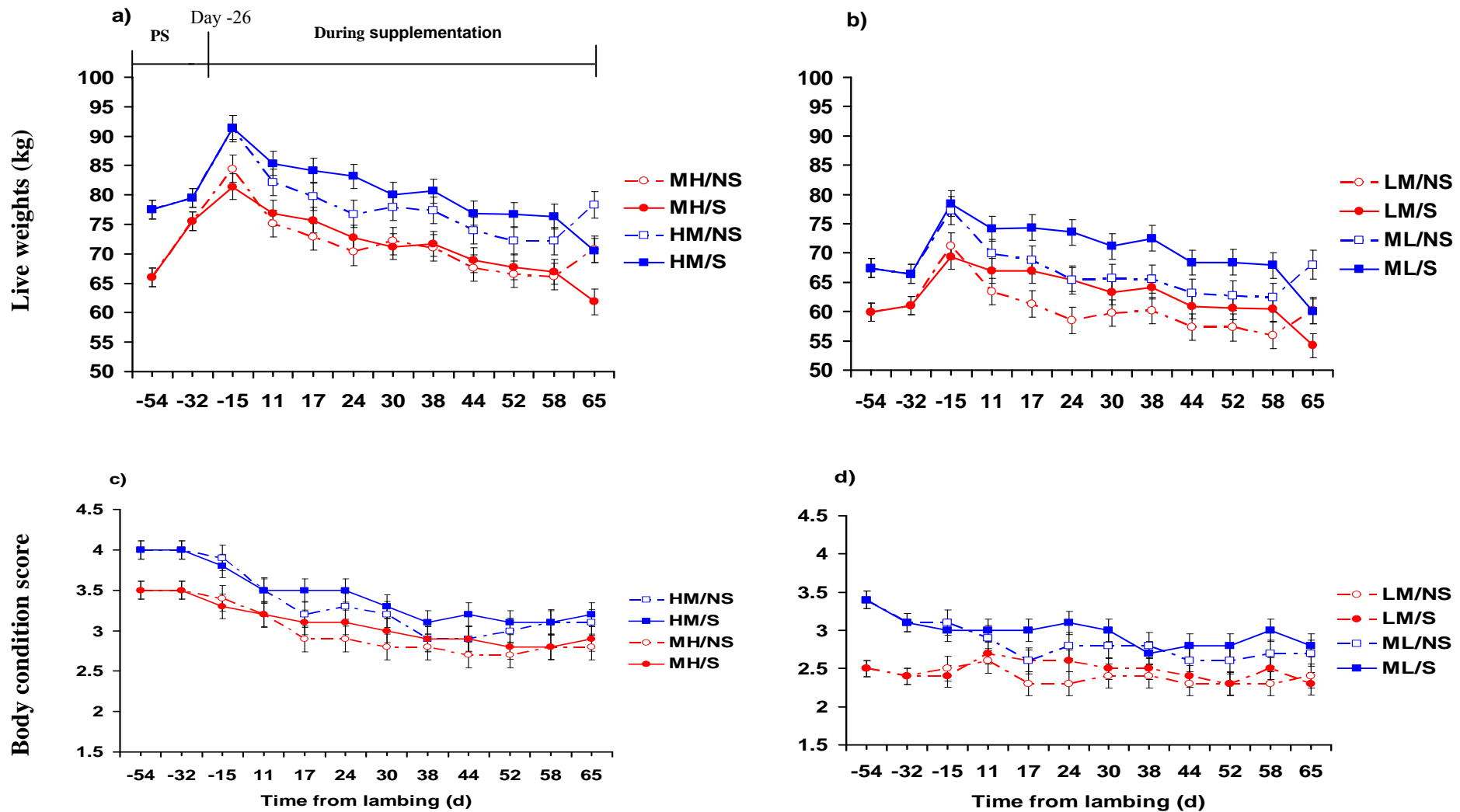


Figure 6 2: Live weights (a, b) and body condition score (c, d) of parasitized pregnant- and suckling ewes of different body condition grazing spring pasture with or without a protein supplement. PS = prior supplementation

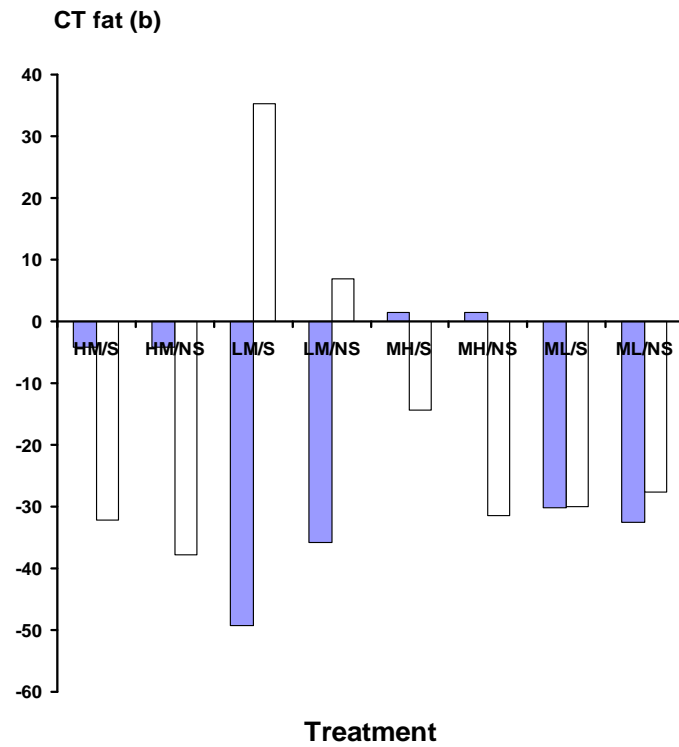
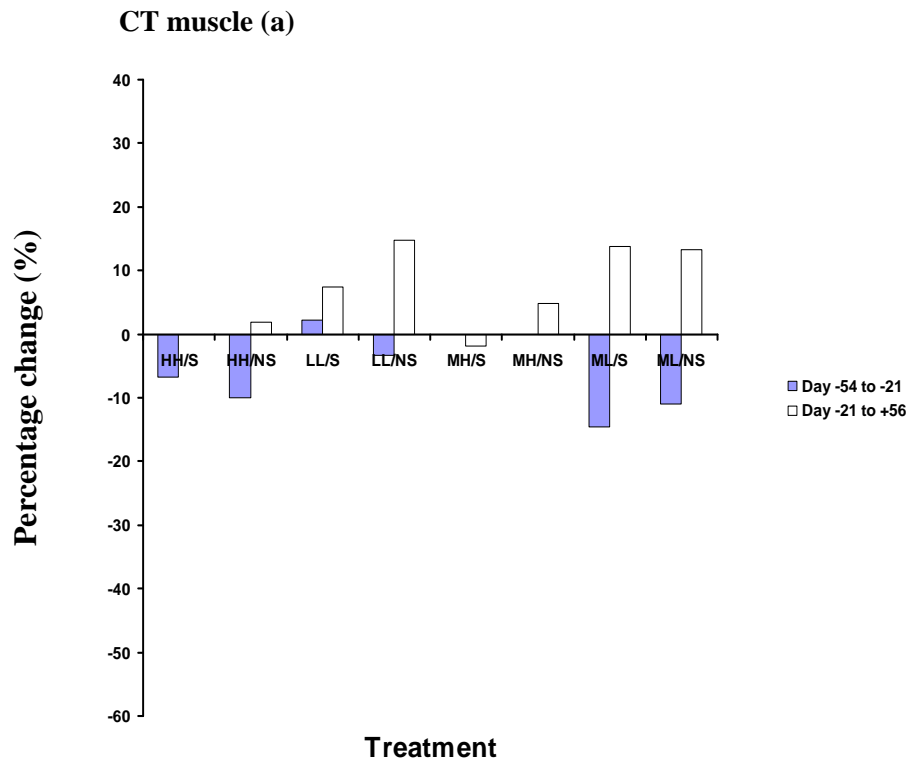


Figure 6 3: Percentage changes in CT estimated muscle (a) and fat (b) contents of the treatment groups measured between week -8 and week -3 and between week -3 and week 8 relative to lambing

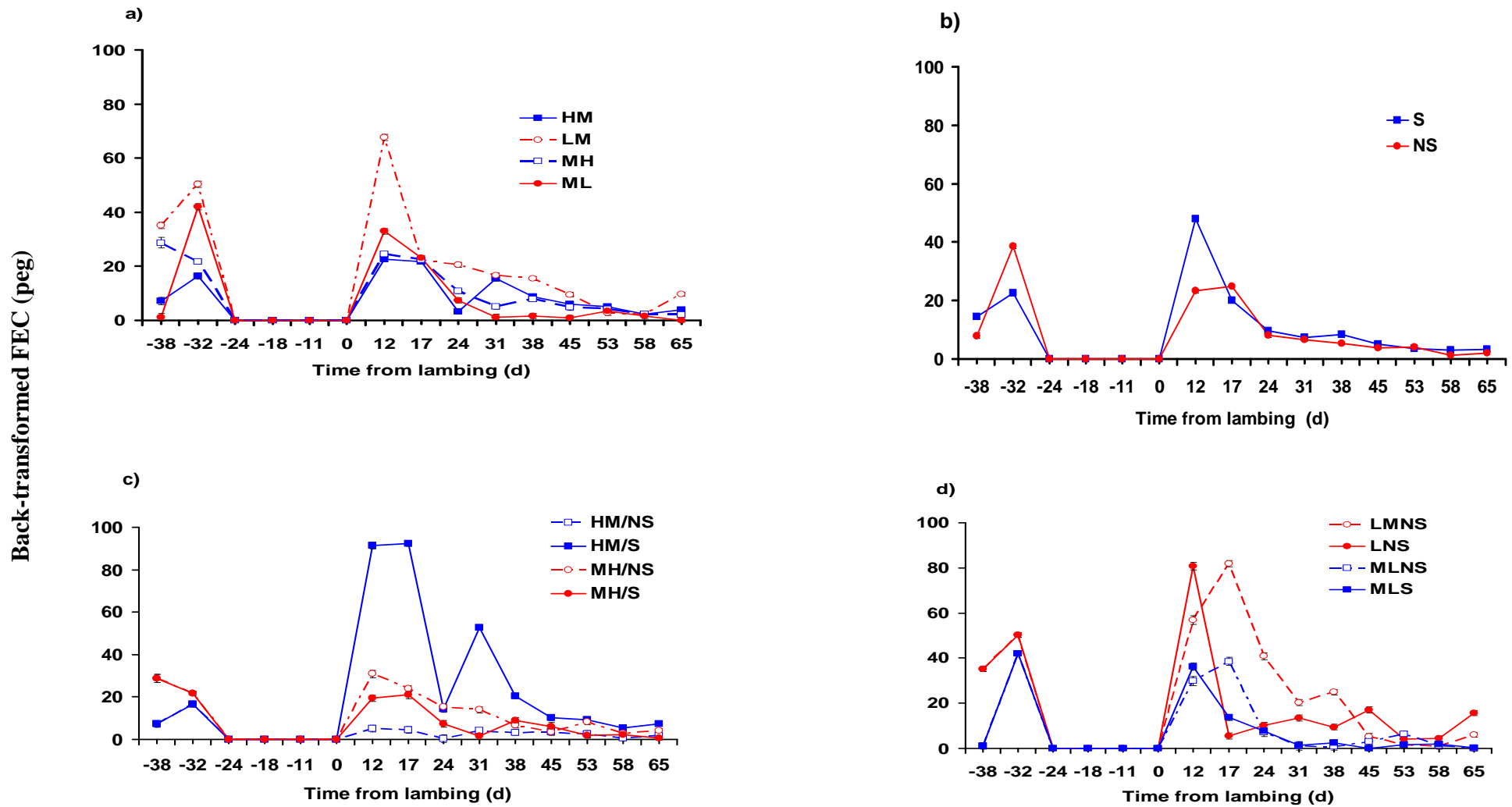


Figure 6 4: Effects of body condition (a), protein supplementation (b) and combination of body condition and supplementation for HM and MH (c) and for LM and ML (d) on FEC of ewes grazing spring pasture during pregnancy and lactation.

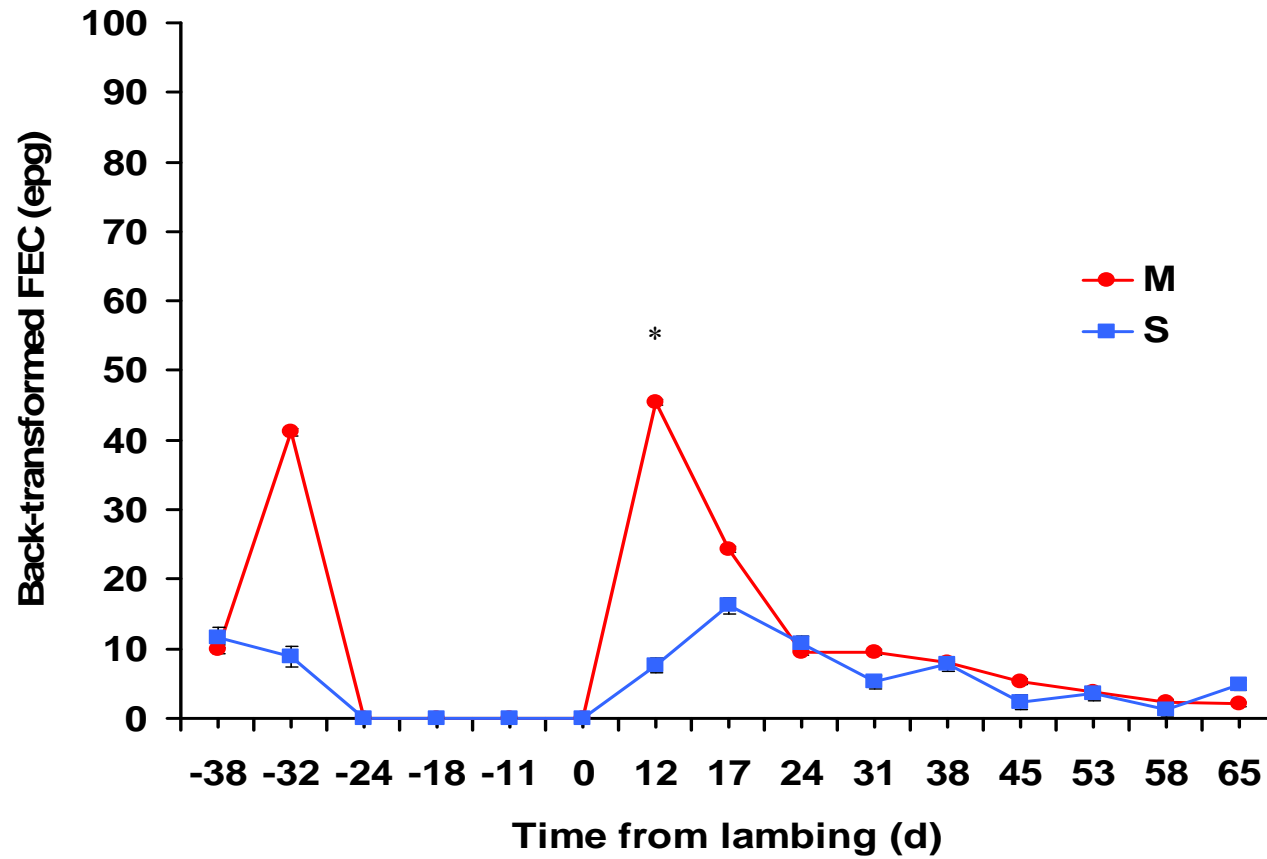


Figure 6 5: Back-transformed FEC of ewes grazing spring pasture during pregnancy and lactation and either bearing multiple (M) or single lambs (S)

6.3.3 Glucose tolerance test

a) All the animals

Prior to day -26 relative to parturition, none of the S groups were receiving the supplement but they were during the second GTT on day -12 relative to lambing. Trends in blood glucose responses to glucose infusion of all groups of sheep are given in Figure 6.6. Most indexes of blood glucose response were not influenced by period when GTT was determined and so GTT 1 and GTT 2 were combined during the analysis and these responses are presented in Tables 6.4. Basal plasma glucose was different ($P < 0.001$) between groups, being highest in HM/S and least in ML/NS ewes and was higher ($P < 0.001$) during GTT 2 than GTT 1 (2.6 ± 0.07 vs. 1.3 ± 0.07 mmol/L). Ewes carrying a single foetus had higher ($P < 0.001$) basal glucose than those carrying multiple lambs (2.2 ± 0.10 vs. 1.7 ± 0.05 mmol/L). Peak glucose occurred 5 min after glucose infusion (Figure 6.6) and was not different ($P > 0.05$) between the groups (Table 6.4). Blood glucose response as indexed by increment, AUC, turnover rate, half life and clearance rate were similar ($P > 0.05$) between groups (Table 6.4). However, glucose clearance rate were similar during GTT2 and GTT 1 (285.0 ± 15.80 vs. 262.1 ± 13.58 min) and between ewes carrying multiples than those with a single lamb (284.2 ± 9.06 vs. 263.0 ± 18.44 min). The differences were not significant ($P > 0.05$). Glucose response to the infusion, in terms of AUC, was higher ($P < 0.05$) during GTT 1 than GTT 2 (703 ± 30.4 vs. 630 ± 30.4 mmol/L x min; Figure 6.5 and Table 6.4).

b) Subsample of 12 ewes

The blood glucose and insulin responses for the subgroups for which insulin was determined are given in Table 6.5 and Figures 6.7, 6.8 and 6.9. There was tendency for higher, though non-significant, basal insulin concentrations in HM ewes (Table 6.5). AUC was slightly higher in HM and MH ewes, but these differences were not significant. Trends in Figure 6.7 suggest greater insulin responses at GTT 1. Insulin concentration trends are

consistent with results obtained during GTT 1 and GTT 2 (Table 6.6). When the data was examined according to the supplemented and non-supplemented groups during GTT 2 (Figure 6.8), no effect (22.8 ± 3.49 vs. 18.4 ± 2.95 pmol/L; $P > 0.05$) of supplementation was observed. An effect ($P < 0.001$) of time x supplement was due to higher insulin concentrations of sub-sampled supplemented ewes 80 min after infusion ($P < 0.05$) during GTT 2 than in non-supplemented ewes.

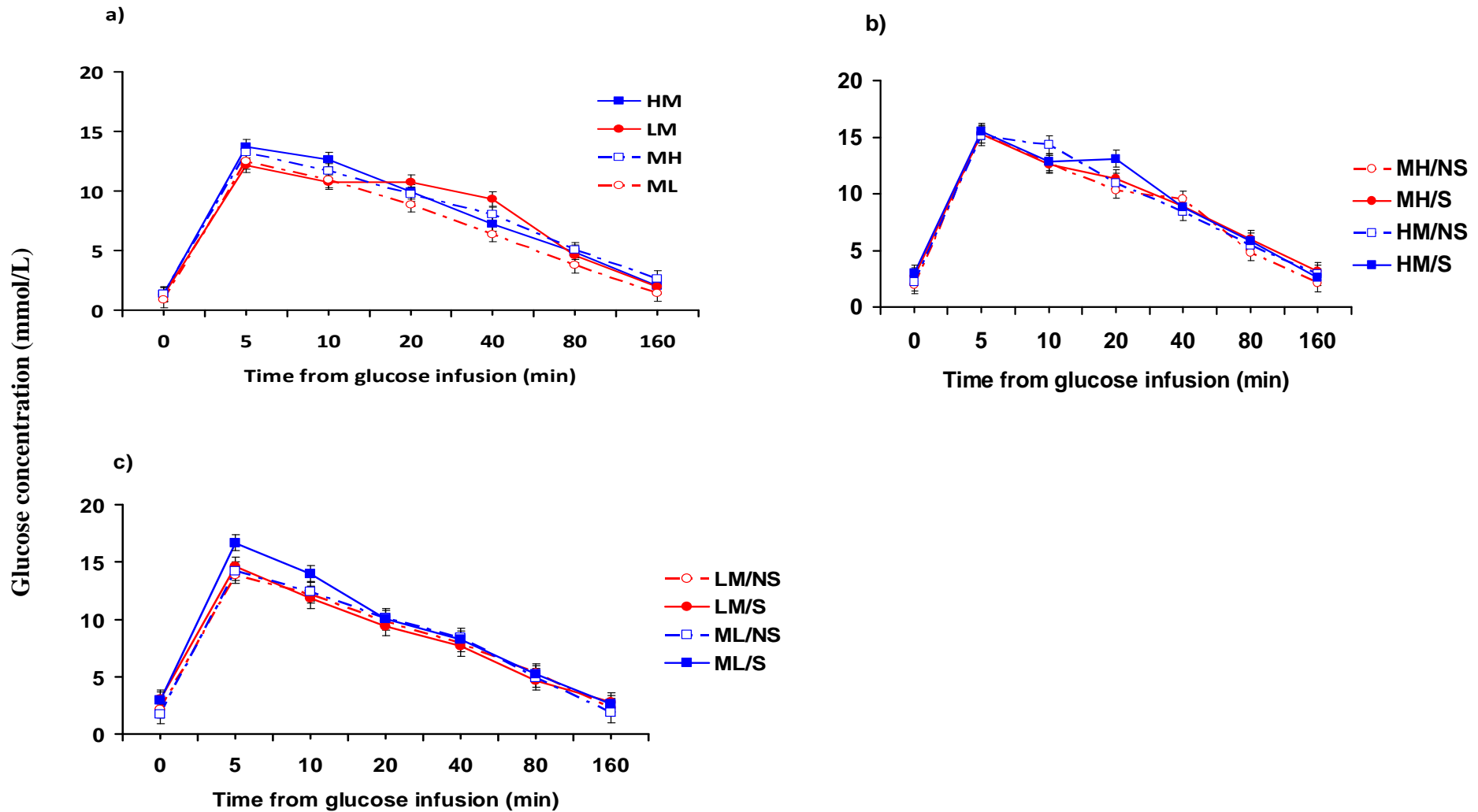


Figure 6 6: Whole-blood glucose profiles after glucose infusion in parasitized pregnant ewes of different body condition while grazing spring pasture with or without a protein supplement at day -26 (a; before supplementation commence) and at day -12 (b, c; after supplementation was started) relative to lambing

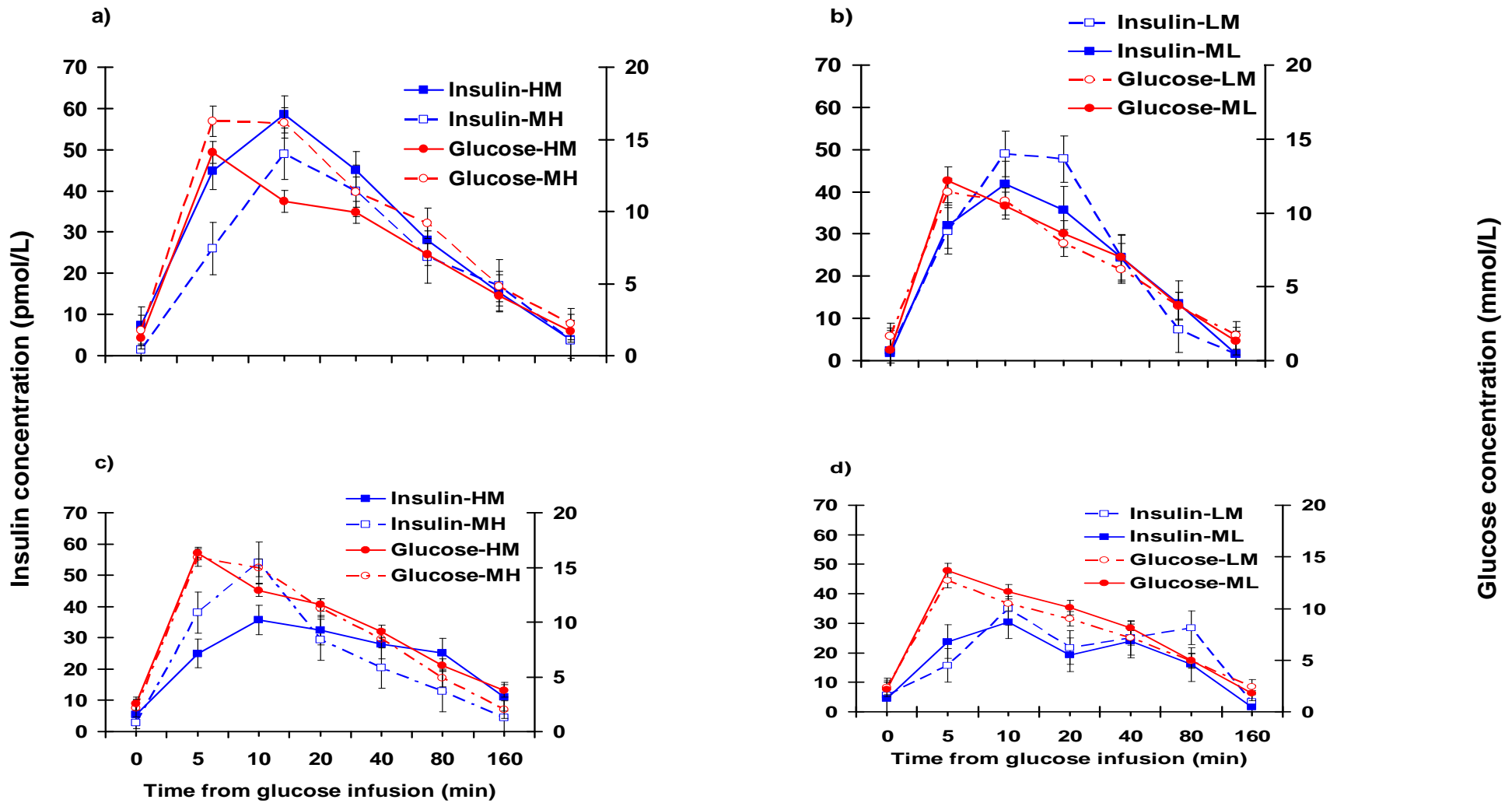


Figure 6 7: Whole-blood glucose and insulin profiles after glucose infusion in parasitized pregnant ewes of different body and grazing spring pasture at day -26 (a, b) and day -12 (c, d) relative to lambing for a subsample of 12 ewes.

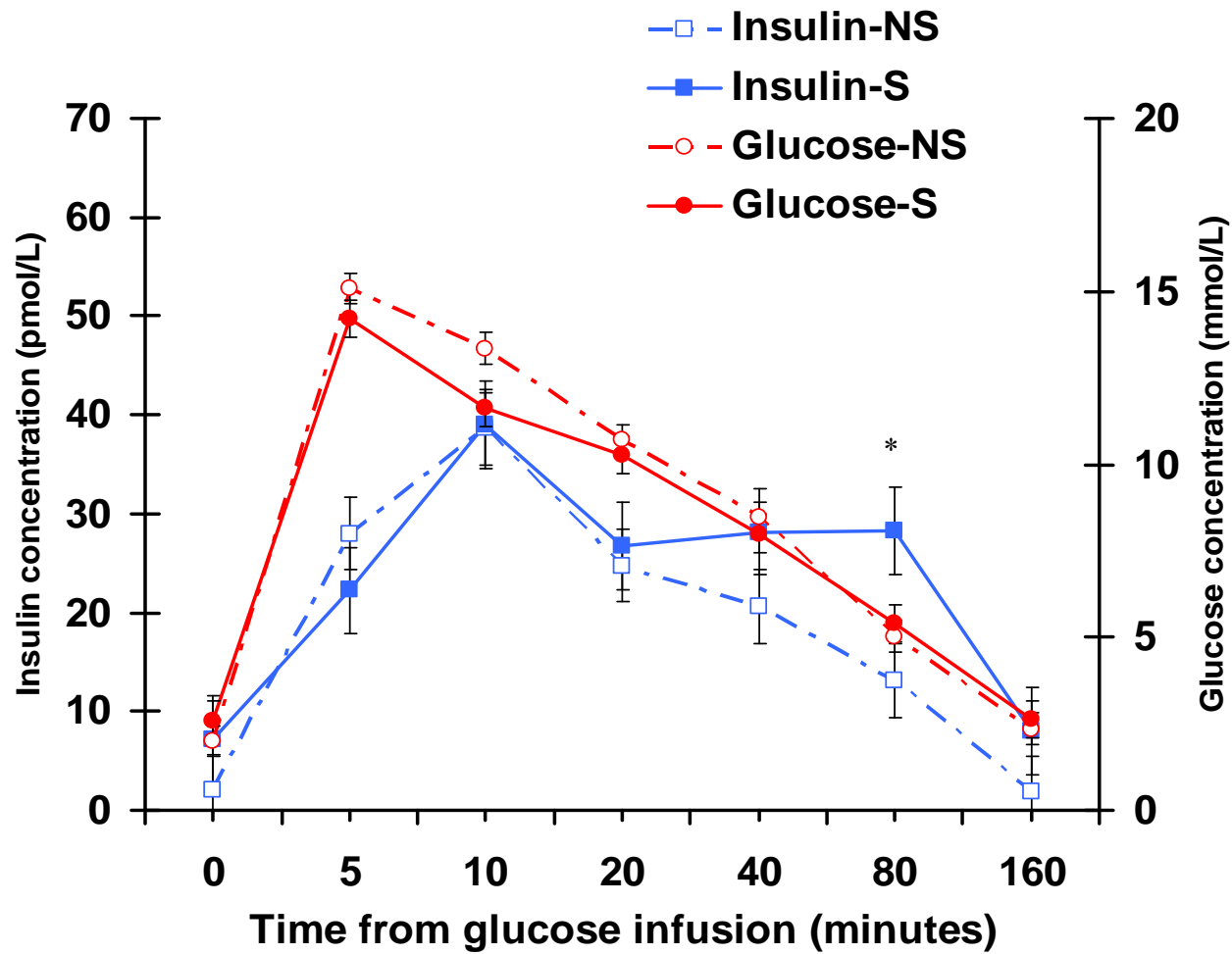


Figure 6 8: Effects of protein supplementation on blood glucose and insulin profiles after glucose infusion in parasitized pregnant ewes of different body condition at day -12 relative to lambing and grazing spring pasture

Table 6 4: Basal glucose (mmol /L whole blood), glucose increment (mmol/L), peak glucose (mol/L whole blood), glucose AUC (mmol/L x min), turnover rate (k, % /min), half life ($t_{1/2}$, min), and CLR (min) of parasitized pregnant ewes of different body reserves grazing spring pasture with or without a protein supplement during GTT 1 and GTT 2.

TrtSup	HM/NS	HM/S	LM/NS	LM/S	MH/NS	MH/S	ML/NS	ML/S	Main effects		
									Trt	Period	Btype
Basal	2.0±0.15 ^{ab}	2.3±0.14 ^a	1.7±0.11 ^{cd}	2.2±0.11 ^a	1.8±0.14 ^{bcd}	2.1±0.14 ^{ab}	1.4±0.14 ^d	2.0±0.12 ^{abc}	***	***	***
Increm	10.9±0.95	9.2±0.91	10.5±0.85	9.9±0.86	10.5±0.82	9.6±0.81	10.4±0.87	10.6±0.76	NS	NS	*
Peak	12.9±0.96	11.6±0.93	12.3±0.86	12.1±0.86	12.3±0.83	11.7±0.82	11.8±0.88	12.6±0.76	NS	NS	NS
AUC*	719±57.4	643±55.2	665±53.6	632±55.8	720±55.3	717±54.3	659±60.6	575±52.6	NS	*	NS
k	1.9±0.18	2.0±0.18	2.0±0.16	2.0±0.17	2.0±0.17	1.6±0.17	2.0±0.19	2.2±0.16	NS	NS	NS
$T_{1/2}$	38.9±5.39	39.3±5.18	44.2±5.03	38.7±5.39	38.8±5.18	46.1±5.08	39.7±5.68	33.6±4.93	NS	NS	NS
CLR	277±26.0	317±26.1	253±20.4	264±21.5	278±23.6	239±23.5	262±25.2	296±35.3	NS	NS	NS

*AUC = area under the curve; k = turnover rate; $T_{1/2}$ = half life; CLR = clearance rate.

Table 6 5: Plasma basal insulin (pmol/L), peak insulin (pmol/L), insulin increment (pmol/L), insulin turnover rate (%/min), insulin half life (min) and insulin AUC pmol/L x min) after glucose infusion in parasitized pregnant ewes of different body reserves grazing spring pasture with or without a protein supplement at -26 and -12 day relative to lambing .

Parameter	Treatment				GTT		Main effects (P-value)		
	HM	LM	MH	ML	1	2	Treatment	Supplement	GTT
Basal	5.9±1.71	3.8±1.31	2.1±2.01	3.4±1.64	3.4±1.00	4.2 ±1.05	0.57	0.09	0.53
Peak	47.2±6.88	39.9±5.62	51.7±8.27	35.7±6.75	49.3 ±4.22	37.9 ±4.40	0.46	0.23	0.06
Increment	41.2±5.65	35.7±5.48	49.4±7.26	32.3±5.97	45.7 ±4.00	33.6 ±4.07	0.36	0.46	0.06
k	1.7±0.39	1.6±0.46	1.9±0.55	1.4±0.46	2.1 ±0.33	1.2 ±0.33	0.88	0.98	0.08
T _{1/2}	71.0±21.15	50.8±17.43	44.1±18.57	56.7±15.41	42.9 ±11.00	68.3 ±11.70	0.66	0.71	0.16
AUC	2343±326.3	2289±303.7	2450±411.2	2106±337.8	1906 ±130.70	1611 ±137.60	0.92	0.18	0.93

Table 6 6: Correlations between body tissue mass and between body condition score, basal blood glucose and plasma insulin (day -26) with mass body tissue (kg) at day -54, -21 and +65 from lambing

	Muscle			Fat			Bone		
	-54	-21	+65	-54	-21	+65	-54	-21	+65
Muscle				0.69***	0.60***	0.41***	0.49***	0.56***	0.67***
Fat							0.30*	0.41***	0.12 ^{NS}
BCS	0.57***		0.35**	0.77***		0.83***			
Basal glucose		0.20 ^{NS}			0.04 ^{NS}				
Basal insulin ¹		0.46 ^{NS}			0.24 ^{NS}				

¹Sample size (n = 13 ewes); * = P<0.05; ** = P<0.01; *** = P<0.001; NS = P>0.05

6.4 Discussion

A limitation imposed by the small sample size on insulin data may have led to a less variation in insulin and the attempt to demonstrate the ability to control FEC may be linked to insulin status was not achieved. The reason for testing the association between insulin and the ability to overcome FEC rise was that insulin has been implicated in immune function by Campos and Baumann (1992), insulin resistance has been associated with the outcome of disease and infection (Hotamisligil, 1999; Hotamisligil et al., 1994). Insulin has also been implicated with leptin in nutrient regulation and partitioning in the peri-parturient period (Block et al., 2003). It was unfortunate that lack of variation in FEC and in glucose and insulin response to glucose infusion made it difficult to test the possibility of such hormone- immune response interactions.

The present study attempted to tease out the dynamics of insulin during the peri-parturient period, to coincide with nutrient supplementation which was previously shown to attenuate the peri-parturient breakdown of immunity to parasitic nematodes (Xie, 2003). As part of the array of normal metabolic adaptations to late pregnancy, sheep develop insulin resistance in peripheral glucose-utilizing maternal tissues in order to give a competitive advantage in channeling of glucose to the uterus (Pettersen et al., 1993). The present study did not demonstrate an existence of any insulin resistance. The protocol used in the present study whereby a single intravenous injection of glucose was administered to evaluate the response of glucose to insulin was adopted to provide the ability to screen large numbers of animals to allow examination of associations with indirect indicators of immune status (i.e. FEC). Clearly, a more precise method of measuring insulin status such as the euglycemic insulin clamp (Pettersen et al., 1993) may have provided more definite data on insulin status, but would have limited the numbers of animals that could have been used. The average insulin half life was similar to that reported by Regnault et al. (2004) in pregnant

sheep but basal plasma insulin concentration was not different between groups. The fact that these data revealed a tendency for greater insulin responses (peak, increment, and k) at GTT 1 than GTT 2 (see Table 6.5 and Figure 6.7), and for the supplemented than non-supplemented groups during GTT 2 (Figure 6.8), though not significantly so, implies changes in insulin-glucose metabolism dynamics as animal approaches term and or when exposed to protein supplement, which may imply nutrient partitioning as would be anticipated from studies of Block et al. (2003), and Petterson et al. (1993; 1994). Insulin has anti-catabolic effects on body nutrients, and according to Petterson et al. (1994), the general development of insulin resistance during late pregnancy includes a reduction in the anti-lipolytic response to insulin. When this phenomenon is viewed against body composition data in the present study, which show that HM ewes lost more protein than fat while LM gained body protein but lost fat at day -54 to -21 relative to lambing, there is a possibility of altered nutrient metabolism due to re-setting of insulin effects in ewes differing in body condition.

Glucose tolerance ($T_{1/2}$) in the present study was comparable to the 38 min recorded by (Wastney, 1980) but faster than 57.4 min for pregnant sheep reported by Regnault et al. (2004) at 135 d gestation age. Insulin responsiveness (IR) was estimated by dividing insulin increment (mean of the subsamples for each body condition treatment) by glucose increment to determine how much insulin above fasting levels responded to one mmol/L of glucose. Elevation of basal insulin concentration during a glucose tolerance test indicates normal glucose tolerance (Bagdade et al., 1967). Insulin responsiveness, based on data from subsample ewes, was different ($P < 0.001$) between groups, being highest in MH/S (5.3 pmol/mmol of glucose) and least in ML/S (3.5 pmol/mmol of glucose) ewes. HM/NS, HM/S, LM/S and LM/NS had similar IR (4.1, 4.3, 4.2 and 3.8 pmol/mmol of glucose, respectively). Similar IR was also observed between MH/NS and ML/NS ewes (4.8 and

4.9 pmol/mmol). Insulin responsiveness tended ($P = 0.06$) to be lower during GTT 1 than GTT 2 (4.2 ± 0.13 vs. 4.5 ± 0.13 pmol/mmol), but was higher ($P < 0.01$) in ewes carrying singles than multiples (4.7 ± 0.20 vs. 4.0 ± 0.10 pmol/mmol). The index IR was calculated based on data of sub-sampled ewes and was applied both to animals carrying multiple and to singleton lambs. Therefore there is likelihood of differences in insulin concentrations between ewes carrying multiples and singleton lamb, and this could affect the results of IR differently. In addition, the protocol was limited in that it did not allow for the glucose to be regulated at constant levels so as to allow monitoring of response of insulin sensitivity more precisely.

In general the effects of body condition or supplementation could not be shown by the present study even though Petterson et al. (1993) observed that undernutrition attenuated the increase in glucose clearance rate associated with pregnancy. The lack of effect of body condition and protein supplementation on glucose responses may well be explained by the lack of sensitivity of the GTT technique but the tendency for differences in peak glucose and insulin response between GTT 1 and GTT 2 and between ewes carrying multiples and those carrying a single lamb is expected due to increased usage of glucose by the multiple foetuses as parturition approaches.

An attempt was made to tease out an association between rise in FEC with insulin and glucose responses. On that basis ewes were categorised as having high (H) or low (L) glucose CLR *per* kg CT fat during GTT 2 thus resulting in H and L groups, relative to the mean minus an arbitrary $\frac{1}{4}$ standard deviation (stddev) (i.e. higher or lower than 37.2 ml/min). Ewes which had higher or lower IR than the mean (0.53 pmol/mmol) minus $\frac{1}{4}$ stddev were categorised into H and L respectively. A combination of IR and glucose CLR groups was constituted to result in HH, HL, LL, LH groupings. In addition, ewes which

had insulin AUC (AUC_{in}) *per* kg LW higher or lower than the mean minus $\frac{1}{4}$ stddev (i.e. 26.9 pmol x min/L) were categorised as either high (H) or low AUC_{in} (L) respectively. FEC was then analysed based on these CLR, IR and AUC_{in} groupings and results are shown in Figure 6.9. Ewes which had low insulin responsiveness but high clearance rate (LH) had >300epg and 200 peg compared to 28 and 9 peg of HH, 13 and 13 peg of HL and 28 and 26epg of LL ewes during 12 and 17 days after lambing respectively (Figure 6.9c). High glucose clearance rate probably indicates greater foetal metabolic demand in twin-bearing ewes that resulted in low maternal plasma glucose during glucose tolerance test in a study by Rumball et al. (2008). Maternal insulin resistance would presumably bring forth much of the glucose sparing effect of twin-pregnant ewes (Bell and Baumann, 1997). The breakdown of immunity to nematode parasite with a consequent rise in FEC would be expected to be higher in multiple-rearing ewes according to the studies by Donaldson et al. (1998) and Houdijk et al. (2006). Plasma glucose disposal by multiple-bearing ewes would also be expected, due to more than doubled foetal glucose requirements in late-pregnant ewes carrying twins (Bell and Baumann, 1997). These factors may suggest a likelihood of a link between insulin metabolism and immune function in highly productive nematode infected peri-parturient ewes.

Though the present study was an attempt to search for an underlying mechanism involved in achieving restoration of immune function during the peri-parturient period with diets (Sykes, unpublished data) and body conditions (Houdijk et al., 2001a) previously found to be effective in reducing rise in FEC, parasitological responses were also evaluated.

Differences in body condition and protein supplementation were not associated with differences in parasitological response in terms of FEC (Figures 6.4 and 6.5). The lack of response may be because the larval challenge was too low or all groups were well fed above a minimal threshold where immunity would be penalised. In addition, they may

have been in too good a body condition. Comparing body fat and protein of ewes used in the present study, between day -54 and -21 relative to lambing, with those used by Houdijk et al. (2001a) during approximately the same period, reveals similarity in body fat and protein percentage changes between ML ewes in this study and ewes manipulated by dietary means to lose body reserves by Houdijk et al. (2001a) (31.3 vs. 26% fat loss and 13% vs. 11.2% muscle loss, respectively). Similarities were also observed between ewes with high body condition and allowed to maintain condition (HM) in the present study and those allowed to maintain body reserves in the afore-mentioned study (4.2 vs. 2.4% fat loss and 8.3 vs. 8.5% muscle loss). Despite this matching fat and protein changes, at day -21 relative to parturition, ewes used by Houdijk et al. (2001a) were in a lower body condition than those in the present study at a corresponding time (day -15) suggesting that perhaps the present ewes were in too good body condition to show a peri-parturient breakdown in immunity.

Maternal weight loss during pregnancy may be a practical indicator of the likely magnitude of the peri-parturient rise and subsequent immuno-responsiveness to increased MP supply, even though it does not provide an indication as to the specific importance of body protein mass in the maintenance of immunity to infection (Kahn, 2003). In the current study, it was possible to get a more direct evaluation of compositional changes by the use of computed tomographic scanning. This revealed some interesting differences between groups in body tissue changes during pregnancy and lactation. Though all animals lost body weight during the peri-parturient period, CT scanning revealed that different groups mobilised both body protein and fat to different extents. Russell et al. (1968) have observed that body protein is preferentially mobilised in the first stages of nutrient loss during pregnancy in hill ewes. This was in accordance with the present HM ewes which lost proportionately more protein than fat early in the trial at day -54 to -21 relative to lambing. In contrast, LM ewes gained

protein but lost fat. The present data confirm that body composition can be manipulated by dietary means during lactation, as reported by Robinson (1986) whereby low levels of a high protein diet resulted in an increase of body fat without major shift in protein. This was observed in LM/S ewes though in this present case it occurred at day -54 to -21 relative to lambing. During Day -21 to +56 relative to lambing LM ewes gained both muscle and fat, a phenomenon related to the findings of Sykes (unpublished data) whereby ewes of low condition were heavier during lactation than those previously on high allowance during mid-pregnancy. These differences may be indicating variation in metabolic settings in inherently fat versus lean ewes. On the other hand, ewes which were caused to either (MH) gain or lose (ML) body condition by dietary manipulation at the beginning of the study mobilised both protein and fat differently from either HM or LM ewes, respectively. This may implies the possibilities of two operating mechanisms and also may be suggesting an indirect involvement of dietary nutrients in metabolic setting. Houdijk et al. (2003) contended that protein studies do not always show consistent results in terms of affecting the degree of breakdown of immunity to parasites due to the use of inappropriate levels of MP intake. However, based on the finding of Houdijk et al. (2001a) that body protein was important in overcoming the limitation of MP scarcity on peri-parturient breakdown of immunity, it may well be that variable responses of groups of animals with different body protein reserves, also contribute to the inconsistency reported by Houdijk et al. (2003). This suggests that care will be needed in the use of body weight to assess nutritional changes during pregnancy which may affect immune responses. More especially that during pregnancy conceptus growth would mask changes in maternal weight. In addition, an increase in tissue hydration, particularly in the amounts extracellular fluid, which in sheep, a ratio of 10% in water:protein is possible (Robinson, 1986). It is thus argued that the influence of past and current body composition on the animal's future responses to nutritional changes and future immune response be recognised. In addition, the idea of

indirect involvement of dietary nutrients in metabolic setting may be in line with a hypothesis by Sykes (2008) that responses to nutrition (observed during restoration of immunity) may be driven by indirect effects of nutrition on metabolic setting rather than on a strict immune system-nutrient demand-supply basis.

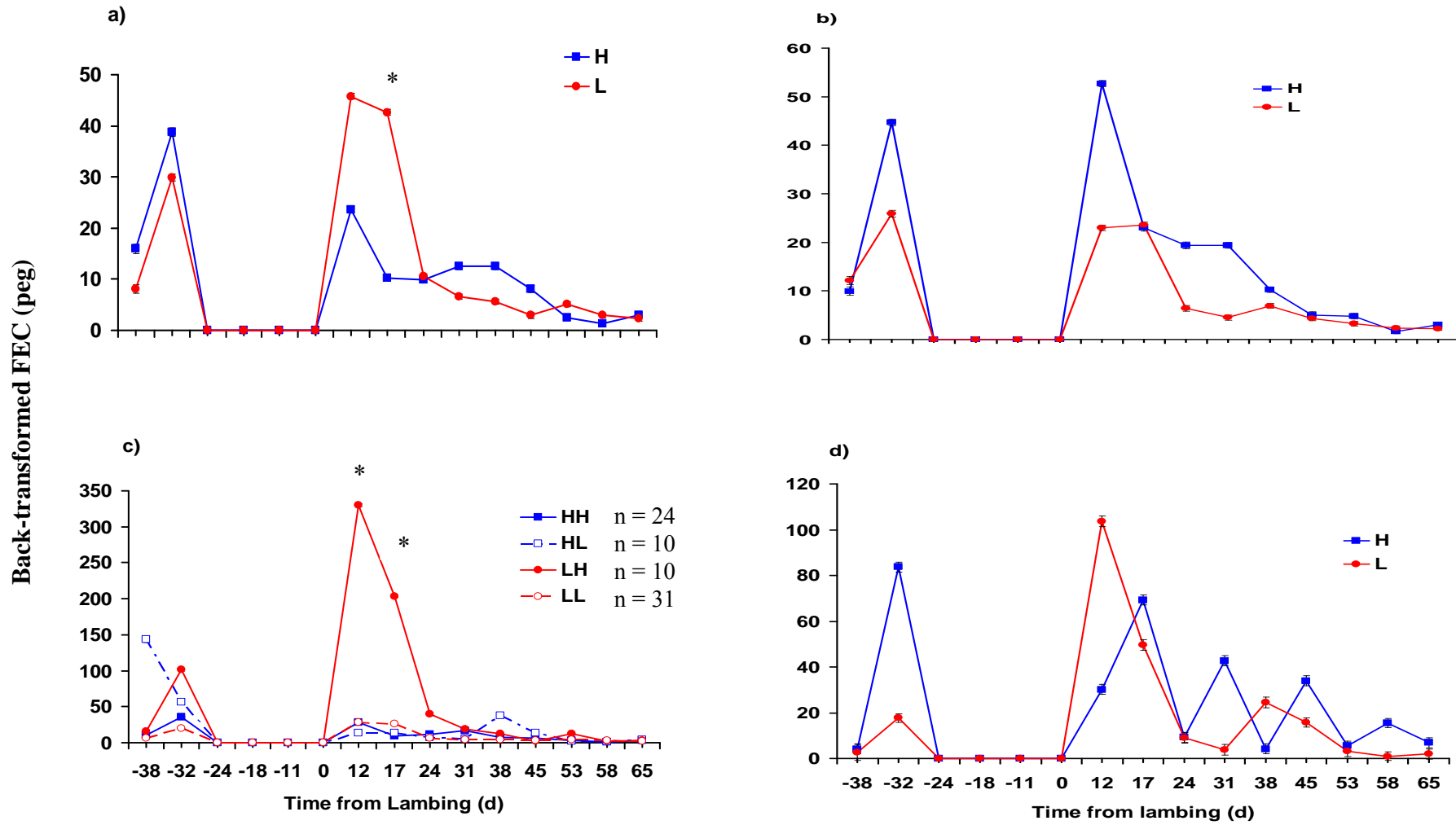


Figure 6 9: Back-transformed FEC of parasitized ewes grazing spring pasture during pregnancy and lactation with H or L insulin Resp/kg fat (a), H or L glucose CLR/kg fat (b) and combination of insulin Resp/kg fat and glucose CLR/kg fat (c) and AUCin/kg LW (d) at day -12 relative to lambing

Conclusions

No differences were detected in glucose response due to body condition and protein supplementation but were observed between GTT1 and GTT2 and between ewes of different lamb ranking. Taken together with the greater insulin response during GTT 2 than GTT 1, and between the supplemented and non-supplemented ewes, these data, though limited by sample size, imply the existence of hormonal metabolism which may explain differences in body protein and fat mobilisation. If insulin resistance does exist in periparturient nematode infected ewes, the natural drive of the animals to secure nutrients for the foetus(es) may be the results of such hormonal impairment. The current study failed to demonstrate effects of protein supplementation and body condition on FEC or establish a link between FEC and insulin status. However, differences in glucose tolerance due to ewes' foetal ranking and differences in FEC rise between ewes carrying or suckling multiples and those carrying a single lamb may suggest an existence of such a link for lamb ranking. The data presented here suggest the need body composition studies to give a full perspective on the nature of changes in body weight.

CHAPTER 7

GENERAL SUMMARY

The issue of the adequacy of the protein supply at pasture has been controversial for some time. On the one hand, the large intake of readily degradable protein and the consequences of dealing with excessive nitrogen have provided concern for the survival and development of embryos at early pregnancy. On the other hand and reflecting the fact that in a nutritional sense it is the quality and quantity of specific amino acid which matters, the role of the rumen in modulating protein supply has raised the question of the adequacy of the (largely) microbial protein supply in meeting the amino acid requirements. This has been the case particularly at times of high demand for nutrients for production (growth, pregnancy and lactation). More recently, it has been appreciated that components of maintenance requirement may be more complex and in grazing animals need to include a specific component for immune system function.

Incidence of large-offspring syndrome from in vitro fertilization (Young et al., 1998) led to interrogation of components of culture medium used and resulted in the suggestion that excessive nitrogen may be associated with reduced fertility, embryo development and viability. This was explored in the first two studies. On the other hand, increasing nutritional intake through supplementation is effective in restoring immune function. However, though data on the supply of amino acid beyond the rumen in sheep grazing tropical grasses is available (McMeniman et al., 1986) none have investigated in sheep grazing ryegrass-white clover. Neither is the mechanism underlying the channelling of nutrients from dietary or endogenous body reserves to the immune system. The last two experiments investigated the supply of amino acids and the mechanism involved.

The first two experiments have suggested that urea from high-protein pasture does not impair reproductive performance of livestock grazing high-quality spring ryegrass/clover pasture or lush autumn re-growth. This is despite the fact that in both studies (Chapter 3 and 4) plasma urea was consistently high during breeding. No correlation was observed between plasma urea concentration and reproductive indicators. The fact that cows with high plasma urea concentrations had similar pregnancy rates, calving to conception interval, calving interval and conception to first service interval to cows with low plasma urea concentration suggests similar effects on both groups. In fact, repeatability of urea concentration was low, even though these may be attributed to less sampling from individual cows. The concern for detrimental effects of plasma urea on fertility arose from studies utilising total mixed rations in the Northern Hemisphere in which highly degradable protein affected reproduction of dairy cows (Canfield et al., 1990; Elrod and Butler, 1993; Folman et al., 1981) and sheep (Bishonga et al., 1996; McEvoy et al., 1997). However, some dairy studies (Dawuda et al., 2004; Laven et al., 2004) did not find any damage to embryo development when cows were fed diets that caused elevation of blood urea and ammonia. Pastures in NZ during spring contain high protein (Chaves et al., 2002; Moller et al., 1993) and it was conceivable that when animals are turned out to spring pasture during mating they would be negatively affected. Though plasma urea sampling was not done after insemination/conception but instead spot sampled during the breeding circle, the results of the present dairy study point to the contrary. Cows selected for high milk yields may reflect change in endocrine setting, predisposing them to detrimental effects of high urea and ammonia levels. This may be in line with a study by Ferguson et al. (1993) in which cows in herds with high conception rates were less affected by increasing urea nitrogen than cows from a herd with low conception rates. Other factors

may include high negative energy balance; high-producing cows with high negative energy balance at early lactation may have inherently reduced chance of re-breeding activities. The study with sheep (Chapter 4) also showed that grazing lush autumn re-growth does not affect reproductive performance. Despite the high plasma urea concentrations in these studies, the observed reproductive wastage was consistent with normal reproductive wastage reported by Kelly (1982) and by Kleemann and Walker (2005a) for NZ and Australian sheep. It was observed that sheep offered a high-protein diet consisting of lush autumn pasture had similar ovulation rate, conception rate, foetal loss, neonatal loss and mean lamb birth weight as ewes offered a low-protein diet consisting of hay and barley. Even when ewes were categorized as having relatively high (HU) or low plasma urea concentrations (LU), embryo mortality, foetal death rate and neonatal viability were similar. In addition, the proportion of lambs categorized as oversize was similar in both the high- and low-protein diets and at high and low plasma urea concentrations, suggesting lack of an effect on development. Implications from the present results with cows and sheep support the recent suggestion by Ordonez et al. (2007) that ruminants grazing high-protein NZ pasture may be adapted to high circulating concentrations of urea. Alternatively, the results may imply that incidence of deleterious effects from controlled studies suggests a more complex aetiology in those studies and that high protein supply from pastoral settings may not compromise reproductive performance.

The study on supply of amino acids is the first to report the flow of amino acids in parasitized twin-rearing ewes on pasture. Increased MP intake has previously been reported to reduce the rise in FEC and restore immune function in peri-parturient ewes indoors (Donaldson et al., 2001; Houdijk et al., 2000). The present work (Chapter 5) quantified the changes in amino acid flow through the abomasum when pasture was supplemented with fishmeal-based supplement which had previously been shown to be

effective in maintaining immunity. Flow of all amino acids including diaminopimelic acid (DAPA) was increased on average by 16% due to protein supplementation. Sulphur amino acids were the most enhanced by 21%, an interesting observation since cysteine and methionine and glutamine are important in the synthesis of glutathione, a component of anti-oxidant defences (Grimble et al., 1992). Sequestration of leucine across the GIT has been shown to increase by 24% (Yu et al., 2000) in nematode-infested sheep. Interestingly leucine flow was increased by 20% by supplementation and could have had positive implication for mucosal immune function. These flows may be suggesting a maximum MP that needs to be supplied, however to identify specific limiting amino acids, graded amounts of MP would need to be fed to provide scaled levels of AA which would perhaps indicate which AAs are limiting. If increases in amino acid flows are hypothetically considered to represent the needs of the immune function, it may be of interest to know how much pasture intake would need to be increased to match these without a need for supplementation. On this basis, dry matter intake would have to increase more than the generally accepted predicted maximum intake of 4 % of body weight. These results therefore put a case for supplementation of nematode-infested peri-parturient sheep on pasture particularly in twin-bearing sheep. During the design of this experiment, the complex demands to enable measurement of AA flows being incompatible with the large numbers of animals needed for valid parasitological data were recognised. However, a more rapid increase in FEC in unsupplemented ewes following anthelmintic treatment and its reduction when these ewes were supplemented as treatments were reversed suggests that the beneficial effects of increased protein supply were operating. Lamb performance was similar between S and C ewes indicating that reproduction was not penalised in line with Coop and Kyriazakis (1999)'s nutrient-partitioning framework. But, given the trend for greater FEC in C ewes, it suggests that immune responses are more sensitive than lactation to increase MP supply, as previously observed by Houdijk et al. (2003). There is a

scarcity of information on plasma free AA in parasitized pregnant ewes on pasture. The current data would contribute to the understanding of mobilisation of nutrients in infected ewes in the peri-parturient period. The reduced plasma amino acids at day +21 after lambing in S ewes may indicate a sparing of skeletal muscle from degradation. Masters et al. (1993) recorded increases in plasma arginine, lysine and threonine during the third week of lactation in unsupplemented ewes. This was considered necessary to support milk production. On the other hand skeletal muscle degradation was reduced by increased dietary crude protein (Sano et al., 2004).

The difference in blood glucose between S and C ewes may be suggesting hormonal involvement in nutrient partitioning as an explanation for relaxation of immunity in peri-parturient ewes. This theory was investigated in the last experiment (Chapter 6). After reviewing Donaldson et al. (1998)'s data, Houdijk et al. (2000) suggested the existence of an interaction between body reserves, protein nutrition and expression of immunity to parasites. The possible existence of such interactions was suggested in data in supplemented grazing ewes (Sykes, unpublished data) as effects of body condition score on relaxation of immunity and response of FEC to protein supplementation were inconsistent during late pregnancy in ewes in different body condition. Data obtained from computed tomography (CT) showed that ewes entering late pregnancy in poor body condition score (i.e. LM group) mobilised less muscle than those entering with higher body condition score (i.e. HM group). On the other hand, ewes which were fed to either gain or lose body condition, at start of experiment, mobilised body nutrients differently. Unlike HM, MH ewes maintain body muscle 3 weeks before lambing while ML, in contrast to LM ewes, lost body protein. MH gain CT fat while ML ewes lost body fat 3 weeks before lambing. Data from LM and ML grouping show that supplemented groups lost the most fat, which was consistent with the literature (Robinson, 1986). Houdijk et al. (2003)

contended that protein studies do not always show consistent results in terms of affecting the degree of breakdown of immunity to parasites due to the use of inappropriate levels of MP intake. However, based on the finding by Houdijk et al. (2001a) that body protein was important in overcoming the limitation of MP scarcity on peri-parturient breakdown of immunity, it may be that, body protein partitioning by groups of animals with different body reserves would also contribute to the inconsistency was reported by Houdijk et al. (2003). An attempt to demonstrate that the ability to control FEC may be linked to insulin status provided equivocal data. The lack of significant effects due to protein supplementation or body condition on parasites may be indicating low parasite challenge or lack of MP scarcity for immunity to be penalised. This experiment involved three shortcomings; possible low parasite challenge, small sample size for insulin data and ineffective experimental protocol for testing insulin response. Future experiments may consider using the hyperinsulinemic euglycemic clamp approach to more precisely estimate body responsiveness to insulin.

These experiments were set up to determine the effects of protein supply from pasture. The first two experiments investigated the previously reported detrimental effects of highly degradable protein on fertility. Since mating of dairy cows and ewes occurs during turnout to high-quality spring pasture and lush autumn re-growth, it was imperative to test this theory on pastoral conditions of NZ. The results of these first two studies suggest no impairment of reproductive performance. The last two trials however, were not necessarily testing the nutrition-parasitology interaction but rather the possible underlying mechanism that could be responsible for restoration of immunity due to supplementation and/or mobilization of body protein. The results indicate that protein supplementation previously associated with overcoming the limitation of MP scarcity in the peri-parturient ewe actually enhanced the supply of amino acids to the gastrointestinal tract. This enhancement

was accompanied by reduced skeletal muscle degradation and may not be met by increasing pasture intake. Animals of different body condition may mobilise different body nutrients to different extents. The differences appear to exist in animals inherently fat or lean and between those fed to gain or lose condition. These differences may result in variation in immune response to protein supplementation and also implicate hormonal involvement.

REFERENCES

- Abdoun, K., Wolf, K., Arndt, G., Martens, H., 2003, Effect of ammonia on Na⁺ transport across isolated rumen epithelium of sheep is diet dependent. *British Journal of Nutrition* 90, 751-758.
- Adamiak, S.K., Mackie, K., Watt, R.G., Webb, R., Sinclair, K.D., 2005, Impact of nutrition on oocyte quality: cumulative effects of body composition and diet leading to hyperinsulinaemia in cattle. *Biology of Reproduction* 73, 918-926.
- AFRC, 1993, Energy and protein requirements of ruminants. An advisory manual prepared by the AFRC technical committee on responses to nutrients. Cab International, Wallingford.
- Aguilar-Caballero, A.J., Torres-Acosta, J.F., Vera-Ayala, C.E., Espana-Espana, E., 2002. Long and short term supplementary feeding and the resilience of browsing Criollo goats to gastrointestinal nematodes. In: *Responding to the Livestock Revolution*, Merida, Mexico, pp. 213-214.
- Ali, D.N., Hennessy, D.R., 1995, The effect of level of feed intake on the pharmacokinetic disposition of oxfendazole in sheep. *International Journal for Parasitology* 25, 63-70.
- Allison, G.G., Rees Stevens, P., Jackman, R., Moorby, J.M., 2008, Normal ranges and temporal variation in plasma concentrations of L-lactate and free amino acids in adult sheep. *Research in Veterinary Science* 85, 22-25.
- Altmann, M., Sauerwein, H., von Borell, E., 2005, Relationship between plasma leptin concentrations and carcass composition in fattening mutton: a comparison with ultrasound results. *Journal of Animal Physiology and Animal Nutrition* 89, 326-330.
- Altmann, M., Von Borell, E., 2007, Leptin as an indicator for carcass composition in farm animals. *Animal Science Journal* 78, 449-459.
- Armour, J., 1980, The epidemiology of helminth disease in farm animals. *Veterinary Parasitology* 6, 7-46.
- Ashworth, C.J., Antipatis, C., 2001, Micronutrient programming of development throughout gestation. *Reproduction* 122, 527-535.
- Attwood, G.T., Klieve, A.V., Ouwerkerk, D., Patel, B.K.C., 1998, Ammonia-hyperproducing bacteria from New Zealand ruminants. *Applied and Environmental Microbiology* 64, 1796-1804.
- Auldust, M.J., Prosser, C.G., 1998, Differential effects of short-term once daily milking on milk yield, milk composition and concentrations of selected blood metabolites in cows with low or high pasture intake. *Proceedings of the New Zealand Society of Animal Production* 58, 41-43.
- Bagdade, J.D., Bierman, E.L., Porte, D.J., 1967, The significance of basal insulin levels in the evaluation of the insulin response to glucose in diabetic and nondiabetic subjects. *The Journal of Clinical Investigation* 46, 1549-1557.
- Barger, I.A., 1993, Influence of sex and reproductive status on susceptibility of ruminants to nematode parasitism. *International Journal for Parasitology* 23, 463-469.
- Bargo, F., Muller, L.D., Delahoy, J.E., Cassidy, T.W., 2002, Milk response to concentrate supplementation of high producing dairy cows grazing at two pasture allowances. *Journal of Dairy Science* 85, 1777-1792.
- Barnes, F.L., 2000, The effects of the early uterine environment on the subsequent development of embryo and fetus. *Theriogenology* 53, 649-658.

- Baskin, D.G., Figlewicz, L.D., Seeley, R.J., Woods, S.C., Porte, D.J., Schwartz, M.W., 1999, Insulin and leptin: dual adiposity signals to the brain for the regulation of food intake and body weight. *Brain Research* 848, 114-123.
- Behm, C.A., Ovington, K.S., 2000, The role of eosinophils in parasitic helminth infections: insights from genetically modified mice. *Parasitology Today* 16, 202-209.
- Bell, A.W., 1995, Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *Journal of Animal Science* 73, 2804-2819.
- Bell, A.W., Baumann, D.E., 1997, Adaptations of glucose metabolism during pregnancy and lactation. *Journal of Mammary Gland Biology and Neoplasia* 2, 265-278.
- Berardinelli, J.G., Weng, J., Burfening, P.J., Adair, R., 2001, Effect of excess degradable intake protein on early embryonic development, ovarian steriods, and blood urea nitrogen on days 2, 3, 4, and 5 of estrous cycle in mature ewes. *Journal of Animal Science* 79, 193-199.
- Bermingham, E.N., McNabb, W.C., Sutherland, I.A., Sinclair, B.R., Treloar, B.P., Roy, N.C., 2005, Adult *Trichostrongylus colubriformis* infection did not affect protein synthesis rate in whole-body, intestinal, hepatic and skeletal muscle tissues of lambs fed fresh Lucerne (*Medicago sativa*). *Canadian Journal of Animal Science* 87, 315-325.
- Bermingham, E.N., McNabb, W.C., Sutherland, I.A., Sinclair, B.R., Treloar, B.P., Roy, N.C., 2006, Whole-body valine and cysteine kinetics and tissue fractional protein synthesis rates in lambs fed Sula (*Hedysarum coronarium*) and infected or not infected with adult *Trichostrongylus colubriformis*. *British Journal of Nutrition* 96, 28-38.
- Bermingham, E.N., Roy, N.C., Sutherland, I.A., Waghorn, G.C., Sinclair, B.R., Peters, J.S., McNabb, W.C., 2008, Intestinal amino acid absorption in lambs fed fresh lucerne (*Medicago sativa*) and during an established *Trichostrongylus colubriformis* infection. *Animal* 7, 1037-1044.
- Berry, D.P., Buckely, F., Dillon, P., Evans, R.D., Rath, M., Veerkamp, R.F., 2003, Genetic relationships among body condition score, body weight, milk yield, and fertility in dairy cows *Journal of Dairy Science* 86, 2193-2204.
- Berzaghi, P., Herbein, J.H., Polan, C.E., 1996, Intake, site and extent of nutrient digestion of lactating cows grazing pasture. *Journal of Dairy Science* 79, 1581-1589.
- Billo, E.J., 2007, Excel for Scientists and Engineers; Numerical Methods. Wiley, New Jersey.
- Bishonga, C., Robinson, J.J., McEvoy, T.G., Findlay, P.A., Aitken, R.P., Robertson, I.S., 1996, Excess dietary urea intake in ewes and its effect on ovulation rate and embryo development. *Japanese Journal of Veterinary Research* 44, 139-151.
- Blanchard, G., Nguyen, P., Gayet, C., Leriche, I., Siliart, B., Paragon, B., 2004, Rapid weight loss with high-protein low-energy diet allows the recovery of ideal body composition and insulin sensitivity in obese dogs. *Journal of Nutrition* 134, 2148S-2150S.
- Blanchard, T., Ferguson, J.D., Love, L., Takeda, T., Henderson, B., 1990, Effect of dietary crude-protein type on fertilization and embryo quality in dairy cattle. *American Journal of Veterinary Research* 51, 905-908.
- Block, S.S., Rhoads, R.P., Baumann, D.E., Ehrhardt, R.A., McGuire, M.A., Crooker, B.A., Griinari, J.M., Mackle, T.R., Weber, W.J., van Amburgh, M.E., Boisclair, Y.R., 2003, Demonstration of the role for insulin in the regulation of leptin in lactating dairy cows. *Journal of Dairy Science* 86, 3508-3515.
- Blouet, C., Moriatti, F., Azzout-Marniche, D., Bos, C., Mathe, V., Tome, D., Huneau, J., 2006, The reduced energy intake of rats fed a high-protein low-carbohydrate diet explains the lower fat deposition, but macronutrient substitution accounts for the improved glycemic control. *Journal of Nutrition* 136, 1849-1854.

- Bonnet, M., Delavaud, C., Rouel, J., Chilliard, Y., 2005, Pregnancy increases plasma leptin in nulliparous but not primiparous goats while lactation depresses it. *Domestic Animal Endocrinology* 28, 216-223.
- Bousquet, D., Bouchard, E., DuTremblay, D., 2004. Decreasing fertility in dairy cows: Myth or reality. In: 23rd World Buiatrics Congress, Quebec, Canada, 11 July 2004.
- Bown, M.D., Poppi, D.P., Sykes, A.R., 1991a, The effect of post-ruminal infusion of protein or energy on the pathophysiology of *Trichostrongylus colubriformis* infection and body composition in lambs. *Australian Journal of Agricultural Research* 42, 253-267.
- Bown, M.D., Poppi, D.P., Sykes, A.R., 1991b, Nitrogen transactions along the digestive tract of lambs concurrently infected with *Trichostrongylus colubriformis* and *Ostertagia circumstincta*. *British Journal of Nutrition* 66, 237-249.
- Buckely, F., O'Sullivan, K., Mee, J.F., Evans, R.D., Dillon, P., 2003, Relationships among milk yield, body condition, cow weight and reproduction in spring-calved Holstein-Friesians. *Journal of Dairy Science* 86, 2308-2319.
- Butler, W.R., Calaman, J.J., Beam, S.W., 1996, Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle. *Journal of Animal Science* 74, 858-865.
- Campos, S.P., Baumann, H., 1992, Insulin is a prominent modulator of the cytokine-stimulated expression of acute-phase plasma protein genes. *Molecular and Cellular Biology* 12, 1789-1797.
- Canfield, R.W., Sniffen, C.J., Butler, W.R., 1990, Effects of excess degradable protein on postpartum reproduction and energy balance in dairy cattle. *Journal of Dairy Science* 73, 2342-2349.
- Carducci, C., Birarelli, M., Leuzzi, V., Santagata, G., Serafini, P., Antonozzi, I., 1996, Automated method for the measurement of amino acids in urine by high-performance liquid chromatography. *Journal of Chromatography A* 729, 173-180.
- Cecava, M.J., Merchen, N.R., Berger, L.L., Nelson, D.R., 1990, Effect of energy level and feeding frequency on site of digestion and post-ruminal nutrient flows in steers. *Journal of Dairy Science* 73, 2470-2479.
- Chartier, C., Etter, E., Hoste, H., Pors, I., Mallereau, M.P., Broqua, C., Mallet, S., Koch, C., Masse, A., 2000, Effects of the initial level of milk production and dietary protein intake on the course of natural nematode infection in dairy goats. *Veterinary Parasitology* 92, 1-13.
- Chaves, A.V., Waghorn, G.C., Brookes, I.M., Hedderley, D., 2002, Digestion kinetics of ryegrass. *Proceedings of the New Zealand Society of Animal Production* 62, 157-162.
- Coffey, K.P., Paterson, J.A., Saul, C.S., Coffey, L.S., Turner, K.E., Bowman, J.G., 1989, The influence of pregnancy and source of supplemental protein on intake, digestive kinetics and amino acid absorption by ewes. *Journal of Animal Science* 67, 1805-1814.
- Colditz, I.G., 2002, Effects of the immune system on metabolism: implications for production and disease resistance in livestock. *Livestock Production Science* 75, 257-268.
- Colditz, I.G., 2003, Metabolic effects of host responses during gastrointestinal parasitism in sheep. *Australian Journal of Experimental Agriculture* 43, 1437-1443.
- Colditz, I.G., 2004, Some mechanisms regulating nutrient utilisation in livestock during immune activation: an overview. *Australian Journal of Experimental Agriculture* 44, 453-457.
- Colditz, I.G., 2008, Six costs of immunity to gastrointestinal nematode infections. *Parasite Immunology* 30, 63-70.
- Coop, R.L., Holmes, P.H., 1996, Nutrition and parasite interaction. *International Journal for Parasitology* 26, 951-962.

- Coop, R.L., Kyriazakis, I., 1999, Nutrition-parasite interaction. *Veterinary Parasitology* 84, 187-204.
- Coop, R.L., Sykes, A.R., 2002, Interactions between gastrointestinal parasites and nutrients, In: Freer, M., Dove, H. (Eds.) *Sheep Nutrition*. CABI Publishing and CSIRO Publishing Wallingford, pp. 313-331.
- Cottle, D.J., Velle, W., 1989, Degradation and outflow of amino acids from the rumen of sheep. *British Journal of Nutrition* 61, 397-408.
- Cruickshank, G.J., 1986. Nutritional constraints to lamb growth at pasture, PhD thesis. University of Canterbury, Christchurch.
- D'Mello, J.P.F., 2003, Amino acids as multifunctional molecules, In: D'Mello, J.P.F. (Ed.) *Amino acids in animal nutrition*. CABI Publishing, Wallingford, pp. 1-14.
- Dal Zotto, R., De Marchi, M., Cecchinato, A., Penasa, M., Cassandro, M., Carnier, P., Gallo, L., Bittante, G., 2008, Reproducibility and repeatability of measures of milk coagulation properties and predictive ability of mid-infrared reflectance spectroscopy. *Journal of Dairy Science* 91, 4103-4112.
- Datta, F.U., Nolan, J.V., Rowe, J.B., Gray, G.D., 1998, Protein supplementation improves the performance of parasitised sheep fed straw-based diet. *International Journal for Parasitology* 28, 1269-1278.
- Datta, F.U., Nolan, J.V., Rowe, J.B., Gray, G.D., Crook, B.J., 1999, Long-term effects of short-term provision of protein-enriched diets on resistance to nematode infection, and live-weight gain and wool growth in sheep. *International Journal for Parasitology* 29, 479-488.
- Davis, G.H., McEwan, J.C., Howarth, M.V., Kelly, R.W., Paterson, A.T., 1987, Reproductive performance of progeny of prolific Romney, Coopworth and Perendale sheep. *Proceedings of the New Zealand Grassland Association* 47, 89-91.
- Dawuda, P.M., Scaramuzzi, R.J., Drew, S.B., Biggadike, H.J., Laven, R.A., Allison, R.D., Collins, C.F., Wathes, D.C., 2004, The effect of a diet containing excess quickly degradable nitrogen (QDN) on reproductive and metabolic hormonal profiles of lactating dairy cows. *Animal Reproduction Science* 81, 195-208.
- Dawuda, P.M., Scaramuzzi, R.J., Leese, H.J., Hall, C.J., Peters, A.R., Drew, S.B., Wathes, D.C., 2002, Effect of timing of urea feeding on the yield and quality of embryos in lactating dairy cows. *Theriogenology* 58, 1443-1455.
- de Oliveira, C.C., Acedo, S.C., Pedrazzoli, J., Saad, M.J., Gambero, A., 2009, Depot-specific alterations to insulin signaling in mesenteric adipose tissue during intestinal inflammatory response. *International Immunopharmacology* 9, 396-402.
- Dehority, B.A., 2003, *Rumen Microbiology*. Nottingham University Press, Nottingham.
- Delavaud, C., Bocquier, F., Baumont, R., Chaillou, E., NBan-Tokada, T., Chilliard, Y., 2007, Body fat content and feeding level interact strongly in short- and medium-term regulation of plasma leptin during underfeeding and re-feeding in adult sheep. *British Journal of Nutrition* 98, 106-115.
- Demas, G.E., Sakaria, S., 2005, Leptin regulates energetic tradeoffs between body fat and humoral immunity. *Proceedings of the Royal Society B* 272, 1845-1850.
- Donaldson, J., van Houtert, M.F.J., Sykes, A.R., 1998, The effect of nutrition on the periparturient parasite status of mature ewes. *Animal Science* 67, 523-533.
- Donaldson, J., van Houtert, M.F.J., Sykes, A.R., 2001, The effect of fish-meal supplementation on parasite burdens of periparturient sheep. *Animal Science* 72, 149-158.
- Dove, H., Mayes, R.W., 1991, The use of plant wax alkanes as marker substances in studies of the nutrition of herbivores; a review. *Australian Journal of Agricultural Research* 42, 913-952.

- Dove, H., Mayes, R.W., Freer, M., 1996, Effects of species, plant part and plant age on the n-alkane concentrations in the cuticular wax of pasture plants. *Australian Journal of Agricultural Research* 47, 1333-1347.
- Dove, H., Milne, J.A., 1994, Digesta flow and rumen microbial protein production in ewes grazing perennial ryegrass. *Australian Journal of Agricultural Research* 45, 1229-1245.
- Dove, H., Milne, J.A., Lamb, C.S., McCormack, H.A., Spence, A.M., 1985, The effects of supplementation on non-ammonia nitrogen flows at the abomasum of lactating grazing ewes. *Proceedings of the Nutrition Society* 44, 63A.
- Dove, H., Milne, J.A., Sibbald, A.M., Lamb, C.S., McCormack, H.A., 1988, Circadian variation in abomasal digesta flow in grazing ewes during lactation. *British Journal of Nutrition* 60, 653-668.
- Ehrhardt, R.A., Slepatis, R.M., Bell, A.W., Boisclair, Y.R., 2001, Maternal leptin is elevated during pregnancy in sheep. *Domestic Animal Endocrinology* 21, 85-96.
- El-Kadi, S.W., Baldwin, R.L.V., Sunny, N.E., Owens, S.L., Bequette, B., 2006, Intestinal protein supply alters amino acid, but not glucose, metabolism by the sheep gastrointestinal tract. *The Journal of Nutrition* 136, 1261-1268.
- Elrod, C.C., Butler, W.R., 1993, Reduction of fertility and alteration of uterine pH in heifers fed excess ruminally degradable protein. *Journal of Dairy Science* 71, 694-701.
- Elrod, C.C., van Amburgh, M., Butler, W.R., 1993, Alterations of pH in response to increased dietary protein in cattle are unique to the uterus. *Journal of Animal Science* 71, 702-706.
- Faichney, G.J., 1980, The use of markers to measure digesta flow from the stomach of sheep fed once daily. *Journal of Agricultural Science, Cambridge* 94, 313-318.
- Faichney, G.J., White, G.A., 1987, Effects of maternal nutritional status on fetal and placental growth and on fetal urea synthesis in sheep. *Australian Journal of Biological Science* 40, 365-377.
- Ferguson, J.D., Chalupa, W., 1989, Impact of protein nutrition on reproduction in dairy cows. *Journal of Dairy Science* 72, 746-766.
- Ferguson, J.D., Galligan, D.T., Blanchard, T., Reeves, M., 1993, Serum urea nitrogen and conception rate: The usefulness of test information. *Journal of Dairy Science* 76, 3742-3746.
- Fisher, M.W., 2004, A review of the welfare implications of out-of-season extensive lamb production systems in New Zealand. *Livestock Production Science* 85, 165-172.
- Folman, Y., Neumark, H., Kaim, M., Kaufmann, W., 1981, Performance, rumen and blood metabolites in high yielding cows fed varying protein percents and protected soybean. *Journal of Dairy Science* 62, 58-63.
- Fountoulakis, M., Lahm, H., 1998, Hydrolysis and amino acids composition analysis of proteins: Review. *Journal of Chromatography A* 826, 109-134.
- Friggens, N.C., Newbold, J.R., 2007, Towards a biological basis for predicting nutrient partitioning: the dairy cow as an example. *Animal* 1, 87-97.
- Geenty, K.G., Sykes, A.R., 1986, The effect of herbage allowance during pregnancy and lactation on feed intake, milk production, body composition and energy utilisation of ewes at pasture. *Journal of Agricultural Science, Cambridge* 106, 351-367.
- Gehrke, C.W., Rexroad, P.R., Schisla, R.M., Absheer, J.S., Zumwalt, R.W., 1987, Quantitative analysis of cystine, methionine, lysine, and nine other amino acids by a single oxidation-4 hour hydrolysis method. *Journal - Association of Official Analytical Chemists* 70, 171-174.
- Gibbs, H.C., 1982, Mechanisms of survival of nematode parasites with emphasis on hypobiosis. *Veterinary Parasitology* 11, 25-48.

- Gill, M., Robinson, P., 1995, Diurnal patterns in crude protein and amino acid flow in the duodenum and the influence of time of feeding a protein meal to lactating cows. *Annales de Zootechnie* 44, Suppl, 230.
- Godfrey, K.M., 2002, The role of the placenta in fetal programming: A Review. *Placenta* 23, 520-527.
- Gonzalez, J.S., Robinson, J.J., Fraser, C., 1985, The effects of physiological state on digestion in the ewe and its influence on the quantity of protein reaching the abomasum. *Livestock Production Science* 12, 57-68.
- Greer, A.W., 2005. Estimates of nutritional cost of the development of immunity to gastrointestinal parasites in sheep. PhD thesis. Lincoln University, Christchurch.
- Greer, A.W., Stankiewicz, M., Jay, N.P., McAnulty, R.W., Sykes, A.R., 2005, The effect of concurrent corticosteroid induced immuno-suppression and infection with the intestinal parasite *Trichostrongylus colubriformis* on food intake and utilization in both immunology naive and competent sheep. *Animal Science* 80, 89-99.
- Grimble, R.F., 1992, Dietary manipulation of inflammatory response. *Proceedings of the Nutrition Society* 51, 285-294.
- Grimble, R.F., 1998, Nutritional modulation of cytokine biology. *Nutrition* 14, 634-640.
- Grimble, R.F., Grimble, G.K., 1998, Immunonutrition: Role of sulfur amino acids, related amino acids, and polyamines. *Nutrition* 14, 605-610.
- Grimble, R.F., Jackson, A.A., Persaud, C., Wride, M.J., Delers, F., Engler, R., 1992, Cysteine and glycine supplementation modulate the metabolic response to tumor necrosis factor alpha in rats fed a low protein diet. *The Journal of Nutrition* 122, 2066-2073.
- Gundersen, H.J.G., Bendtsen, T.F., Korbo, L., Marcussen, N., Moller, A., Nielsen, K., Nyengaard, U.R., Pakkenberg, B., Sorensen, F.B., Vesterby, A., West, M.J., 1988, Some new, simple and efficient stereological methods and their use in pathological research and diagnoses. *Acta Pathologica Microbiologica et Immunologica Scandinavica* 96, 379-394.
- Haile-Mariam, M., Morton, J.M., Goddard, M.E., 2003, Estimates of genetic parameters for fertility traits of Australian Holstein-Friesian cattle. *Animal Science* 76, 35-42.
- Hammon, D.S., Holyoak, G.R., Dhimann, T.R., 2005, Association between blood plasma urea nitrogen levels and reproductive fluid urea nitrogen and ammonia concentrations in early lactation dairy cows. *Animal Reproduction Science* 86, 195-204.
- Hammon, D.S., Wang, S., Liu, G., Wiedmeier, R.D., Holyoak, G.R., 1997, Effects of ammonia on in vitro development of bovine embryos. *Theriogenology* 47, 321 abstract.
- Harris, B.L., Kolver, E.S., 2001, Review of Holsteinization on intensive pastoral dairy farming in New Zealand. *Journal of Dairy Science* 84 (Suppl. E), E56-E61.
- Hecker, J.F., 1969, A simple rapid method for inserting rumen cannulae in sheep. *Australian Veterinary Journal* 45, 293-294.
- Heems, D., Luck, G., Fraudeau, C., Verette, E., 1998, Fully automated percolumn derivatization, on-line dialysis and high-performance liquid chromatographic analysis of amino acids in food, beverages and feedstuff. *Journal of Chromatography A* 798, 9-17.
- Hill, R.A., 2004, The role of leptin axis in modulating energy partitioning and nutrient utilization in livestock species, In: Garnsworthy, P.C., Wiseman, J. (Eds.) *Recent Advances in Animal Nutrition*. Nottingham University Press, Nottingham, pp. 149-184.
- Holland, J.B., 2006, Estimating genotypic correlations and their standard errors using multivariate restricted maximum likelihood estimation with SAS Proc Mixed. *Crop Science* 46, 642-654.

- Hoskin, S.O., Lobley, G.E., Coop, R.L., Jackson, F., 2002, The effect of cysteine and glutamine supplementation on sheep infected with *Trichostrongylus colubriformis*. Proceedings of the New Zealand Society of Animal Production 62, 72-76.
- Hotamisligil, G.S., 1999, The role of TNF alpha and TNF receptors in obesity and insulin resistance. Journal of Internal Medicine 245, 621-625.
- Hotamisligil, G.S., Murray, D.L., Choy, L.N., Spiegelman, B.M., 1994, Tumor necrosis factor alpha inhibits signaling from the insulin receptor. Proceedings of National Academy of Sciences of USA 91, 4854-4848-4858.
- Hotamisligil, G.S., Shargill, N.S., Spiegelman, B.M., 1993, Adipose expression of tumor necrosis factor alpha: Direct role in obesity-linked insulin resistance. Science 259, 87-90.
- Houdijk, J.G.M., Jackson, F., Coop, R.L., Kyriazakis, I., 2006, Rapid improvement of immunity to *Teladorsagia circumcincta* is achieved through a reduction in the demand for protein in lactating ewes. International Journal for Parasitology 36, 219-227.
- Houdijk, J.G.M., Kyriazakis, I., Coop, R.L., Jackson, F., 2001a, The expression of immunity to *Teledorsagia circumcincta* in ewes and its relationship to protein nutrition depend on body protein reserves. Parasitology 122, 661-672.
- Houdijk, J.G.M., Kyriazakis, I., Jackson, F., Coop, R.L., 2001b, The relationship between protein nutrition, reproductive effort and breakdown in immunity to *Teladorsagia circumcincta* in periparturient ewes. Animal Science 72, 595-606.
- Houdijk, J.G.M., Kyriazakis, I., Jackson, F., Huntley, J.F., Coop, R.L., 2000, Can an increased intake of metabolizable protein affect the periparturient relaxation in immunity against *Teladorsagia circumcincta* in sheep? Veterinary Parasitology 91, 43-62.
- Houdijk, J.G.M., Kyriazakis, I., Jackson, F., Huntley, J.F., Coop, R.L., 2003, Is the allocation of metabolisable protein prioritised to milk production rather than to immune functions in *Teladorsagia circumcincta* infected lactating ewes? International Journal for Parasitology 33, 327-338.
- Houdijk, J.G.M., Kyriazakis, I., Jackson, F., Huntley, J.F., Coop, R.L., 2005, Effects of protein supply and reproductive status on local and systemic immune responses to *Teladorsagia circumcincta* in sheep. Veterinary Parasitology 129, 105-117.
- Huntley, J.F., Jackson, F., Coop, R.L., Macalodowie, C., Houdijk, J.G.M., Familton, A.S., Xie, H.L., Stankiewicz, M., Sykes, A.R., 2004, The sequential analysis of local inflammatory cell during abomasal nematode infection in periparturient sheep. Veterinary Immunology and Immunopathology 97, 163-176.
- Ingvartsen, K.L., Boisclair, Y.R., 2001, Leptin and the regulation of food intake, energy homeostasis and immunity with special focus on periparturient ruminants. Domestic Animal Endocrinology 21, 215-250.
- Israfi, D.A., Coop, R.L., Stevenson, L.M., Jones, D.G., Jackson, F., Jackson, E., MacKellar, A., Huntley, J.F., 1996, Dietary protein influences upon immunity to *Nematodirus battus* infection in lambs. Veterinary Parasitology 61, 273-286.
- Johnson, R.W., Arkins, S., Dantzer, R., LKelley, K.W., 1997, Hormones, lymphohemopoietic cytokines and the neuroimmune axis. Comparative Biochemistry and Physiology 116A, 183-201.
- Jones, H.E., Lewis, R.M., Young, M.J., Simm, G., 2004, Genetic parameters for carcass composition and muscularity in sheep measured by X-ray computer tomography, ultrasound and dissection. Livestock Production Science 90, 167-179.
- Jordan, E.R., Chapman, T.E., Holtan, D.W., Swanson, L.V., 1983, Relationships of dietary crude protein to composition of uterine secretions and blood in high-producing postpartum dairy cows. Journal of Dairy Science 66, 1854-1862.

- Jordan, E.R., Swanson, L.V., 1979a, Effect of crude protein on reproductive efficiency, serum total protein and albumin in the high-producing dairy cow. *Journal of Dairy Science* 62, 58-63.
- Jordan, E.R., Swanson, L.V., 1979b, Serum progesterone and luteinizing hormone in dairy cattle fed varying levels of crude protein. *Journal of Animal Science* 48, 1154-1158.
- Kahn, L.P., 2003, Regulation of resistance and resilience of periparturient ewes to infection with gastrointestinal nematode parasites by dietary supplementation. *Australian Journal of Experimental Agriculture* 43, 1477-1485.
- Kahn, L.P., Knox, M.R., Gray, G.D., 1999, Enhancing immunity to nematode parasites in pregnant and lactating sheep through nutrition and genetic selection. *Recent Advances in Animal Nutrition in Australia* 12, 15-22.
- Kahn, L.P., Knox, M.R., Gray, G.D., Lea, J.M., Walkden-Brown, S.W., 2003b, Enhancing immunity to nematode parasite in single-bearing Merino ewes through nutrition and genetic selection. *Veterinary Parasitology* 112, 211-225.
- Kahn, L.P., Knox, M.R., Walkden-Brown, S.W., Lea, J.M., 2003a, Regulation of resistance to nematode parasite of single- and twin-bearing Merino ewes through nutrition and genetic selection. *Veterinary Parasitology* 114, 15-31.
- Kambara, T., McFarlane, R.G., Abell, T.J., McAnulty, R.W., Sykes, A.R., 1993, The effect of age and dietary protein on immunity and resistance in lambs vaccinated with *Trichostrongylus colubriformis*. *International Journal for Parasitology* 23, 471-476.
- Kaneko, J.J., 1989, *Clinical biochemistry of domestic animals*. Academic Press, San Diego. pp 64-68.
- Kearney, J.F., Schutz, M.M., Boettcher, P.J., 2004, Genotype x Environment interaction for grazing vs confinement. II Health and Reproduction traits. *Journal of Dairy Science* 87, 510-516.
- Kelley, K.W., Weigent, D.A., Kooijman, R., 2007, Protein hormones and immunity. *Brain, Behavior, and Immunity* 21, 384-392.
- Kelly, R.W., 1982, Reproductive performance of commercial sheep flocks in South Island districts 1. Flock performance and sources of wastage between joining and tailing. *New Zealand Journal of Agricultural Research* 25, 175-183.
- Kenny, D.A., Boland, M.P., Diskin, M.G., Sreenan, J.M., 2001, Effect of pasture crude protein and fermentable energy supplementation on blood metabolite and progesterone concentrations and embryo survival in heifers. *Animal Science* 73, 501-511.
- Kenny, D.A., Humpherson, P.G., Leese, H.J., Morris, D.G., Tomos, A.D., Diskin, M.G., Sreenan, J.M., 2002, Effect of elevated systematic concentrations of ammonia and urea on metabolite and ionic composition of oviductal fluid in cattle. *Biology of Reproduction* 66, 1797-1804.
- Kimambo, A.E., MacRae, J.C., Walker, A., Watt, C.F., Coop, R.L., 1988, Effect of prolonged subclinical infection with *Trichostrongylus colubriformis* on performance and nitrogen metabolism of growing lambs. *Veterinary Parasitology* 28, 191-203.
- Kleemann, D.O., Walker, S.K., 2005a, Fertility in South Australian commercial Merino flocks; sources of reproductive wastage. *Theriogenology* 63, 2075-2088.
- Kleemann, D.O., Walker, S.K., 2005b, Fertility in South Australia commercial Merino flocks; relationships between reproductive traits and environmental cues. *Theriogenology* 63, 2416-2433.
- Klusmeyer, T.H., McCarthy Jr, R.D., Clark, J.H., 1990, Effects of source and amount of protein on ruminal fermentation and passage of nutrients to the small intestine of lactating cows. *Journal of Dairy Science* 73, 3526-3537.

- Knox, M., Steel, J., 1996, Nutritional enhancement of parasite control in small ruminant production systems in developing countries of South-East Asia and the Pacific. *International Journal for Parasitology* 26, 963-970.
- Knox, M.R., Torres-Acosta, J.F., Aguilar-Caballero, A., 2006, Exploiting the effect of dietary supplementation of small ruminants on resilience and resistance against gastrointestinal nematodes. *Veterinary Parasitology* 139, 385-393.
- Kolver, E.S., 2003, Nutritional limitations to increased production on pasture-based systems. *Proceedings of the Nutrition Society* 62, 291-300.
- Kolver, E.S., Carruthers, V.R., Neil, P.G., De Veth, M.J., Jansen, E.B.L., Phipps, D.E., 1999, Amino acid supply to the small intestine of dairy cows fed pasture. *Proceedings of the New Zealand Society of Animal Production* 59, 180-183.
- Kolver, E.S., Macmillan, K.L., 1993, Short term changes in selected metabolites in pasture fed dairy cows during peak lactation. *Proceedings of the New Zealand Society of Animal Production* 53, 77-81.
- Kolver, E.S., Macmillan, K.L., 1994, Variation in selected blood plasma constituents during the post-partum and breeding periods in dairy cows. *New Zealand Veterinary Journal* 42, 161-166.
- Kolver, E.S., Napper, A.R., Copeman, P.J.A., Muller, L.D., 2000, Comparison of New Zealand and overseas Holstein Friesian heifers. *Proceedings of the New Zealand Society of Animal Production* 60, 265-269.
- Konashi, S., Takahashi, K., Akiba, Y., 2000, Effects of dietary essential amino acid deficiencies on immunological variables in broiler chickens. *British Journal of Nutrition* 83, 449-456.
- Lane, M., Bavister, B.D., 1998, Hamster 2-cell embryo utilise the Na⁺/H⁺ antiporter to regulate intracellular pH. *Theriogenology* 49, 184 abstract.
- Laven, R.A., Biggadike, H.J., Allison, R.D., 2002, The effect of pasture nitrate concentration and concentrate intake after turnout on embryo growth and viability in lactating dairy cows. *Reproduction Domestic Animal* 37, 111-115.
- Laven, R.A., Dawuda, P.M., Scaramuzzi, R.J., Wathes, D.C., Biggadike, H.J., Peters, A.R., 2004, The effects of feeding diets high in quickly degradable nitrogen on follicular development and embryo growth in lactating Holstein dairy cows. *Animal Reproduction Science* 84, 41-52.
- Lavigne, C., Tremblay, F., Asselin, G., Jacques, H., Marette, A., 2001, Prevention of skeletal muscle insulin resistance by dietary cod protein in high fat-fed rats. *American Journal of Physiology, Endocrinology and Metabolism* 281, E62-E71.
- Leury, B.J., Baumgard, L.H., Block, S.S., Segoale, N., Ehrhardt, R.A., Rhoads, R.P., Baumann, D.E., Bell, A.W., Boisclair, Y.R., 2003, Effect of insulin and growth hormone on plasma leptin in perparturient dairy cows. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* 285, R1107-R1115.
- Liu, S.M., Karlsson, L.J.E., 2004, Nematode Parasitism in sheep production. *Annals of Arid Zone* 43, 111-128.
- Liu, S.M., Masters, D.G., Adams, N.R., 2003, Potential impact of nemadote parasitism on nutrient partitioning for wool production, growth and reproduction in sheep. *Australian Journal of Experimental Agriculture* 43, 1409-1417.
- Liu, S.M., Mata, G., Figliomeni, S., Powell, B.C., Nesci, A., Masters, D.G., 2000, Transsulfuration, protein synthesis rate and follicle mRNA in the skin of young Merino lambs in response to infusions of methionine and serine. *British Journal of Nutrition* 83, 401-409.
- Liu, S.M., Smith, T.L., Karlsson, L.J.E., Palmer, D.G., Besier, R.B., 2005a, The costs for protein and energy requirements by nemadote infection and resistance in Merino sheep. *Livestock Production Science* 97, 131-139.

- Liu, S.M., Smith, T.L., Plamer, D.G., Karlsson, L.J.E., Besier, R.B., Greeff, J.C., 2005b, Biochemical differences in Merino sheep selected for resistance against gastrointestinal nematodes and genetic and nutritional effects on faecal worm egg output. *Animal Science* 81, 149-157.
- Lobley, G.E., 1998, Nutritional and hormonal control of muscle and peripheral tissue metabolism in farm species. *Livestock Production Science* 56, 91-114.
- Lobley, G.E., Shen, X., Le, G., Bremmer, D.M., Milne, E., Calder, A.G., Anderson, S.E., Dennison, N., 2003, Oxidation of essential amino acids by ovine gastrointestinal tract. *British Journal of Nutrition* 89, 617-629.
- Lucy, M.C., 2001, Reproductive physiology and management of high-yielding dairy cattle. *Proceedings of the New Zealand Society of Animal Production* 61, 120-127.
- MacMillan, K.L., Lean, I.J., Westwood, C.T., 1996, The effects of lactation on fertility of dairy cows. *Australian Veterinary Journal* 73, 141-147.
- MacRae, J.C., Bruce, L.A., Brown, D.S., Calder, A.G., 1997a, Amino acid use by the gastrointestinal tract of sheep given lucerne forage. *American Journal of Physiology* 273, G1200-G1207.
- MacRae, J.C., Walker, A., Brown, D., Lobley, G.E., 1993, Accretion of total protein and individual amino acids by organs and tissues of growing lambs and ability of nitrogen balance techniques to quantitate protein retention. *Animal Production* 57, 237-245.
- Madibela, O.R., McEvoy, T.G., Robinson, J.J., Findlay, P.A., Aitken, R.P., Robertson, I.S., 1995, Excess rumen degradable protein influences the rate of development and glucose metabolism of fertilized sheep ova. *Animal Science* 60, 536-537 abstract.
- Madibela, O.R., Sykes, A.R., Nicol, A.M., Logan, C.M., 2008. There is no evidence of impairment of reproductive function of dairy cows on spring pasture during mating in New Zealand. (NZ). In: *British Society of Animal Science Annual Conference, Scarborough, England, 31 March - 2 April 2008*, p. 4.
- MAFF 1979. *Manual of veterinary parasitological laboratory techniques*. In Ministry of Agriculture, Fisheries and Food (London).
- Malmizat, T., Breuille, D., Pouyet, C., Buffiere, C., Denis, P., Mirand, P.P., Obled, C., 2000, Methionine transsulfuration is increased during sepsis in rats. *American Journal of Physiology and Endocrinology* 279, E1391-E1397.
- Mann, G.E., Green, M.P., Sinclair, K.D., Demmers, K.P., Fray, M.D., Gutierrez, C.G., Garnsworthy, P.C., Webb, R., 2003, Effects of circulating progesterone and insulin on early embryo development in beef heifers. *Animal Reproduction Science* 79, 71-79.
- Masters, D.G., Stewart, C.A., Connell, P.J., 1993, Changes in plasma amino acid patterns and wool growth during pregnancy and early lactation in the ewe. *Australian Journal of Agricultural Research* 44, 945-957.
- Matarese, G., Leiter, E.H., La Cava, A., 2007, Leptin in autoimmunity: many questions, some answers. *Tissue Antigens* 70, 87-95.
- McDougall, S., 2006, Reproduction performance and management of dairy cattle. *Journal of Reproduction and Development* 52, 185-194.
- McEvoy, T.G., Robinson, J.J., Aitken, R.P., Findlay, P.A., Robertson, I.S., 1997, Dietary excesses of urea influence the viability and metabolism of preimplantation sheep embryos and may affect fetal growth among survivors. *Animal Reproduction Science* 47, 71-90.
- McKelvey, W.A.C., Robinson, J.J., Aitken, R.P., Robertson, I.S., 1986, Reaped recoveries of embryos from ewes by laparoscopy. *Theriogenology* 25, 855-865.
- McMeniman, N.P., Kondos, A.C., Beale, I.F., 1986, Nutritional evaluation of South-west Queensland Pastures. III The amino acid composition of pasture plants in a

- Mitchell grass Association and their digestibility by grazing sheep. *Australian Journal of Agricultural Research* 37, 315-322.
- McNabb, W.C., Waghorn, G.C., Barry, T.N., Shelton, I.D., 1993, The effect of condensed tannins in *Lotus pedunculatus* on the digestion and metabolism of methionine, cystine and inorganic sulphur in sheep. *British Journal of Nutrition* 70, 647-661.
- McNeill, D.M., Slepetic, R., Ehrhardt, R.A., Smith, D.M., Bell, A.W., 1997, Protein requirements of sheep in late pregnancy: Partitioning of nitrogen between gravid uterus and maternal tissues. *Journal of Animal Science* 75, 809-816.
- Mellor, D.J., 1983, Nutritional and placental determinants of foetal growth rate in sheep and consequences for the newborn lamb. *British Veterinary Journal* 139, 307-324.
- Meyer, H.H., Piper, L.R., Bindon, B.M., Woolaston, R.R., 1994, Litter size and uterine efficiency of Booroola Merinos, control Merinos and their crosses with Border Leicester and Dorset. *Livestock Production Science* 38, 217-223.
- Miller, B.G., Moore, N.W., 1976, Effects of progesterone and oestradiol on endometrial metabolism and embryo survival in the ovariectomized ewe. *Theriogenology* 6, 636 Abstract.
- Miller, F.M., Blair, H.T., Birtles, M.J., Reynolds, G.W., Gill, H.S., Revell, D.K., 2000, Cysteine may play a role in the immune response to internal parasites in sheep. *Australian Journal of Agricultural Research* 51, 793-799.
- Moller, S., Matthew, C., Wilson, G.F., 1993, Pasture protein and soluble carbohydrate level in spring dairy pasture and associations with cow performance. *Proceedings of the New Zealand Society of Animal Production* 53, 83-86.
- Muhlhausler, B.S., Roberts, C.T., McFarlane, J.R., Kauter, K.G., McMillen, I.C., 2002, Fetal leptin is a signal of fat mass independent of maternal nutrition in ewes fed at or above maintenance energy requirements. *Biology of Reproduction* 67, 493-499.
- Murthy, G.K., Rhea, V., Peeler, T.J., 1971, Levels of antimony, cadmium, chromium, cobalt, manganese, and zinc in institutional total diets. *Environmental Science and Technology* 5, 436-442.
- NRC, 1985, Nutrient Requirements of sheep. National Academy Press, Washington DC.
- Nsoso, S.J., 1995. Genetic control of lean tissue growth rate in sheep: Genetic parameters and responses to selection. PhD thesis. Lincoln University, Christchurch. PhD Thesis.
- O'Reagain, P.J., McMeniman, N.P., 2002, Nutrition of sheep under rangeland conditions, In: Freer, M., Dove, H. (Eds.) *Sheep Nutrition*. CABI Publishing and CSIRO Publishing, Wallingford, pp. 263-284.
- Ordóñez, A., Parkinson, T.J., Matthew, C., Holmes, C.W., Miller, R.D., Lopez-Villalobos, N., Burke, J., Brookes, I., 2007, Effects of application in spring of urea fertiliser on aspects of reproductive performance of pasture-fed dairy cows. *New Zealand Veterinary Journal* 55, 69-76.
- Pallares-Trujillo, J., Carbo, N., Lopez-Soriano, F.J., Argiles, J.M., 2000, Does the mechanism responsible for TNF-mediated insulin resistance involve the proteasome? *Medical Hypothesis* 54, 565-569.
- Peart, J.N., 1968, Some effects of live weight and body condition on milk production of Blackface ewes. *Journal of Agricultural Science, Cambridge* 70, 331-338.
- Petterson, J.A., Dunshea, F.R., Ehrhardt, R.A., Bell, A.W., 1993, Pregnancy and undernutrition alter glucose metabolic response to insulin in sheep. *Journal of Nutrition* 123, 1286-1295.
- Petterson, J.A., Slepetic, R., Ehrhardt, R.A., Dunshea, F.R., Bell, A.W., 1994, Pregnancy but not moderate undernutrition attenuates insulin suppression of fat mobilisation in sheep. *The Journal of Nutrition* 124, 2431-2436.
- Piatti, P.M., Monti, L.D., Magni, F., Fermo, I., Baruffaldi, L., Nasser, R., Santambrogio, G., Librenti, M.C., Galli-Kienle, M., Pontiroli, A.E., Pozza, G., 1994, Hypocaloric

- high-protein diet improves glucose oxidation and spares lean body mass: Comparison to hypocaloric high-carbohydrate diet. *Metabolism* 43, 1481-1487.
- Pico, C., Oliver, P., Sanchez, J., Palou, A., 2003, Gastric leptin: a putative role in the short-term regulation of food intake. *British Journal of Nutrition* 90, 735-741.
- Pitta, D.W., Barry, T.N., Lopez-Villalobos, N., Attwood, G.T., 2009, Effect of willow supplementation upon plasma amino acid concentration in ewes grazing drought pastures of low nutritive value. *Animal Feed Science and Technology* 148, 183-191.
- Poppi, D.P., MacRae, J.C., Brewer, A., Coop, R.L., 1986, Nitrogen transactions in the digestive tract of lambs exposed to the internal parasite, *Trichostrongylus colubriformis*. *British Journal of Nutrition* 55, 593-602.
- Preiser, J., Peres-Bota, D., Eisendrath, P., Vincent, J., van Gossum, A., 2003, Gut mucosal and plasma concentration of glutamine: a comparison between two enriched enteral feeding solutions in critically ill patients. *Nutrition Journal* 2. <http://www.nutritionj.com/content/2/1/13>
- Qu, Y., Slocum, R.H., Fu, J., Rasmussen, W.E., Rector, H.D., Miller, J.B., Coldwell, J.G., 2001, Quantitative amino acid analysis using a Beckman system gold HPLC 126AA analyser. *Clinica Chimica Acta* 312, 153-162.
- Ranilla, M.J., Carro, M.D., 2003, Diet and procedures used to detach particle-associated microbes from ruminal digesta influence chemical composition of microbes and estimation of microbial growth in Rusitec fermenters. *Journal of Animal Science* 81, 537-544.
- Reeds, P.J., Burrin, D.G., 2000, The gut and amino acid homeostasis. *Nutrition* 16, 666-668.
- Reeds, P.J., Burrin, D.G., 2001, Glutamine and the bowel. *The Journal of Nutrition* 131, 2505S-2508S.
- Reeds, P.J., Burrin, D.G., Stoll, B., Jahoor, F., Wykes, L., Henry, J., Frazer, M.E., 1997, Enteral glutamate is the preferential source for mucosal glutathione synthesis in fed piglets. *American Journal of Physiology* 273, E408-E415.
- Rees, S., Harding, R., 2004, Brain development during fetal life: influences of the intra-uterine environment. *Neuroscience Letters* 361, 111-114.
- Regnault, T.R.H., Oddy, V.H., Nancarrow, C., Sriskandarajah, N., Scaramuzzi, R.J., 2004, Glucose-stimulated insulin response in pregnant sheep following acute suppression of plasma non-esterified fatty acid concentrations. *Reproductive Biology and Endocrinology*. doi:10.1186/1477-7827-2-64.
- Rhoads, M.L., Rhoads, R.P., Gilbert, R.O., Toole, R., Butler, W.R., 2006, Detrimental effects of high plasma urea nitrogen levels on viability of embryos from lactating dairy cows. *Animal Reproduction Science* 91, 1-10.
- Robinson, J.J., 1986, Changes in body composition during pregnancy and lactation. *Proceedings of the Nutrition Society* 45, 71-80.
- Robinson, J.J., Ashworth, C.J., Rooke, J.A., Mitchell, L.M., McEvoy, T.G., 2006, Nutrition and fertility in ruminant livestock. *Animal Reproduction Science* 126, 259-276.
- Robinson, J.J., McDonald, I., McHattie, I., Pennie, K., 1978, Studies on reproduction in prolific ewes: 4. Sequential changes in the maternal body during pregnancy. *Journal of Agricultural Science, Cambridge* 91, 291-304.
- Robinson, J.J., Sinclair, K.D., McEvoy, T.G., 1999a, Nutritional effects on foetal growth. *Animal Science* 68, 315-331.
- Robinson, J.J., Sinclair, K.D., Randel, R.D., Sykes, A.R., 1999b. Nutritional management of the female ruminant: mechanistic approaches and predictive models. In: *Nutritional Ecology of Herbivores*, San Antonio, Texas, USA, pp. 550-608.

- Robinson, P.H., Gill, M., Kennelly, J.J., 2002, Influence of time of feeding a protein meal on diurnal patterns of amino acids in duodenal digesta protein of lactating dairy cows. *Animal Feed Science and Technology* 97, 115-121.
- Rogero, M.M., Tirapegui, J., Vinolo, M.A.R., Borges, M.C., de Castro, I.A., Pires, I.S.O., Borelli, P., 2008, Dietary glutamine supplementation increases the activity of peritoneal macrophages and hemopoiesis in early-weaned mice inoculated with *Mycobacterium bovis* Bacillus Calmette-Guerin. *The Journal of Nutrition* 138, 1343-1348.
- Roseby, F.B., 1977, Effects of *Trichostrongylus colubriformis* (Nematoda) on the nutrition and metabolism of sheep. III Digesta flow and fermentation. *Australian Journal of Agricultural Research* 28, 155-164.
- Royal, M.D., Darwash, A.O., Flint, A.P.F., Webb, R., Wooliams, J.A., Lamming, G.E., 2000, Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility. *Animal Science* 70, 487-501.
- Rumball, C.W.H., Harding, J.E., Oliver, M.H., Bloomfield, F.H., 2008, Effects of twin pregnancy and peri-conceptional undernutrition on maternal metabolism, fetal growth and glucose-insulin axis function in ovine pregnancy. *Journal of Physiology* 586, 1399-1411.
- Russel, A.J.F., Gunn, R.G., Doney, J.M., 1968, Components of weight loss in pregnant hill ewes during winter. *Animal Production* 10, 43-51.
- Sankey, C., 2005. Comparative digestibility by cattle versus sheep; effect of forage quality. Oklahoma State University, Oklahoma. PhD Thesis.
- Sano, H., Kajita, M., Fujita, T., 2004, Effect of dietary protein intake on plasma leucine flux, protein synthesis, and degradation in sheep. *Comparative Biochemistry and Physiology* 139, 163-168.
- Sano, H., Terashima, Y., 2001, Effects of dietary protein level and cold exposure on tissue responsiveness and sensitivity to insulin in sheep. *Journal of Physiology and Animal Nutrition* 85, 349-355.
- SAS, 1999, User's Guide. Statistics. Version 8. SAS Institute Inc., Cary, NC, USA.
- SAS, 2002-2003, User's Guide. Statistics. Version 9. SAS Institute Inc., Cary, NC, USA.
- Scholljegerdes, E.J., Ludden, P.A., Hess, B.W., 2004, Site and extent of digestion and amino acid flow to the small intestine in beef cattle consuming limited amounts of forage. *Journal of Animal Science* 82, 1146-1156.
- Scott, I., Stear, M.J., Irvine, J., Dick, A., Wallace, D.S., McKellar, Q.A., 1998, Changes in the zymogenic cell populations of the abomasa of sheep infected with *Haemonchus contortus*. *Parasitology* 116, 569-577.
- Siddons, R.C., Paradine, J., Beaver, D.E., Cornell, P.R., 1985, Ytterbium acetate as a particulate-phase digesta-flow marker. *British Journal of Nutrition* 54, 509-519.
- Sinclair, K.D., Maxfield, E.K., Robinson, J.J., Maltin, C.A., McEvoy, T.G., Dunne, L.D., Young, L.E., Broadbent, P.J., 1997, Culture of sheep zygotes can alter fetal growth and development. *Theriogenology* 47, 380 abstract.
- Sinclair, K.D., McEvoy, T.G., Maxfield, E.K., Maltin, C.A., Young, L.E., Wilmut, I., Broadbent, P.J., Robinson, J.J., 1999, Aberrant fetal growth and development after in vitro culture of sheep zygotes. *Journal of Reproduction and Fertility* 116, 177-186.
- Smith, J.F., Beaumont, S., Hagemann, L., McDonald, R.M., Peterson, A.J., Xu, Z.Z., Duganzich, D.M., 2001, Relationship between bulk milk urea nitrogen and reproductive performance of New Zealand dairy herds. *Proceedings of the New Zealand Society of Animal Production* 61, 192-194.
- Stewart, C.A., Masters, D.G., Williams, I.H., Connell, P.J., 1993, Changes in plasma amino acid patterns and wool growth in response to abomasal injections of amino

- acids during late pregnancy and early lactation. *Australian Journal of Agricultural Research* 44, 959-971.
- Sykes, A.R., 1978, An assessment of the value of plasma urea nitrogen and albumin concentrations as monitors of the protein status in sheep, In: Lister, D. (Ed.) *Blood profiles in Animal Production*. British Society of Animal Production, pp. 143-154.
- Sykes, A.R., 2008, Manipulating host immunity to improve parasite control - Quo vadit. *Parasite Immunology* 30, 71-77.
- Sykes, A.R. unpublished data.
- Sykes, A.R., Coop, R.L., 2001, Interaction between nutrition and gastrointestinal parasitism in sheep. *New Zealand Veterinary Journal* 49, 222-226.
- Sykes, A.R., Xie, H.L., Stankiewickz, M., Huntley, J.F., MacKellar, A., Sedcole, J.R., R.W., M., Green, R., 2007, The effect of vaccinating infection during pregnancy and dietary protein supply on the peri-parturient immune response of sheep to infection with *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* larvae. *Animal* 1, 249-260.
- Tamminga, S., 2006, The effect of the supply of rumen degradable protein and metabolisable protein on negative energy balance and fertility in dairy cows. *Animal Reproduction Science* 96, 227-239.
- Thompson, J.G., 2000, In vitro culture and embryo metabolism of cattle and sheep embryos - a decade of achievement. *Animal Reproduction Science* 60-61, 263-275.
- Thomson, B.C., Cruickshank, G.J., Poppi, D.P., Sykes, A.R., 1985, Diurnal patterns of rumen fill in grazing sheep. *Proceedings of the New Zealand Society of Animal Production* 45, 117-120.
- Torres-Acosta, J.F., Jacobs, D.E., Aguilar-Caballero, A., Sandoval-Castro, C., Cob-Galera, L., May-Martinez, M., 2006, Improving resilience against natural gastrointestinal nematode infections in browsing kids during the dry season in tropical Mexico. *Veterinary Parasitology* 135, 163-173.
- Torres-Acosta, J.F., Jacobs, D.E., Aguilar-Caballero, A., Sandoval-Castro, C., May-Martinez, M., Cob-Galera, L., 2004, The effect of supplementary feeding on the resilience and resistance of browsing Criollo kids against natural gastrointestinal nematode infections during the rainy season in tropical Mexico. *Veterinary Parasitology* 124, 217-238.
- Valderrabano, J., Delfa, R., Uriarte, J., 2002, Effect of level of feed intake on the development of gastrointestinal parasitism in growing lambs. *Veterinary Parasitology* 104, 327-338.
- Valderrabano, J., Gomez-Rincon, C., Uriarte, J., 2006, Effect of nutritional status and fat reserves on the periparturient immune response to *Haemonchus contortus* infection in sheep. *Veterinary Parasitology* 141, 122-131.
- Valderrabano, J., Uriarte, J., 2003, Effect of nutrition in early pregnancy on periparturient relaxation of immunity to gastro-intestinal parasitism in prolific ewes. *Animal Science* 76, 481-489.
- van Goudoever, J.B., Stoll, B., Henry, J.F., Burrin, D.G., Reeds, P.J., 2000, Adaptive regulation of intestinal lysine metabolism. *Proceedings of the National Academy of Sciences of the United States of America* 97, 11620-11625.
- van Houtert, M.F.J., Barger, I.A., Steel, J.W., Windon, R.G., Emery, D.L., 1995, Effects of dietary protein intake on responses of young sheep to infection with *Trichostrongylus colubroformis*. *Veterinary Parasitology* 56, 163-180.
- van Winkle Lon, J., Haghighat, N., Campione, A.L., 1990, Glycine protects preimplantation mouse conceptuses from detrimental effect on development of inorganic ions in oviductal fluid. *Journal of Experimental Zoology* 253, 215-219.
- Verkerk, G., 2003, Pasture-based dairying: challenges and rewards for New Zealand producers. *Theriogenology* 59, 553-561.

- Volden, H., 1999, Effects of level of feeding and ruminally undegraded protein on ruminal bacterial protein synthesis, escape of dietary protein, intestinal amino acid profile and performance of dairy cows. *Journal of Animal Science* 77, 1905-1918.
- Waghorn, G.C., Clark, D.A., 2004, Feeding value of pastures for ruminants. *New Zealand Veterinary Journal* 52, 320-331.
- Washburn, S.P., Silvia, W.J., Brown, C.H., McDaniel, B.T., McAllister, A.J., 2002, Trends in reproductive performance in South-eastern Holstein and Jersey DHI herds. *Journal of Dairy Science* 85, 244-251.
- Wastney, M.E., 1980. Glucose metabolism of fed, starved and toxæmic pregnant sheep. University of Canterbury, Christchurch. PhD Thesis.
- Westwood, C.T., Lean, I.J., Garvin, J.K., 2002, Factors influencing fertility of Holstein dairy cows: A multivariate description. *Journal of Dairy Science* 85, 3225-3237.
- Wittwer, F.G., Gallardo, P., Reyes, J., Opitz, H., 1999, Bulk milk urea concentrations and their relationship with cow fertility in grazing dairy herds in Southern Chile. *Preventive Veterinary Medicine* 38, 159-166.
- Wolfinger, R., Chang, M., 1998, Comparing the SAS GLM and Mixed Procedures for repeated measures. SAS Institute, Inc., Cary, NC, USA.
- Wu, G., 1998, Intestinal mucosal amino acid catabolism. *Journal of Nutrition* 128, 1249-1252.
- Xie, H.L., 2003. Nutritional and immunological interrelationships in response to nematode infections in periparturient ewes. Lincoln University, Christchurch. PhD Thesis.
- Xie, H.L., Stankiewicz, M., Huntley, J.F., Sedcole, J.R., McAnulty, R.W., Green, R.S., Sykes, A.R., 2004, The effects of cold exposure, food allowance and litter size on immunity of peri-parturient sheep to *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. *Animal Science* 78, 149-158.
- Young, L.E., Guttierrez, C.G., Butterwith, S.C., Robinson, J.J., Broadbent, P.J., McEvoy, T.G., Wilmut, J., Sinclair, K.D., 1999, Altered IGF binding protein expression is associated with large offspring syndrome in fetal sheep. *Theriogenology* 51, 196.
- Young, L.E., Sinclair, K.D., Wilmut, I., 1998, Large offspring syndrome in cattle and sheep. *Reviews of Reproduction* 3, 155-163.
- Young, M.J., Nsoso, S.J., Logan, C.M., Beatson, P.R., 1996, Prediction of carcass tissue weight *in vivo* using liveweight, ultrasound or X-ray CT measurements. *Proceedings of the New Zealand Society of Animal Production* 56, 205-211.
- Yu, F., Bruce, L.A., Calder, A.G., Milne, E., Coop, R.L., Jackson, F., Horgan, G.W., MacRae, J.C., 2000, Subclinical infection with the nematode *Trichostrongylus colubriformis* increases gastrointestinal tract leucine metabolism and reduces availability of leucine for other tissues. *Journal of Animal Science* 78, 380-390.

APPENDICES

Appendix 5.1: Processing of rumen digesta for rumen microbes

Rumen samples were homogenised with a Polytron homogeniser at 15.000 rpm x 1 min repeated 3 times on the day of collection. The homogenised samples were stored at 4°C for 24 h. The following day samples were placed in centrifuge tubes and spun for 15 min at 25000 x g at 4°C (Beckman Instruments, California, USA). The supernatant was discarded and a similar amount of rumen buffer (\approx 100 ml) was added and samples vortexed (Model 250VM, Hwashin Technology, Korea). After vortexing, samples were incubated for 30 min at 39°C and then placed in a freezer (-20°C) for further 30 min and then left at 4°C until the following day. The following day samples were centrifuged at 25000 x g for 15 min at 4°C. The supernatant was discarded and 100 ml saline used to reconstitute the pellet by vortexing. The above cleaning process was repeated. After letting the sample rest for 5 min, 10 ml of 50% sucrose was added between the layer of the rough material and the supernatant. Samples were centrifuged again at 1000 x g for 5 min at 4°C. The supernatant was obtained in a clean container, avoiding contamination with debris. The supernatant was poured back into a clean centrifuge container and spun at 25000 x g for 15 min at 4°C. The supernatant was discarded and saline (\approx 100 ml) added to the pellet and centrifugation was repeated. After discarding the supernatant, the bacterial pellet was reconstituted with 15 ml saline and the suspension placed in 70 ml containers and frozen, then freeze dried. Before analysis the samples were pooled together across subjects due too small quantities of samples.

Appendix 5.2: Amino acid analysis in abomasal digesta, ruminal bacteria, pasture and protein nuts samples (Hydrolysis method)

Ten microliters of internal standard (α -amino butyric acid; 0.5 M) and 5.0 ml of 6M hydrochloric acid was added into each tube. Tubes were put in an ultrasound bath (Transsonic 460, Geprüfte Sicherheit, Germany) for 5 min and purged with nitrogen gas and the lids screwed tightly. After 20 h in a block heater (Ratex DBH30D, Ratek Instruments PTY LTD, Australia) at 110°C, tubes were allowed to cool to room temperature. After cooling, samples were transferred into a round bottom flask using nanopure water. Anti-bumping granules were added into the flask and the contents evaporated to dryness in a water bath under a rotary evaporator (Buchi Labortechnik, Switzerland). After drying, samples were reconstituted by nanopure water and washed down using a bent-tipped glass rod. The sample was then transferred to a 50 ml volumetric flask and topped up with nanopure water. The contents were mixed well and passed through a 0.45 μ m syringe filter (13 mm diameter, Labserv, Biolab, New Zealand) into HPLC vials. Vials were frozen at -20°C pending analysis.

Appendix 5.3: Oxidation method for analysis of cysteine and methionine in abomasal digesta, ruminal bacteria, pasture and protein nuts samples.

Freeze-dried abomasal digesta, the rumen bacterial pellet, pasture and protein nuts samples were weighed (0.10 g) into 50 ml round-bottom flasks. Performic acid was generated under a fume hood by mixing 90:10 v:v of formic acid and hydrogen peroxide in a flat-bottom flask for 1 h then placing the flask in an ice bath for 15 min. Each round-bottom flask, with sample, received 5 ml performic acid and was mixed well. Parafilm-sealed flasks were placed on plastic racks inside the ice box and the flasks surrounded with ice. The box was covered and placed in a cold room (4 °C) for 17 h. On the next day, 0.75 ml hydrobromic acid (HBr) was added into each flask while still in ice box to react with excess performic acid. After the reaction had slowed down, flasks were placed at room temperature. Samples were then dried in a vacuum evaporator at 40 °C. Dry samples were dissolved in 25.0 ml 6N HCL and then flasks placed in an oil bath at 115-120 °C under reflux for 23 h. After 23 h reflux the apparatus was flushed with nanopure water and the flasks taken out from the oil bath. Samples were then dried in the vacuum evaporator at 40°C. Dry samples were re-dissolved by adding nanopure water and transferred into a 50 ml volumetric flask. The solution was filtered through a 0.45 µm filter and stored in vials at -20°C until analysis.

Appendix 5.4: Example of calculations for flow rates

Ewe	Infusion rate (mg/d)		Conc in whole digesta (mg/kg)		Conc in filtrate		DM of whole digesta
	Yb	Cr	Yb	Cr	Yb	Cr	
120	136.9	2366.8	50.3	53.4	0	43.8	3.51

Proportion of Cr in whole digesta as a fraction of that infused = $53.4/2366.8 = 0.0226$
[1]

Proportion of Cr in filtrate as a fraction of that infused = $43.8/2366.8 = 0.0185$
[2]

Proportion of Yb in whole digesta as a fraction of that infused $50.3/136.9 = 0.367$
[3]

Reconstitution factor as defined by (Faichney, 1980) is as follows

$$R = (\text{digesta Yb} - \text{digesta Cr}) / (\text{filtrate Cr} - \text{filtrate Yb})$$

[4]

For Ewe 120; $R = ([3] - [1]) / ([2] - 0.001)$ or $(0.367 - 0.0226) / (0.0185 - 0.001) = 19.68$

True digesta Cr = $(\text{digesta Cr} + (R \times \text{filtrate Cr})) / (1 + R)$ (Faichney, 1980)

For Ewe 120; True digesta = $(0.0226 + (19.68 \times 0.0185)) / (1 + 19.68) = 0.0187$
[5]

True digesta flow = $1 / \text{true digesta Cr}$ (Faichney, 1980)

For Ewe 120; True digesta flow = $1/0.0187 = 53.48$ liters/day

Express as dry matter of whole digesta; Dry matter flow = $(3.51/100) \times 53.48 = 1.88$ kg/day

There fore water flow = $(96.49/100) \times 53.48 = 51.60$ kg/day

Example for amino acid flow;

Flow of Aspartic acid (concentration in abomasal digesta = 16.7g/kg)

Substitute in Equation 5; $(16.7 + (19.68 \times 16.7)) / (1 + 19.68) = 10.32$

Converting to dry matter = $10.32 \times 1.88 = 19.4$ g/day

PUBLICATIONS AND CONFERENCES DURING THE COURSE OF THE STUDY

Madibela O. R., Nicol, A. M., Sykes A. R. and Jay N. P. (2008) High crude protein in autumn pasture does not impair reproductive performance in sheep. *Processings of the New Zealand Society of Animal Production* 68:158-161 (*short communication*)

Madibela O. R., Sykes A. R., Nicol A. M., Logan C. M. (2008) There is no evidence of impairment of reproductive function of dairy cows on spring pasture during mating in New Zealand (NZ). *Proceedings of British Society of Animal Science Annual Conference*. Paper # 4 (*One page summary*).

Madibela, O. R., Gibbs, J., Sykes A. R., Laporte J., and J. Zhao (2008) New Zealand parasitized sheep went “backpacking” in search of a protein supplement. Paper presented at Lincoln University Post Graduate Conference. Lincoln, Canterbury. 26th to 27th August 2008

Madibela O. R., Gibbs, J., Sykes, A. R., Laporte-Urbe, J., Zhao, J. (2009) Amino acid flow at the abomasum in twin-suckling ewes at pasture and the effect of a fishmeal supplement. *Proceedings of the New Zealand Society of Animal Production* 69: 233-237