

Chapter 5

THE EFFECT OF A MOLASSES-UREA SUPPLEMENT ON PARASITISM OF SHEEP FED A LOW QUALITY ROUGHAGE DIET.

5.1 Abstract

Seventy-two 4-5 month-old Merino wethers were individually confined and given free access to oaten chaff and a molasses-mineral supplement (MS) for 6 weeks. The sheep were then divided into 2 groups; one was offered MS and the other was offered MS containing 10% urea (UM). Within each group 3 subgroups were then orally infected with either 200 H. contortus and 1000 T. colubriformis (LOW) thrice weekly, twice this rate of infection (HIGH) or were not infected (CON). Weight gain, wool production, parasite burden, blood parameters and rumen function were assessed over an 18 week period to compare treatments.

Intake of chaff was affected by dietary supplement and parasite infection with UM sheep consuming more than MS sheep while for the supplements the intake of MS was greater than that of UM. Within dietary groups CON sheep ingested more feed than LOW sheep which also consumed more than the HIGH sheep. Over time MS-CON sheep changed their feed selection towards consuming more MS than chaff but in MS-LOW sheep this change was not as evident and no change occurred in MS-HIGH sheep. A large number of HIGH sheep died in both MS and UM dietary treatment groups. Parasitised sheep gained less weight than CON sheep but there were no effects of dietary supplement on growth rate. Wool production was also affected by infection with CON sheep producing more wool of higher fibre diameter than LOW and HIGH sheep. Faecal egg counts did not differ between dietary treatments. The UM diet significantly reduced worm numbers

when all subgroups were considered but there was no significant interaction of diet and parasitism. The UM diet caused a significant elevation of plasma protein, albumin and urea concentrations in infected and uninfected sheep. Parasitism caused significant changes in parameters indicative of enteric blood loss but there was no interaction of diet and parasitism. Changes were observed in rumen parameters due to the addition of urea and parasitism affected rumen ammonia levels and numbers of protozoa but there was no interaction between diet and parasitism.

Results obtained for the productivity measures were confounded by a change in feed selection behaviour of those sheep offered MS to meet their nutritional requirements for protein and possibly energy. Further experimentation must restrict intake of supplements to levels akin to those recommended in pastoral situations and determine whether such supplements can yield nutritional and parasitological benefits.

5.2 Introduction

Urea supplementation can assist in increasing the production of ruminant animals when the forage base is of low quality (Preston & Leng, 1987). Many livestock producers are however, reluctant to feed urea because of “feeding out” problems and fears of urea toxicity from over consumption (Leng *et al*, 1991). This has led to the development of various feed supplements which are based on molasses but have urea as the primary source of digestible nitrogen. The advantage of these mixtures is that they can be prepared from locally available ingredients and can assist in optimising rumen function by providing nitrogen, energy and many essential minerals .

Previous experimentation (Chapter 4) showed beneficial effects of urea supplementation of weaner Merino sheep fed a low quality oaten chaff diet in terms of increased bodyweight gain and wool production and enhanced regulation of parasitic infection. In order to further investigate this finding it was necessary to test whether a similar result could be derived from the delivery of urea to the animals via a molasses-minerals feed supplement rather than by spraying the basal chaff diet with urea. Since the most pronounced effects of urea treatment were observed in the groups receiving mixed

infections of *Haemonchus contortus* and *Trichostrongylus colubriformis*, the present experiment compared concurrent infections of these species at two levels of larval intake.

5.3 Materials and Methods

5.3.1 Experimental Procedure

72 wether Merino sheep (4-5 months) were confined in individual pens for 6 weeks to allow them to adapt to a basal diet of oaten chaff and molasses-mineral supplement (without urea). Throughout the adaptation and experimental periods feed intakes were monitored daily through recording of residues with individual sheep being offered 120% of the preceding day's consumption. Intake of the molasses-mineral supplement (MS) was recorded twice weekly. After the adaptation period the sheep were randomly allocated into 6 equal groups according to liveweight (range 14.4-30.6kg; average 21.9kg) and feed and supplement intake over the preceding week. The sheep were then given the treatments described in Table 5.1 for 16 weeks before being slaughtered at week 18 to enable estimation of total worm burdens. Liveweights of the sheep were recorded each two weeks after allocation to treatment groups. Dietary treatments were given for the full 18 weeks.

Table 5.1 Dietary and parasite infection groups within Experiment 2

<u>Group</u>	<u>Diet</u>	<u>Parasite challenge (3 times per week)</u>
MS CON	Chaff + MS ^a	None
MS LOW	Chaff + MS	200 <i>H.contortus</i> and 1000 <i>T. colubriformis</i>
MS HIGH	Chaff + MS	400 <i>H.contortus</i> and 2000 <i>T.colubriformis</i>
UM CON	Chaff + UM ^b	None
UM LOW	Chaff + UM	200 <i>H.contortus</i> and 1000 <i>T.colubriformis</i>
UM HIGH	Chaff + UM	400 <i>H.contortus</i> and 2000 <i>T.colubriformis</i>

^a MS = molasses-mineral supplement (semi-solid "block" type mixture)

^b UM = urea (10%) molasses-mineral supplement (semi-solid "block" type mixture)

5.3.2 Parasitology

Strains of parasites used were supplied from cultures originating at CSIRO McMaster Laboratory, Glebe, NSW which were isolated from sheep, maintained in culture and are susceptible to treatment with benzimidazole anthelmintics. From 3 weeks after commencing infections, fresh faecal samples were taken weekly from individual sheep for egg count estimation using the modified McMaster technique (Whitlock, 1948).

Total worm burdens of parasitised groups were estimated after slaughter at the end of week 18 of infection or earlier for those showing clinical signs of disease (see section 5.4.2.1). Before slaughter feed was withheld for 24 hours. The sheep were stunned with a captive bolt pistol and exsanguinated. Abomasa and the first 10m of small intestine distal to the pylorus were retrieved from all sheep. Contents were collected and gut sections washed 3 times with warm water. The contents and washings were made up to 400ml and preserved in 5% formaldehyde solution. Numbers of worms in gut contents were estimated from a 2.5% aliquot of this preserved material. After washing, the gut sections were split longitudinally with a knife and cut into small sections and incubated for 2 hours at 37°C with approximately 250ml of an acid-pepsin solution containing 10g pepsin-A (1:2500) and 17g 12M HCl per litre of H₂O. The remaining tissue was washed and the washings made up to 1litre and a 2.5% sample preserved in 5% formaldehyde solution for later enumeration of worms.

5.3.3 Blood parameters

Each 3 weeks blood samples were collected into 10 ml vacutainers (Becton Dickinson Ltd, Australia) containing sodium-heparin for haematological assessment. Fresh whole blood was mixed thoroughly and packed cell volume (PCV) estimated using the microhaematocrit method. The remaining blood was centrifuged at 1000g for 15 minutes and the plasma collected and stored at -20°C for later estimation of protein, albumin and urea -N content using standard Auto-Analyser methods.

5.3.4 Wool production

Greasy wool production was assessed using the dye-banding technique of Wheeler *et al.* (1977). Dye-bands were applied before the 6 week period of adaptation to basal feed, prior to the introduction to the urea diet, and at week 9 of the infection period. Collection of dye-banded staples was carried out at week 17 and all sheep were shorn prior to slaughter at week 18. Total fleece weights were recorded and estimates made of the quantity of wool produced in each of the 3 periods by calculating the proportions for each period between the dye bands. Mean fibre diameter was assessed from 2000 wool snippets per sample for each of these periods using a Sirolan-Laserscan Fibre Diameter Analyser (Glass & Dabbs, 1992).

5.3.5 Nutritional procedures

Samples of rumen liquor were collected 4 hours after feeding from each sheep by stomach tube on 1 occasion at the end of the experimental period (week 18). Rumen liquor (15ml) was prepared for assessment of levels of rumen ammonia by acidification with 5 drops (ca. 0.4ml) of H_2SO_4 and stored at -20°C . Samples were thawed and centrifuged at 3000g for 10 minutes and the supernatant kept for analysis. Samples were analysed for the concentration of $\text{NH}_3\text{-N}$ using an Autoanalyser (Technicon Equipment Co., New Jersey, USA) according to the method of Crooke & Simpson (1971) modified by Bietz (1974).

For enumeration of rumen protozoa, 4ml of ruminal fluid was added to 16ml of 4% formol saline (11.1% formaldehyde and 0.9% saline). Samples were thoroughly shaken and an aliquot pipetted onto a counting chamber of 0.2mm depth (Fuchs-Rosenthal; Brand, West Germany) and protozoa counted.

Samples of the chaff and supplements were collected at regular intervals for estimation of crude protein content using the micro-Kjeldahl method (Crooke and Simpson, 1971). The composition of the molasses-mineral supplements are shown in Table 5.2. Components were those commonly available in rural produce outlets except for the phosphoric acid (Food grade 85%) which was obtained from Albright & Wilson,

Wetherill Park, NSW. After preparation the supplement mixtures were allowed to harden to semi-solid consistency before being offered to the sheep.

Table 5.2 Ingredients used to formulate the molasses-mineral supplements used in Experiment 2.

<u>Ingredient</u>	<u>No Urea</u>	<u>Urea</u>
Molasses	50%	43%
Urea	NIL	10%
Salt	5%	5%
Agricultural Lime	8.3%	6.7%
Wheat Bran	21.8%	20.3%
Calcium Oxide	3.3%	3.3%
Triple superphosphate	2%	2%
Magnesium Oxide	7%	4.7%
Phosphoric acid	3%	3%

5.3.6 Statistical Analyses

Statistical analyses were carried out using the SYSTAT Statistical Package (Systat Inc., 1992). Treatment comparisons were made using analysis of variance where only one or two measurements were recorded (wool measurements, worm counts, rumen parameters). For wool production and fibre diameter, measurements in the 6 week adaptation period were used as covariates in the analysis. Multiple measurements (feed intake, liveweight gain, faecal egg count, blood parameters) were analysed using a repeated measures analysis of variance. Where required logarithmic ($\log x + 1$) or square root transformation was carried out prior to analysis in order to stabilise variance within groups.

5.4 Results

5.4.1 Feed and Feeding

5.4.1.1 Feed analysis

The oaten chaff consistently showed N concentrations indicative of a crude protein content of around 6.9% ($CP = 6.25 \times N$). Analysis of the molasses-mineral supplements indicated a crude protein content of 8.5% for the supplement without urea and 40.8% for that containing urea. The latter value confirmed that minimal loss of urea occurred during the supplement production process.

5.4.1.2 Feed intake

Figure 5.1 shows the mean chaff intake during the experiment for the different treatment groups. Chaff consumption was significantly affected by diet ($p=0.005$) and the effect of infection tended towards significance ($p=0.078$) but there was no interaction of diet X infection. Over time chaff consumption changed ($p<0.0005$) and there was a significant interaction of time X infection ($p<0.0005$) and time X diet X infection ($p<0.0005$) while the interaction of time X diet was not significant ($p=0.077$). The chaff was readily consumed by all sheep but those with access to the urea-molasses supplement (UM) tended to consume more chaff than those offered the molasses-mineral supplement (MS). Chaff consumption increased progressively in the CON sheep and in the LOW and HIGH sheep during the first 9 weeks but thereafter declined to a level similar to that at commencement of infection. CON sheep generally tended to eat more chaff than LOW sheep while HIGH sheep ate the least.

5.4.1.3 Supplement intake

All animals readily ingested the supplements offered during experimentation. Intake was affected by diet ($p<0.0005$) and infection ($p<0.0005$) and there was a significant interaction of diet X infection ($p=0.049$). Intake changed over time ($p<0.0005$) and there were significant interactions of time X diet ($p<0.0005$), time X infection ($p<0.0005$) and time X diet X infection ($p<0.0005$). As shown in Figure 5.2, groups offered MS tended

Figure 5.1 Chaff consumption (g/day; mean $\pm$ SE) for parasite treatment groups of young sheep offered a molasses supplement without (-●-) or with 10% urea (-○-).

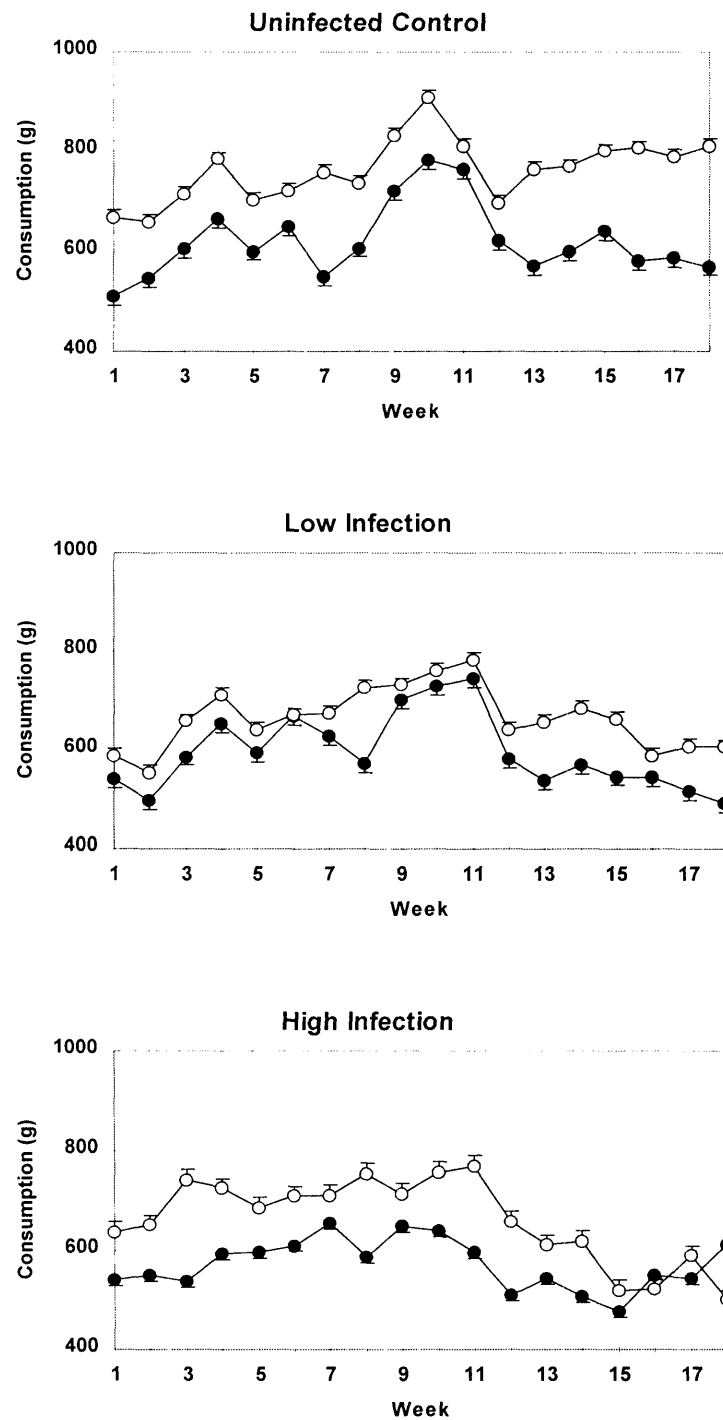
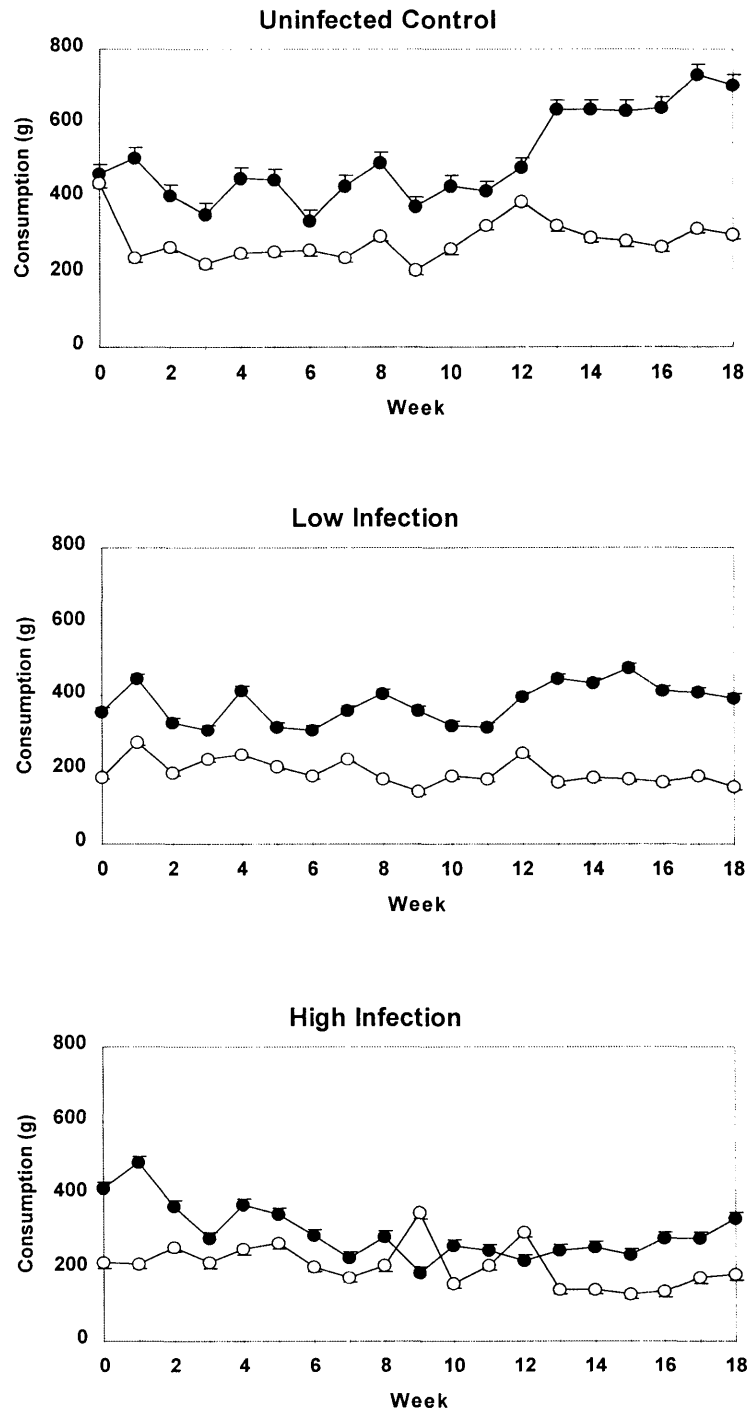


Figure 5.2 Consumption (g/day; mean $\pm$ SE) of a molasses-mineral supplement without (-●-) or with 10% urea (-○-) for parasite treatment groups of young sheep.



to consume more block than those offered UM while parasitised sheep ate less supplement than CON sheep. Within the parasitised groups LOW sheep consumed more supplement than HIGH sheep. Over time supplement consumption increased in both CON groups with the greater increase occurring in sheep offered MS and although consumption fluctuated between recordings, in general there was no trend for the parasitised groups.

5.4.1.4 Diet selection

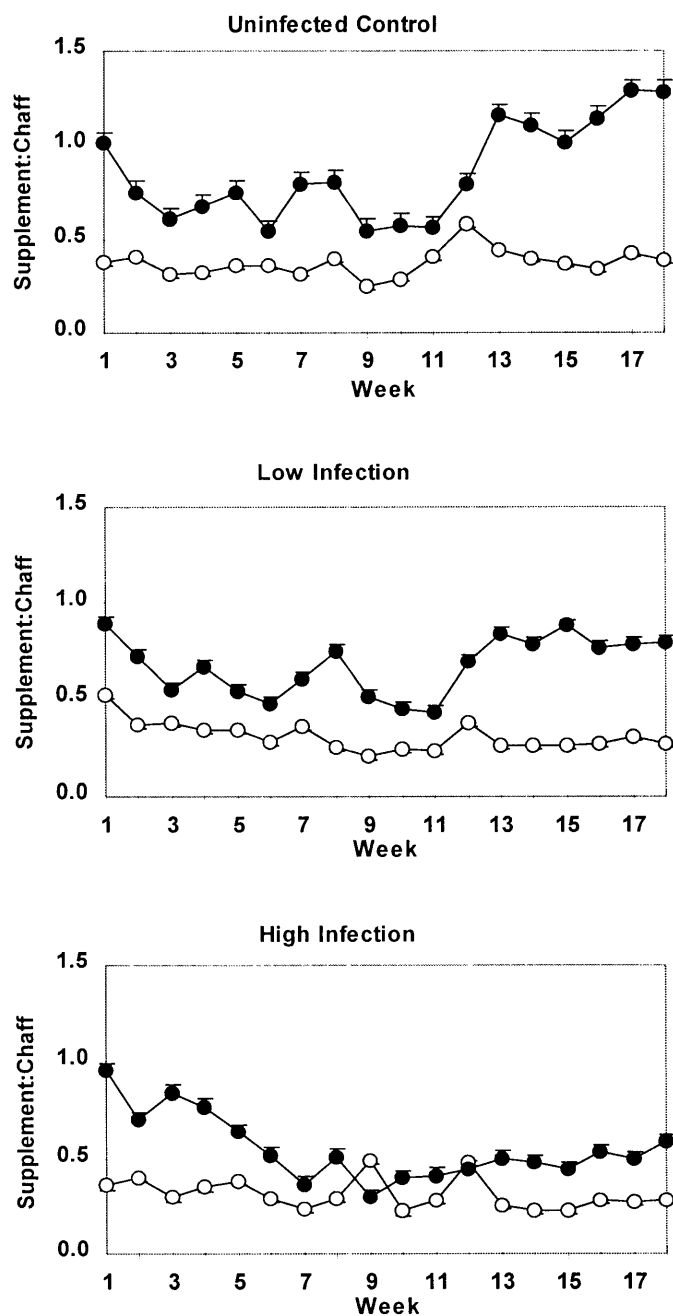
There were substantial differences between the treatment groups of sheep in the proportion of molasses-mineral supplement to chaff consumed during the course of the experiment (Figure 5.3). Analysis of the square root of this ratio showed that there was a significant effect of diet ($p < 0.0005$) and infection ($p = 0.001$) but that the diet X infection interaction was not significant ($p = 0.090$). The effects were also evident over time ($p < 0.0005$) and there were significant interactions of time X diet ($p < 0.0005$), time X infection ($p < 0.0005$) and time X diet X infection ($p < 0.0005$). In general the ratio of supplement to chaff was higher in those sheep offered MS than those offered UM. Parasitism affected the ratio for MS groups with CON having a higher ratio than LOW and HIGH having the lowest ratio. This was not the case for the UM groups for which the ratios did not differ. Over time the MS groups showed a change in supplement to chaff ratios, ie. the CON sheep consumed a greater amount of supplement to chaff during the second half of the experiment, the LOW sheep displayed a similar but smaller change whereas the HIGH sheep showed a reduction over time.

5.4.2 Animal Productivity

5.4.2.1 Mortalities

A considerable number of animals became chronically affected during the course of the experiment and had to be slaughtered for welfare reasons. Animals that displayed high faecal egg counts, low PCV and feed consumption below sustainable levels were euthanased and total worm counts determined. Seven animals “died” in each of the

Figure 5.3 Ratio of molasses-mineral feed supplement consumption to chaff consumption (mean $\pm$ SE) for groups of young sheep subjected to parasite treatments and offered the supplement without (-●-) or with 10% urea (-○-).



HIGH groups (see Table 5.5 for details of total worm counts) with the majority of these “deaths” occurring during the final four weeks. One animal “died” at week 16 in the UM-LOW group but this was due to causes other than helminth infection.

5.4.2.2 Liveweight gain

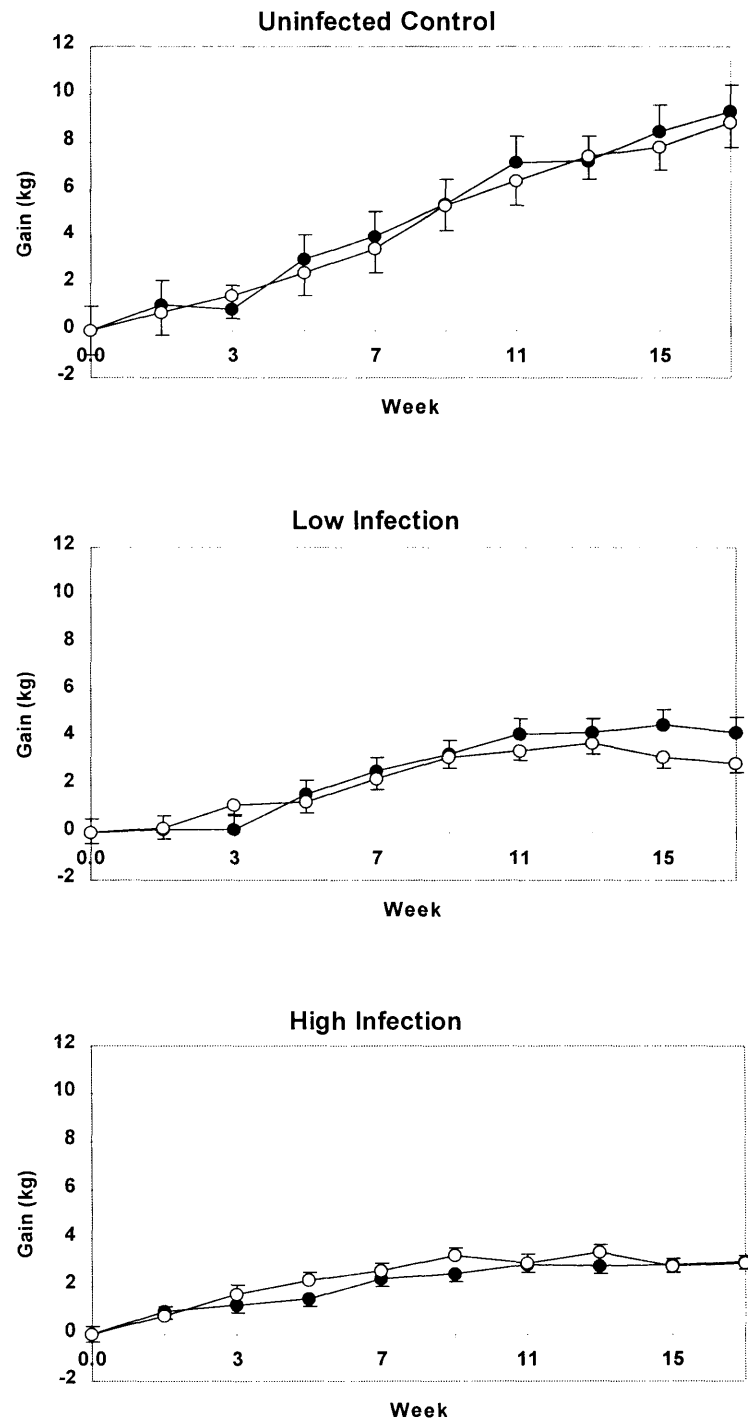
Figure 5.4 shows the cumulative weight gains for the treatment groups over time. Cumulative liveweight gain was affected by infection ($p < 0.0005$) but there was no effect of diet or diet X infection interaction. Over time rates of liveweight change differed ($p < 0.0005$) and there were significant interactions of time X diet ($p < 0.0005$) and time X infection ($p < 0.0005$) but no interaction between time X diet X infection. Over 18 weeks rates of liveweight gain were greatest in the CON sheep (74 and 70 g/head/day for MS-CON and UM-CON, respectively) but there was no difference between dietary groups. Parasitised sheep gained less weight than the CON sheep and there was no difference in gain between the different dietary groups or infection levels (34, 24, 23 to 23 g/head/day for MS-LOW, MS-HIGH, UM-LOW and UM-HIGH, respectively).

5.4.2.3 Wool growth and fibre diameter

Wool growth and fibre diameter results are presented in Tables 5.3 and 5.4, respectively. During the first 9 weeks of infection (P1) there were no significant differences between treatment groups. During the second 9 weeks of infection (P2) a significant difference was shown for diet and infection but there was no diet X infection interaction. Overall CON sheep tended to produce most wool while HIGH sheep produced least wool and, when parasites were present MS sheep produced more wool than those offered UM.

For P1 there was no difference in fibre diameter between the treatment groups. For P2 fibre diameter was affected by infection but there was no effect of diet or diet X infection interaction. CON sheep produced increased fibre diameter during this period whereas no change occurred in the parasitised sheep. Analysis across both periods (P1 and P2) using the pre-experimental measurement as a covariate showed a significant effect of infection ($p = 0.013$), a significant time X diet interaction ($p = 0.049$) and a significant time X

Figure 5.4 Cumulative weight gain (mean $\pm$ SE) for parasite treatment groups of young sheep offered a molasses supplement without (-●-) or with 10% urea (-○-)



infection interaction ($p < 0.0005$) but no effect of diet or time or interaction between diet X infection or time X infection X diet.

Table 5.3 Average daily wool production (g/head/day; mean $\pm$ SE) for young sheep offered a molasses supplement without (MS) or with 10% urea (UM) while infected thrice weekly with 200 *H. contortus* and 1000 *T. colubriformis* (LOW), with 400 *H. contortus* and 2000 *T. colubriformis* (HIGH) or uninfected (CON).

	<u>Pre-Experiment</u>	<u>Period 1</u>	<u>Period 2</u>	<u>n</u>
		<u>(0-9 weeks)</u>	<u>(9-18 weeks)</u>	
MS CON	6.52 $\pm$ 0.28	6.17 $\pm$ 0.23	5.90 $\pm$ 0.28	12
MS LOW	6.33 $\pm$ 0.47	5.97 $\pm$ 0.48	5.24 $\pm$ 0.39	12
MS HIGH	5.59 $\pm$ 0.38	5.68 $\pm$ 0.53	4.01 $\pm$ 0.48	5
UM CON	6.19 $\pm$ 0.34	6.28 $\pm$ 0.42	5.79 $\pm$ 0.43	12
UM LOW	6.11 $\pm$ 0.31	5.91 $\pm$ 0.34	3.79 $\pm$ 0.24	11
UM HIGH	5.88 $\pm$ 0.58	5.21 $\pm$ 0.37	3.35 $\pm$ 0.31	5
<u>Statistical Effects</u>				
Diet (D)	p=0.803	p=0.780	p=0.026	
Infection (I)	p=0.402	p=0.598	p<0.0005	
D X I	p=0.788	p=0.378	p=0.102	
Covariate	-----	p<0.0005	p<0.0005	

5.4.3 Parasitology

5.4.3.1 Faecal egg counts

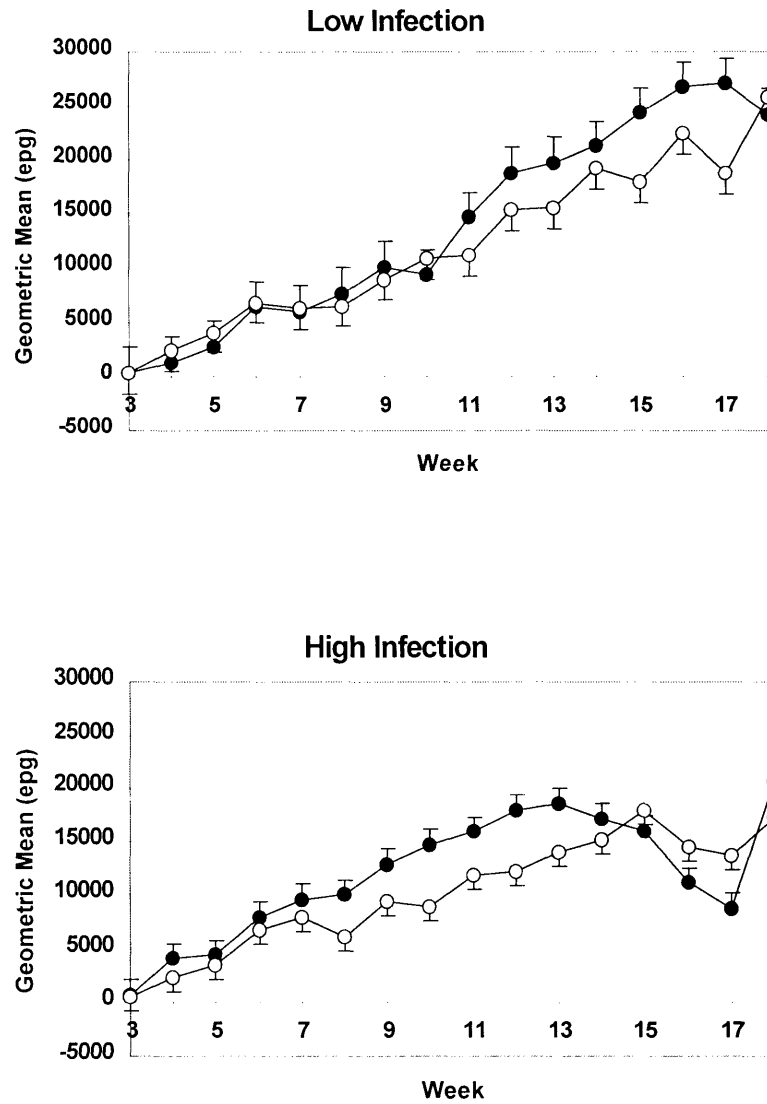
Faecal egg counts are presented as geometric means for treatment groups over time in Figure 5.5. Infection comparisons were between LOW and HIGH groups. Analysis of log (x +1) transformed faecal egg counts between weeks 3 and 18 indicated significant effects of infection ($p=0.027$) and a significant time X infection interaction ($p < 0.0005$)

Table 5.4 Average fibre diameter (μm ; mean $\pm$ SE) for young sheep offered a molasses supplement without (MS) or with 10% urea (UM) while thrice weekly infected with 200 *H. contortus* and 1000 *T. colubriformis* (LOW), with 400 *H. contortus* and 2000 *T. colubriformis* (HIGH) or uninfected (CON).

	<u>Pre-Experiment</u>	<u>Period 1</u> <u>(0-9 weeks)</u>	<u>Period 2</u> <u>(9-18 weeks)</u>	<u>n</u>
MS CON	14.2 $\pm$ 0.5	14.8 $\pm$ 0.4	16.0 $\pm$ 0.5	12
MS LOW	13.9 $\pm$ 0.4	14.5 $\pm$ 0.4	15.4 $\pm$ 0.4	12
MS HIGH	14.1 $\pm$ 0.5	14.5 $\pm$ 0.5	14.8 $\pm$ 0.3	5
UM CON	14.5 $\pm$ 0.4	15.2 $\pm$ 0.4	16.4 $\pm$ 0.4	12
UM LOW	13.6 $\pm$ 0.3	14.1 $\pm$ 0.3	14.4 $\pm$ 0.3	11
UM HIGH	14.7 $\pm$ 0.7	15.3 $\pm$ 0.7	15.2 $\pm$ 0.6	5
<u>Statistical Effects</u>				
Diet (D)	p=0.648	p=0.396	p=0.372	
Infection (I)	p=0.258	p=0.636	p=0.001	
D X I	p=0.572	p=0.745	p=0.206	
Covariate	-----	p<0.0005	p<0.0005	

whereas diet and the interactions of time X diet, diet X infection and time X diet X infection were not significant ($p>0.05$). The main effect of infection described in this comparison has resulted from differing egg count trajectories for the different infection levels within dietary treatment. This difference was caused by the decreased faecal egg counts after week 14 in the HIGH groups, attributable to the “death” of those animals with highest levels of parasite infection.

Figure 5.5 Faecal egg counts (geometric mean $\pm$ SE) for parasite-infected groups of young sheep offered a molasses supplement without (-●-) or with 10% urea (-○-)



5.4.3.2 Worm counts

Details of worm counts for each species and the total worm counts for sheep that “died” during the course of the experiment are presented in Table 5.5. The numbers of parasites did not differ between diets ($p>0.05$) even though the data suggests that there were less *Trichostrongylus* in the animals offered UM.

Table 5.5 Total worm counts (mean $\pm$ SE) for young sheep offered a molasses supplement without (MS) or with 10% urea (UM) while infected thrice weekly with 200 *H. contortus* and 1000 *T. colubriformis* (LOW) or with 400 *H. contortus* and 2000 *T. colubriformis* (HIGH) - animals which “died” during experimentation.

Group	n	<i>T. colubriformis</i>	<i>H. contortus</i>	Total worms
MS HIGH	7	16183 $\pm$ 2804	4043 $\pm$ 690	20226 $\pm$ 3086
UM LOW	1	15020	2080	17100
UM HIGH	7	12417 $\pm$ 866	3334 $\pm$ 655	15751 $\pm$ 682

Details of worm counts for each species are presented in Table 5.6 for sheep slaughtered at the end of experimentation. . Infection comparisons were between LOW and HIGH groups. There were no significant effects of diet, infection or diet X infection interaction ($p>0.05$) for *H. contortus* except for higher numbers of *H. contortus* larvae in HIGH sheep. There was a significant effect of diet on male ($p=0.019$), female ($p=0.006$) and total ($p=0.009$) *T. colubriformis* numbers with the MS sheep having the greater numbers of this species. There were no other significant effects for this species although there was an apparent interaction of diet X infection for *T. colubriformis* larvae ($p=0.063$). For total worm numbers there was a significant effect of diet ($p=0.032$) which mirrored the effects for *T. colubriformis* alone. There were no effects of infection or diet X infection interaction for total worm numbers ($p>0.05$).

Table 5.6 Worm counts at slaughter (mean $\pm$ SE) of sheep offered oaten chaff and molasses-mineral supplement without (MS) or with 10% urea (UM) and infected thrice weekly with 200 *H. contortus* (H) and 1000 *T. colubriformis* (T) (LOW) or 400 H and 2000 T (HIGH).

Group	H male	H female	H larvae	H total	T male	T female	T larvae	T total	Total Worms
MS LOW	1437 $\pm$ 120	1453 $\pm$ 135	157 $\pm$ 29	3047 $\pm$ 232	3383 $\pm$ 461	4672 $\pm$ 451	502 $\pm$ 128	8557 $\pm$ 848	11603 $\pm$ 888
MS HIGH	1632 $\pm$ 502	1960 $\pm$ 607	356 $\pm$ 88	3948 $\pm$ 1139	4568 $\pm$ 838	4840 $\pm$ 847	908 $\pm$ 263	10316 $\pm$ 1814	14264 $\pm$ 2516
UM LOW	1645 $\pm$ 170	1796 $\pm$ 141	165 $\pm$ 48	3607 $\pm$ 319	2505 $\pm$ 570	2744 $\pm$ 608	704 $\pm$ 173	5953 $\pm$ 1242	9560 $\pm$ 1319
UM HIGH	1864 $\pm$ 275	1812 $\pm$ 465	332 $\pm$ 79	4008 $\pm$ 701	2008 $\pm$ 987	2468 $\pm$ 1079	356 $\pm$ 183	4832 $\pm$ 2211	8840 $\pm$ 2548
<u>Statistics</u>									
Diet	p=0.366	p=0.734	p=0.892	p=0.553	p=0.019	p=0.006	p=0.376	p=0.009	p=0.032
Infection	p=0.395	p=0.365	p=0.003	p=0.218	p=0.624	p=0.941	p=0.881	p=0.828	p=0.562
D X I	p=0.962	p=0.394	p=0.770	p=0.632	p=0.236	p=0.760	p=0.063	p=0.331	p=0.315

5.4.4 Blood analyses

Mean results of analyses of whole blood and plasma are presented in Figures 5.6 to 5.9 for each parameter. Details of means and standard errors for individual treatment groups at each collection time are presented in Appendix Tables A5.1 to A5.4.

5.4.4.1 *Packed Cell Volume (PCV)*

Mean PCV levels over time are shown in Figure 5.6. There was no effect of diet on square root PCV level ($p=0.250$) nor was there any interaction between time X diet ($p=0.825$), diet X infection ($p=0.913$) or time X diet X infection ($p=0.803$). There were significant effects of infection ($p<0.0005$) and time ($p<0.0005$) and a significant time X infection interaction ($p<0.0005$) which reflects the pathogenic effects of parasitic infection when compared with uninfected controls. The observed increase in PCV in some groups towards the end of the experiment has resulted from the "deaths" of those animals showing the greatest pathological effects of parasite infection.

5.4.4.2 *Plasma Protein and Albumin*

For square root plasma protein concentration there was a significant effect of diet ($p=0.001$), infection ($p<0.0005$) and time ($p<0.0005$) and significant interactions between time X diet ($p<0.0005$) and time X infection ($p<0.0005$) but no significant interaction of diet X infection ($p=0.507$) or time X diet X infection ($p=0.305$). As shown in Figure 5.7 those animals receiving UM had higher plasma protein concentrations than those offered MS. Plasma protein concentrations in both HIGH and LOW groups declined over time while those of CON sheep remained relatively stable.

For square root plasma albumin concentration there was a significant effect of diet ($p=0.001$), infection ($p<0.0005$) and time ($p<0.0005$) and significant interactions between time X infection ($p<0.0005$) and time X diet X infection ($p=0.028$) but no significant interaction of diet X infection ($p=0.392$) or time X diet ($p=0.074$). As shown in Figure 5.8 those animals receiving UM had higher plasma albumin concentrations than those

Figure 5.6 Mean packed cell volume (PCV; %) for young sheep offered a molasses supplement without (MS) or with 10% urea (UM) while infected thrice weekly with 200 *H. contortus* and 1000 *T. colubriformis* (LOW), with 400 *H. contortus* and 2000 *T. colubriformis* (HIGH) or uninfected (CON).

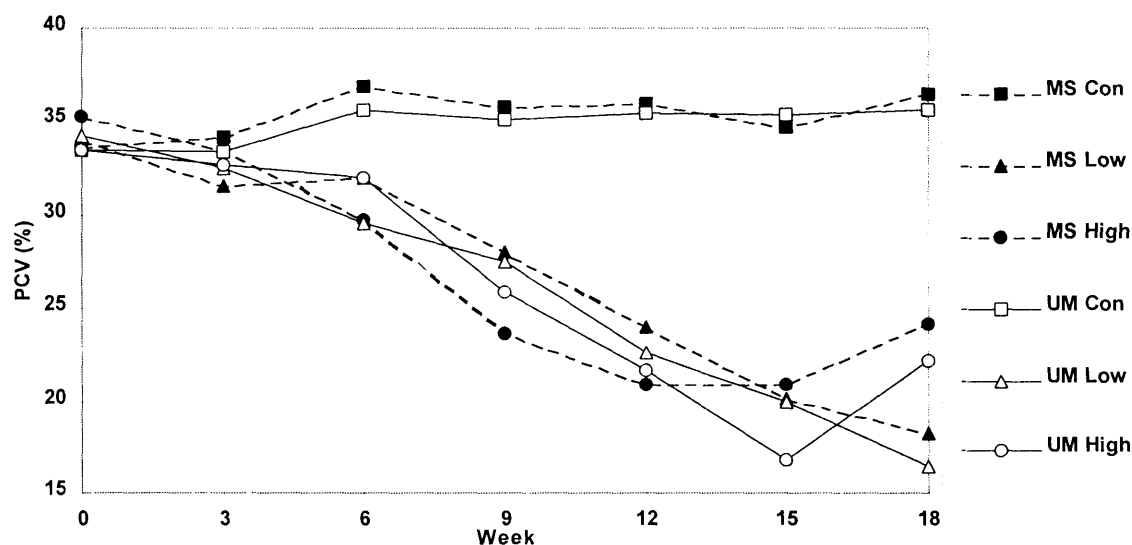


Figure 5.7 Mean plasma protein concentration (g/l) for young sheep offered a molasses supplement without (MS) or with 10% urea (UM) while infected thrice weekly with 200 *H. contortus* and 1000 *T. colubriformis* (LOW), with 400 *H. contortus* and 2000 *T. colubriformis* (HIGH) or uninfected (CON).

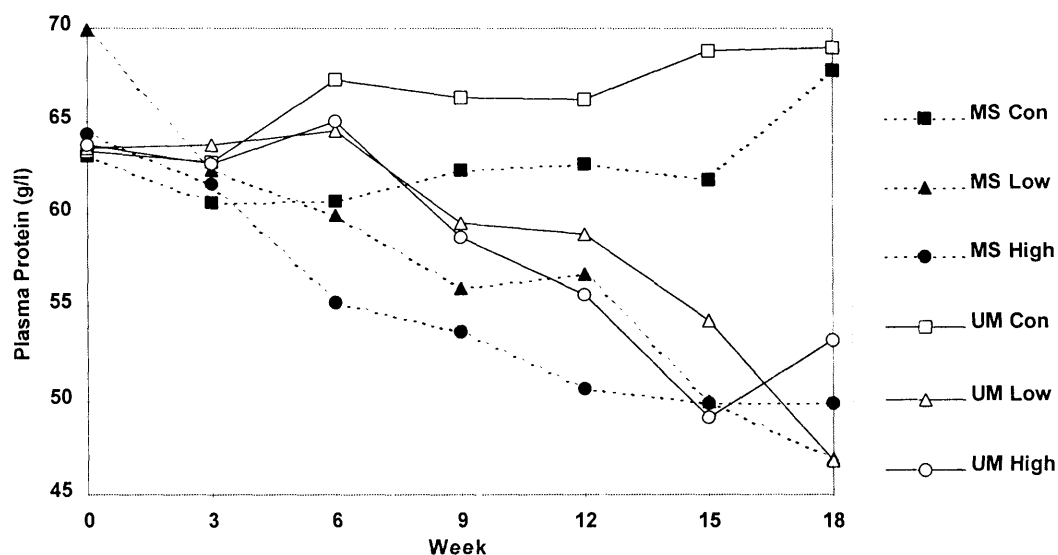


Figure 5.8 Mean plasma albumin concentration (g/l) for young sheep offered a molasses supplement without (MS) or with 10% urea (UM) while infected thrice weekly with 200 *H. contortus* and 1000 *T. colubriformis* (LOW), with 400 *H. contortus* and 2000 *T. colubriformis* (HIGH) or uninfected (CON).

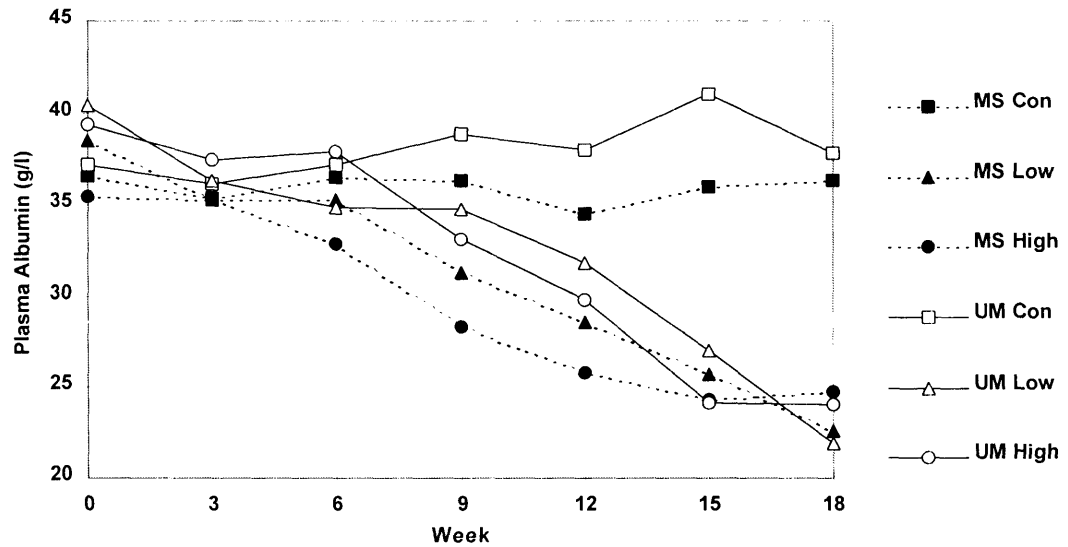
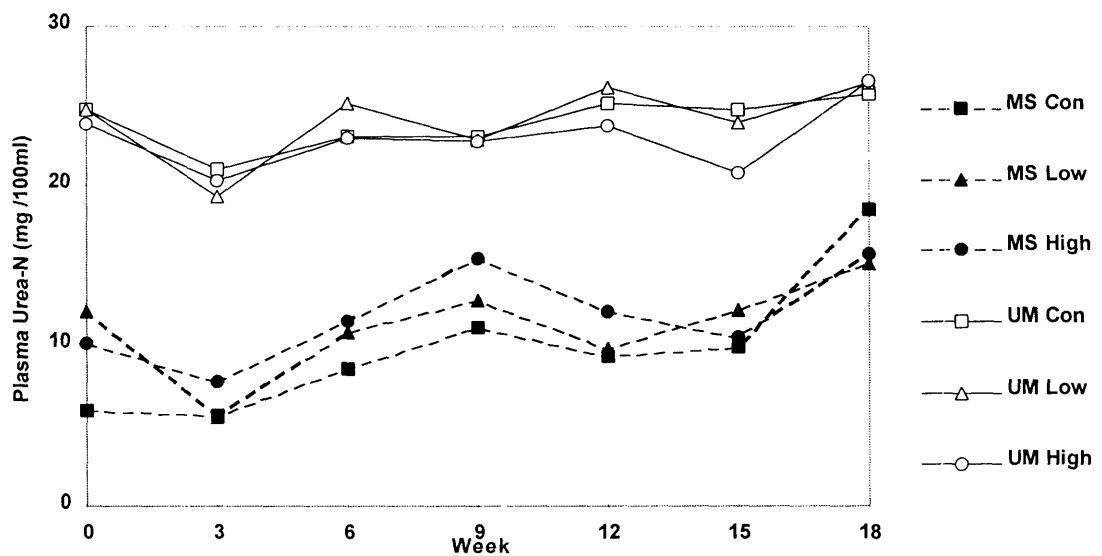


Figure 5.9 Mean plasma urea nitrogen concentration (mg/100ml) for young sheep offered a molasses supplement without (MS) or with 10% urea (UM) while infected thrice weekly with 200 *H. contortus* and 1000 *T. colubriformis* (LOW), with 400 *H. contortus* and 2000 *T. colubriformis* (HIGH) or uninfected (CON).



offered MS. Plasma albumin concentrations in parasitised groups declined over time while those of uninfected animals remained relatively stable.

5.4.4.3 Plasma Urea

Mean plasma concentrations of urea over time are shown in Figure 5.9. Plasma urea concentration was affected by diet ($p < 0.0005$) but there was no effect of infection ($p = 0.588$) or diet X infection interaction ($p = 0.486$). Concentration changed with time ($p < 0.0005$) and there was a significant interaction of time X diet ($p < 0.0005$) but no interaction between time X infection ($p = 0.225$) or time X diet X infection ($p = 0.206$). Sheep offered UM had higher concentrations of plasma urea than those offered MS throughout the experiment. Plasma urea concentration fluctuated over the 18 week period in all groups but for MS sheep there was a steady increase from below 10mgN/100ml to above 15mgN/100ml while for UM sheep fluctuations were around the 25mgN/100ml level with only a very slight increase to just over 26mgN/100ml over the same period.

5.4.5 Rumen fluid analyses

5.4.5.1 Rumen $\text{NH}_3\text{-N}$

Rumen $\text{NH}_3\text{-N}$ concentrations are presented in Table 5.7. There were significant effects of diet ($p < 0.0005$) and infection ($p = 0.020$) but no interaction of diet X infection. Those sheep receiving UM had higher rumen $\text{NH}_3\text{-N}$ levels than MS sheep. Within the MS groups CON and LOW sheep had the same level which was higher than for HIGH sheep. Within UM groups CON sheep had highest levels, LOW sheep were intermediate and HIGH sheep had the lowest levels.

Table 5.7 Rumen $\text{NH}_3\text{-N}$ concentrations (Mean $\pm$ SE) for young sheep offered a molasses feed supplement without (MS) or with 10% urea (UM) while thrice weekly infected with 200 *H. contortus* and 1000 *T. colubriformis* (LOW), with 400 *H. contortus* and 2000 *T. colubriformis* (HIGH) or uninfected (CON).

<u>Rumen $\text{NH}_3\text{-N}$ (mgN/l)</u>		
<u>Infection</u>	<u>MS</u>	<u>UM</u>
CON	63.8 $\pm$ 14.4	354.2 $\pm$ 34.4
LOW	66.1 $\pm$ 11.2	292.0 $\pm$ 35.4
HIGH	33.4 $\pm$ 12.2	180.7 $\pm$ 35.2

5.4.5.2 Protozoa

Table 5.8 shows the results of counts of rumen protozoa at the end of the experimental period. There was a significant effect of infection ($p=0.001$) on protozoa numbers but no effect of diet ($p>0.05$) or diet X treatment interaction ($p>0.05$). Highest numbers of protozoa were observed in CON sheep while numbers in LOW and HIGH sheep were the same.

Table 5.8 Total rumen protozoa counts (thousands/ml; mean $\pm$ SE) for parasite groups of young sheep offered a molasses feed supplement without (MS) or with 10% urea (UM) while infected with 200 *H. contortus* and 1000 *T. colubriformis* thrice weekly (LOW), with 400 *H. contortus* and 2000 *T. colubriformis* thrice weekly (HIGH) or remaining uninfected (CON).

	<u>MS</u>	<u>UM</u>
CON	68.9 $\pm$ 53.1	74.6 $\pm$ 62.1
LOW	13.7 $\pm$ 9.2	33.9 $\pm$ 27.2
HIGH	18.3 $\pm$ 11.6	25.4 $\pm$ 15.0

5.5 Discussion

5.5.1 Feed and Feeding

Supplementation of a low quality oaten chaff diet with UM increased feed intake in both infected and uninfected sheep in the present experiment. This observation is in agreement with previous studies where urea supplementation increased digestibility and intake of low quality fibrous diets (Stephenson *et al.*, 1981; Krebs & Leng, 1984; Leng *et al.*, 1995; Hogan, 1996b). In accordance with previous findings (see review by Symons, 1985) intake of the basal ration was reduced by parasitism with the greatest reduction occurring in those sheep given the HIGH rate of infection while the LOW sheep were intermediate between the HIGH and CON sheep.

The molasses-mineral supplements were readily ingested by all sheep but levels and patterns of consumption varied greatly between groups. Intakes of UM were relatively uniform in all groups with only a slight increase throughout the experiment but were reduced by infection, with LOW sheep consuming less than CON sheep and the HIGH sheep consuming least of all. A similar influence of infection was observed in the sheep offered MS but changes occurred over time which differed between groups. From week 11 of experimentation the intake of MS by CON sheep increased substantially, the LOW sheep showed a similar but lesser increase in intake of MS during the same period whereas intake of MS by the HIGH group tended to decrease over time.

It has been shown in sheep infected with monospecific infections of *T. colubriformis* (Kyriazakis *et al.*, 1994; Kyriazakis *et al.*, 1996a,b) and *O. circumcincta* (Kyriazakis & Coop, cited by Coop & Holmes, 1996) that sheep select higher protein feeds during infection to compensate for protein losses due to the parasites. During the present experiment, feed selection changed in those sheep offered MS with the CON sheep preferentially choosing to consume a higher proportion of MS to oaten chaff during the latter stages of experimentation. It appears that these sheep may have recognised MS as a source of easily digestible protein (from the wheat bran) and energy and shifted their consumption away from the less digestible oaten chaff to meet their nutritional demands. Seasonal change during that stage of experimentation may also offer an explanation with

colder environmental temperatures possibly increasing the energy demand to maintain body temperature and MS being a source of high energy molasses. Similar selective behaviour to the MS-CON sheep was shown by the MS-LOW sheep but parasitism appeared to limit the response. Sheep given the HIGH level of infection showed no change in food preference during the experiment other than a decline in the proportion of MS consumed during the early stage of infection. If protein demand is the driving force behind the change in food preference as suggested by Kyriazakis & Oldham (1993), the results of the present study do not agree with previous studies in parasitised sheep (Kyriazakis *et al.*, 1994; Kyriazakis *et al.*, 1996a,b; Kyriazakis & Coop, cited in Coop & Holmes, 1996) since the change in food preference did not occur in sheep given HIGH parasite levels and only moderately affected those given LOW parasite infection. Similar levels of protein were offered in the low protein diets in all experiments but the high protein diet offered in previous studies is considerably different from the MS offered in the present experiment and may initiate a greater food selection response in infected animals. The use of *H. contortus* in the mixed species infection in the present experiment is also a major difference particularly since pathological changes appeared to be dominated by this species.

5.5.2 Animal Productivity

Mortality is the most severe form of production loss that can be experienced in a livestock rearing enterprise (Barger, 1982). During the present experiment more than half the sheep in the HIGH infection groups had to be euthanased because of severe clinical haemonchosis. In similar sheep given this rate of larval infection other workers observed no mortalities during 15 weeks of infection but the diet offered was lucerne pellets (14-17% crude protein) given *ad libitum* (Barger *et al.*, 1985). The low quality diet offered and the imposition of concurrent infection with *T. colubriformis* was clearly responsible for the high level of “mortality” observed during the present study.

Chronic infection of Merino sheep with nematode parasites commonly reduces liveweight gain and wool production (see review by Barger, 1982). In the present experiment mixed infection with *H. contortus* and *T. colubriformis* resulted in greater

than 50% reduction in liveweight gain and an almost 20% reduction in wool production. The sheep surviving the HIGH infection produced less wool than those given LOW infection as would be expected in animals suffering more acute clinical disease and increased anorexia due to parasitism. There was no difference in liveweight gain or wool production between the uninfected MS and UM sheep. This indicates that the altered feeding behaviour of the MS sheep (as discussed above) enabled this group to overcome the deficiencies of the basal oaten chaff diet sufficiently for them to perform as well as the UM sheep.

5.5.3 Parasitology

Faecal egg counts in all infected groups increased to progressively high levels after 18 weeks of infection even though there was a decline in the HIGH groups after those sheep suffering severe infection were removed. Similar egg count trajectories have been observed previously when a low protein diet was offered during a trickle infection of *H. contortus* (Abbott *et al.*, 1988) or when the quantity of a moderate quality (14-17% crude protein) diet was restricted to less than maintenance levels (Roberts & Adams, 1990). Although these were mixed species infections it is likely that *H. contortus* would be the predominant contributor to overall egg count since they have a greater egg output per female worm than *T. colubriformis*.

After 18 weeks of infection total worm counts of the remaining sheep were significantly affected by diet with UM sheep having fewer worms than MS sheep. This difference was due to 30 and 50% lower numbers of *T. colubriformis* in the UM sheep than in the MS sheep for LOW and HIGH groups, respectively. It is thought that this difference is due to improved immunological regulation arising from the improved diet offered by UM compared to the MS since improved rumen function (see section 5.5.5) would lead to greater availability of microbial protein for intestinal absorption. Similar effects have been noted in *T. colubriformis* infected sheep when an oaten chaff diet was supplemented with additional bypass protein (van Houtert *et al.*, 1995a). Alternatively there could be a direct effect of urea on *T. colubriformis* survival and establishment. This is highly unlikely since the urea would be rapidly degraded to ammonia in the rumen and unless

the associated rise in rumen pH affects the *T. colubriformis* larvae the impact of urea seems likely to be minimal.

5.5.4 Blood analyses

The effect of experimental treatment on blood constituents was dominated by the effect of parasites, particularly *H. contortus*, on red blood cell and plasma loss. This is exemplified by the progressive reduction in PCV and protein and albumin concentrations in plasma in all infected sheep whereas these parameters remained relatively stable in the uninfected groups. These results accord previous studies describing the pathophysiology of infection with *H. contortus* (Abbott *et al.*, 1984, 1988) and *T. colubriformis* (Steel *et al.*, 1980; Coop *et al.*, 1976). Inclusion of urea in the molasses-mineral supplement led to higher PCVs and plasma concentrations of protein and albumin in both infected and uninfected sheep which agrees with observations in the previous experiment (see Chapter 4).

As expected, plasma urea concentration was higher in sheep offered UM than in sheep offered MS. No effect of infection was recorded for either dietary treatment with all parasite treatment groups having similar levels of plasma urea within dietary treatment. This observation is contrary to previous studies where plasma urea concentration was increased after high level infection with a single dose of *H. contortus* (Rowe *et al.*, 1988) or *T. colubriformis* (Roseby, 1973; Roseby & Leng, 1974). Differences in rate and mode (single large dose versus long term trickle dosing) of infection may explain this inconsistency.

5.5.5 Rumen fluid analyses

Rumen ammonium nitrogen concentration (RAN) was five to six times greater in UM sheep than MS sheep and was a direct effect of the urea supplement providing a considerably higher level of rumen degradable nitrogen. RAN level was influenced by infection in both groups and was directly related to the amount of molasses-mineral supplement consumed. Within dietary treatment HIGH sheep consumed the least supplement and had the lowest RAN, CON sheep consumed the most supplement and had the highest RAN. Levels of RAN in the UM sheep were sufficient to optimise

rumen function and production of microbial protein whereas levels in MS sheep were suboptimal (Kanjapruithipong, 1995).

Numbers of rumen protozoa were not affected by dietary supplement but were affected by infection. This is not likely to be a direct effect of the parasites on protozoa numbers but is probably due to the depressive effects of infection on feed intake which in turn reduced the substrate availability in the rumen thereby reducing growth rate and total populations of all rumen micro-organisms including protozoa.

5.5.6 Conclusion

This experiment has confirmed the beneficial effects of urea supplementation in terms of controlling *T. colubriformis* numbers in sheep but no such benefits were seen for the *H. contortus* population. The high level of “mortality” in sheep given the HIGH rate of infection clearly illustrates that in situations where low quality forages predominate greater care must be taken to control parasites below the threshold for clinical helminthosis since the impact of parasites is much greater when nutrient intake is suboptimal. The impact of supplementation on other productivity measures was confounded by the high intake of the MS supplement which suggested some change in diet selection/preference by these sheep shifting their preference away from chaff to MS to satisfy protein and/or energy requirements. However, overall feed consumption was clearly higher in the uninfected animals and chaff consumption was stimulated by the addition of urea to the supplement. Further experimentation is required to determine whether the beneficial effects of urea are solely due to increased feed intake or if there are additional nutritional benefits in terms of efficiency of microbial protein production. Such experiments should utilise the types of supplements currently available for use in the developing nations and in pastoral regions of Australia where low quality roughages are the predominant forage. Further experimentation must restrict intake of supplements to levels akin to those recommended in pastoral situations and determine whether such supplements can yield the nutritional and parasitological benefits suggested by experimentation so far completed under pen conditions.

Chapter 6

THE EFFECTS OF UREA-MOLASSES BLOCKS ON FEED INTAKE AND RESPONSES TO PARASITISM IN SHEEP FED A LOW QUALITY ROUGHAGE DIET.

6.1 Abstract

Seventy-two 4-5 month-old Merino wethers were individually confined and fed oaten chaff and minerals (NU) for 6 weeks. The sheep were then divided into 3 groups which were fed either NU, pair fed to NU and offered a urea-molasses block (UP) or fed chaff ad libitum and offered a urea-molasses block (UA). Within each group one subgroup was then infected per os with 200 H. contortus and 1000 T. colubriformis thrice weekly (INF) and one subgroup remained uninfected (CON). Weight gain, wool production, parasite burden and blood parameters were assessed over a 21 week. During the final 3 weeks 4 sheep from each treatment were placed in metabolism cages to measure digestibility, water and nitrogen balance and urinary purine excretion.

The UA sheep consumed more feed, gained more weight and grew more wool than the NU sheep whereas UP sheep performed similarly to NU sheep. In all groups parasitism decreased feed intake, liveweight gain and wool production. Faecal egg counts increased progressively during the experiment with NU sheep having highest counts, UP sheep the lowest counts and UA sheep being intermediate. No difference was observed between the groups in total worm counts. Blood loss associated with H. contortus infection caused considerable decline in PCV and protein and albumin concentration over time. The greatest decline in PCV occurred in NU sheep, the least in UP sheep while UA sheep were intermediate. Plasma urea concentrations were higher in UP and

UA sheep and tended to increase in infected groups between 12 and 18 weeks. Dry matter, organic matter and nitrogen (N) digestibilities were decreased by infection and adding urea-molasses block (UMB) to the diet increased N digestibility. Infection decreased water and N intakes and retention with increased urine volume and increased urinary N. Availability of UMB increased water intake in UP and UA sheep. Similarly UMB increased N intakes of UP and UA sheep but N retention was increased only in UA sheep. Urinary purine excretion was highest in UA sheep, intermediate in UP sheep and lowest in NU sheep.

Although there appeared to be beneficial effects of UMB supplementation, as indicated by lowered faecal egg counts, higher PCV and plasma urea and increased urinary purine excretion, no benefits were seen in production when intake of the basal diet was restricted. Only when UMB supplementation was given in conjunction with ad libitum access to the basal diet was productivity improved and the detrimental effects of nematode parasites reduced. It is concluded that free access to sufficient feed is necessary to fulfil additional requirements for dry matter intake resulting from increased ruminal efficiency generated by non-protein nitrogen supplementation.

6.2 Introduction

It has long been established that urea supplementation can improve utilisation of low quality forages (Leng, 1986; Taiwo *et al.*, 1992; Sansoucy, 1995) and a popular means of delivering urea to grazing animals is via feed blocks. These blocks are supplied by various manufacturers but are generally prepared from molasses, urea, salt, protein meal and binders specific to each manufacturer. Positive nutritional benefits of urea-molasses blocks (UMB) have been obtained in sheep when fed predominantly on low quality forages.

Earlier experiments reported in this thesis have shown that feeding young Merino sheep low quality diets supplemented with urea increased bodyweight gain and wool production and ameliorated the effects of parasitic infection (see Chapters 4 and 5). To

apply this finding in the field it was desirable to test whether a similar result could be derived from the delivery of urea via a feed supplement block rather than as an in-feed preparation.

6.3 Materials and Methods

6.3.1 Experimental Procedure

72 wether Merino sheep (4-5 months) were confined in individual pens and allowed 6 weeks to adapt to a basal diet of oaten chaff and essential minerals (7g CaCO₃, 1g elemental sulphur, 1g mineral premix Q422SE, BASF, Sydney, NSW). Throughout the adaptation and experimental periods, feed intakes were monitored daily with individual sheep being offered 120% of the preceding day's consumption. After the 6 week adaptation period the sheep were divided into 6 equal groups according to liveweight (range 16.0-28.3kg; average 21.3 kg) and feed intake over the preceding week. The groups were then given the treatments described in Table 6.1 for 18 weeks and then slaughtered at week 21 for estimation of total worm burdens. Dietary treatments were given for the full 21 weeks. Liveweights were recorded each two weeks from the time of allocation to treatment groups.

4.3.2 Parasitology

Strains of parasites used were supplied from cultures at CSIRO McMaster Laboratory, Glebe. These parasites were isolated from sheep, maintained in culture and were susceptible to treatment with benzimidazole anthelmintics. From 3 weeks after commencing infections, fresh faecal samples from individual sheep were taken weekly for egg count estimation using the modified McMaster technique (Whitlock, 1948).

Table 6.1 Parasite and dietary treatments in Experiment 3.

Group	Diet	Parasite challenge (3 times per week)
NU CON	Chaff <i>ad libitum</i>	Nil
UP CON	Pair fed to NU CON + UMB ^a	Nil
UA CON	Chaff <i>ad libitum</i> + UMB ^a	Nil
NU INF	Chaff <i>ad libitum</i>	200 <i>H. contortus</i> + 1000 <i>T. colubriformis</i>
UP INF	Pair fed to NU INF + UMB ^a	200 <i>H. contortus</i> + 1000 <i>T. colubriformis</i>
UA INF	Chaff <i>ad libitum</i> + UMB ^a	200 <i>H. contortus</i> + 1000 <i>T. colubriformis</i>

^a UMB = 15% urea, 43% molasses, 5% NaCl, 23% wheat bran, 9% CaO (fine powder, DMM, Tamworth, NSW) and 5% mineral premix (Q422SE, BASF, Sydney, NSW)

Total worm burdens of parasitised groups were estimated after slaughter at week 21 of infection. Prior to slaughter feed was withheld for 24 hours. The sheep were stunned with a captive bolt pistol and exsanguinated. The abomasum and first 10m of small intestine distal to the pylorus were retrieved from each sheep. Contents were collected and gut sections washed 3 times with warm water. The contents and washings were made up to 400ml and preserved in 5% formaldehyde solution. Numbers of worms in gut contents were estimated from a 2.5% aliquot of this preserved material. After washing, the gut sections were split longitudinally and cut into small sections and incubated for 2 h. at 37°C with approximately 250ml of an acid-pepsin solution containing 10g pepsin-A (1:2500) and 17g 12M HCl per litre of H₂O. The remaining tissue was washed and the washings made up to 1litre and a 2.5% sample preserved in 5% formaldehyde solution for later enumeration of worms.

4.3.3 Blood parameters

Each 3 weeks blood samples were collected into 10ml vacutainers (Becton Dickinson Ltd, Australia) containing sodium-heparin. Fresh whole blood was mixed thoroughly and packed cell volume (PCV) estimated by the microhaematocrit method. The remaining blood was centrifuged at 1000g. for 15 minutes and the plasma collected and

stored at -20°C for later estimation of protein, albumin and urea-N content using standard Auto-Analyser methods.

4.3.4 Wool production

Greasy wool production was assessed using the dye-banding technique of Wheeler *et al.* (1977). Dye-bands were applied prior to the 6 week period of adaptation to the basal diet and at weeks 1 and 10 of the infection period. Collection of dye-banded staples was carried out at week 20 and all sheep were shorn prior to slaughter at week 21. Total fleece weights were recorded and estimates made of the quantity of wool produced in each of the 3 periods by calculating the proportions for each period between the dye bands. Mean fibre diameter was assessed from 2000 wool snippets per sample for each of these periods using a Sirolan-Laserscan Fibre Diameter Analyser (Glass & Dabbs, 1992).

4.3.5 Nutritional procedures

Samples of the basal diet chaff (NU) and of urea-molasses blocks (UMB) were collected at regular intervals for estimation of crude protein content using the micro-Kjeldahl method (Crooke & Simpson, 1971).

At week 18 of infection 24 animals (4 per treatment group) were moved to metabolism crates for the final 3 weeks of experimentation to enable estimation of feed digestibility, water balance and nitrogen retention using standard techniques. Microbial protein flow into the small intestine was predicted from purine excretion in urine by the method of Balcells *et al.* (1992) as modified by Kahn (1996). Collections for all parameters were made over 7 days and the daily means calculated for individual sheep.

4.3.6 Statistical Analyses

Statistical analyses were carried out using the SYSTAT Statistical Package (Systat Inc., 1992). Treatment comparisons were made using analysis of variance where only one or two measurements were recorded (wool measurements, worm counts, metabolism

estimates). For wool production and fibre diameter, measurements in the pre-experimental period were used as covariates in the analysis. Multiple measurements (feed intake, liveweight gain, faecal egg count, blood parameters) were analysed using a repeated measures analysis of variance. Where required logarithmic ($\log x + 1$) or square root transformation was carried out prior to analysis in order to stabilise variance within groups.

6.4 Results

6.4.1 Feed and Feeding

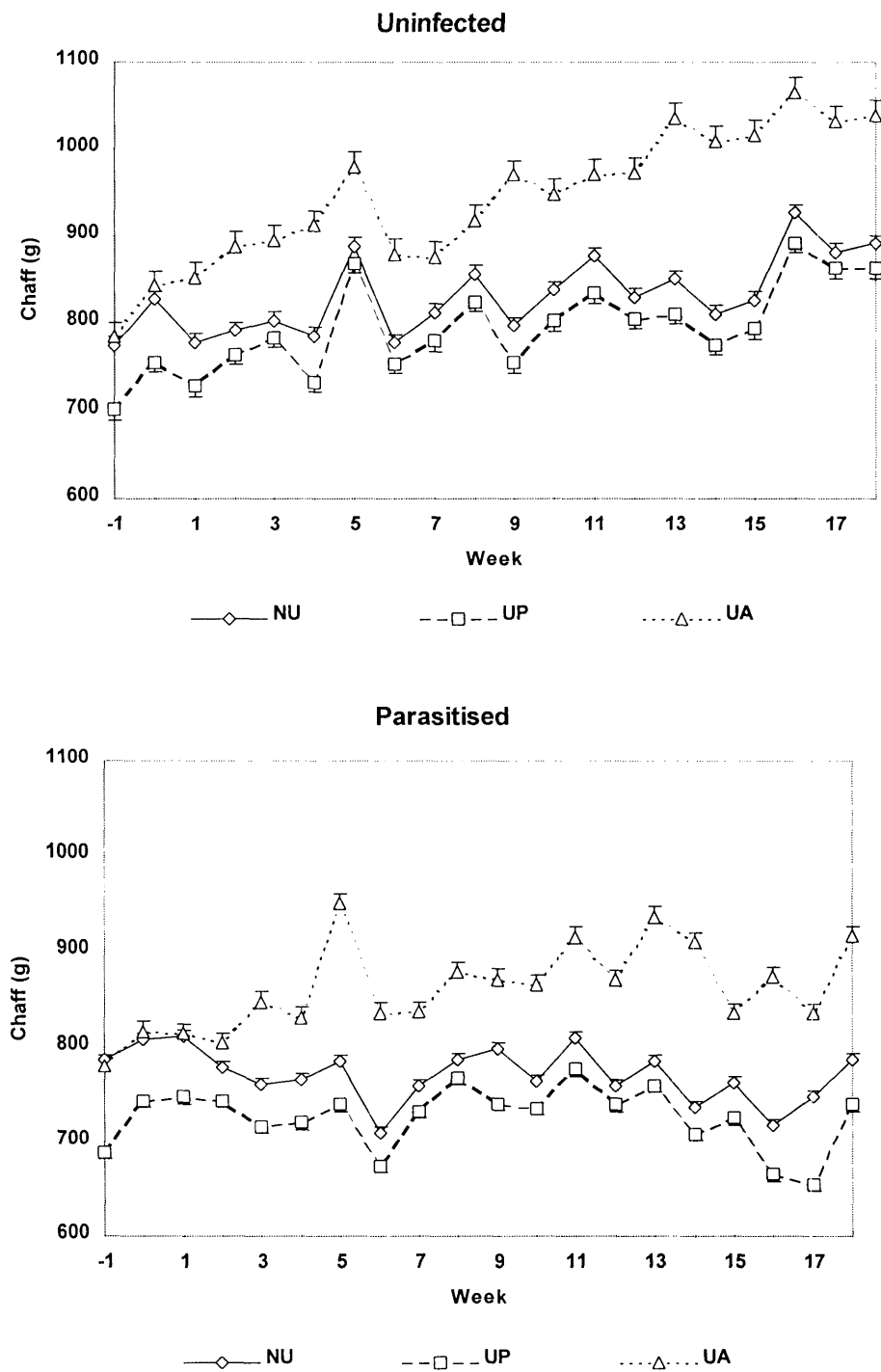
6.4.1.1 *Feed analysis*

The basal diet of oaten chaff (chaffed oaten hay) contained 1.07% N which is indicative of a crude protein content of around 6.7% ($CP = 6.25 \times N$). UMB contained 8.6%N, equivalent to a crude protein content of 53.8%, of which a high proportion was derived from urea (15% of UMB mix).

6.4.1.2 *Chaff intake*

As shown in Figure 6.1 the oaten chaff was readily consumed by all animals. Chaff consumptions for the final three weeks of the study were substantially affected by movement of some sheep into metabolism crates and this period has been excluded from the statistical analyses. Chaff consumption was affected by diet ($p < 0.0005$) and infection ($p = 0.021$) but there was no diet X infection interaction. Over time consumption of chaff tended to change ($p < 0.0005$) and there were significant interactions between time and diet ($p < 0.0005$) and time and infection ($p < 0.0005$) but there was no time X diet X infection interaction. Similar results were obtained when chaff consumption was calculated on metabolic liveweight basis (ie. $\text{g/kg liveweight}^{0.75}$) with the exceptions that the time X diet interaction was not significant ($p = 0.644$) and the time X diet X infection interaction approached significance ($p = 0.058$). Within infection treatments, the sheep offered the UA diet tended to consume more chaff while the UP sheep consumed

Figure 6.1 Daily oaten chaff intake (mean $\pm$ SE) for sheep offered *ad libitum* chaff (NU), pair fed to NU and offered UMB (UP) or offered *ad libitum* chaff and UMB (UA) and infected with 200 *H. contortus* and 1000 *T. colubriformis* thrice weekly or uninfected.



slightly less chaff than the NU sheep. Over time chaff consumption increased in all but the NU INF and UP INF groups. Within dietary treatment, infection with parasites depressed actual chaff intake when CON and INF groups were compared. However, no difference was observed over time between the UA CON and UA INF sheep when intake was expressed on a metabolic bodyweight basis whereas the NU and UP groups differed significantly.

6.4.1.3 Block consumption

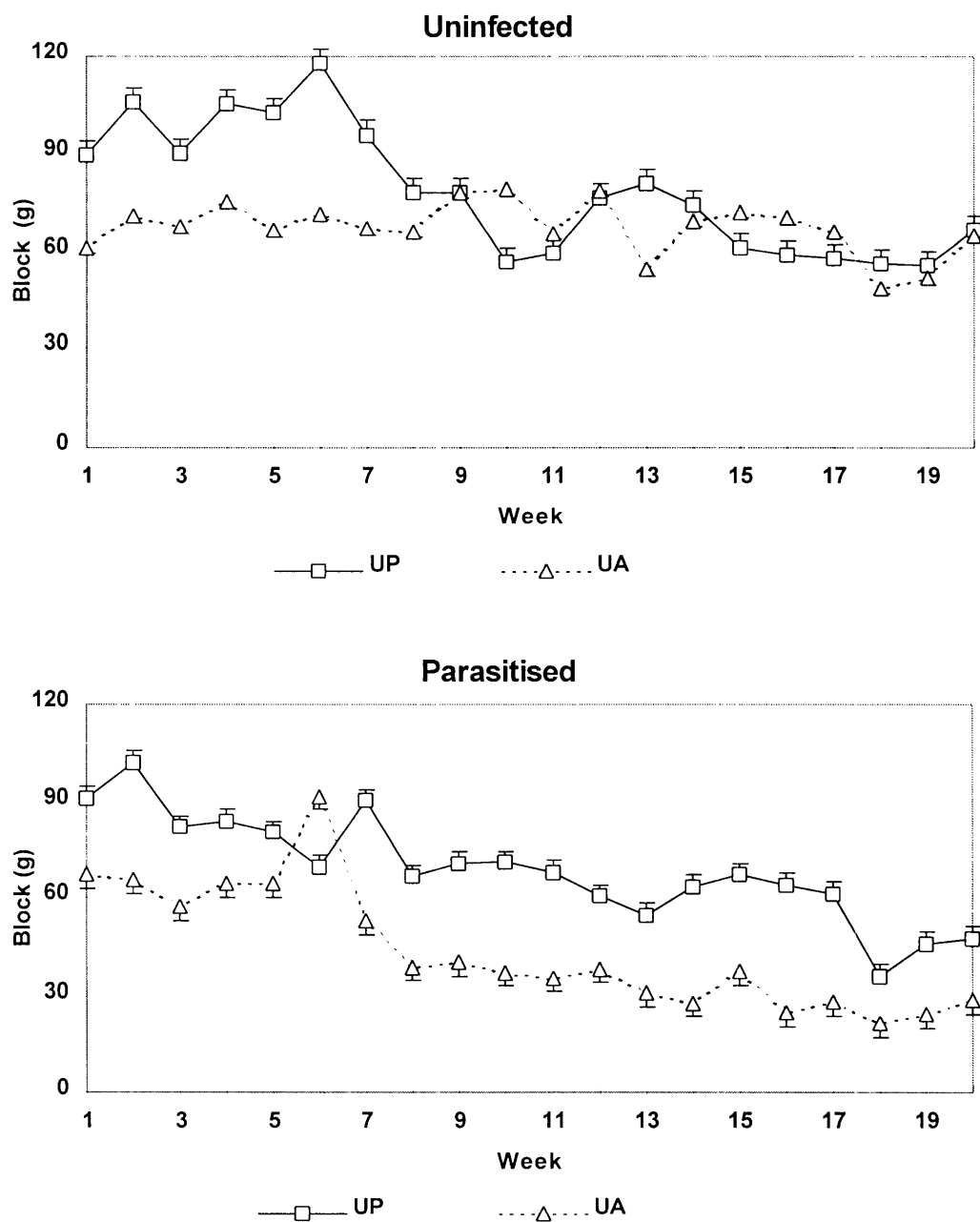
Block consumption for UA and UP groups are shown in Figure 6.2. Block consumption was affected by infection ($p=0.051$) and diet ($p=0.021$) but there was no infection X diet interaction ($p=0.338$). Over time, block consumption changed significantly ($p<0.0005$) but there was no interaction of time X infection ($p=0.712$) or time X diet ($p=0.598$). However, the interaction of time X infection X diet was significant ($p<0.0005$). Across all groups there was a general tendency for INF sheep to consume less block than CON sheep and for UP sheep to consume more block than UA sheep particularly during the early stages of experimentation. Over time consumption by UA INF sheep declined to less than 30g/head/day while the consumption of the other three groups fluctuated but remained around 60g/head/day throughout the experiment.

6.4.2 Animal Productivity

6.4.2.1 Mortalities

Three animals became clinically affected during the course of the experiment displaying high faecal egg counts, low PCV and feed consumption below sustainable levels and were euthanased due to animal welfare concerns. One animal died in the UP INF group and 2 animals died in the UA INF group. No mortalities occurred in the other groups.

Figure 6.2 Daily urea-molasses block intake (mean $\pm$ SE) for sheep pair fed to NU and offered UMB (UP) or offered *ad libitum* chaff and UMB (UA) and uninfected or infected with 200 *H. contortus* and 1000 *T. colubriformis* thrice weekly.



6.4.2.2 Liveweight gain

Figure 6.3 shows the cumulative weight gains for the treatment groups over time. Analysis of squareroot liveweight gain indicated a significant effect of infection ($p<0.0005$) and diet ($p<0.0005$) but no interaction of infection X diet. Liveweight gain changed over time ($p<0.0005$) and there were significant interactions of time X infection ($p<0.0005$), time X diet ($p<0.0005$) and time X infection X diet ($p<0.0005$). Over time CON sheep gained about 50% more weight than INF sheep within dietary treatment. Irrespective of parasite infection, those sheep offered the UA diet grew faster than UP and NU sheep which grew at similar rates. By the end of the experiment infected NU, UP and UA sheep had gained 41, 39 and 59 g/head/day, respectively while the uninfected groups had gained 69, 69 and 90 g/head/day, respectively.

6.4.2.3 Wool growth

Results of wool growth estimates and their statistical comparisons using the pre-experimental measurement as a covariate are presented in Table 6.2. During the experiment INF groups produced around 10% less wool than their CON counterparts. No difference in wool production was shown between the groups offered the NU and UP diets. In INF sheep wool production was increased by more than 20% when animals were offered the UA diet compared to the other diets. Analysis across both 10 week experimental periods confirmed these comparisons and overall wool production for CON sheep was 6.4, 6.4 and 8.6 g/head/day for NU, UP and UA, respectively while for INF sheep estimates were 6.2, 6.0 and 7.5 g/head/day, respectively.

6.4.2.4 Fibre diameter

Fibre diameter measurements and their statistical comparisons using the pre-experimental measurement as a covariate are shown in Table 6.3. Fibre diameter differed between groups prior to experimentation. For the individual fibre diameter estimate periods, no significant differences between treatment groups were observed. When analysis was carried out across both periods there was a significant effect of diet ($p=0.021$) but no effect of infection ($p=0.260$) or diet X infection interaction ($p=0.617$). Between periods there was a significant decrease in fibre diameter in all groups ($p<0.0005$) and significant

Figure 6.3 Cumulative liveweight gain (kg; mean + SE) for sheep offered *ad libitum* chaff (black bars), pair fed to NU and offered UMB (clear bars) or offered *ad libitum* chaff and UMB (striped bars) and infected with 200 *H. contortus* and 1000 *T. colubriformis* thrice weekly or uninfected.

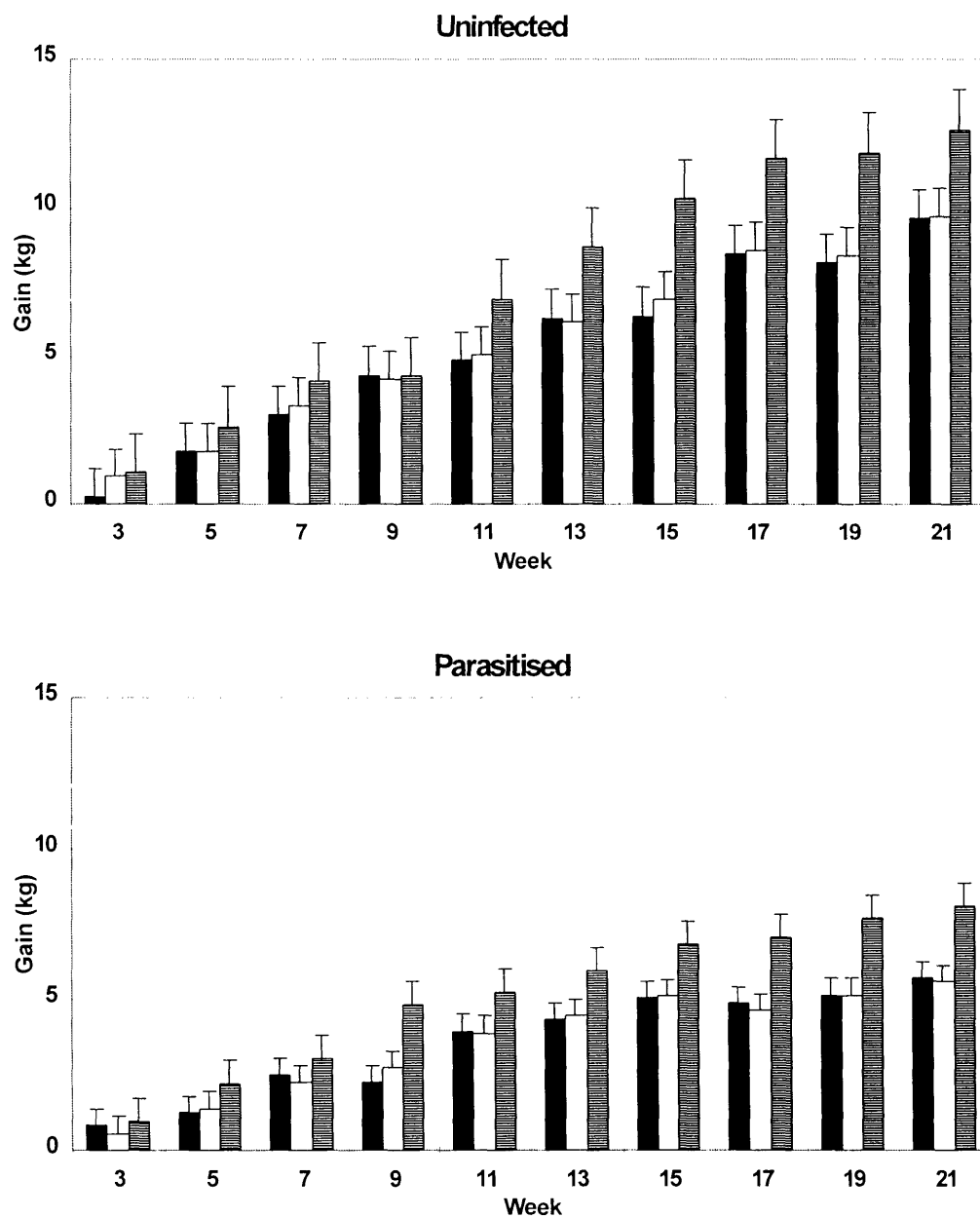


Table 6.2 Wool production (g/h/day; mean $\pm$ SE) in young sheep infected with 200 *H. contortus* and 1000 *T. colubriformis* (INF) thrice weekly or uninfected (CON) and offered either *ad libitum* oaten chaff (NU), urea-molasses block and pair fed to NU (UP) or urea-molasses block and fed *ad libitum* chaff (UA).

Group	Pre -experiment	Weeks 0-10	Weeks 10-21
NU CON	8.3 $\pm$ 0.7	7.6 $\pm$ 0.4	5.3 $\pm$ 0.3
UP CON	9.2 $\pm$ 0.6	8.2 $\pm$ 0.3	5.5 $\pm$ 0.4
UA CON	9.6 $\pm$ 0.9	10.1 $\pm$ 0.3	7.2 $\pm$ 0.6
NU INF	8.6 $\pm$ 0.3	7.5 $\pm$ 0.2	5.0 $\pm$ 0.3
UP INF	7.9 $\pm$ 0.6	7.0 $\pm$ 0.3	5.0 $\pm$ 0.4
UA INF	9.4 $\pm$ 0.7	8.9 $\pm$ 0.5	6.3 $\pm$ 0.4
<u>Statistical analyses</u>			
Infection (I)	p=0.223	p=0.012	p=0.068
Diet (D)	p=0.422	p<0.0005	p<0.0005
I X D	p=0.442	p=0.243	p=0.728
Covariate	-----	p=0.206	p=0.460

Table 6.3 Wool fibre diameter (μ m; mean $\pm$ SE) in young sheep (abbreviations as in Table 6.2).

Group	Pre-experiment	Weeks 0-10	Weeks 10-21
NU CON	16.8 $\pm$ 0.4	16.6 $\pm$ 0.3	16.2 $\pm$ 0.3
UP CON	17.0 $\pm$ 0.3	16.9 $\pm$ 0.3	16.0 $\pm$ 0.3
UA CON	18.8 $\pm$ 0.5	18.3 $\pm$ 0.5	16.4 $\pm$ 0.4
NU INF	16.7 $\pm$ 0.5	16.9 $\pm$ 0.4	16.1 $\pm$ 0.4
UP INF	17.8 $\pm$ 0.4	17.6 $\pm$ 0.4	16.7 $\pm$ 0.5
UA INF	18.8 $\pm$ 0.3	18.1 $\pm$ 0.3	17.1 $\pm$ 0.3
<u>Statistical analyses</u>			
Infection (I)	p=0.688	p=0.388	p=0.095
Diet (D)	p<0.0005	p=0.699	p=0.100
I X D	p=0.356	p=0.617	p=0.156
Covariate	-----	p<0.0005	p<0.0005

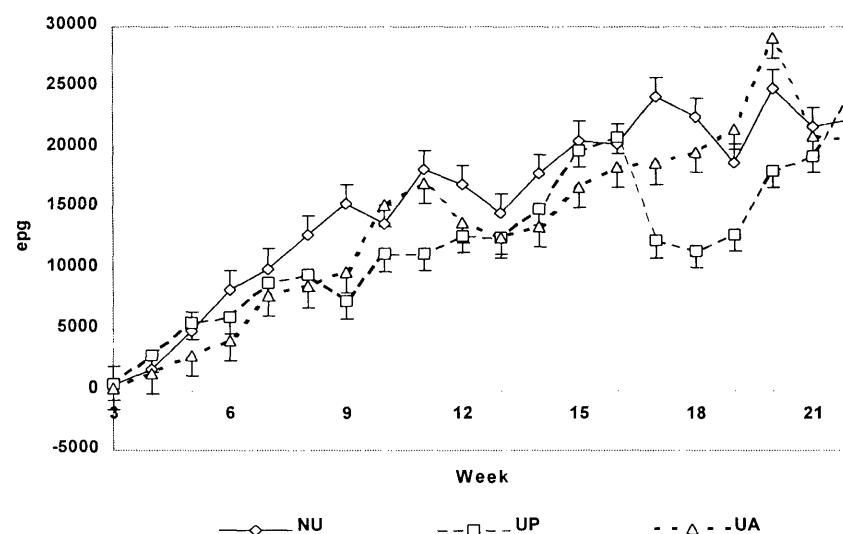
interactions between period X diet ($p=0.001$) and period X infection X diet ($p=0.020$) but no interaction of period X infection ($p=0.210$). Overall it appeared that the greater decline in fibre diameter occurred in the CON sheep than the INF sheep while the UA sheep showed smaller reductions than the NU and UP sheep. There was a large reduction in fibre diameter in the UA CON sheep during the second measurement period compared to the other treatments.

6.4.3 Parasitology

6.4.3.1 Faecal egg counts

Faecal egg counts are presented as geometric means for treatment groups over time in Figure 6.4. Considerable variation within groups occurred and faecal egg counts increased in all groups over time ($p<0.0005$). There was a significant effect of diet ($p=0.038$) and a significant time X diet interaction ($p<0.0005$) for log faecal egg counts. Despite fluctuations over time in all groups, there was a general tendency for NU sheep to have the highest faecal egg counts, UP sheep to have the lowest egg counts while UA sheep were intermediate.

Figure 6.4 Faecal egg counts (geometric mean $\pm$ SE) for sheep offered *ad libitum* chaff (NU), pair fed to NU and offered UMB (UP) or offered *ad libitum* chaff and UMB (UA) and infected with 200 *H. contortus* and 1000 *T. colubriformis* thrice weekly.



6.4.3 2 Worm counts

Details of worm counts for each species and the total worm counts are presented in Table 6.4. No statistical differences were observed between dietary treatment groups for male, female, immature or total parasites for either species nor for total worm count.

Table 6.4 Worm counts (mean $\pm$ SE) of young sheep infected with 200 *H contortus* (H) and 1000 *T colubriformis* (T) thrice weekly and offered *ad libitum* oaten chaff (NU), offered urea-molasses block and pair fed to NU (UP) or offered urea-molasses block and fed *ad libitum* chaff (UA).

	NU	UP	UA
H male	1445 $\pm$ 134	1200 $\pm$ 137	1418 $\pm$ 214
H female	1558 $\pm$ 140	1275 $\pm$ 178	1576 $\pm$ 213
H larvae	23 $\pm$ 8	20 $\pm$ 8	14 $\pm$ 7
H total	3025 $\pm$ 250	2476 $\pm$ 306	3002 $\pm$ 414
T male	5482 $\pm$ 756	6258 $\pm$ 900	6034 $\pm$ 790
T female	5927 $\pm$ 747	6242 $\pm$ 884	5954 $\pm$ 800
T larvae	8 $\pm$ 4	16 $\pm$ 6	6 $\pm$ 4
T total	11425 $\pm$ 1496	12635 $\pm$ 1776	12014 $\pm$ 1573
Total Worms	14450 $\pm$ 1550	15111 $\pm$ 1852	15016 $\pm$ 1719

6.4.4 Blood analyses

Results of analyses of whole blood and plasma are presented in Figures 6.6.5-6.8. Details of means and standard errors for individual treatment groups at each collection time are given in Appendix Tables A6.1 to A6.4.

6.4.4.1 Packed Cell Volume (PCV)

PCV estimates over time are shown in Figure 6.5. There was a significant effect of infection ($p<0.0005$) and diet ($p=0.002$) on PCV and the interaction infection X diet was also significant ($p=0.007$). Over time PCV changed ($p<0.0005$) and there were significant interactions of time X infection ($p<0.0005$), time X diet ($p=0.003$) and time X infection X diet ($p<0.0005$). Parasitised groups showed a reduction in PCV over time but apart from a temporary reduction at week 6 the CON groups did not change. Within the INF groups, NU sheep showed the greatest decline in PCV over time, UA sheep were intermediate while UP sheep showed the least decline but were still substantially lower than the CON sheep.

6.4.4.2 Plasma Protein and Albumin

Plasma protein concentration was affected by infection ($p<0.0005$) but not by diet ($p=0.989$) nor was there an interaction of infection X diet ($p=0.768$). Protein concentration declined over time in all groups ($p<0.0005$) and there were significant interactions of time X infection ($p<0.0005$) and time X diet ($p<0.0005$) but no interaction of time X infection X diet ($p=0.108$). As shown in Figure 6.6 plasma protein concentration declined during the experiment by around 5g/l in CON sheep but in INF the decline was by around 20g/l. Within infection groups concentrations fluctuated but no consistent effects of diet were observed.

Plasma albumin concentration followed a similar pattern to that for plasma protein. Concentration was affected by infection ($p<0.0005$) and diet ($p=0.020$) but there was no interaction of infection X diet. Plasma albumin concentration declined over time ($p<0.0005$) and there were significant interactions of time X infection ($p<0.0005$) and time X diet ($p<0.0005$) but the interaction time X infection X diet was not significant ($p=0.524$). There was a greater decline in plasma albumin concentrations in INF sheep (>15 g/l decline) compared to CON sheep (around 5 g/l decline) during the experiment. For both INF and CON sheep it appeared that UP sheep had highest plasma albumin concentrations NU sheep had the lowest and UA sheep were intermediate.

Figure 6.5 Packed cell volume (PCV; %; mean $\pm$ SE) of young sheep infected with 200 *H. contortus* and 1000 *T. colubriformis* (INF) thrice weekly or uninfected (CON) and offered *ad libitum* oaten chaff (NU), offered urea-molasses block and pair fed to NU (UP) or offered urea-molasses block and fed *ad libitum* chaff (UA).

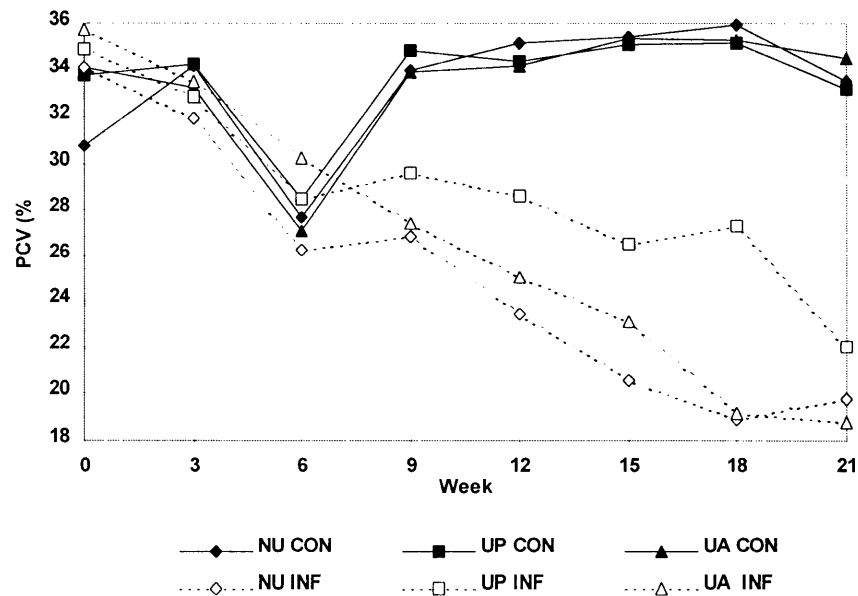


Figure 6.6 Plasma protein concentration (g/l; mean $\pm$ SE) of young sheep infected with 200 *H contortus* and 1000 *T colubriformis* (INF) thrice weekly or uninfected (CON) and offered *ad libitum* oaten chaff (NU), offered urea-molasses block and pair fed to NU (UP) or offered urea-molasses block and fed *ad libitum* chaff (UA).

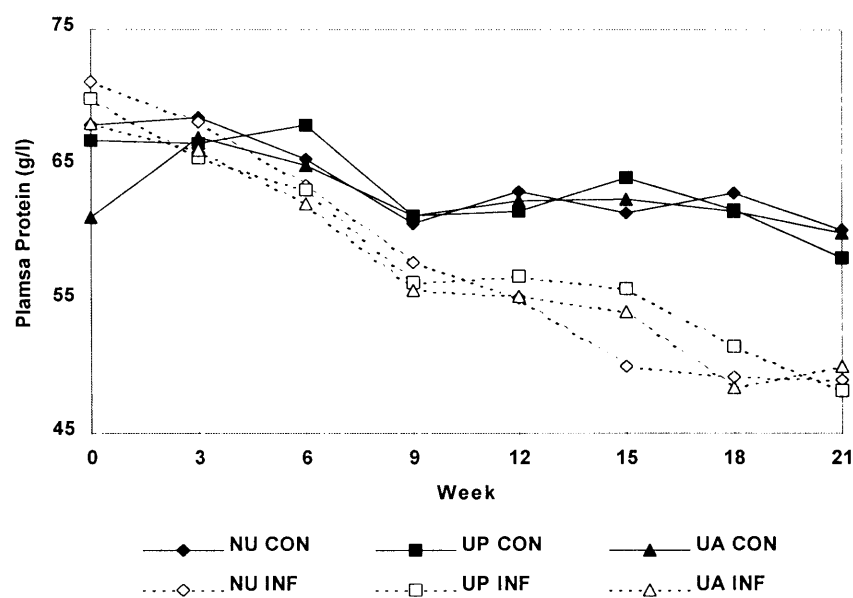


Figure 6.7 Plasma albumin concentration (g/l; mean $\pm$ SE) of young sheep infected with 200 *H. contortus* and 1000 *T. colubriformis* (INF) thrice weekly or uninfected (CON) and offered either *ad libitum* oaten chaff (NU), urea-molasses block and pair fed to NU (UP) or urea-molasses block and fed *ad libitum* chaff (UA).

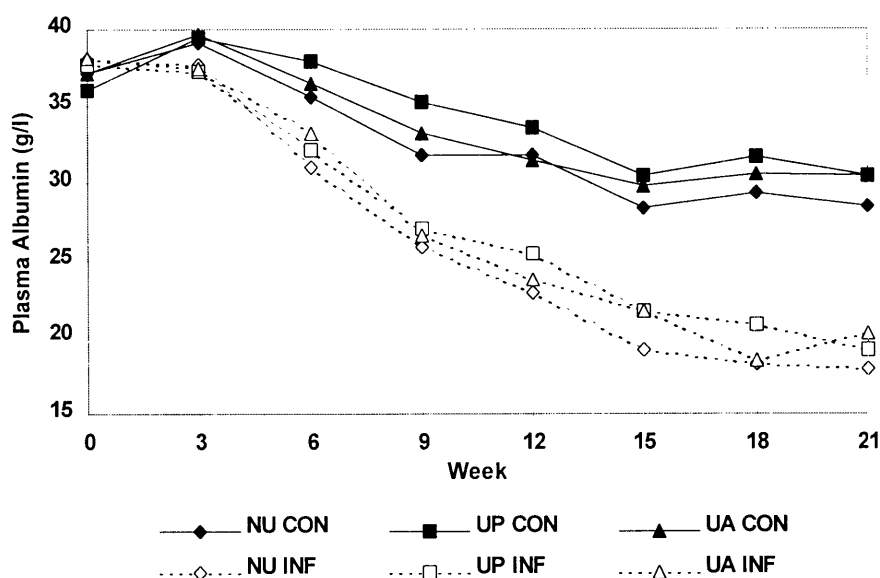
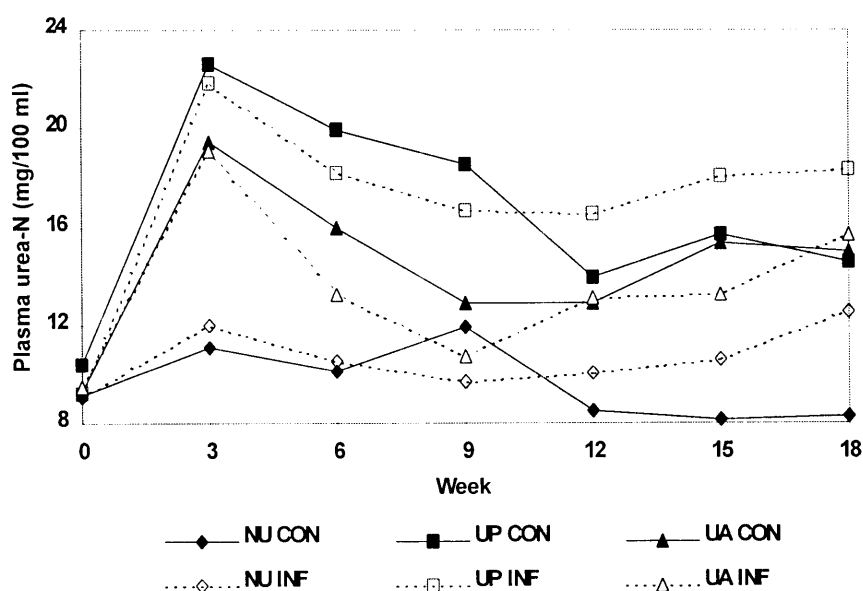


Figure 6.8 Plasma urea-N concentration (mg/100ml; mean $\pm$ SE) of young sheep infected with 200 *H. contortus* and 1000 *T. colubriformis* (INF) thrice weekly or uninfected (CON) and offered *ad libitum* oaten chaff (NU), offered urea-molasses block and pair fed to NU (UP) or offered urea-molasses block and fed *ad libitum* chaff (UA).



6.4.4.3 Plasma Urea

Results for plasma urea-N concentration are shown in Figure 6.8. The samples from week 21 were accidentally lost during processing and are therefore not included in the results. Plasma urea-N concentration was affected by diet ($p < 0.0005$) but there was no effect of infection ($p = 0.612$) or infection X diet interaction ($p = 0.266$). Plasma urea-N concentration changed over time ($p < 0.0005$) and there were significant interactions of time X infection ($p < 0.0005$) and time X diet ($p < 0.0005$) but time X infection X diet was not significant ($P = 0.328$). Groups receiving UMB (UP and UA) had higher plasma urea-N levels than NU sheep. During the first four samplings UP CON and UA CON sheep had higher plasma urea-N concentrations than their respective INF groups while NU CON and NU INF sheep had similar levels. From week 12 onwards plasma urea-N levels appeared to increase in the NU INF and UP INF sheep compared to their respective CON groups while UA CON and UA INF sheep had similar levels.

6.4.5 Metabolism studies

6.4.5.1 Digestibility

Results of dry matter digestibility (DMD), organic matter digestibility (OMD) and nitrogen digestibility (ND) estimates and the statistical comparison for different treatments are shown in Table 6.5. Infection caused a reduction in DMD and OMD in all dietary treatments but there was no effect of diet or infection X diet interaction for either parameter. Infection also caused a reduction in ND for NU and UA sheep but not for UP sheep while ND was increased by having UMB available. There was a significant interaction of infection X diet for ND indicating that sheep offered the different diets responded differently to infection.

6.4.5.2 Water Balance

Water balance estimates and the results of statistical comparisons of treatments are shown in Table 6.6. For these analyses water intake has been used as a covariate in the analysis for the respective urinary, faecal and retention estimates. Infection decreased intake of water, urine volume and water retention. Diet affected water intake with those sheep having access to UMB drinking more water. The effect of UMB availability on

Table 6.5 Dry matter (DM), organic matter (OM) and nitrogen (N) digestibility estimates (mean $\pm$ SE) of sheep infected with 200 *H contortus* and 1000 *T colubriformis* (INF) thrice weekly or remaining uninfected (CON) and offered either *ad libitum* oaten chaff (NU), urea-molasses block and pair fed to NU (UP) or urea-molasses block and fed *ad libitum* chaff (UA).

Group	DM Digestibility	OM Digestibility	N Digestibility
NU CON	0.61 $\pm$ 0.00	0.64 $\pm$ 0.01	0.64 $\pm$ 0.01
UP CON	0.61 $\pm$ 0.01	0.63 $\pm$ 0.01	0.69 $\pm$ 0.04
UA CON	0.61 $\pm$ 0.01	0.63 $\pm$ 0.01	0.71 $\pm$ 0.02
NU INF	0.58 $\pm$ 0.01	0.60 $\pm$ 0.01	0.58 $\pm$ 0.01
UP INF	0.60 $\pm$ 0.01	0.62 $\pm$ 0.01	0.73 $\pm$ 0.02
UA INF	0.58 $\pm$ 0.01	0.60 $\pm$ 0.01	0.64 $\pm$ 0.02
<u>Statistics</u>			
Infection (I)	p = 0.007	p = 0.009	p = 0.054
Diet (D)	p = 0.613	p = 0.521	p < 0.0005
I X D	p = 0.511	p = 0.421	p = 0.020

urine volume also tended towards significance (p=0.090). There were no significant interactions of infection X diet for any of the parameter estimates.

6.4.5.3 Nitrogen Balance

Nitrogen balance estimates and the results of statistical comparisons of treatments are shown in Table 6.6. For these analyses N intake has been used as a covariate in the analysis for the respective urinary, faecal and retention estimates. Infection decreased N intake and N retention and tended towards significance for urinary-N (p=0.071). Diet affected N intake and faecal-N with the NU sheep having the lowest levels, UP sheep having intermediate levels and UA sheep having the highest levels. Diet affected urinary N but the effect varied with infection grouping with NU CON sheep having the lowest levels and UA CON sheep the highest and UP CON sheep being intermediate whereas UP INF was highest, NU INF lowest and UA INF intermediate. Diet also affected N retention with UA sheep having the higher N retentions while NU and UP sheep

Table 6.6 Water and nitrogen balance estimates (mean $\pm$ SE) of young sheep infected with 200 *H contortus* and 1000 *T colubriformis* (INF) thrice weekly or uninfected (CON) and offered either *ad libitum* oaten chaff (NU), urea-molasses block and pair fed to NU (UP) or urea-molasses block and fed *ad libitum* chaff (UA).

Group	H₂O Intake (ml/day)	Urine Volume (ml/day)	Faecal H₂O (ml/day)	H₂O Retention (ml/day)	N Intake (g/day)	N Faeces (g/day)	N Urine (g/day)	N Retention (g/day)
NU CON	2511 $\pm$ 167	379 $\pm$ 26	634 $\pm$ 63	1499 $\pm$ 86	11.01 $\pm$ 0.42	3.98 $\pm$ 0.25	2.75 $\pm$ 0.07	4.28 $\pm$ 0.13
UP CON	2696 $\pm$ 184	520 $\pm$ 67	586 $\pm$ 71	1590 $\pm$ 69	13.94 $\pm$ 1.52	4.21 $\pm$ 0.30	5.59 $\pm$ 1.07	4.14 $\pm$ 0.55
UA CON	3077 $\pm$ 151	617 $\pm$ 63	822 $\pm$ 90	1639 $\pm$ 149	18.74 $\pm$ 0.63	5.36 $\pm$ 0.24	6.17 $\pm$ 0.96	7.22 $\pm$ 0.71
NU INF	2187 $\pm$ 147	470 $\pm$ 62	513 $\pm$ 76	1204 $\pm$ 27	8.64 $\pm$ 0.65	3.59 $\pm$ 0.29	2.55 $\pm$ 0.21	2.50 $\pm$ 0.35
UP INF	2148 $\pm$ 109	711 $\pm$ 85	379 $\pm$ 41	1057 $\pm$ 103	13.22 $\pm$ 0.53	3.62 $\pm$ 0.29	6.94 $\pm$ 0.56	2.66 $\pm$ 0.51
UA INF	2857 $\pm$ 90	729 $\pm$ 131	765 $\pm$ 87	1363 $\pm$ 66	14.36 $\pm$ 1.69	5.17 $\pm$ 0.38	5.06 $\pm$ 0.53	4.14 $\pm$ 0.97
<i>Statistics</i>								
Infection (I)	p = 0.006	p = 0.010	p = 0.662	p = 0.005	p = 0.009	p = 0.625	p = 0.071	p = 0.021
Diet (D)	p = 0.001	p = 0.090	p = 0.106	p = 0.178	p < 0.0005	p = 0.007	p = 0.002	p = 0.015
I X D	p = 0.524	p = 0.526	p = 0.901	p = 0.553	p = 0.236	p = 0.086	p = 0.652	p = 0.848
Covariate	-----	p = 0.073	p = 0.017	p < 0.0005	-----	p = 0.001	p = 0.004	p = 0.001

were the same within infection grouping. There was no infection X diet interaction for any of the parameters measured although faecal-N may have been tending towards significance ($p=0.086$).

6.4.5.4 Urinary Purines

Estimates of urinary purine concentrations are shown in Table 6.7. Infection had no effect on actual urinary purine production. Diet affected urinary purine production ($p=0.051$) with NU sheep having lowest levels, UP sheep intermediate levels and UA sheep having the highest levels. No interaction between infection X diet was observed. Urinary purine production was also calculated with relation to dry matter intake (DMI) of individual sheep (ie. mmol/kg DMI) and similar statistical results were observed from comparisons but the effect of diet was not as strongly demonstrated ($p=0.093$).

Table 6.7 Total excretion of purine derivatives in urine (mean $\pm$ SE) of young sheep infected with 200 *H. contortus* and 1000 *T. colubriformis* (INF) thrice weekly or uninfected (CON) and offered either *ad libitum* oaten chaff (NU), urea-molasses block and pair fed to NU (UP) or urea-molasses block and fed *ad libitum* chaff (UA).

Group	Purine Excretion (mmol/day)	Purine excretion/DMI (mmol/kg DMI)
NU CON	7.78 $\pm$ 0.32	9.11 $\pm$ 0.28
UP CON	9.55 $\pm$ 1.05	11.60 $\pm$ 0.93
UA CON	10.90 $\pm$ 1.54	10.68 $\pm$ 1.76
NU INF	7.28 $\pm$ 1.61	10.63 $\pm$ 1.76
UP INF	9.81 $\pm$ 1.03	14.51 $\pm$ 1.41
UA INF	12.70 $\pm$ 3.52	15.80 $\pm$ 5.75
<u>Statistics</u>		
Infection (I)	$p = 0.691$	$p = 0.243$
Diet (D)	$p = 0.051$	$p = 0.093$
I X D	$p = 0.771$	$p = 0.722$

6.5 Discussion

6.5.1 Feed and Feeding

Urea-molasses blocks offer a safe and convenient means of delivering urea (non-protein nitrogen), energy and minerals to livestock when nutritional supplementation is necessary to maximise utilisation of low quality roughage diets (Leng, 1986; Taiwo *et al.*, 1992; Sansoucy, 1995). In the present experiment supplementation with UMB increased the *ad libitum* intake of the basal oaten chaff diet (ie. UA CON and UA INF). This stimulatory effect of urea supplementation on feed intake is well documented (Stephenson *et al.*, 1981; Hogan, 1996b) and confirms results obtained previously (Chapters 4 and 5, respectively).

Pair fed sheep (UP) consumed slightly (5-10%) less chaff than their counterparts (ie. NU). This could be due to UMB consumption being compensated for by lower chaff consumption. However, this would be contradictory to the stimulatory effect of urea on chaff intake seen in *ad libitum* fed sheep and is therefore unlikely. When offered chaff and other roughages sheep preferentially select the leaf material over the coarser stems and stalks and this is what comprised a large proportion of the residues in the UP groups. To overcome this behaviour in future experiments involving pair feeding sheep with roughage diets additional basal ration (5-10%) should be given so that actual intakes will be the same both in quantity and quality of the basal diet.

As observed previously (Chapters 4 & 5) infection with parasites reduced actual chaff consumption in agreement with the well established anorectic effects of nematode parasites (see review by Symons, 1985). It is noteworthy, however, that when intake was expressed on a metabolic liveweight basis then reduction in intake with infection did occur in the NU sheep but not in the UA sheep. This suggests that the stimulatory effect of urea on intake counteracted the depressive effect of parasites on appetite. Similar results have been observed in sheep infected with *T. colubriformis* (van Houtert *et al.*, 1995a) and *H. contortus* (Datta *et al.*, 1996) when protein supplements were offered to sheep on low quality roughage based diets.

Australian manufacturers of urea-molasses block recommend consumption rates of 25-100g/head/day for young sheep at pasture (eg. Olsson Industries Ltd, Weanermaster Product Bulletin, 1992). In the present experiment consumption of block averaged around 60g/head/day except for the UA INF sheep which consumed less than 30g/head/day from week 8 of infection onwards. Since the UMB contained 15% urea the majority of the sheep consumed about 9g urea/day and the UA INF sheep 4.5g of urea/day.

6.5.2 Animal Productivity

At the rate of infection in the present experiment only 3 sheep developed clinical signs of haemonchosis and required euthanasia before the end of the experiment. In sheep populations there is substantial genetic variation in resistance to nematode parasites (Woolaston, 1996) and it is thought that the sheep which “died” in the present experiment were at the susceptible end of the resistance distribution curve.

Productivity of ruminant livestock is closely related to feed availability and as detailed above (section 6.5.1), UA sheep consumed more of the available diet than the NU sheep and cumulative weight gain and wool production reflects that increase. For CON sheep, UA sheep gained 30% more weight and produced 34% more wool than the NU and UP sheep while for INF sheep, UA sheep gained 44% more weight and produced 22% more wool than NU and UP sheep. Parasitism decreased the amount of weight gained and wool produced by 35% and 12% respectively for the UA sheep. Similarly infection produced a greater than 40% reduction in weight gain and 3% and 12% reduction in wool produced in the NU and UP sheep, respectively. These results accord with previous findings in sheep offered a low quality diet while infected with nematode parasites (see review by Barger, 1982).

6.5.3 Parasitology

Faecal egg counts increased to high levels over time in all groups. Similar egg count trajectories have been observed in previous experiments in sheep offered a low protein diet

during a trickle infection of *H. contortus* (Abbott *et al.*, 1988) or when the quantity of a moderate quality (14-17% crude protein) diet was restricted to less than maintenance levels (Roberts & Adams, 1990). Faecal egg counts tended to be higher in NU sheep, lower in UP sheep while UA sheep were intermediate. This result agrees with the previous observations of higher faecal egg counts in sheep not receiving a urea supplement and lower faecal egg counts in those sheep receiving a urea supplement (Chapters 4 and 5). The observed difference between the UA and UP sheep is more difficult to explain. Total worm numbers did not differ statistically between the treatments but the UA sheep had on average >15% more *H. contortus* than the UP sheep and since faecal egg counts closely correlate with worm numbers (Roberts & Swan, 1981) this may account for the difference.

The lack of difference between the treatment groups for worm numbers retrieved at slaughter was contrary to previous observations (Chapters 4 and 5) that *T. colubriformis* numbers were reduced in sheep offered urea supplements. Explanation of this anomaly may lie with the mode of delivery of the urea and the amounts consumed in each experiment. In the previous experiments sheep consumed the equivalent of 20-25g urea/day. In the present experiment UA INF sheep only consumed around 30g of UMB which would provide around 4.5g urea/day. Although this amount stimulated appetite and increased microbial protein production as indicated by urinary purine excretion (see section 6.4.5.4), it may be below that required for maximum efficiency of ruminal function (Kanjapruithipong, 1995). Therefore less microbial protein would have been available to the UA INF sheep than to those in the previous experiments which would decrease the impact of supplementation on the ability of the UA INF sheep to respond immunologically to infection.

6.5.4 Blood Analyses

Effects on blood constituents were dominated by the effects of the parasites, particularly *H. contortus*, on blood loss as exemplified by reduction in PCV and protein and albumin concentrations in plasma during the course of the experiment in all infected sheep whereas these parameters remained relatively stable in the uninfected groups. These results are in accordance with previous studies describing the pathophysiology of

infection with *H. contortus* (Abbott *et al.*, 1984, 1988) and *T. colubriformis* (Steel *et al.*, 1980; Coop *et al.*, 1976). The UP INF sheep had higher PCVs and plasma concentrations of albumin than the UA INF sheep while the NU sheep had the lowest levels and it appears that urea may help to maintain these blood parameters at higher levels during infection. The difference noted between the infected UA and UP sheep is contrary to expectation but this may be due to the slightly lower numbers of *H. contortus* in the latter since blood loss is closely correlated with worm numbers (Le Jambre, 1995).

Provision of UMB increased levels of plasma urea and on average doubled the concentration from 8mg/100ml to 16mg/100ml throughout the experiment but did not exceed 20mg/100ml attained in previous experiments (see Chapters 4 & 5). From week 9 of infection onwards, parasites tended to increase the plasma urea concentration in agreement with previous findings during infections with *H. contortus* (Rowe *et al.*, 1988) and *T. colubriformis* (Roseby, 1973; Roseby & Leng, 1974).

6.5.5 Metabolism Studies

Results of studies to determine the effects of nematode infection on apparent digestibility of feed are somewhat equivocal with some demonstrating a reduction (Barger, 1973; Sykes *et al.*, 1979; Steel *et al.*, 1980; Abbott *et al.*, 1985b; Rowe *et al.*, 1988) but others showing no effect (Roseby, 1973; Sykes & Coop, 1976; Poppi *et al.*, 1981; Abbott *et al.*, 1986b, 1988). In the present experiment dry matter (DMD) and organic matter (OMD) digestibility were reduced by 1-4% by infection. Apparent nitrogen digestibility (ND) was also reduced by infection in the NU and UA sheep but not in the UP sheep. The latter observation may be due to the relatively high proportion of UMB to chaff consumed by the UP INF sheep compared to their controls since the highly soluble urea-nitrogen in the UMB would be rapidly digested and absorbed whereas the chaff-nitrogen is not as readily available. As pointed out earlier (see Section 2.4.1.3) there can be considerable loss of endogenous protein into the gastrointestinal tract during nematode infection and the results for ND must be interpreted accordingly. Although ND was apparently decreased by infection endogenous nitrogen is indistinguishable from faecal nitrogen derived from undigested feed and this results in an underestimation of the

amount of nitrogen absorbed. Other authors have also discussed the problems of conventional digestibility studies in this regard (Steel *et al.*, 1980; Parkins & Holmes, 1989; Stevenson, 1989).

The availability of UMB substantially increased ND in both supplemented groups presumably due to the increased fermentative digestion of the basal diet in the presence of additional non-protein nitrogen provided by the urea (Leng *et al.*, 1995).

Water balance was affected by infection with decreased water intake and retention and increased urine volume in INF sheep compared to CON sheep. Intake was also affected by diet with UA sheep consuming more water than the other dietary groups. These results conflict with previous findings where water intake and retention were increased by infection with *H. contortus* (Abbott *et al.*, 1986b; Rowe *et al.*, 1988). In these latter studies moderately high numbers of larvae were given on one occasion at the start of experimentation and this may account for the anomalous findings. Another contributing factor could be that sheep were exposed to two species of parasites in the present study and not *H. contortus* alone.

In sheep given dry feeds water intake and feed intake are closely related and in this experiment considerable differences in feed intake were observed between the different treatments. Water intake closely mirrored feed intake and regression analysis showed there was a highly significant relationship between these two parameters ($r^2 = 0.594$, $p < 0.0005$). Those sheep consuming UMB had higher water intakes than the NU sheep which would be partly due to the influence of the salt content in the blocks (5%) and its effect on water requirements.

In the present study infection with parasites not only reduced N intake but increased the proportion of N appearing in the faeces and urine thereby lowering overall N retention by 36-43%. The gross efficiency of utilisation of absorbed N (N retained/N apparently digested) was lower in those sheep receiving UMB and was also reduced by parasitism. The availability of UMB increased N intake but only increased N retention in those groups with *ad libitum* access to feed (ie. UA sheep) whereas the gross efficiency of N

retention (N retained/N intake) did not differ between NU and UA sheep. The negative effects of nematode parasites on N retention have been previously observed in studies of *T. colubriformis* (Sykes & Coop, 1976; Roseby, 1973) and *H. contortus* (Abbott *et al.*, 1985b, 1986b; Rowe *et al.*, 1988). As discussed above caution must be used when interpreting these results because the effects of endogenous losses due to parasitism on faecal and urinary N outputs cannot be separated from losses due to decreased digestive function. However, it can be concluded that the overall effects of decreased intakes and increased losses on the N economy of the host would be substantial.

Estimates of the yield of rumen microbial protein can be based on the levels of purine derivatives in urine (Balcells *et al.*, 1992; Chen & Gomes, 1992; Kahn, 1996) and in the present study urinary purine concentrations were higher in UP than NU sheep while UA sheep showed a further increase. This finding agrees with previous studies where urea infusion led to increased urinary purine production in sheep offered low quality basal diets (Balcells *et al.*, 1993; Kanjanapruthipong, 1995). Although not statistically significant, infected sheep tended to have higher urinary purine levels than their respective uninfected control groups. Purines of endogenous origin are usually present in very low concentration in uninfected sheep (Chen *et al.*, 1990a) but in parasitised sheep losses of gastrointestinal cellular components are considerable, and although most would be salvaged by lower gastrointestinal absorption, endogenous purines may contribute to elevated urinary purine excretion. Further work in this area is obviously warranted.

6.5.5 Conclusion

As in the previous experiments presented in this thesis supplementation with urea yielded significant benefits to sheep infected with *T.colubriformis* and *H. contortus*. Consumption of the UMB supplement by parasitised animals led to lowered faecal egg counts, higher PCV and protein and albumin concentration in plasma and increased microbial protein availability for intestinal absorption as indicated by increased urinary purine production. Liveweight gain and wool production benefits were however, restricted to those groups which had access to *ad libitum* quantities of the basal ration of oaten chaff. It is concluded that the benefits derived from UMB supplements can only be

fully exploited if feed is freely available to meet the increased requirement for dry matter intake which is stimulated by increased efficiency of ruminal function after supplementation.

The difference of block intake in UA INF sheep (approx. 30g/day) compared to UA CON sheep (approx. 60g/day) in this experiment is reason for some concern for, if parasitised sheep do not obtain sufficient supplement to obtain maximum benefit while infected, the beneficial effects on host resilience and resistance will be reduced. It is, therefore, suggested that further work is necessary to ensure adequate supplement intake occurs but, in the interim, provision of “softer” blocks may prevent the occurrence of this problem. Target intakes for commercial supplements would also need to be adjusted accordingly.