Chapter 3

NUTRITIONAL SUPPLEMENTATION WITH UREA-MOLASSES BLOCKS FOR IMPROVED RUMINANT PRODUCTION: A REVIEW

3.1 Introduction

Ruminant livestock are an important source of human food and draught power throughout the developing nations of the world. Their importance lies in their ability to supply these essential resources without competing directly for the food requirements of the human population. The ruminant's ability to utilise low quality feed resources for production of meat and milk for human consumption therefore sets them apart from monogastric livestock which rely for sustenance on more readily digestible feed resources in direct competition with humans.

At present the most underutilised feed resources in the developing nations of Asia are the fibrous residues which are the by-products of crop cultivation and the native herbage understorey in tree crop plantations (Leng & Devendra, 1995). Both of these feed resources have low crude protein content, low digestibility and are often deficient in minerals (Minson, 1981; Leng & Devendra, 1995) but these problems can be overcome if the correct nutritional approaches are taken to ensure efficient rumen function.

This review briefly describes the processes by which the ruminant animal derives its sustenance from consumed plant material. Discussion of how these processes can be manipulated through supplementation of deficient nutrients to increase rumen efficiency, with reference to relevant recent research, will then lead to a description of how urea-molasses blocks can be used to satisfy needs for specific nutrients. This synopsis will

enable establishment of the nutritional principles that are to be exploited in the program of research embodied in this thesis.

3.2 Ruminant Nutrition

Of the feed consumed by the ruminant animal, only a small proportion can be directly digested and absorbed and the ruminant animal relies on the microbial population in the rumen to convert the carbohydrates in feed into useable nutritional resources by fermentative processes. The following sections briefly describe the organisms involved in rumen fermentation and the processes involved in transformation of fibrous plant material into the components which sustain ruminant maintenance and growth.

3.2.1 Rumen Organisms and their Interactions

The rumen provides an anaerobic environment which is maintained at constant temperature and pH and is ideal to support large populations of micro-organisms, the varieties of which are highly dependant on the type of feed consumed (Hungate 1966). Numerically the predominant micro-organisms in the rumen are the anaerobic bacteria which attach to the feed particles, remain free in the ruminal fluid or are associated with the epithelium (see Hungate, 1966). Ciliate and flagellate protozoa are the next most abundant and under some dietary conditions can equal the bacteria in total biomass (see Hungate, 1966; Leng, 1982a; Bird, 1991). Phytomycetous fungi (Bauchop, 1981; Soetanto, 1986; Orpin & Ho, 1991) are often present while mycoplasmas, viruses and bacteriophages can also be present but usually in lesser numbers (Preston & Leng, 1987).

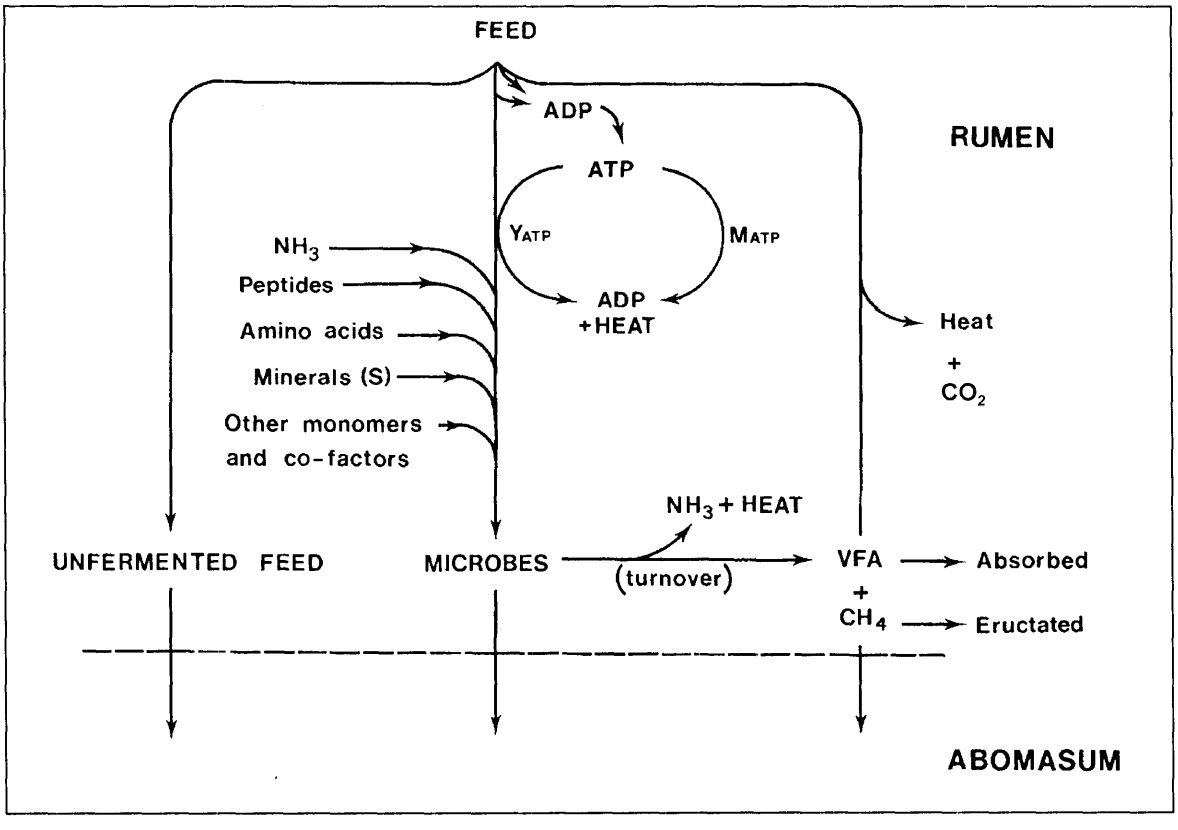
Single species of micro-organisms can rarely carry out the complex chemical processes required for complete digestion of feed materials (Cheng *et al.*, 1991). Therefore digestion requires significant interaction between those species involved in the digestion of a particular feed resource. Interaction may involve cooperation (commensalism, mutualism) or competition between different species and benefit one or both, or may involve parasitism or predation where one benefits at the expense of the other (Mackie, 1987; Theodorou & France, 1993). The overall outcome of these interactions is a well

adapted population of organisms which is dependent on the physical and chemical characteristics of the ecosystem provided by a particular feed resource.

3.2.2 Rumen Fermentation

As shown in Figure 3.1, volatile fatty acids (VFAs), carbon dioxide and methane are the end-products of fermentation of carbohydrate in the rumen whereas feed protein is either degraded to peptides, amino acids and eventually to NH_3, (all of which may be utilised for microbial growth) or passes through the rumen undegraded. Through the fermentation process energy is lost as both heat and methane. During the fermentation of carbohydrate to produce VFAs, ATP is generated and is the main source of energy for growth and maintenance of rumen micro-organisms. Details of the stoichiometry involved in energy metabolism and ATP release are provided in the literature and for sake of brevity will not be repeated here (see McDonald *et al.*, 1973; Czerkawski, 1986; Preston & Leng, 1987; Beever, 1993; Russell & Strobel, 1993).

Figure 3.1 The energetics of rumen function (adapted from Preston & Leng, 1987).



3.2.3 Nitrogen Transactions in the Rumen

As stated in the preceding section much of the protein in feed is degraded by rumen fermentation and the byproducts of this process are utilised in the production of microbial cells. Non-protein nitrogen sources in feed can also contribute to the ammonia pool in the rumen and are also utilised by rumen microbes. Undegraded protein and endogenous microbial protein passes to the lower gastrointestinal tract to be subjected to intestinal digestion and absorption or are passed in the faeces. As shown in Figure 3.2, ruminal nitrogen may also be derived from endogenous sources and ammonia may be absorbed directly from the rumen. Precise details of nitrogen metabolism in ruminants are available in the literature and will not be presented here (see Leng & Nolan, 1984; Preston & Leng, 1987; Nolan, 1993).

3.3 Efficiency of Microbial Growth in the Rumen

Since most ruminant animals rely on microbial protein as their major source of amino acids and peptides for growth and production, factors which determine the efficiency of microbial growth are of considerable importance in ruminant nutrition. The following section will discuss the key elements which affect microbial protein production efficiency or Y_{ATP} (g of microbial cell dry matter produced per mole of ATP available; Bauchop & Elsden, 1960).

3.3.1 Growth and Maintenance Requirements for ATP

Maximum efficiency of microbial cell production occurs when conditions are favourable for steady and vigorous growth since both growth and maintenance requirements for ATP influence Y_{ATP} (Hespell & Bryant, 1979; Preston & Leng, 1987). At low growth rates there will be a proportionally high maintenance requirement for ATP and conversely at high growth rates maintenance requirements will be proportionally low (Russell & Strobel, 1993). As greater amounts of intermediary carbohydrate fermentation products (eg. glucose from cellulose) are utilised for cell synthesis during vigorous microbial growth, less will be fermented to VFAs and therefore, Y_{ATP} increases (see Figure 3.3). When Y_{ATP} increases there will also be a decline in the amount of

Figure 3.2 A model of nitrogen transactions in the rumen. The ovals delineate the microbial cell wall and numbers adjacent to arrows refer to individual pathways as follows: 1, proteolysis by bacterial, protozoal and fungal proteases; 2, carrier-mediated peptide uptake across microbial cell walls; 3, peptidolysis; 4, amination/deamination; 5, protein synthesis; 6, microbial assimilation/excretion or equilibration of amino acids and ammonia; 7, protein not hydrolysed before efflux from rumen; 8, microbial protein efflux; 9, efflux of extracellular peptides and amino acids; 10, efflux of extracellular ammonia; 11, absorption of ammonia through rumen wall; 12, movement of endogenous urea through the rumen wall; 13, N compounds excreted by living cells and debris of lysed cells; 14, engulfment of proteinaceous particles by protozoa. (from Nolan, 1993)

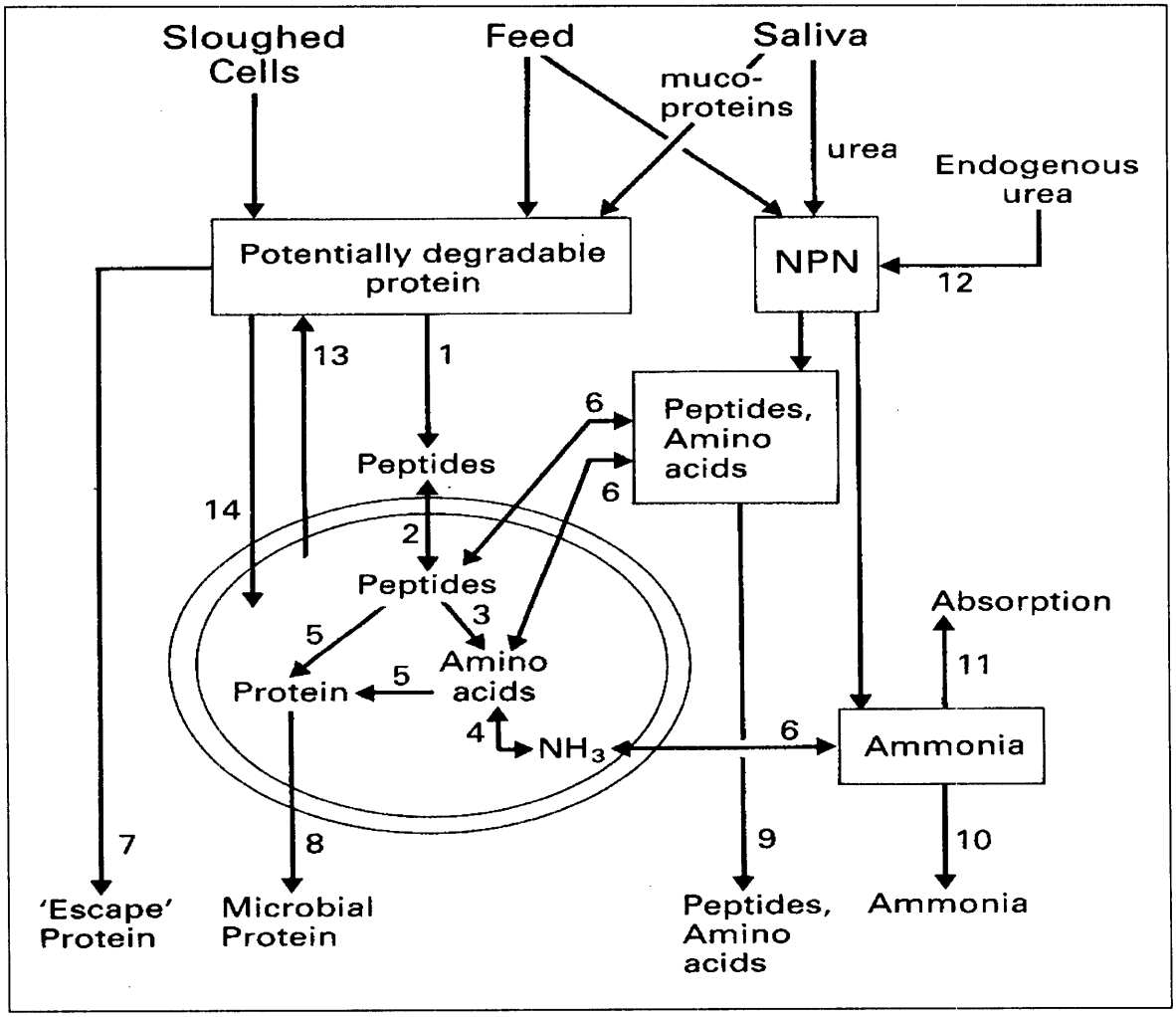
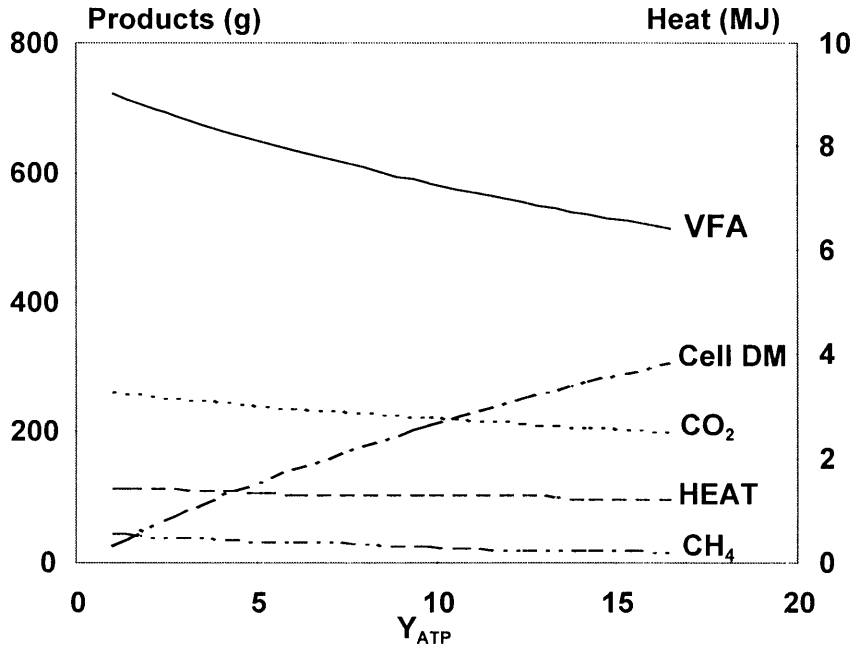


Figure 3.3 Relationship between microbial growth efficiency (Y_{ATP}) and the products of organic matter fermentation (VFA —— ; Cell Dry Matter -·-·-· ; CO_2 ····· ; Heat ----- ; CH_4 --·--).



energy lost to heat and methane which are byproducts of VFA production (Leng, 1991a). Microbial growth efficiency is a major factor in ensuring a high ratio of microbial cells produced relative to VFA (protein to energy or P/E) and is therefore, critical to efficient feed utilisation.

3.3.2 Availability of Fermentable Substrate

Growth of microbial populations is highly dependent on the characteristics and availability of the fermentable portion of the diet (Schwartz & Gilchrist, 1975; Cottle, 1991; Hogan, 1996a). Availability of fermentable dietary nitrogen, carbohydrates and minerals all contribute to efficiency of microbial growth and will be discussed in more detail.

3.3.2.1 Nitrogen

Ammonia is the major source of nitrogen for microbial protein synthesis and its concentration in ruminal fluid has a major impact on the efficiency of microbial growth. At low ammonia concentrations synthesis of bacterial amino acids is by a two step process involving glutamine synthetase and glutamate synthase which requires ATP and thereby contributes to lower Y_{ATP}. At higher ammonia concentrations, assimilation of ammonia into bacterial amino acids is thought to be via glutamate dehydrogenase which does not require ATP and hence Y_{ATP} is increased (Preston & Leng, 1987). Optimal concentrations of ammonia for microbial synthesis in the rumen have been shown to be between 50 and 80 mgN/l (Satter & Slyter, 1974). More recently, concentrations of 150-200 mgN/l have been suggested to be highly effective in increasing microbial synthesis in animals fed low quality fibrous diets (Leng & Nolan, 1984; Boniface *et al.*, 1986; Perdok & Leng, 1989).

Although ammonia is the major source of nitrogen for microbial protein synthesis the availability of other nitrogenous components (peptides and amino acids) can contribute to increasing Y_{ATP} (Maeng *et al.*, 1976). When this *in vitro* observation is applied *in vivo* the response appears to be highly dependent on the other components available in the ruminal fluid and in particular the amount of true protein in the available dietary substrate (Russell *et al.*, 1990).

3.3.2.2 Carbohydrates

Carbohydrates from different feed resources differ in their degradability and ability to be utilised by rumen microbes which are highly adapted to utilise particular types of carbohydrate (Hungate, 1966; Cottle, 1991). Maximum Y_{ATP} will not be achieved unless the amount and type of fermentable carbohydrate is sufficient to supply the needs of microbial synthesis and the rate of production and utilisation of fermented ammonia keeps pace with carbohydrate fermentation (Smith, 1979). If the activity of either component (or any other necessary growth factor) is reduced then Y_{ATP} will decrease to the detriment of the nutrition of the animal.

3.3.2.3 Minerals

Inadequate availability of sufficient minerals in the diet can limit microbial protein synthesis (Durand & Kawashima, 1979; Durand & Komisarczuk, 1988). The macronutrients phosphorus (Komisarczuk-Bony & Durand, 1991; Gunn & Ternouth, 1994) and sulphur (Komisarczuk-Bony & Durand, 1991; Preston & Leng, 1987) are of particular importance since deficiencies of these nutrients can severely inhibit microbial growth. In general, if the ruminant animal appears deficient in a particular mineral then it is highly likely that rumen microbes are also deficient and supplementation will be necessary.

3.3.3 Other Factors Affecting Y_{ATP}

Physical and chemical factors acting on the rumen can affect Y_{ATP}. Chemical changes (pH, osmolarity, buffering capacity, oxidation-reduction potential) within the rumen can affect microbial synthesis directly when different dietary substrates are utilised by the animal. Physical function of the rumen can affect Y_{ATP} through affecting the dilution rate of ruminal contents and the specific growth rate of rumen microbes. At increased dilution rates specific growth rate may increase and Y_{ATP} increases accordingly (Kennedy & Milligan, 1978) while in other instances increased dilution rate can have no effect (Leng, 1982b). The Y_{ATP} response is dependant on the dietary substrate and on the specific components of the microbial population in the rumen. Dilution effects are not normally a problem where low quality roughages are fed and rumen retention is sufficiently long for microbial growth to be maintained provided the nutritional substrates are adequate.

Large populations of protozoa in the rumen can impact negatively on Y_{ATP} since they prey on bacteria and are preferentially retained in the rumen (Preston & Leng, 1987). Studies have established that significant production increases occur in animals after removal of the protozoa population which is presumably due to increased microbial protein availability for intestinal absorption (see review by Bird, 1991).

3.4 Manipulation of Rumen Function with Non-Protein Nitrogen

In livestock rearing enterprises which are reliant on low quality roughage for the provision of digestible carbohydrate, the most critical nutritional deficiency is nitrogen. As stated above efficient rumen function is dependent on having sufficient degradable nitrogen in the diet to provide adequate ruminal ammonia for microbial growth. In situations where deficiency occurs provision of additional nitrogen can have a dramatic impact on digestibility and productivity from low quality diets. Protein sources can provide additional nitrogen through rumen fermentation but unfortunately in most developing countries supplies are inadequate and costly. Non-protein nitrogen supplements are less expensive, readily available and can assist the animal to meet its nutritional requirements from low quality roughages.

3.4.1 Non-Protein Nitrogen Supplements

Non-protein nitrogen (NPN) has long been identified as being able to provide the nitrogen requirements for microbial growth in ruminants (Stangel, 1967). A broad range of compounds can provide NPN (Hendrickx, 1967; Doyle, 1987) but since the 1920's most research has concentrated on the use of urea as a NPN source since it is relatively inexpensive and readily available in most agricultural situations. In animals offered low quality diets supplementation with urea increases microbial decomposition of feed carbohydrates which results in increased feed intake and increased protein availability for intestinal absorption (Bruggemann & Giesecke, 1967; Kempton & Leng, 1979; Boniface *et al.*, 1986; Preston & Leng, 1987). Delivery of urea supplements to grazing animals has been successfully attempted by many means including in drinking water (Stephenson *et al.*, 1981), in molasses mixtures (Nolan *et al.*, 1975; Langlands & Bowles, 1976; Mulholland & Coombe, 1979; Coombe & Mulholland 1983) and in feed supplement blocks (Leng *et al.*, 1991; Taiwo *et al.*, 1992). The latter means of delivery appears most practical for widespread use and will be discussed further below (see Section 3.5).

3.4.2 Urea Supplementation and Efficiency of Microbial Growth

When urea is fed to the ruminant animal it is rapidly metabolised to ammonia in the ruminal fluid (Schwartz, 1967). Numerous studies have attempted to determine the optimal concentration of ammonia for microbial synthesis in the rumen (see Section

3.3.2.1). Recent studies by Balcells *et al.* (1993) established that by increasing amounts of urea (3, 6, 9 & 12 g/d; giving rumen ammonia levels of 50-110mgN/l) infused into the rumen of sheep, increased concentrations of allantoin were excreted in the urine. Prior studies had established that allantoin excretion could be used to determine the response of microbial yield to changes in rumen-degradable N supply (Chen *et al.*, 1990a,b; Balcells *et al.* 1991). Therefore, with increased urea supplementation more microbial protein became available to the sheep and it was suggested that most of this increase was due to enhanced feed intake stimulated by an improved rate of rumen fermentation (Balcells *et al.*, 1993).

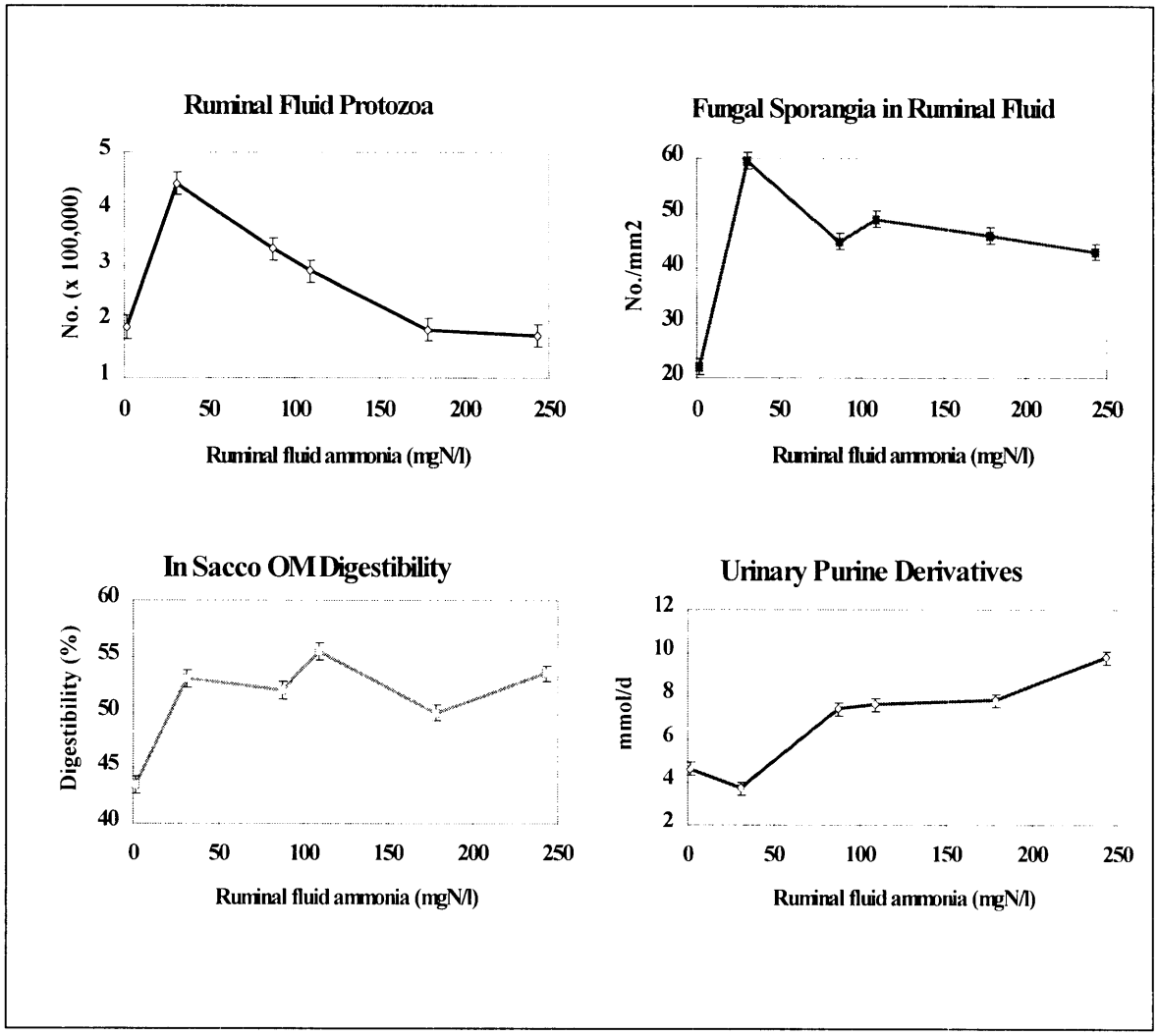
Further studies conducted with similar methodology but restricting feed intake to 800g/d showed that the greater the rumen ammonia concentration the greater the influence on microbial protein production (Kanjanapruthipong, 1995). As shown in Figure 3.4 with increased rates of urea infusion digestibility increased, protozoa numbers increased and then declined, fungal sporangia numbers plateaued while urinary purine production increased as rumen ammonia levels increased. It was suggested that, at the higher concentrations of ammonia, increased microbial protein availability resulted from decreased turnover of microbial cells within the rumen due to lower numbers of protozoa (Kanjanapruthipong, 1995). From this study it can be concluded that for situations where low quality forages are to be utilised that supplementation of NPN to give a ruminal fluid ammonia concentration of at least 200mgN/l would be appropriate.

3.5 Urea-Molasses Blocks (UMB)

3.5.1 Composition of UMB

One means of delivering NPN supplements in areas where low quality feeds are the primary feed resource is through feed supplement blocks. These blocks have varied compositions (Kunju, 1986; Sansoucy, 1986; Leng, 1986; Leng *et al.*, 1991; Taiwo *et al.*, 1992; Hadjipanyiotou *et al.*, 1993; Sansoucy, 1995) but the primary ingredients are urea to provide NPN and molasses to provide energy and attract the animal to eat the supplement. Molasses is also a good source of various macro and micro-minerals and can be further

Figure 3.4 Effects of levels of ruminal fluid ammonia obtained through urea supplementation on ruminal fluid protozoa numbers, fungal sporangia, *in sacco* digestibility and urinary excretion of purine derivatives in sheep fed a low protein roughage-based diet (adapted from Kanajanapruthipong, 1995).



fortified with minerals appropriate to the particular production system during the preparation procedure (Leng, 1986; Kunju, 1986; Preston & Leng, 1987).

3.5.2 Application of UMB

Urea-molasses blocks have been shown to increase feed intake and improve the digestibility of roughage-based diets (Krebs & Leng, 1984; Kunju, 1986; Sudana & Leng, 1986; Soetanto, 1986; Soetanto *et al.*, 1987). Field trials have established that access to UMB results in productivity increases in small and large ruminant livestock in the

developing nations of Asia and the Pacific Islands (Kunju, 1986; Hendratno *et al.*, 1991; Leng *et al.*, 1991; Manueli *et al.*, 1995; Salman, 1996) and in Australia (Butler *et al.*, 1994). It is expected that further use of UMB will occur throughout the developing nations of the world as the human population expands and demand increases for ruminant products from the limited feed resources available, since UMB enables rumen function and ruminant productivity to be optimised cost effectively (Leng *et al.*, 1991).

3.5.3 Consumption of UMB

Variability of consumption of block supplements is commonly observed when individual animals within a flock or herd are compared (Lobato & Pearce, 1980; Lobato *et al.*, 1980; Kendall *et al.*, 1983) and this can cause some concern for livestock producers who desire uniform productivity from all animals. Factors such as block constituents, particularly nitrogen content, and hardness can affect rate of consumption but of equal importance are animal-related factors such as exposure time, previous experience and social behaviour and factors relating to pasture quality and availability (see review by Bowman & Sowell, 1997). It is desirable for the majority of animals to consume sufficient block to compensate for deficiencies in the available diet and therefore blocks should be tailor made to the local environment and animals conditioned to consume them. Inadequate block intake does not appear to be as great a problem in developing nation livestock production systems where supplements are usually readily consumed. Over consumption should also be avoided to reduce the risk of urea toxicity (Preston & Leng, 1987) and to maintain supplementation cost at an economic level.

3.6 Conclusion

Rumen function can be dramatically changed by optimising the rumen environment with mineral and/or NPN supplements (Leng, 1991a,b). Recent research has shown that substantially improved efficiency of utilisation of a low quality fibrous feed base can be achieved by increasing rumen ammonia levels to greater than 200mgN/l with urea supplementation (Kanjanapruthipong, 1995). The research in this thesis will determine the impact of similar levels of urea supplementation on the ability of sheep to resist the effects of gastrointestinal nematode parasites by adding the urea to a low quality roughage diet and

measuring productivity and pathological responses. Successful completion of this assessment will lead to application of the same nutritional principles by delivery of the urea through supplements, such as UMB, which are more relevant to field application. Through these studies it is anticipated that soundly based advice can then be offered on the potential role for UMB in nematode parasite control programs currently being developed in the countries collaborating in ACIAR Project 9132.

Chapter 4

THE EFFECT OF UREA SUPPLEMENTATION ON NEMATODE PARASITISM OF SHEEP FED A ROUGHAGE BASED DIET.

4.1 Abstract

Seventy-two 5-month-old Merino wethers were individually confined and fed oaten chaff and essential minerals for 6 weeks. The sheep were then divided into 2 groups which were fed untreated chaff (NU) or chaff containing 3% Urea (U). Within each group 4 subgroups were orally infected with either 200 H.contortus (H), 1000 T. colubriformis (T) or both species (HT) thrice weekly or remained uninfected (C). Weight gain, wool production, parasite burden, blood parameters and rumen function were measured over a 19 week period to compare treatments.

Feed intake was affected by diet with U sheep consuming more than NU sheep and over time this difference became more pronounced. Within dietary groups uninfected animals consumed more feed than infected animals and on the NU diet HT sheep ate considerably less than all other groups. Sheep on the U diet gained more weight than those on NU and parasitised sheep gained less weight than C sheep with NU HT sheep gaining least of all. Wool production was also affected by diet with U sheep producing more wool of higher fibre diameter than NU sheep. Faecal egg counts were lower in U sheep for H and HT sheep but no difference was observed for T sheep. The U diet significantly reduced worm numbers when all subgroups were considered but there was no significant interaction of diet and parasitism. The U diet caused a significant elevation of plasma urea concentration and rumen NH_3-N concentration and parasitism caused significant changes in blood parameters indicative of blood loss but there was no interaction of diet and parasitism for the rumen function and blood parameters measured.

Supplementation with urea reduced the effects of parasitic infection by increasing weight gain and wool production and reducing faecal egg output and parasite burden in young sheep on low quality roughage diets. This boost to productivity can be partly attributed to a greater intake of the basal diet, presumably due to enhanced digestibility, but more importantly to increased microbial protein availability arising from enhanced rumen NH_3-N levels and their effect on microbial fermentation.

4.2 Introduction

Gastrointestinal nematode parasitism of sheep results in reduced growth and productivity and sometimes increased mortality due to the pathogenic effects of infection (Barger, 1982). Once the level of parasite challenge is above a threshold determined by breed, age and sex of the host, infection most importantly causes a reduction in voluntary feed intake and increases the losses of endogenous protein via excreta. Infection also causes a diversion of nutritional resources from synthesis of muscle, bone and fibre to the synthesis of specific proteins for repair and replacement of lost components and for immunological reaction to infection.

Several reviews have concluded that sheep offered a high plane of nutrition are better able to withstand the detrimental effects of nematode parasite infection than those less adequately nourished (see Steel, 1978; Parkins & Holmes, 1989; Poppi *et al.*, 1990; Coop & Holmes, 1996). It has been shown that an adequate supply of dietary protein enables infected sheep to withstand the pathophysiological consequences of infection through compensating for parasite-induced protein deficiency resulting from increased endogenous protein loss into the gastrointestinal tract. Improved dietary protein supply improves the capacity of infected sheep to mount an effective immunological response to infection and enhances the onset of parasite rejection (Steel *et al.*, 1982; Abbott *et al.*, 1988; Roberts & Adams, 1990). More recently it has been demonstrated that the greatest benefits are derived from the post-duodenal provision of protein either by post-ruminal infusion (Bown *et al.*, 1986) or by feeding protein which is not degraded by rumen microbial activity (van Houtert et al., 1995a; Smith *et al.*, 1996).

This experiment investigates whether such a response is achievable in sheep being fed low quality roughage diets through supplementation with urea as a source of non-protein-nitrogen (NPN). Supplementation with urea has been shown to increase digestibility of fibre by the rumen microflora and increase availability of microbial protein for absorption in the small intestine (Kanjanapruthipong, 1995). Published research into the effects of nutritional supplementation on parasitised hosts has concentrated on single species infections or combinations of species particular to temperate climates. This experiment focuses on the predominant species involved in parasitic disease of small ruminants in humid tropical and sub-tropical environments (ie. *Haemonchus* spp. and *Trichostrongylus* spp.) both as monospecific infections as well as concurrent infections. Previous research has shown that during infection with more than one species, effects are minimally additive and frequently multiplicative.

4.3 Materials and Methods

4.3.1 Experimental Procedure

Seventy-two wether Merino sheep aged 5 months were confined in individual pens and allowed 6 weeks to adapt to a basal diet of oaten chaff and essential minerals (7g $CaCO_3$, 1g elemental sulphur and 1g Pfizer 422 mineral premix daily). 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. After the 6 week adaptation period the sheep were divided into 8 equal groups according to liveweight (range 15.5-27.5 kg; average 20.1kg) and feed intake over the preceding week. Half the groups were then given a diet containing 1.5% urea for a period of 2 weeks after which the urea content was increased to 3% (to give rumen ammonia > 200 mgN/l; see section 3.4.2) and a further 2 weeks allowed for the rumen to equilibrate. Urea treated chaff was prepared by dissolving the urea in a minimum amount of hot water and spraying the solution onto the chaff during continual mixing with a mechanical mixer. The other 4 groups received untreated chaff. All chaff used during experimentation was from a single batch of oaten hay. Dietary treatments were given for the full 19 weeks of parasite infection.

The groups were allocated to the parasite treatments described in Table 4.1 and infected for 17 weeks before being slaughtered at week 19 to enable estimation of total worm burdens. Liveweights were recorded weekly from the time of allocation into treatment groups.

Table 4.1 Parasite and dietary treatments for sheep in Experiment 1.

Group	Diet	Parasite challenge (3 times per week)
NU Con	No Urea	None
NU H	No Urea	200 *Haemonchus contortus*
NU T	No Urea	1000 *Trichostrongylus colubriformis*
NU H+T	No Urea	200 *H.contortus* and 1000 *T.colubriformis*
U Con	3% Urea	None
U H	3% Urea	200 *H.contortus*
U T	3% Urea	1000 *T.colubriformis*
U H+T	3% Urea	200 *H.contortus* and 1000 *T.colubriformis*

4.3.2 Parasitology

Strains of parasites used were derived from cultures originating at CSIRO McMaster Laboratory, Glebe 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 from individual sheep were taken weekly for egg count estimation using the modified McMaster technique (Whitlock, 1948).

Total worm burdens of parasitised groups were estimated after slaughter at week 19 of infection. Prior to slaughter feed was withheld for 24 hours. The sheep were stunned with a captive bolt pistol and exsanguinated. Abomasa, 10m of small intestine distal to the pylorus or both sections were retrieved from those sheep with *H. contortus, T. colubriformis* and mixed species infections, respectively. 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% (v/v) 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 and cut into small sections and incubated for 2hours at 37°C with approximately 250ml of an acid-pepsin solution containing 10g pepsin-A (1:2500) and 17g 12M HCl per litre of H_2O. The remaining tissue was washed and the washings made up to 1litre and a 2.5% sub-sample preserved in 5% (v/v) formaldehyde solution for later enumeration of worms. To compare the size of *H.contortus* from the groups infected with this species 100 intact adults of each sex were collected at random from aliquoted subsamples of preserved washings, dried at 65°C for 24 hours and then weighed.

4.3.3 Blood parameters

Each 3 weeks blood samples were collected into 10ml vacutainers (Becton Dickinson Ltd, Australia) containing K-EDTA for haematological assessment. Fresh whole blood was mixed thoroughly and estimates of packed cell volume (PCV), red blood cells (RBC), white blood cells (WBC), mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets (PLT) and haemoglobin (HB) concentration made using a Model S880 Coulter Counter (Coulter Electronics, Brookvale, NSW). An aliquot of 100µl of mixed whole blood was added to 900µl of Carpentiers fixative and samples stored for later eosinophil (EOS) counts using a haemocytometer (Dawkins *et al.*, 1989). 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 basal feed ration, prior to the introduction to the urea diet, and at weeks 1 and 10 of the infection period. Collection of dye-banded staples was carried out at week 18 and all sheep were shorn prior to slaughter at week 19. Total fleece weights were recorded and estimates made of the quantity of wool produced in each of the 4 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 rumen liquor were collected 4 h after feeding from each sheep by stomach tube on 2 occasions, ie. prior to commencing parasite infections and at the end of the experimental period. Rumen liquor was prepared for assessment of levels of rumen ammonia and volatile fatty acids (VFA) by acidification with 5 drops 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 NH_3-N using an Autoanalyser (Technicon Equipment Co., New Jersey, USA) according to the method of Crooke & Simpson (1971) modified by Bietz (1974). The VFA concentration and molar proportions of acetic, proprionic, butyric, isobutyric, isovaleric and valeric acids were determined by gas liquid chromatography (Model 427, Packard Instrument Co., USA) according to the method of Erwin *et al.* (1961) and using iso-caproic acid as the internal standard (Geissler *et al.*, 1976).

For enumeration of rumen protozoa, 4ml of ruminal fluid was added to 16ml of 4% formol saline (11.1% formalin 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 basal diet chaff (NU) and of urea-treated chaff (U) were collected at regular intervals for estimation of crude protein content using the micro-Kjeldahl method (Crooke & Simpson, 1971).

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, rumen parameters). 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 squareroot transformation was carried out prior to analysis in order to stabilise variance within groups.

4.4 Results

4.4.1 Feed

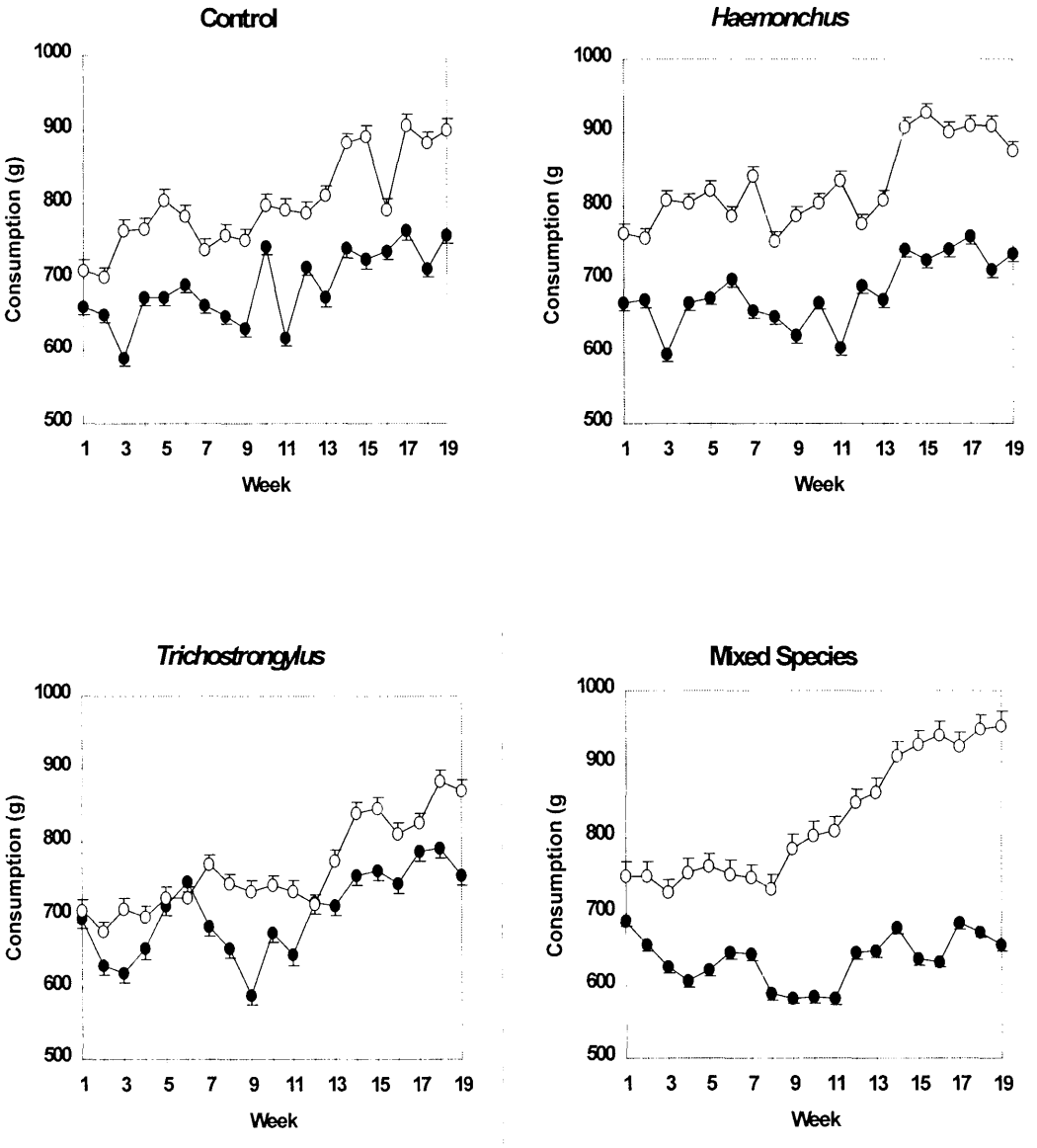
4.4.1.1 Feed analysis

The basal ration of oaten chaff had a N concentration of 0.80 ± 0.02% (Mean ± SE) indicative of a crude protein (CP) content of around 5.0% (CP = 6.25 X N). Analysis of U chaff gave a N concentration of 2.14 ± 0.03% (13.4% CP) consistent with a minimal loss of urea during the treatment process.

4.4.1.2 Feed intake

Mean daily intakes of chaff for each treatment group are presented in Fig. 4.1. Sheep offered the U diet ate significantly more chaff ($p<0.0005$) than those offered the NU diet. There was no significant effect of infection on feed intake nor was there an interaction between diet X infection. Over time consumption of feed changed significantly ($p<0.0005$) and there was a significant time X diet interaction ($p<0.0005$) which relates to the feed consumption of the U diet groups showing a greater increase over time than the NU diet groups. There was no time X infection interaction but there was a significant time X diet X infection interaction ($p<0.0005$) indicating that the change in feed intake over time was not consistent in all dietary and infection treatment groups. This is shown when infected groups are compared to their respective controls with the mixed species group showing significantly lower feed intake ($p=0.001$) for the NU diet but, on the U diet, the mixed infection group consumed more than other infection treatments ($p=0.048$).

Figure 4.1 Feed intake (mean ± SE) for parasite treatment groups of young sheep offered oaten chaff unsupplemented (-●-) or with 3% urea (-O-).



4.4.2 Animal Productivity

4.4.2.1 Liveweight gain

Fig. 4.2 shows the cumulative liveweight gains for the treatment groups over time. Since in the period prior to week 4 of infection there was a considerable number of animals showing negative weight gains statistical analyses have been performed only for the period from week 4 to 18 of infection. During this period there was a significant effect of diet ($p=0.001$) but no effect of infection or diet X infection interaction. Repeated measures ANOVA indicated a significant change in squareroot liveweight gain over time ($p<0.0005$), a significant time X diet interaction ($p<0.0005$), a significant time X infection interaction ($p<0.0005$) and a significant time X diet X infection interaction ($p=0.001$) which indicates that sheep grew at different rates depending on the diet offered and that infection affected the rate of growth on those diets. For the NU diet, comparison of individual treatment groups revealed that the parasitised groups grew slower than the uninfected control and that those sheep harbouring mixed species infections grew at a slower rate than those with single species infections. For the U diet comparison of individual treatment groups revealed that the parasitised groups grew slower than the uninfected control group.

4.4.2.2 Wool growth and fibre diameter

Estimates of average daily wool production for each experimental group for each of the periods between application of dye bands are shown in Table 4.2. For Period 1 (0-9 weeks of infection) there was a significant effect of diet with the U diet groups producing more wool than the groups receiving the NU diet. An effect of infection was also observed for this period with the mixed infection group on the NU diet producing considerably less wool than other groups on the same diet. During Period 2 (10-18 weeks of infection) there was still a significant effect of diet but there was no infection effect. No significant interaction of diet X infection was observed during either period.

Results of fibre diameter analysis with the pre-experimental measurement used as a covariate are shown in Table 4.3. There was a significant effect of diet during both measurement periods with animals receiving the U diet producing wool of greater fibre

Figure 4.2 Liveweight gain (mean ± SE) for parasite treatment groups of young sheep offered unsupplemented oaten chaff (-●-) or oaten chaff with 3% urea (-O-).

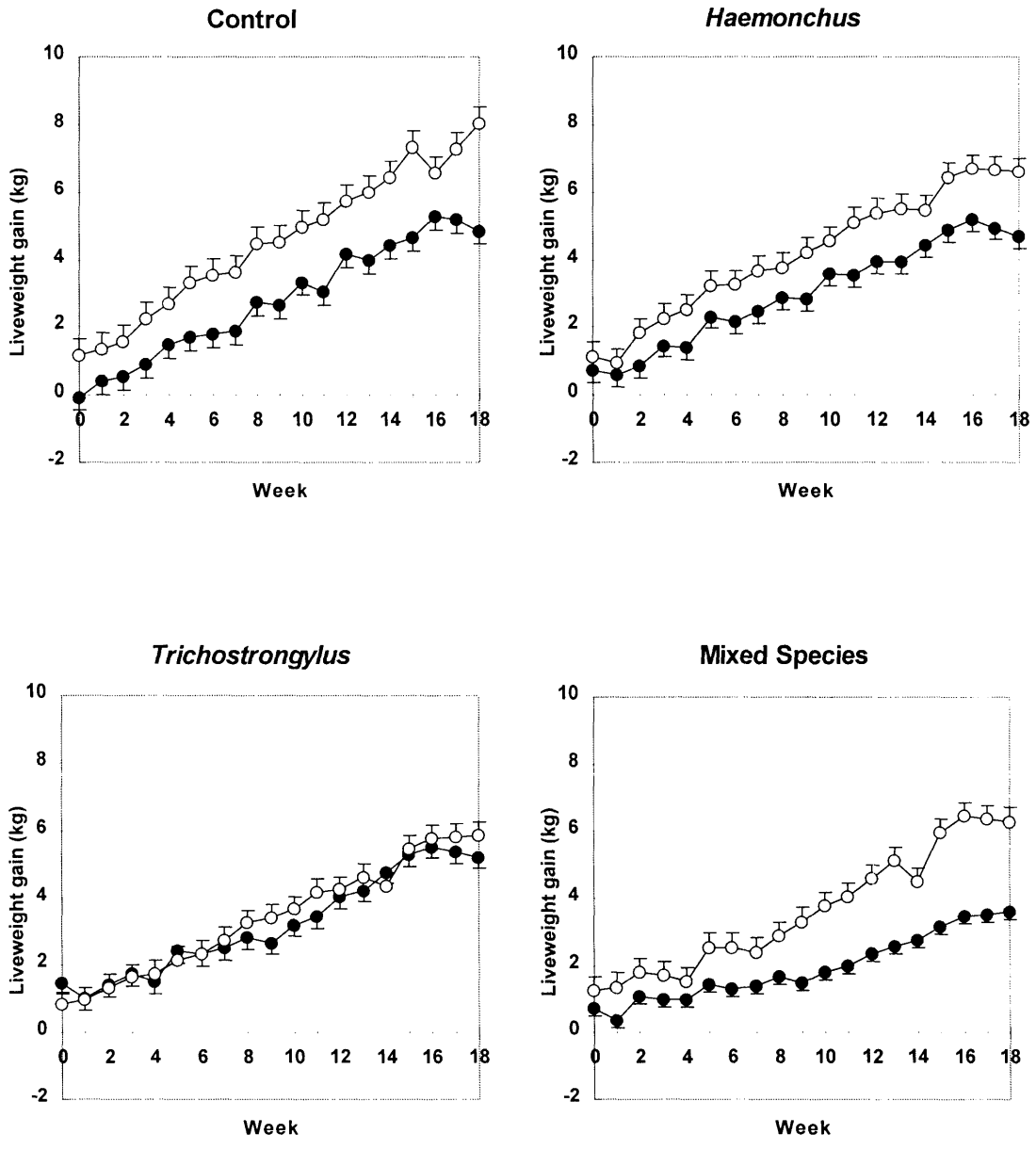


Table 4.2 Wool production (g/head/day; mean ± SE) of sheep offered oaten chaff unsupplemented (NU) or 3% urea (U) and infected with *H. contortus* (H), *T.colubriformis* (T), both species (H+T) or remaining uninfected (Con).

Group	Pre-experiment	Week 0-9	Week 10-18
NU Con	6.4 ± 0.5	4.1 ± 0.3	4.2 ± 0.4
NU H	6.1 ± 0.4	4.2 ± 0.2	4.1 ± 0.3
NU T	6.4 ± 0.5	4.3 ± 0.2	4.5 ± 0.3
NU H+T	6.7 ± 0.6	3.6 ± 0.3	4.0 ± 0.3
U Con	6.2 ± 0.2	5.0 ± 0.5	5.5 ± 0.3
U H	6.3 ± 0.3	5.3 ± 0.3	6.1 ± 0.3
U T	6.7 ± 0.4	4.8 ± 0.2	5.5 ± 0.5
U H+T	7.1 ± 0.2	4.7 ± 0.3	5.9 ± 0.5
Statistical Effects			
Diet (D)	p=0.637	p<0.0005	p<0.0005
Infection (I)	p=0.299	p=0.049	p=0.803
D X I	p=0.888	p=0.663	p=0.602
Covariate	--------	p=0.004	p=0.012

diameter than those offered the NU diet. When diet and infection effects were analysed across both time periods there was a significant effect of diet, a significant time X diet interaction and a significant time X diet X infection interaction while other comparisons were not significant.

4.4.3 Parasitology

4.4.3.1 Faecal egg counts

Faecal egg counts are presented as geometric means for treatment groups over time in Fig. 4.3. Repeated measures ANOVA of log (n + 1) transformed faecal egg counts showed significant differences between the diets for the *Haemonchus* (Week 6 to Week 18: p=0.041) and mixed species (Week 3 to Week 18: p=0.005) infections but no significant difference for the *Trichostrongylus* infected groups. For the mixed species infections there was also a significant time X diet interaction (p=0.020).

Table 4.3 Fibre diameter of wool produced (μm; mean ± SE) of sheep offered oaten chaff unsupplemented(NU) or with 3% urea (U) and infected with *H. contortus* (H), *T.colubriformis* (T), both species (H+T) or remaining uninfected (Con).

Group	Pre-experiment	Week 0-9	Week 10-18
NU Con	14.0 ± 0.3	13.6 ± 0.4	14.0 ± 0.4
NU H	14.1 ± 0.4	13.8 ± 0.3	14.0 ± 0.3
NU T	13.8 ± 0.3	13.3 ± 0.2	14.0 ± 0.3
NU H+T	14.0 ± 0.5	13.6 ± 0.2	13.8 ± 0.2
U Con	14.5 ± 0.5	14.6 ± 0.4	15.2 ± 0.4
U H	14.5 ± 0.4	14.7 ± 0.4	15.7 ± 0.3
U T	14.8 ± 0.5	14.8 ± 0.6	15.3 ± 0.6
U H+T	15.1 ± 0.6	14.2 ± 0.3	15.3 ± 0.3
Statistical Effects			
Diet (D)	p = 0.230	p = 0.005	p = 0.000
Infection (I)	p = 0.922	p = 0.408	p = 0.647
D X I	p = 0.801	p = 0.467	p = 0.769
Covariate	------	p < 0.0005	p < 0.0005

4.4.3.2 Worm counts

Details of worm counts for each species and the total are presented in Table 4.4. For *Haemonchus* infected sheep no significant difference between infection type or diet groups were observed for numbers of either sex or for total numbers. For *Trichostrongylus* infected animals there were significantly less male worms in those sheep offered the U diet and there was a suggestion of a difference between diets for female and total numbers. There was a significant difference in larvae (L_4) numbers between diets with those animals offered the U diet having least larvae. There was a significant difference in larvae (L_4) numbers between infection groups with those receiving *Trichostrongylus* alone having very few larvae. There was also a significant interaction of diet X infection for larvae numbers. Comparison of total worms across all treatments showed a significant effect of diet, an effect of infection but no diet X infection interaction.

Figure 4.3 Faecal egg counts (geometric mean ± SE) of groups of young sheep offered oaten chaff unsupplemented (-●-) or with 3% urea (-O-).

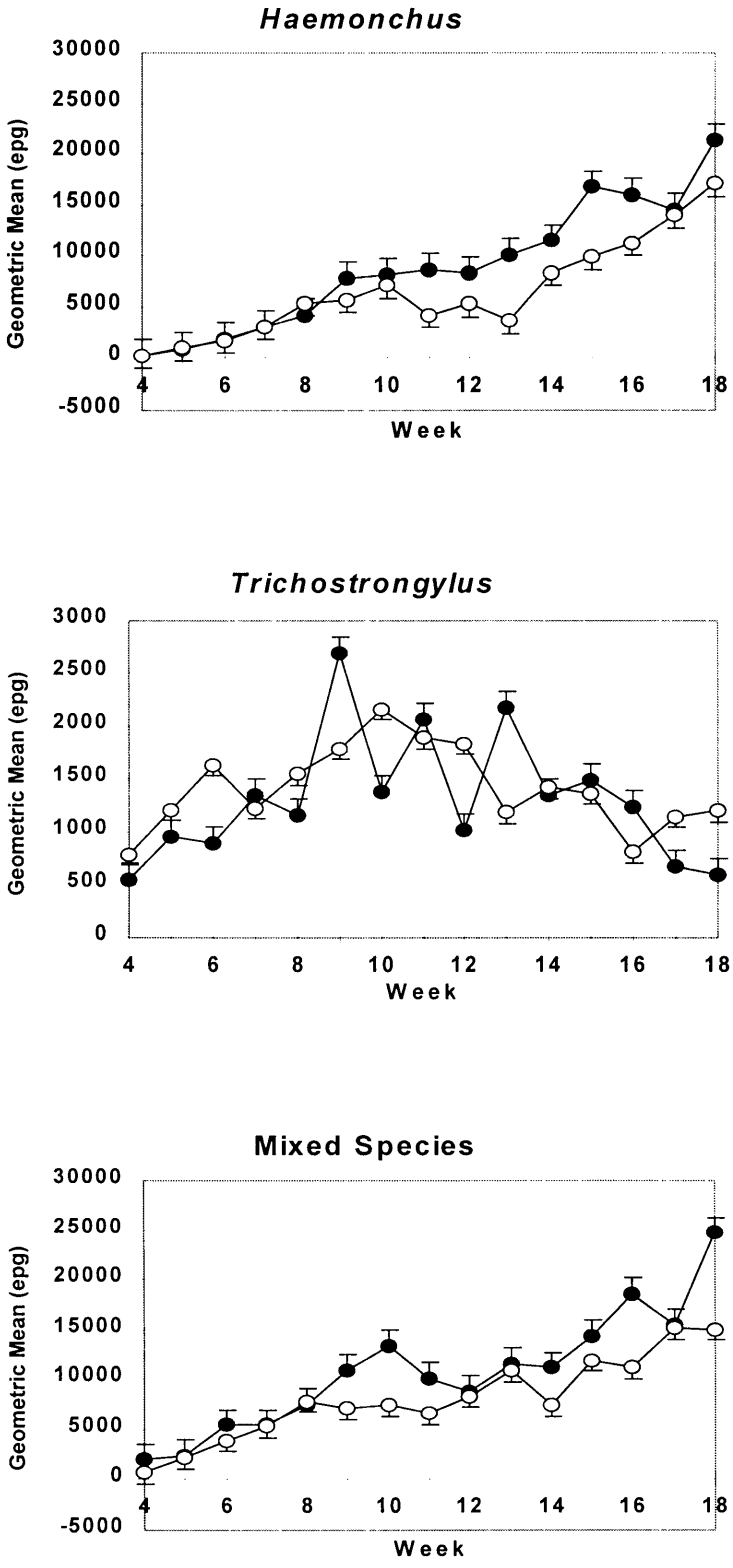


Table 4.4 Worm counts (mean ± SE) of young sheep offered oaten chaff unsupplemented (NU) or with 3% urea (U) and infected with *Haemonchus* (H), *Trichostrongylus* (T) or both species (H+T).

Group	H Male	H Female	H All	T Male	T Female	T All	L_4	Total
NU H	1684 ± 204	1813 ± 280	3498 ± 462	---	---	---	271 ± 60	3769 ± 462
NU T	---	---	---	3821 ± 634	4101 ± 723	7923 ± 1344	6 ± 6	7929 ± 1347
NU H+T	1742 ± 104	1917 ± 101	3659 ± 171	3372 ± 363	3569 ± 329	6941 ± 673	434 ± 64	11599 ± 533
U H	1729 ± 249	1739 ± 279	3468 ± 515	---	---	---	238 ± 56	3706 ± 534
U T	---	---	---	2713 ± 434	2861 ± 477	5574 ± 895	0 ± 0	5574 ± 895
U H+T	1850 ± 254	1783 ± 224	3633 ± 470	2528 ± 421	2963 ± 550	5491 ± 948	176 ± 47	9818 ± 962
Statistics								
Diet (D)	p = 0.721	p = 0.658	p = 0.949	p = 0.047	p = 0.097	p = 0.065	p = 0.019	p = 0.044
Infection (I)	p = 0.675	p = 0.753	p = 0.705	p = 0.507	p = 0.692	p = 0.595	p < 0.0005	p < 0.0005
D X I	p = 0.882	p = 0.900	p = 0.996	p = 0.782	p = 0.560	p = 0.654	p = 0.026	p = 0.358

The presence of *Ostertagia* spp. was noted in animals with mixed species infection and was the result of low level contamination of the *Trichostrongylus* culture used. Numbers of *Ostertagia* at slaughter (271 ± 59; mean ± SE) were, however, well below the pathogenic effect level.

4.4.3.3 Worm size

Dry weights of adult *Haemonchus* recovered from sheep with monospecific and mixed infections are presented in Table 4.5. There were no significant differences between diet or infection treatments but there was a significant interaction between these factors for both male and female worms. Those worms derived from monospecific infections on the NU diet were larger than those from sheep receiving the U diet. The reverse was the case for worms recovered from sheep receiving the mixed species infection.

Table 4.5 Dried weight of 100 *H. contortus* adults (g; mean ± SE) from sheep offered oaten chaff unsupplemented (NU) or with 3% urea (U) and infected with *H. contortus* (H) or *H. contortus* and *T. colubriformis* (H+T).

Group	**Male**	**Female**
NU H	0.015 ± 0.001	0.035 ± 0.002
NU H+T	0.012 ± 0.001	0.026 ± 0.002
U H	0.013 ± 0.001	0.031 ± 0.001
U H+T	0.014 + 0.001	0.035 + 0.002
Statistics		
Diet (D)	$p = 0.809$	$p = 0.155$
Infection (I)	$p = 0.281$	$p = 0.109$
D X I	$p = 0.015$	$p < 0.0005$

4.4.4 Blood parameters

Results of analyses of whole blood and plasma carried out during experimentation are presented as figures for each parameter measured and summary tables of results of repeated measures statistical comparisons. Details of means and standard errors for individual treatment groups from each collection for each assessed parameter readers are given in Appendix Tables A4.1 to A4.12.

4.4.4.1 Haematological changes

Table 4.6 gives a summary of the results of repeated measures statistical comparisons for the haematological parameters measured. For PCV, RBC and haemoglobin concentration effect of infection, time and time X infection interaction were all highly significant while for RBC and haemoglobin the effect of diet was approaching significance. All other effects were not significant for these three parameters. The major factor underlying this result was the effect of *Haemonchus* and mixed species infection which reduced levels of all three parameters as the experiment progressed (see Figures 4.4, 4.5 and 4.6, respectively). Conversely increased levels of RBC and haemoglobin were observed in sheep offered the U diet compared to the NU diet.

Numbers of WBC were not affected by diet or infection and there was no diet X infection interaction. There were significant effects of time, time X diet, and time X infection but no interaction of time X diet X infection. As Figure 4.7 shows there was a decline in WBC over time in the *Haemonchus* and mixed species infected animals and there was a tendency for the sheep offered the U diet to have higher WBC counts than those offered the NU diet for those groups not infected with *Haemonchus*.

There was a significant effect of infection on squareroot eosinophil counts with counts tending to be higher in infected animals than in uninfected controls (see Figure 4.8). All other effects were not statistically significant for this parameter.

Table 4.6 Probability values of differences in treatment effects of diet (D), infection (I) and time (T) and their interactions from repeated measures analysis of variance of haematological parameters measured in experimental sheep.

Effect	PCV	RBC	Haemoglobin	WBC	MCV	MCH	MCHC	Platelets	Eosinophils
D	0.141	0.054	0.064	0.966	0.900	0.318	0.280	0.833	0.824
I	< 0.0005	< 0.0005	< 0.0005	0.225	0.082	0.366	0.185	0.034	0.006
D X I	0.842	0.840	0.924	0.532	0.371	0.220	0.941	0.402	0.576
T	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005	0.180
T X D	0.107	0.138	0.162	0.054	< 0.0005	0.003	0.177	0.434	0.978
T X I	< 0.0005	< 0.0005	< 0.0005	0.001	< 0.0005	< 0.0005	0.004	< 0.0005	0.141
T X D X I	0.580	0.568	0.437	0.206	< 0.0005	0.028	0.395	0.003	0.971

Figure 4.4 Mean packed cell volume (PCV; %) in young sheep offered oaten chaff unsupplemented (NU) or with 3% urea (U) and infected with *Haemonchus* (H), *Trichostrongylus* (T), both species (H+T) or uninfected (Con).

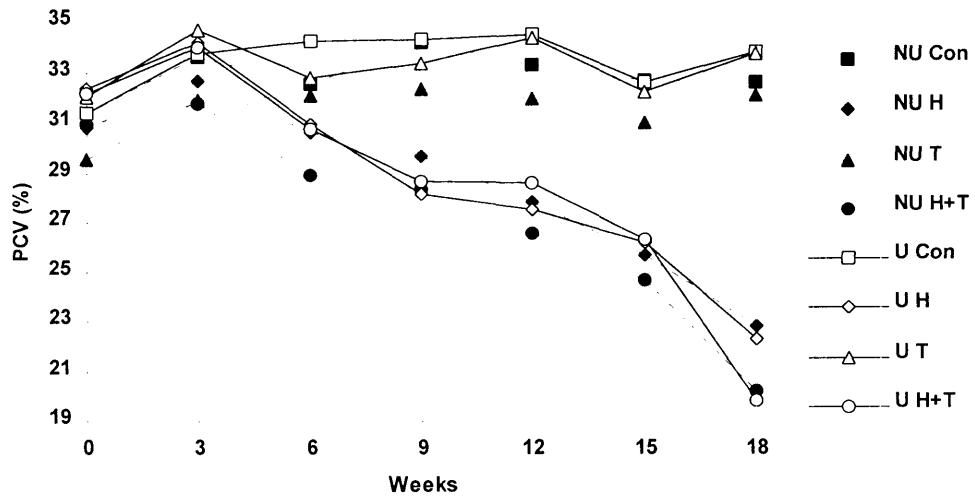


Figure 4.5 Mean red blood cells (no. X 10^{12}/l) in young sheep offered oaten chaff unsupplemented (NU) or with 3% urea (U) and infected with *Haemonchus* (H), *Trichostrongylus* (T), both species (H+T) or uninfected (Con).

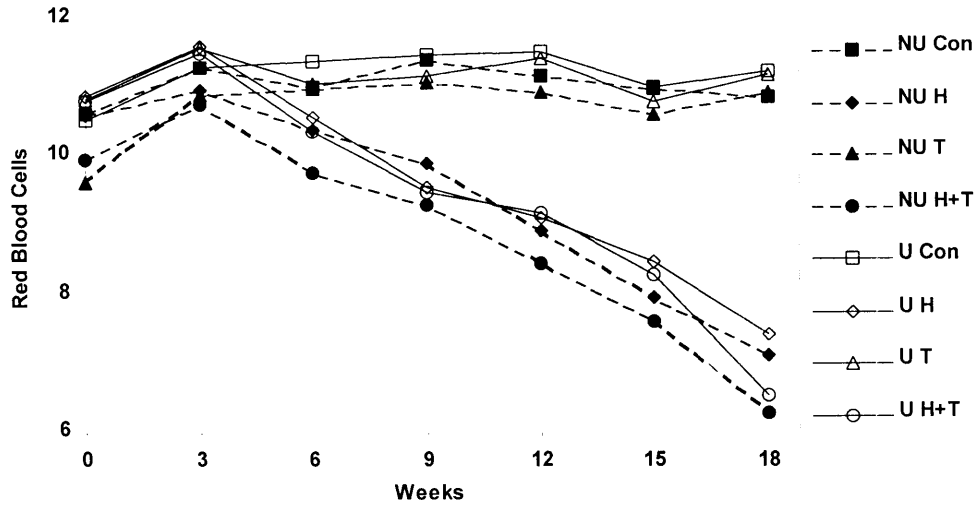


Figure 4.6 Mean haemoglobin concentration (g/dl) in young sheep offered oaten chaff unsupplemented (NU) or with 3% urea (U) and infected with *Haemonchus* (H), *Trichostrongylus* (T), both species (H+T) or uninfected (Con).

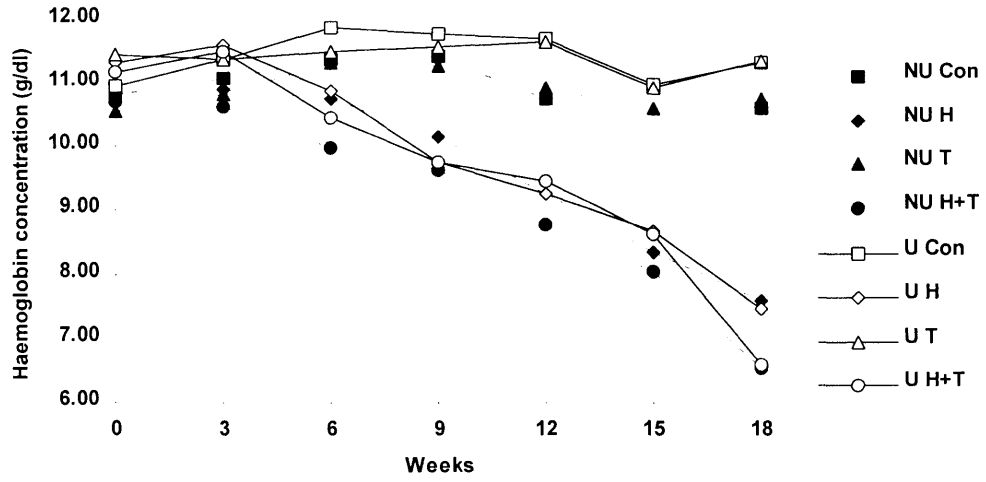


Figure 4.7 Mean white blood cell count (no. X10^9/l) in young sheep offered oaten chaff unsupplemented (NU) or with 3% urea (U) and infected with *Haemonchus* (H), *Trichostrongylus* (T), both species (H+T) or uninfected (Con).

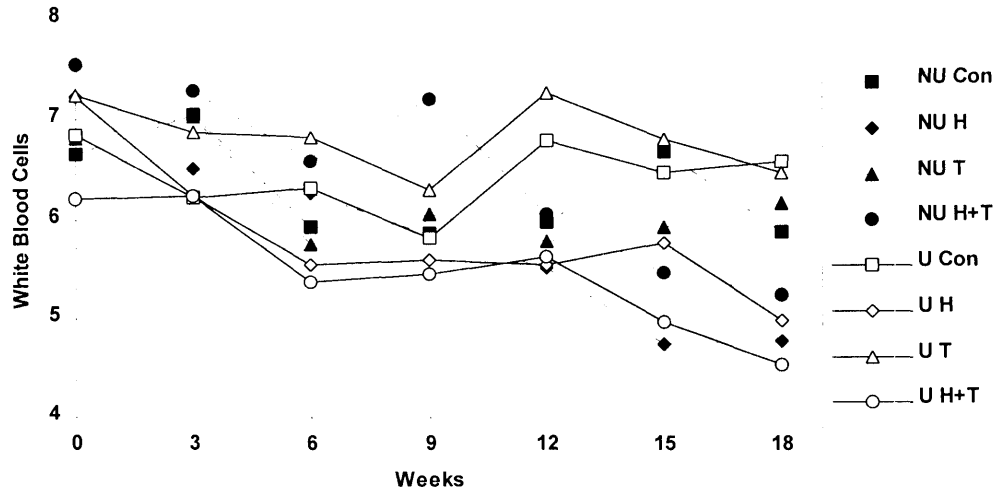


Figure 4.8 Mean eosinophil count (no. X 10^4/ml) in young sheep offered oaten chaff unsupplemented (NU) or with 3% urea (U) and infected with *Haemonchus* (H), *Trichostrongylus* (T), both species (H+T) or uninfected (Con).

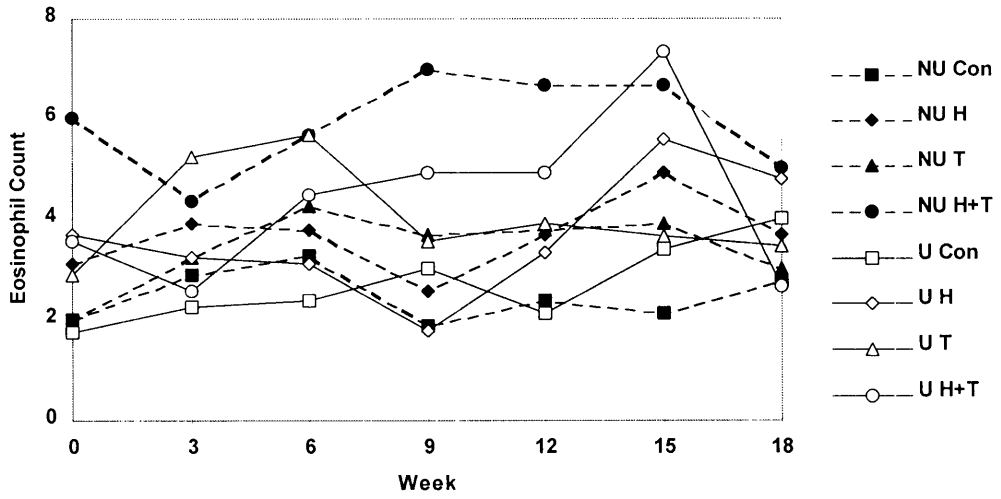
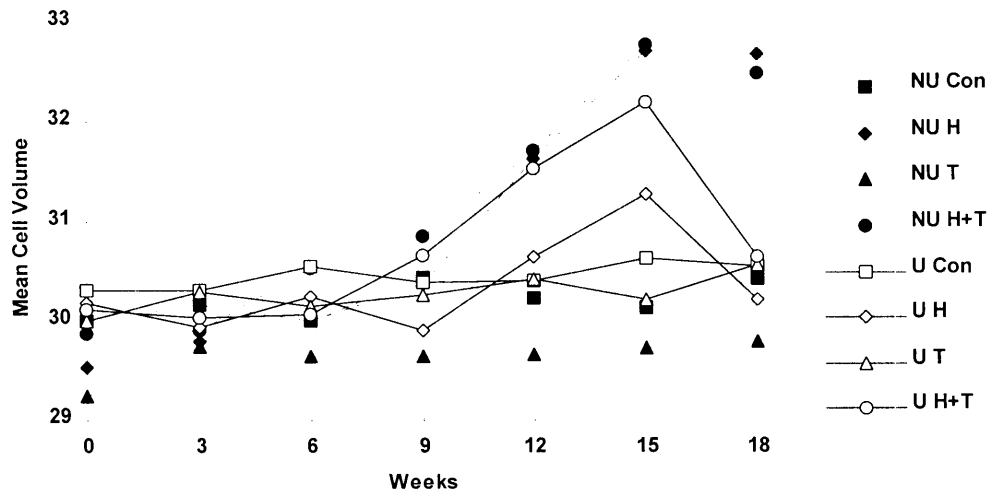


Figure 4.9 Mean cell volume of whole blood (X10^{-15}l) in young sheep offered oaten chaff unsupplemented (NU) or with 3% urea (U) and infected with *Haemonchus* (H), *Trichostrongylus* (T), both species (H+T) or uninfected (Con).



Estimates of MCV, MCH and MCHC were not affected by diet or infection nor was there any interaction of diet X infection. Over time all parameters changed significantly and there was a significant time X infection interaction. For MCV and MCH there were also significant interactions of time X diet and time X diet X infection but these interactions were not significant for MCHC. As figure 4.9 shows, MCV increased over time in those sheep infected with *Haemonchus* and mixed species but not in the other two infection groups. In the sheep infected with *Haemonchus* and mixed species, those offered the NU diet tended to have higher MCV than those offered the U diet. The reverse trend was apparent for the groups infected with *Trichostrongylus* and the uninfected controls. Figure 4.10 shows that MCH changed over time with levels increasing in *Haemonchus* and mixed species infection sheep and declining in the *Trichostrongylus* infected and uninfected control sheep over time for those animals offered the NU diet. MCH of sheep offered the U diet remained relatively constant throughout the experiment. MCHC declined over time with those sheep given the mixed species infection tending to show the greatest decline (see Figure 4.11).

There were significant effects of infection, time and time X infection and time X diet X infection for platelet counts. All other effects were not significant for this parameter. The major factors influencing this effect was the *Haemonchus* and mixed species infection which reduced estimated numbers of platelets as the experiment progressed (see Figure 4.12). For these groups there was also a trend for those sheep offered the NU diet to have lower platelet counts than those offered the U diet while the reverse dietary effect was apparent for those sheep infected with *Trichostrongylus*.

4.4.4.2 Biochemical changes

Plasma albumin was significantly affected by diet ($p<0.0005$) and infection ($p<0.0005$) but there was no interaction of diet X infection. There were also significant effects of time ($p<0.0005$), time X diet interaction ($p<0.0005$), time X infection interaction ($p<0.0005$) and time X diet X infection interaction ($p=0.002$). Figure 4.13 shows the change in albumin concentration over time for all treatment groups. Sheep infected with *Haemonchus* or mixed species had lower plasma albumin concentrations than those

Figure 4.10 Mean corpuscular haemoglobin (pg; mean) in young sheep offered oaten chaff unsupplemented (NU) or with 3% urea (U) and infected with *Haemonchus* (H), *Trichostrongylus* (T), both species (H+T) or uninfected (Con).

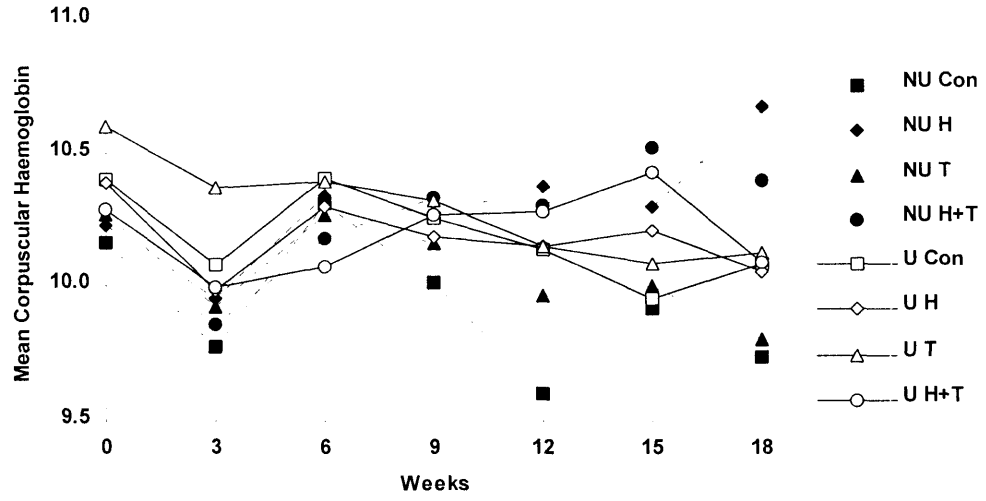


Figure 4.11 Mean corpuscular haemoglobin concentration (MCHC; g/dl; mean) in young sheep offered oaten chaff unsupplemented (NU) or with 3% urea (U) and infected with *Haemonchus* (H), *Trichostrongylus* (T), both species (H+T) or uninfected (Con).

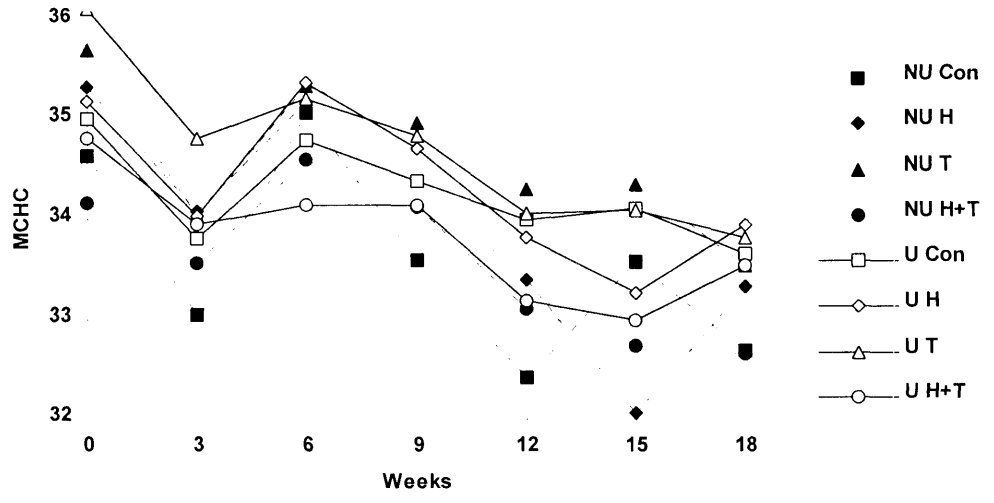


Figure 4.12 Mean platelet count (X10 ^{9}/l) in young sheep offered oaten chaff unsupplemented (NU) or with 3% urea (U) and infected with *Haemonchus* (H), *Trichostrongylus* (T), both species (H+T) or uninfected (Con).

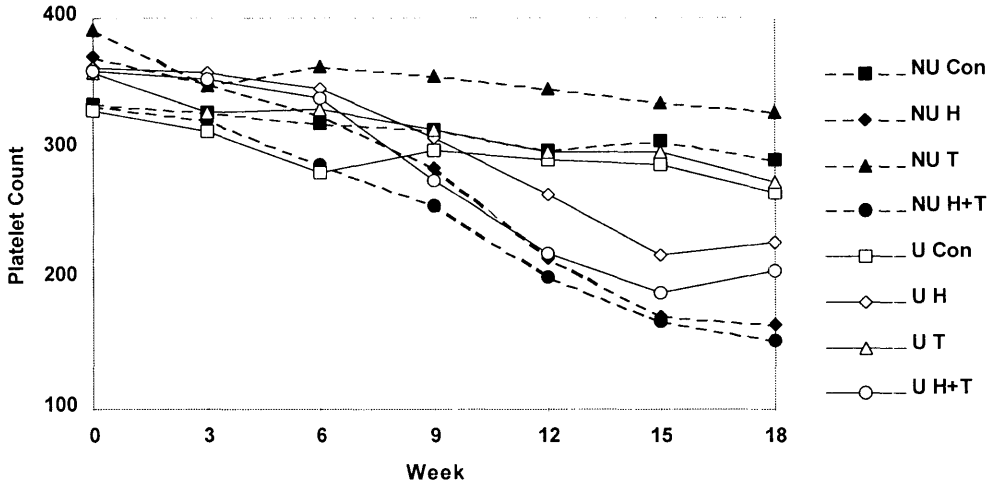


Figure 4.13 Mean plasma albumin concentration (g/l) in young sheep offered oaten chaff unsupplemented (NU) or with 3% urea (U) and infected with *Haemonchus* (H), *Trichostrongylus* (T), both species (H+T) or uninfected (Con).

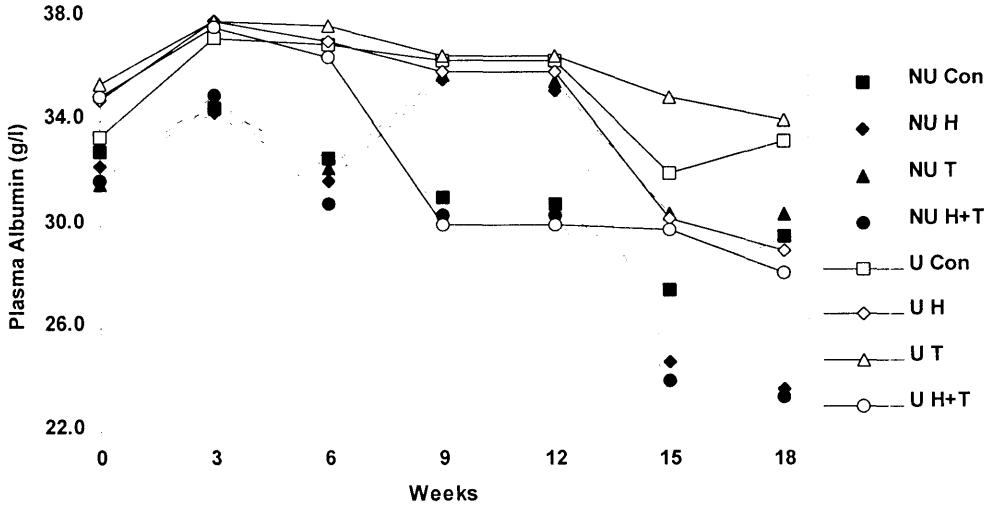
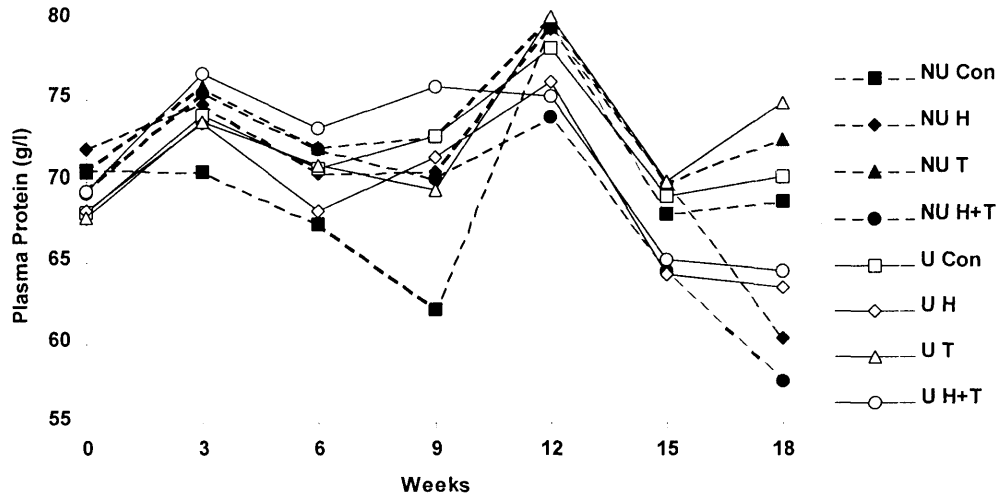


Figure 4.14 Mean plasma protein concentration (g/l) in young sheep offered oaten chaff unsupplemented (NU) or with 3% urea (U) and infected with *Haemonchus* (H), *Trichostrongylus* (T), both species (H+T) or uninfected (Con).

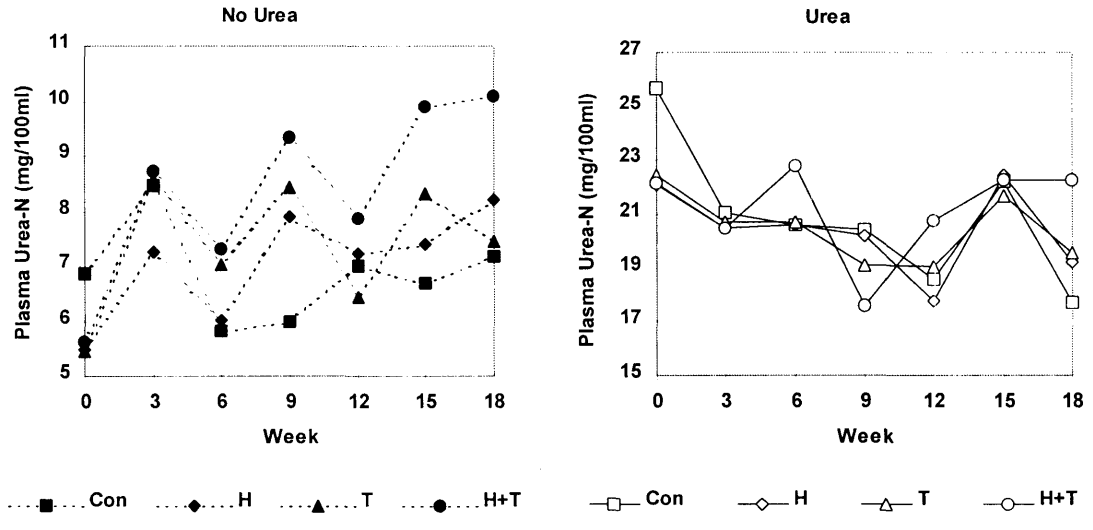


infected with *Trichostrongylus* or the uninfected controls. There was a general tendency within parasite treatment groups for sheep offered the NU diet to have lower plasma albumin concentrations than those offered the U diet.

Plasma protein concentration was significantly affected by infection ($p=0.007$) but there was no effect of diet or any diet X infection interaction. There was also a significant effect of time ($p<0.0005$), time X diet interaction ($p=0.005$), time X infection interaction ($p<0.0005$) and time X diet X infection interaction ($p<0.0005$). Plasma protein concentration fluctuated over time in all groups and sheep infected with *Haemonchus* or mixed species had lower plasma protein concentrations than those infected with *Trichostrongylus* or the uninfected controls (Figure 4.14). There was a general tendency within parasite treatment groups for sheep offered the NU diet to have lower plasma protein concentrations than those offered the U diet on the final sampling occasion.

Statistical analysis of squareroot plasma urea concentration showed that there was a significant effect of diet ($p<0.0005$) but no effect of infection or any infection X diet interaction. There was also a significant change in plasma urea over time ($p<0.0005$), a significant time X diet interaction ($p<0.0005$), a significant time X infection interaction ($p<0.0005$) and a significant time X diet X infection interaction ($p=0.007$). Figure 4.15 shows the changes in urea-N concentration over time in the treatment groups. Sheep offered the U diet had four-fold higher concentrations of urea-N in their plasma when compared to those offered the NU diet. When individual infection treatments were compared for the NU diet, *Trichostrongylus* groups, both as monospecific and mixed infections, had an increase in plasma urea concentration over time when compared to the uninfected controls. For the U diet the group with the mixed species infection showed higher plasma urea concentrations than its control.

Figure 4.15 Mean plasma urea-N concentration (mg/100ml) in young sheep offered oaten chaff unsupplemented or with 3% urea and infected with *Haemonchus* (H), *Trichostrongylus* (T), both species (H+T) or uninfected (Con).



4.4.5 Rumen fluid analyses

4.4.5.1 VFA

Results of VFA analyses are presented in Tables 4.7 and 4.8. From samples collected prior to commencing infections in Week 0 (Table 4.7) rumen fluid analysis showed significant effects of diet on proportions of acetate and propionate while proportion of butyrate and total VFA concentration tended towards significance. For acetate sheep on the U diet had higher levels but for propionate, butyrate and total VFAs the NU sheep had the higher levels. A similar effect of diet was observed during week 18 of experimentation (Table 4.8) with significant effects of diet on proportions of acetate, propionate, butyrate and isovalerate and on total VFAs while there were also significant effects of infection on total VFAs and diet X infection for isobutyrate. At this sampling the U sheep had higher levels of acetate and isovalerate but for propionate, butyrate and total VFAs the NU sheep had the higher levels. Infected sheep generally had lower levels of total VFAs than the uninfected controls with the exception being the U H+T sheep having higher levels than U Con sheep. For isobutyrate Con and T sheep offered the U diet had higher proportions of this VFA than H and H+T sheep but this was not the case for sheep offered the NU diet. Comparison of proportion of VFAs and total concentration between the two samplings (Table 4.9) showed various changes within individual treatments for acetate, propionate and butyrate. The proportion of isobutyrate and isovalerate tended to increase between samples for the U diet sheep but not for the NU diet sheep while the proportion of valerate increased in all groups. The total VFA concentration increased between samples in all NU groups while in the U sheep no change was observed.

4.4.5.2 Rumen NH_3-N

Results of rumen NH_3-N (RAN) analyses are presented in Table 4.10. As expected there was a dramatic effect of the addition of urea to the diet with RAN levels increasing 20 to 30 fold both prior to commencing infections and at Week 18. There was, however, no effect of infection on RAN level and no interaction between diet X infection. When RAN for the different collections were compared, there was no significant difference between any of the treatment groups.

Table 4.7 Proportions of VFAs and total concentration in rumen fluid at Week 0 (mean ± SE) for young sheep offered oaten chaff unsupplemented (NU) or with 3% urea (U) and infected with *H. contortus* (H), *T. colubriformis* (T) or both species (H+T) or uninfected (Con).

	% Acetate	% Propionate	% Isobutyrate	% Butyrate	% Isovaleric	% Valeric	Total Conc. (μM/ml)
NU Con	67.09 ± 3.01	23.12 ± 3.99	0.24 ± 0.28	8.88 ± 3.12	0.20 ± 0.13	0.47 ± 0.12	79.61 ± 4.54
NU H	68.25 ± 2.96	22.19 ± 3.93	0.24 ± 0.15	8.58 ± 1.82	0.32 ± 0.15	0.43 ± 0.14	69.59 ± 9.76
NU T	67.44 ± 1.49	23.26 ± 3.00	0.28 ± 0.26	8.23 ± 2.14	0.34 ± 0.16	0.45 ± 0.08	72.91 ± 9.36
NU H+T	67.26 ± 1.99	22.24 ± 2.71	0.24 ± 0.27	9.51 ± 1.92	0.30 ± 0.19	0.45 ± 0.08	71.48 ± 8.38
U Con	71.95 ± 2.52	19.61 ± 2.47	0.19 ± 0.12	7.49 ± 1.06	0.30 ± 0.15	0.47 ± 0.13	71.90 ± 10.48
U H	71.08 ± 2.41	20.36 ± 2.77	0.21 ± 0.12	7.65 ± 0.85	0.27 ± 0.08	0.43 ± 0.11	66.18 ± 7.36
U T	71.49 ± 3.31	19.47 ± 2.73	0.33 ± 0.29	7.94 ± 1.44	0.34 ± 0.19	0.44 ± 0.10	67.26 ± 10.75
U H+T	69.86 ± 2.01	19.96 ± 1.99	0.41 ± 0.33	8.94 ± 1.34	0.36 ± 0.16	0.47 ± 0.12	70.13 ± 14.92
Statistical Effects							
Diet (D)	p<0.0005	p<0.0005	p=0.562	p=0.076	p=0.468	p=0.911	p=0.062
Infection (I)	p=0.577	p=0.994	p=0.418	p=0.211	p=0.348	p=0.785	p=0.137
D X I	p=0.542	p=0.738	p=0.534	p=0.838	p=0.527	p=0.976	p=0.806

Table 4.8 Proportions of VFAs and total concentration in rumen fluid at Week 18 (mean ± SE) for young sheep offered oaten chaff unsupplemented (NU) or with 3% urea (U) and infected with *H. contortus* (H), *T. colubriformis* (T) or both species (H+T) or uninfected (Con).

	% Acetate	% Propionate	% Isobutyrate	% Butyrate	% Isovaleric	% Valeric	Total Conc. (μM/ml)
NU Con	66.14 ± 3.27	22.79 ± 3.74	0.18 ± 0.09	10.00 ± 2.07	0.30 ± 0.12	0.60 ± 0.12	88.13 ± 6.65
NU H	68.23 ± 2.53	19.87 ± 2.71	0.46 ± 0.25	10.27 ± 1.78	0.48 ± 0.30	0.69 ± 0.12	78.77 ± 6.56
NU T	69.33 ± 3.69	20.36 ± 4.09	0.39 ± 0.27	8.91 ± 0.93	0.41 ± 0.15	0.61 ± 0.12	80.36 ± 10.61
NU H+T	68.98 ± 3.58	19.90 ± 2.94	0.29 ± 0.07	9.75 ± 1.75	0.39 ± 0.16	0.70 ± 0.17	84.64 ± 13.32
U Con	70.43 ± 1.50	19.24 ± 2.14	0.51 ± 0.32	8.70 ± 1.26	0.53 ± 0.21	0.59 ± 0.11	76.09 ± 15.62
U H	71.89 ± 1.49	18.48 ± 1.92	0.29 ± 0.09	8.07 ± 0.73	0.68 ± 0.35	0.59 ± 0.10	64.26 ± 10.13
U T	70.40 ± 2.38	19.34 ± 2.62	0.58 ± 0.33	8.27 ± 0.84	0.71 ± 0.26	0.70 ± 0.09	64.66 ± 13.04
U H+T	71.15 ± 1.40	19.25 ± 1.70	0.29 ± 0.24	8.14 ± 0.52	0.49 ± 0.15	0.67 ± 0.13	81.67 ± 14.23
Statistical Effects							
Diet (D)	p<0.0005	p=0.019	p=0.121	p<0.0005	p<0.0005	p=0.773	p<0.0005
Infection (I)	p=0.167	p=0.284	p=0.117	p=0.411	p=0.091	p=0.210	p=0.005
D X I	p=0.288	p=0.465	p=0.015	p=0.391	p=0.633	p=0.146	p=0.375

Table 4.9 Paired t-test comparisons of Week 0 and Week 18 VFA measurements for young sheep offered oaten chaff unsupplemented (NU) or with 3% urea (U) and infected with *H. contortus* (H), *T. colubriformis* (T) or both species (H+T) or uninfected (Con).

	% Acetate	% Propionate	% Isobutyrate	% Butyrate	% Isovaleric	% Valeric	Total VFA
NU Con							
NU H	p=0.323	p=0.801	p=0.579	p=0.377	p=0.222	p=0.009	p=0.009
NU T	p=0.983	p=0.071	p=0.036	p=0.093	p=0.137	p=0.002	p=0.054
NU H+T	p=0.228	p=0.135	p=0.407	p=0.433	p=0.363	p=0.004	p=0.043
U Con	p=0.148	p=0.034	p=0.527	p=0.603	p=0.274	p<0.0005	p=0.013
U H	p=0.020	p=0.689	p=0.031	p=0.094	p=0.010	p=0.002	p=0.515
U T	p=0.180	p=0.019	p=0.084	p=0.278	p=0.003	p=0.012	p=0.738
U H+T	p=0.203	p=0.863	p=0.017	p=0.576	p=0.001	p<0.0005	p=0.385
	p=0.169	p=0.505	p=0.370	p=0.140	p=0.010	p=0.011	p=0.102

Table 4.10 Rumen NH_3-N concentrations (mgN/l; mean ± SE)in young sheep offered oaten chaff unsupplemented (NU) or with 3% urea (U) and infected with *Haemonchus* (H), *Trichostrongylus* (T), both species (H+T) or uninfected (CON).

Group	Week 0	Week 18
NU CON	12.1 ± 5.8	11.6 ± 3.4
NU H	3.7 ± 0.7	5.9 ± 1.0
NU T	6.8 ± 3.2	9.6 ± 4.4
NU H+T	15.3 ± 7.8	4.8 ± 1.6
U CON	295.7 ± 44.3	302.7 ± 42.7
U H	246.2 ± 25.6	275.6 ± 17.8
U T	279.3 ± 13.6	291.1 ± 39.0
U H+T	302.0 ± 23.5	290.2 ± 28.8

4.4.5.3 Protozoa

As shown in Table 4.11 at Week 0 there was no difference in numbers of protozoa in rumen fluid between the dietary and infection treatment groups. At Week 18 there was a significant effect of diet and the effect of infection was tending towards significance but there was no diet X infection interaction. At Week 18 protozoa numbers in rumen fluid were greater in the sheep offered the U diet and those infected with *Haemonchus* or mixed species had lower protozoa numbers than those infected with *Trichostrongylus* or remaining uninfected. In general protozoa numbers declined between samplings in all parasitised groups with the exception of the *Trichostrongylus* infected group offered the U diet which did not change. Protozoa counts for the uninfected control groups did not change between samplings.

Table 4.11 Total rumen protozoa counts ($x10^5$/ml; mean ± SE) in young sheep offered oaten chaff unsupplemented (NU) or with 3% urea (U) and infected with *Haemonchus* (H), *Trichostrongylus* (T), both species (H+T) or remaining uninfected (CON).

Group	Start (Week 0)	Finish (Week 18)	Start *vs* Finish
NU CON	4.39 ± 1.00	2.53 ± 0.47	p=0.137
NU H	5.14 ± 0.70	2.26 ± 0.48	p=0.005
NU T	8.44 ± 1.63	2.70 ± 0.34	p=0.001
NU H+T	5.04 ± 0.74	2.30 ± 0.39	p=0.003
U CON	4.97 ± 0.64	6.04 ± 1.08	p=0.551
U H	5.03 ± 0.73	2.63 ± 0.36	p=0.004
U T	5.21 ± 1.09	4.28 ± 0.48	p=0.989
U H+T	6.27 ± 1.04	3.71 ± 0.52	p=0.036
Statistical effects			
Diet (D)	p=0.739	p<0.0005	
Infection (I)	p=0.570	p=0.062	
D X I	p=0.130	p=0.337	

4.5 Discussion

4.5.1 Feed

It is well established that supplementation of low quality fibrous feeds with non-protein nitrogen often increases digestibilty of the feed (Krebs & Leng, 1984; Leng *et al.*, 1993) and thereby increases voluntary feed intake (Stephenson *et al.*, 1981; Hogan, 1996b). Addition of 3% urea (w/w) to a diet of low quality oaten chaff (0.8% nitrogen) and essential minerals substantially increased feed intake in both infected and uninfected sheep in the present experiment. During the initial stages of experimentation U sheep consumed about 9% more chaff than NU sheep and this difference increased to 24% by the end of experimentation. On average, urea increased chaff intake by >16% when chaff consumption was expressed per unit metabolic bodyweight (ie. g/kg $W^{0.75}$).

Infection with parasitic nematodes often leads to reduction in voluntary feed intake (see review by Symons, 1985). In the present experiment young sheep given monospecific trickle infections of *H. contortus* or *T. colubriformis* did not exhibit anorectic behaviour when compared to their uninfected counterparts on the same diet. However, when sheep were given both species concurrently and offered the NU diet, feed intake declined soon after commencing infections and remained at low levels throughout with an average of 8% depression of feed intake compared to those supplemented with urea. In the group of sheep offered the U diet while similarly infected, feed intake was not depressed and over the final 9 weeks of experimentation intake tended to exceed that of its uninfected control. It appears that urea supplementation enabled these sheep to overcome the negative effects of the mixed species infection on voluntary feed intake. This may be the result of a reduction in the influence of *T. colubriformis* on initiators of anorexia in the U sheep since *T. colubriformis* numbers were substantially reduced in these animals. During the second half of the experiment the increased intake exhibited by these sheep could then be governed by the predominant species *H. contortus*. If so, there is considerable evidence to suggest that negligible effects of this species on voluntary feed intake occur when higher quality diets are provided (Abbott *et al*, 1986b; Wallace *et al*, 1996).

4.5.2 Animal Productivity

Food consumption is a major factor determining the productivity of sheep (Cottle, 1991) and increased feed intake increased the productivity of sheep offered the U diet. In uninfected sheep liveweight gain was increased from 39g/head/day to 54g/head/day, wool production increased from 4.2g/head/day to 5.3g/head/day and fibre diameter increased from 13.8 to 14.9 μm (for NU sheep and U sheep, respectively). This result accords with previous findings of increased liveweight gain and wool production in sheep given a low digestibility, low N forage and offered a urea supplement (Sudana & Leng, 1986; Butler *et al*., 1994).

Parasitism decreased the liveweight gain of all infected groups relative to their uninfected dietary controls. This was expected as decreased liveweight gain is one of the clinical signs of disease commonly observed during helminth infection (see section 2.3.2). An

increase in liveweight gain in supplemented lambs was evident for the parasitised groups with the NU groups gaining 32, 29 and 23 g/head/day and the U groups gaining 43, 40 and 40 g/head/day for H, T and H+T infections, respectively. It is noteworthy that, on the NU diet, H+T sheep had a lower rate of liveweight gain than either of the groups with single species infections but there was no such difference between these groups when the U diet was offered. Also of importance is the observation that infected sheep offered the U diet gained as much weight as the uninfected sheep offered the NU diet.

Wool production declined in all groups when the pre-experimental period was compared to the experimental period. This was most probably due to the change in day length during the course of the experiment (early Autumn to late Winter) and the innate effect of photoperiod on wool growth (Nagorcka, 1979). There remains the possibility that there were increased nutritional demands on the sheep to maintain body temperature in the colder winter months but it is well established that in well-fleeced sheep wool growth is not usually affected by decreased environmental temperature (Bottomley, 1979). During the first 9 weeks of infection, parasitised sheep produced less wool than uninfected sheep and the H+T sheep offered the NU diet grew the least wool of all during this period. During the latter half of the experiment, there was still an effect of diet on wool growth but no difference due to parasitic infection was observed. Fibre diameter was not affected by infection when the periods were analysed separately but when analysed over both periods there was a significant interaction between diet and infection.

The productivity benefits of supplementation with urea in this study were not as great as those achieved by supplementation of parasitised sheep with high quality protein where, in some instances, the adverse effects of parasites were completely negated (van Houtert *et al.*, 1996; Wallace *et al.*, 1996). High cost prohibits the routine use of supplements high in protein for wool growth whereas urea supplements are less costly and may enable sheep to maintain production when faced with nematode parasite infections in situations where low quality forages predominate.

4.5.3 Parasitology

In the H and HT groups faecal egg counts increased progressively throughout the experiment and by the end of the experiment NU groups had mean faecal egg counts of 21,330 and 24,746 epg for H and HT, respectively and for the U diet groups comparative estimates were 17,026 and 14,980 epg, respectively. Similar egg count trajectories were observed in previous experiments when total available protein in the diet was low during a trickle infection of *H. contortus* (Abbott *et al.*, 1988; Roberts & Adams, 1990). In the T group there was no effect of dietary treatment on faecal egg counts which increased to a maximum of around 2,000 epg at week 10 of infection and declined thereafter to be <1,000 epg in both dietary groups by the end of the experiment.

Urea supplementation resulted in lower faecal egg counts of the H and H+T sheep which is consistent with the response to protein supplementation observed previously with mono-specific infections of *H. contortus* (Abbott *et al.*, 1988; Roberts & Adams, 1990) and *T. colubriformis* (van Houtert *et al.*, 1995a; van Houtert *et al.*, 1995b). The increased feed intake resulting from urea supplementation and its consequent effect on faecal output would not be sufficient to account for the lower egg count and the probable cause is enhanced immunological regulation of the parasite by the host arising from an improved nutritional status. Egg output by *H. contortus* is normally positively correlated to worm size (Le Jambre, 1995) yet in this experiment the larger worms produced the least eggs. This lends further support to the notion that the host sheep were immunologically modulating the fecundity of the worms since the larger worms were producing fewer eggs.

Urea supplementation significantly reduced total worm numbers at slaughter at week 18. The reduced numbers of male *T. colubriformis* and larvae recovered from both T and H+T infections may be the result of the U sheep attaining the immunological competence to partially resist infection with this species. *H. contortus* numbers were not affected by diet or infection type and by the end of experimentation around 3,500 adult worms were present in all H and H+T groups. Similarly, Abbott *et al.* (1985a; 1986b) and Wallace *et al.* (1995; 1996) observed no difference in *H. contortus* burdens even though dietary differences were apparent in productivity and pathological parameters.

4.5.4 Blood Parameters

The predominant feature of the blood parameters was the pathological effect of *Haemonchus* infection. Packed cell volume, RBC, HB, WBC and PLT estimates declined during the experiment as would be expected during continual blood loss due to *Haemonchus* infection. Although decline in these parameters occurred on both diets those groups offered the U diet had higher levels of RBC, HB, WBC and PLT. Similar differences in pathophysiology of infection have been observed in cross-bred sheep offered high and low protein diets and the *Haemonchus* induced anaemia was also evident in the changes in MCV, MCH and MCHC as detailed in previous studies (Abbott *et al.*, 1984, 1988). Diet again influenced these parameters with the U sheep having less severe anaemia.

An increase in circulating eosinophils is commonly observed during infection with parasites and during *T. colubriformis* infection of sheep eosinophil numbers in peripheral blood increased substantially from 6 to 10 weeks after infection (Dawkins *et al.*, 1989; Rothwell *et al.*, 1993). In the present experiment diet had no effect on eosinophil counts but infection caused a slight increase in eosinophil numbers in parasitised groups relative to the uninfected controls but not of the same magnitude observed in previous studies (Dawkins *et al.*, 1989; Rothwell *et al.*, 1993).

Reduction in plasma albumin and protein concentration has been commonly observed during infection of sheep with *H. contortus* (Abbott *et al.*, 1984, 1988) and *T. colubriformis* (Steel *et al.*, 1980; Coop *et al.*, 1976). In the present study plasma albumin concentration was progressively reduced over time by *Haemonchus* infection. Plasma protein concentration fluctuated over time in all groups but tended to decline from week 12 onwards in H and H+T sheep. Plasma albumin and protein concentration in T sheep were not affected in the present experiment which accords with observations by Steel *et al.* (1980) in sheep infected with 3000 L_3/week whereas higher rates of infection reduced these concentrations in the same study. Addition of urea to the diet led to higher levels of plasma albumin and protein in U sheep which again illustrates the nutritional benefits of this supplement.

Plasma urea concentrations responded to the nitrogen content of the diet with the U sheep having four times higher concentrations than the NU sheep. *T. colubriformis* infection is known to increase the concentration of urea in plasma (Roseby, 1973; Roseby & Leng, 1974) and this was confirmed in the present study in T and H+T sheep even though the difference was not as great as in the previous studies. Increased plasma urea concentrations have also been observed during infection with *H. contortus* (Rowe *et al.*, 1988) and this species may have contributed to the higher levels observed in the H+T sheep although in H sheep plasma urea concentration did not increase in the present study. Differences in pathophysiological effects may be attributable to a large single infection (Rowe *et al.*, 1988) compared to the trickle infection used in the present study.

4.5.5 Rumen Fluid

Proportions of VFAs were affected by the addition of urea to the basal diet with the proportion of acetate being higher in U sheep while the proportion of propionate and butyrate was higher in NU sheep. Previous authors have recorded a change in ruminal acetate production (Steel, 1972) or a change in the ratio of acetate to propionate (Rowe *et al.*, 1988) in sheep infected with *T. colubriformis* and *H. contortus*, respectively. No such effects of infection were observed in the present study. Infection did tend to decrease the total concentration of VFAs in both dietary treatments which probably relates directly to the reduced feed intake (ie reduced fermentable substrate) associated with infection. Concentration of total VFAs was greater in NU sheep than in U sheep and this may be due to the increased efficiency of microbial cell synthesis in those sheep offered the U diet decreasing the proportion of fermentable carbohydrate contributing to VFA production (Leng *et al.*, 1995).

As expected the addition of 3% urea to the diet substantially increased RAN levels and previous work has suggested that levels above 200 mgN/l will maximise benefits from a low quality roughage diet (see Section 3.3.2.1). This level was exceeded during the present experiment.

Protozoa numbers in ruminal fluid decreased by 18 weeks in all infected groups except the U T sheep whereas uninfected controls did not change. The U sheep had greater

numbers of protozoa at week 18 than at week 0 and numbers were above that suggested to negatively influence the nutritional status of the host (Kanjanapruthipong, 1995). The reason for this between sample difference is unclear but may relate to the experimental conditions since urea was delivered via ruminal infusion and feed intake was restricted in the cited study while in the present study *ad libitum* access was provided to urea-treated chaff. The effect of nematode parasites on rumen function has not been the subject of rigorous study (see section 2.4.1.2) and further work is needed to explain the results for protozoa numbers in the present study.

4.5.6 Conclusion

In lambs on low digestibility forage there was a beneficial effect of using non-protein nitrogen (NPN) supplements in terms of increased weight gain and wool production. There are indications that NPN can reduce the detrimental effects of parasitic nematode infection and the numbers of parasites and eggs produced. Whether this results simply from increased feed consumption due to increased digestibility when NPN is provided or whether there are other mechanisms operating to ameliorate the effects of parasitism is yet to be determined. The observed wool growth response is indicative of a response to additional protein availability since protein supply from the small intestine is the key factor in wool production (Black *et al.*, 1973; Reis *et al.*, 1992). Therefore increased availability of rumen microbial protein from NPN supplementation, as clearly demonstrated with similar diets by Kanjanapruthipong (1995), was probably a significant contributor to this result.