THE EFFECT OF NUTRITION ON THE PATHOPHYSIOLOGY OF TRYPANOSOMIASIS IN SCOTTISH BLACKFACE SHEEP

by

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SUMMARY

In a series of experiments the relationship between nutrition and trypanosomiasis was investigated in sheep with special emphasis on changes in digestive function. Scottish Blackface lambs were used since these animals had shown to be relatively resistant to trypanosomiasis and the results would therefore be more comparable to the results found in related experiments with trypanotolerant animals conducted in The Gambia and Burkina Faso. The control lambs used in the experiments were pair-fed to the infected lambs in order to distinguish between the direct effects of infection and the effects of anorexia during infection.

In the first experiment the effects of a poor quality and a high quality roughage were compared. Different amounts of concentrate and roughage were fed to the lambs in the second and third experiments. The diets used in these last two experiments contained similar levels of dietary energy and protein. In the fourth experiment the effects of non-protein nitrogen supplementation in the form of urea were investigated. An extra experiment was conducted looking at the effects of an arginine-free diet on a trypanosome infection in mice. In all the experiments *Trypanosoma congolense* was used, except in the third experiment in which a strain of *Trypanosoma vivax* was used.

Some of the traits measured were feed intake, body weight, diet digestibility coefficients, nitrogen balance and mean retention time of the roughage through the digestive tract using chromium as a marker. Some blood haematological and biochemical parameters were also measured.

In general the trypanosome infections led to a reduction in feed intake in Scottish Blackface sheep. The digestibility coefficients of organic matter and gross energy were slightly decreased during trypanosome infections but the changes were

relatively small and appeared to be independent of the type of diet and pathogenicity of the trypanosome infection. The fibre (NDF and ADF) digestibility coefficients were unaffected by trypanosome infections. The trypanosome infections resulted in a small decrease in the digestibility coefficient of nitrogen independent of the type of diet offered to the lambs and the pathogenicity of the infection. In general the mean retention time of the roughage through the digestive tract was significantly longer in trypanosome-infected Scottish Blackface sheep compared with their pair-fed controls. The longer mean retention time appeared to be affected by the level of lower quality roughage intake but was independent of the pathogenicity of the disease. The effect of trypanosome infections on the nitrogen balance appeared to be dependent upon the level of feeding and the pathogenicity of the disease. A higher content of digestible undegraded protein in the diet reduced nitrogen losses in the urine during the *T.vivax* infection, leading to a more positive nitrogen balance.

Supplementation of the diet with urea did not prove to be beneficial to trypanosome-infected Scottish Blackface sheep.

The anaemia observed in the trypanosome-infected Scottish Blackface sheep was relatively mild. The anaemia was independent of the type of diet but was more severe in sheep infected with the more pathogenic strain of *T.vivax* than in the milder strain of *T.congolense*. The erythropoietic response to the anaemia appeared to be inhibited in the *T.vivax* infected lambs by a low intake of dietary protein.

Plasma cholesterol levels were significantly reduced in trypanosome-infected lambs irrespective of the type of diet or infection. There was evidence of a relationship between plasma cholesterol levels and the parasitaemia. The reduction in plasma

albumin levels was greater in the *T.vivax* infected sheep than in the *T.congolense* infected sheep.

Plasma nitric oxide concentrations were significantly increased in lambs displaying a high parasitaemia at the time of sampling. Dietary L-arginine was found to play an important role in the host's defence mechanism against a trypanosome infection, at least in mice.

It was concluded that the effects of trypanosome infections on the diet digestibility coefficients were relatively small and hardly affected by the pathogenicity of the infection and the type of diet fed to the Scottish Blackface lambs. The mean retention time of the roughage through the digestive tract was affected by the trypanosome infections and appeared to be longer in infected lambs with a higher intake of poor quality roughage. The mean retention time was unaffected by the pathogenicity of the disease. In contrast, the nitrogen balance was affected by the pathogenicity of the disease. A higher level of digestible undegraded protein in the diet of *T.vivax* infected lambs decreased urinary nitrogen losses which led to a more positive nitrogen balance. These results indicate that strategic feeding of ruminants at risk from trypanosome infection with diets high in digestible undegraded protein, such as legumes, may increase their ability to withstand the effects of the infection.

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DECLARATION

I hereby declare that the work presented in this thesis is original and was conducted by the author with the exception of some of the work described in Chapter 7, which was carried out by Dr J.M. Sternberg, Department of Zoology, University of Aberdeen.

I also hereby certify that no part of this thesis has been submitted previously in any form to any university for the award of a degree, but has been and will be published in part as scientific abstracts or as papers:

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To the memory of

Hendrika Willemina Wassink-ter Maat

who died in September, 1978

The broad-backed hippopotamus
Rests on his belly in the mud;
Although he seems so firm to us
He is merely flesh and blood

'The Hippopotamus', T. S. Eliot

CHAPTER 1

Introduction

The disease

Tsetse-borne animal trypanosomiasis caused by the protozoa of the genus *Trypanosoma* is widely recognised as one of the biggest obstacles to increased livestock production in Africa. Trypanosomiasis is found over about 10 million km^2, or roughly over one third of Africa. The disease occurs in nearly every country between the deserts of southern Africa and the Sahara. Approximately 7 million km^2 of this area is tropical savannah which could support an estimated 125 million additional cattle (Trail *et al.*, 1983).

Trypanosomiasis has had a great impact on the history of Africa. The disease influenced the distribution and types of agricultural system in almost the entire continent. The cattle-owning tribes who introduced domestic livestock into Africa had to avoid densely infested tsetse fly belts or the livestock had to evolve a capacity to resist the disease. The output of crop production was impaired due to the lack of available draught animals.

Trypanosomiasis not only had an impact on agriculture. The early penetration of alien peoples dependent on the horse or the ox for their transport was much affected by the presence of tsetse. The disease halted the advance of the Islamic faith in Africa because the horses of the islamic missionaries died of trypanosomiasis (Jordan, 1986).

The parasite

Trypanosomiasis is caused by flagellate protozoa of the Order Kinetoplastida, class Mastigophora, family Trypanosomatidae and the genus *Trypanosoma*. The genus *Trypanosoma* is sub-divided into two main groups, namely, the Stercorarian and Salivarian groups of trypanosomes. The Stercorarian group comprises of trypanosomes

that are non-pathogenic to man and livestock except for *Trypanosoma cruzi*, the causative agent of Chagas' disease in man in South and Central America. The development of the Stercorian group of trypanosmes in the vector is completed in the posterior section of the gut and their transmission is basically contaminative.

All those *Trypanosoma* spp. which undergo a cycle of development in the tsetse fly are regarded as members of the Salivarian group. The Salivarian group is divided into four sub-genera, namely, Nannomonas, Duttonella, Trypanozoon and Pycnomonas (Table 1.1). The Nannomonas sub-genus contains *T.congolense* and *T.simiae* and the Duttonella group contains *T.vivax* and *T.uniforme*. The Trypanozoon subgenus consists of *T. brucei gambiense*, *T.b.rhodesiense*, *T.b.brucei*, *T.equiperdum* and *T.evansi*. Of these trypanosomes only *T.b.gambiense* and *T.b.rhodesiense* cause African human trypanosomiasis, whereas *T.b.brucei*, *T.vivax* and *T.congolense* are the important trypanosome species causing African animal trypanosomiasis in cattle, sheep and goats.

Reproduction of trypanosomes occurs both in the vertebrate and invertebrate host. First a second kinetoplast appears and a new flagellum grows. The nucleus then divides and the cytoplasm undergoes a longitudinal fission to produce two daughter trypanosomes (ILRAD, 1986).

Table 1.1 The tsetse-transmitted trypanosomes of African mammals (Adapted from Jordan, 1986)

Subgenus	Species	Site of development in *glossina*	Hosts	Distribution	Importance
Duttonella	*T.vivax*	Proboscis	Wild & domestic mammals (not pigs)	All Tsetse infested areas	Major disease of cattle and other ungulates
	T.uniforme	Proboscis	Wild & domestic mammals (not pigs)	East & Central Africa. Restricted	Localised. Mild disease
Nannomonas	*T.congolense*	Proboscis & midgut	Wild & domestic mammals	All Tsetse infested areas	Major disease of cattle and other ungulates
	T.simiae	Proboscis & midgut	Wild & domestic pigs	All Tsetse infested areas	Acute disease of domestic pigs
Trypanozoon	*T.b.brucei brucei*	Salivary glands & midgut	Wild & domestic mammals	All Tsetse infested areas	Acute in dogs & horses. Chronic in cattle & pigs
	T.b.brucei rhodesiense	Salivary glands & midgut	Man & Wild & domestic mammals	East & South-Central Africa	Acute form of sleeping sickness in man
	T.b.brucei gambiense	Salivary glands & midgut	Man. Wild & domestic mammals probably of some importance	West & North-Central Africa	Chronic form of sleeping sickness in man
Pycnomonas	*T.suis*	Salivary glands & midgut	Wild & domestic pigs	Tanzania, Burundi (? elsewhere)	Very localised. Pathogenic to young domestic pigs

The Vector

Trypanosomes are most often transmitted cyclically by a vector, the tsetse fly (*Glossina* spp.). There are about 30 species and sub-species of tsetse flies in Africa which can be divided into three main groups, namely, the *fusca*, *palpalis* and *morsitans* groups.

Not much is known about the species of tsetse flies of the *fusca* group, partly because none is of major economic importance and partly because many are difficult to detect. Their main habitats are the lowland rain forests and the edges of rain forests.

The distribution of the tsetse of the *palpalis* group is also centred on the lowland rain forest areas, but some species extend far out through the humid savannah and into the drier savannah along rivers and streams. *G.palpalis*, *G.fuscipes* and *G.tachinoides* are well known vectors of human sleeping sickness and animal trypanosomiasis.

All species of the *morsitans* group are restricted to the savannah woodlands surrounding the lowland rain forest. In the wetter areas the flies roam widely over the woodland, but in the drier parts of their range, such as the Sudan savannah of West Africa, they are centred on the mesophytic vegetation of the watercourses, particularly during the dry season. *G.mositans* is the most important *Glossina* species. It infests an enormous area, is a vector of human sleeping sickness in Eastern and Southern Africa, and is a major vector of animal trypanosomiasis. Other tsetse species important to animal trypanosomiasis in this group are *G.longipalpis*, *G.pallidipes* and *G.austeni* (Jordan, 1986).

The tsetse fly transmits trypanosomiasis by virtue of its blood sucking habit. Trypanosomes from an infected animal are ingested by the fly with the animal's blood,

and undergo a cycle of development within the various parts of the fly's digestive tract according to the trypanosome species. The trypanosome species involved in ruminant trypanosomiasis all end this phase of development as metacyclic forms in the salivary glands or the mouthparts. When the infected fly next feeds, infective metacyclic trypanosomes are injected into the skin of the new host along with the fly saliva.

Pathogenesis of African animal trypanosomiasis

Development of the infection in the host

The first host response to trypanosome infections is the development of a local skin reaction, or chancre, at the site of the infected tsetse bite. A dramatic increase in polymorphonuclear leucocytes, lymphocytes, macrophages and mast cells occurs at the site. The largest and most intensely inflamed chancres often occur in animals that are more resistant to the disease and this limits trypanosome growth in the skin more than in susceptible animals (Dwinger, 1985). Some trypanosomes are able to evade this initial immune response and enter the bloodstream.

Metacyclic trypanosomes and the forms which develop in the vertebrate bloodstream have a surface coat composed of glycoproteins. This coat changes during a process known as antigenic variation. The glycoproteins are referred to as variable surface glycoproteins (VSGs). When an animal becomes infected it produces antibodies against the VSGs displayed by the first wave of invading trypanosomes. However, before all parasites can be eliminated, new trypanosomes emerge which are coated with different VSGs. These trypanosmes evade the animal's initial immune response and multiply rapidly. The host produces new antibodies, but then parasites

appear with yet another VSG, always keeping a step ahead of the host response (ILRAD, 1986).

Trypanosomes are predominantly parasites of the bloodstream but depending on the trypanosome species may exist in other sites such as the skin or the central nervous system. In contrast to *T.congolense*, both *T.vivax* (Van den Ingh *et al.*, 1976b; Emery *et al.*, 1980), and to an even greater extent *T.b.brucei* (Morrison *et al.*, 1983) have the capacity to invade tissues in domestic ruminants. Generally, *T.congolense* parasites are confined to the circulation where they remain free or attached to the red blood cells by their anterior or to the endothelial cells lining the blood vessels (Bungener and Muller, 1976).

The haematological and other changes during trypanosome infections are determined by several factors including the virulence of the parasite, the susceptibility of the host and the period of the infection during which the samples are taken. Because of different combinations of these host, parasite and other factors, the pattern of a trypanosome infection can be quite variable. Nevertheless, three recognisable phases of trypanosomiasis can be described:

The acute phase

The acute phase begins with the first appearance of trypanosomes in the blood and lasts several weeks. It is characterised by fluctuating parasitaemia and the presence of microthrombi, which consist of platelets, trypanosomes, monocytoid cells and some fibrin (Van den Ingh *et al.*, 1976a).

The acute phase is also characterised by progressive anaemia. The initial fall in packed cell volume values is associated with the first wave of parasitaemia in the

blood. Progressive decrease in packed cell volume takes place over a period of 4 to 12 weeks (average 6 weeks) after infection by which time values of around 20% are reached (Murray and Dexter, 1988). Death occurs commonly during the acute phase because of a continued fall in packed cell volume (PCV = 15 or less), severe pancytopenia and other deleterious changes.

There are also a number of blood biochemical changes during this period. The increase in metabolic rate (Verstegen *et al.*, 1991), fever, the reduction in feed intake often encountered during infections (Wassink *et al.*, 1993) and the increased rates of protein synthesis for the host defensive mechanisms (Van den Ingh *et al.*, 1976a) lead to increased energy and protein needs. Stores of labile protein present in muscle fibres and somatic tissues serve to provide the required additional supply of amino acid substrate in the absence of sufficient feed nutrients. The deamination of the amino acids lead to an increase in plasma urea (Wassink *et al.*, 1993) and urinary nitrogen excretion (Akinbamijo, 1988).

A rapid decrease in blood lipids such as phospholipids and cholesterol at the time of the first peak parasitaemia has been reported in *T.congolense* infected sheep (Katunguka-Rwakishaya, 1992) and cattle (Traore-Leroux *et al.*, 1987). A similar decrease in plasma albumin is found in infected sheep (Katunguka-Rwakishaya, 1992).

The acute phase appears to be a period when the host immune responses are not yet adequate to deal with the infection and the parasite has the upper hand due to its antigenic variation.

The chronic phase

The chronic phase follows imperceptibly after the acute phase. It is characterised by low frequency and intensity of parasitaemia. The packed cell volume persist, with minor fluctuations, at the low levels attained at the end of the acute phase, for periods ranging from a few weeks to several months.

It appears to be a period when the infected animal has fully mobilised its defence mechanisms to a level that is adequate to depress parasite multiplication but is not yet adequate to completely abort the infection or reverse the pathology that developed during the acute phase.

With the tissue-invading trypanosomes, such as *T.b.brucei* and *T.vivax*, this is the period when the parasites also establish extravascularly and are less numerous in the blood. The development of lesions has been reported in *T.b.brucei* infected cattle in various tissues and organs, such as the heart, brain, testis, skin and eye (Ikede and Losos, 1972). Oedema of the lungs and other tissues and necrotic changes in several organs were also found in *T.vivax* infected goats.

The labile protein stores are expended during this phase and fat depots are mobilised as indicated by the increased non-esterified fatty acid and β-hydroxy-butyrate levels in the plasma. Degenerative atrophy of the muscular tissues at a wasted cachectic level can often be found during trypanosome infections (Van den Ingh *et al.*, 1976a, Tracey *et al.*, 1988). Another factor of importance during the chronic phase is the proliferative glomerulonephritis associated with loss of protein in the urine (Van den Ingh *et al.*, 1976a).

Death could occur at this stage either as a result of the progressive pathology caused by the parasite or due to secondary infections since affected animals are immuno-suppressed.

The recovery phase

A small but significant number of infected animals, and especially those belonging to trypanotolerant breeds, may gradually recover from trypanosome infections. The recovery phase is characterised by aparasitaemia or low very infrequent parasitaemia, symbolising the capitulation of the parasite to the host's defence mechanisms. The leucocyte, erythrocyte and thrombocyte levels begin to recover slowly towards pre-infection levels. Other pathological changes are also slowly reversed (Anosa, 1988).

Impact of the disease

Historically, the presence of *Glossina* spp., particularly the so-called 'fly belts' of the savannah species of the *morsitans* group, has had a profound influence on the routes followed by the cattle-owning tribes who introduced domestic livestock into Africa. The first domesticated cattle, derived from the Euro-Asian humpless longhorn *Bos primigenius*, to enter Africa came from the Sinai peninsula in about 5000 BC and thereafter five successive waves of cattle-owning people migrated into various parts of Africa (Jordan, 1986). During these migrations densely infested tsetse fly belts had to be avoided or the livestock had to evolve over the years a capacity to resist the disease. The distinction between breeds of cattle which are susceptible to trypanosomiasis and hence have to be kept out of tsetse infested areas, and other breeds which have evolved

a degree of resistance to trypanosomiasis (trypanotolerance) and can be kept in contact with at least lower density tsetse populations, still exists today.

The presence of trypanosomiasis also had a major influence on the agricultural systems which developed in Africa south of the Sahara. Apart from the inability of people to keep livestock for meat and milk purposes, animals were not available for draught purposes. Animal power was a major contributor to the development of Europe and Asia - an advantage which was and still is denied to many African peoples. At present, there are approximately 10 million draught animals in Africa which contribute less than 10 % to the labour requirement of crop agriculture (Jordan, 1986). The lack of draught animal power also influences the type of crop production. Cereal crops require more cultivation and more timely seed bed preparation than pulses.

At present, tsetse flies infest a wide range of habitats over about 10 million km^2 of Africa, representing about 37% of the continent (FAO, 1980). Traditionally many African livestock producers used to bring their herds and flocks into tsetse infested areas in search of grazing during the dry season when there were few tsetse flies, and moved quickly back to drier, disease-free areas when the rains began. With the growth of the human population in the region, this type of herding system is becoming less practical, resulting in high grazing pressures in the drier regions of Africa where the local ecology is too fragile to support continuous heavy use. Indirect losses due to the underutilisation of tsetse-infested land and the overutilisation of tsetse-free land in economic terms are far more important than direct mortality and morbidity losses due to trypanosomiasis (Putt and Shaw, 1982).

The loss of productivity in trypanosome-infected animals is also very important. In a longitudinal study in The Gambia of some 2000 N'Dama cattle carried out over 5

years a significant number of cattle, previously found to be infected with trypanosomes, continued to exhibit a low grade anaemia for several months in the absence of parasites (Murray *et al.*, 1979b, c). While some animals suffering from the "chronic trypanosomiasis syndrome" die, many remain alive but in poor health characterised by stunting, wasting and infertility.

Current methods of trypanosomiasis diagnosis and control

Diagnosis of trypanosomiasis

The most important practical field approach for the detection of trypanosomes has been the dark ground/phase contrast buffy coat method (Murray *et al.*, 1977) and quantification of the level of infection on the basis of a parasitaemia score (Paris *et al.*, 1982). However, a high proportion of infections go undetected as the level of parasitaemia fluctuates markedly and is often below the limit of detection of the technique. Furthermore, mixed infections often go undetected (Trail *et al.*, 1992).

Giemsa stained thick blood smears are sometimes used to determine the level of parasitaemia by counting the ratio of trypanosomes to white blood cells. The number of trypanosomes per ml of blood can than be calculated by measuring the number of white blood cells with the Coulter Counter (Wassink, 1990). However, this method is only accurate with high numbers of trypanosomes present in the blood and thick blood smears are not useful to determine whether an animal is infected since no trypanosomes can be detected with low parasitaemias. Both Giemsa stained thick and thin blood smears are useful to determine the species of trypanosome.

Another method of quantifying a trypanosome infection is the use of the Neubauer haemacytometer (Sternberg *et al.*, 1994). This method is mostly used in

rodent infections where trypanosome numbers are high enough to give an accurate estimate of the number of trypanosomes present in the blood.

Blood or tissues of animals suspected of being infected with trypanosomiasis but in which no trypanosomes can be detected with the buffy coat dark ground/phase contrast method is sometimes cultured in rodents to determine whether the animal is infected. However, this is not practical with large numbers of animals.

More recent techniques involve the detection of trypanosomal antibodies with the immunofluorescence antibody test (IFAT; Katende *et al.*, 1987) and the detection of trypanosomal antigens with the antigen detection enzyme immunoassays (antigen ELISA; Nantulya and Lindqvist, 1989). However, the detection of trypanosomal antibodies does not mean that the animal is actually infected at the time of sampling in contrast to the antigen detection enzyme immunoassay. There are several advantages of using the antigen detection enzyme immunoassay over other methods. It accurately identifies the trypanosome species involved, also in mixed infections. Detection of animals antigenaemic without patent parasitaemia could allow individuals with superior ability to control infection to be identified. The immunoassay would also measure more accurately the proportion of time an animal is infected (Trail *et al.*, 1992). However, the antigen ELISA has not been adequately validated. The major disadvantage of both antibody and antigen detection techniques is that good laboratory facilities and practices are required.

Control of trypanosomiasis

In his book "Trypanosomiasis Control and African Rural Development", Jordan (1986) indicates that there are widely differing opinions on how to approach the problems of trypanosomiasis. These range from continent-wide proposals to eradicate the problem by elimination of the tsetse vector, to a belief that the interests of Africa would be best served by doing nothing. He concludes that neither of these extreme approaches is appropriate but that there are a series of middle roads, as possible solutions will vary in different parts of Africa according to different ecological conditions and different political and economic priorities of governments. Some of the methods to fight the disease are discussed in the next paragraphs.

Trypanocidal drugs

Until 1950 tartar emetic was the only readily available trypanocidal drug for the treatment of animal trypanosomiasis. Tartar emetic caused about 6% mortality (Williamson, 1970) and was superseded by quinapyramine (Antrycide) and homidium (Ethidium). There was a great increase in the number of animals treated and following the introduction of mass treatment campaigns cattle numbers increased in many countries (Jordan, 1986). However, resistance to these drugs developed and eventually became widespread. A new curative compound came on the market in the early 1960s, diminazene aceturate (Berenil). Throughout Africa, diminazene aceturate is now widely used. Isometamidium (Samorin, Trypamidium) was also introduced in the early 1960s and has both curative and prophylactic properties. Currently, diminazene aceturate, homidium and isometamidium remain in common use, and 30 million doses are administered per year (Murray, personal communication).

There is no doubt that chemoprophylactic drugs such as samorin can be highly effective in improving animal production in areas of high tsetse challenge (Holmes and Torr, 1988). However, chemoprophylaxis requires stricter supervision of drug administration and conditions of animal husbandry than chemotherapy. After administration the drug concentration in the tissues will eventually drop to a level insufficient to kill trypanosomes. These are ideal conditions for the trypanosomes to develop resistance and it is, therefore important to administer a second dose of drug before the level of drug has dropped to the critical level below which trypanosomes can survive and multiply. Such regime requires the continuing presence of trained staff, reliable transport and access to the animals involved (Leach and Roberts, 1981). Chemoprophylaxis is thus inappropriate for use with nomadic or semi-nomadic livestock. The problem of drug resistance is worsened by the fact that no new drugs for the treatment of tsetse-transmitted trypanosomiasis have been brought into field use since the mid-1950s and none is in prospect.

Tsetse control

Control of trypanosomiasis can be achieved indirectly by attacking the vector, the tsetse fly (*Glossina* spp.) or by avoiding the fly belts. Tsetse control methods in the past included removing the vegetation (Ford *et al.*, 1970) on which the fly depends for shelter and the destruction of wild animals (Wooff, 1968) on which the flies feed. However, these methods are rarely deliberately employed today, although the same effect is achieved unintentionally in places where human populations are rapidly expanding and which have become increasingly denuded of both woody vegetation and wild animals.

Almost all methods of tsetse control employed today involve the use of insecticides. They can be classified in two categories residual (e.g. Dieldrin) and non-residual insecticides (e.g. Endosulfan). The residual insecticide is persistent and remains lethal to *Glossina* spp. for at least 2-3 months when applied to the vegetation. They can be applied by ground spraying or by aerial spraying (Holmes and Torr, 1988). The non-residual insecticide is often sprayed as an aerosol that drifts on the wind and kills any tsetse on which sufficient insecticide impinges.

The effectiveness of the modern traps for catching tsetse, together with the cost and foreign exchange requirements of methods of tsetse control involving the spraying of insecticides, resulted in the increased use of traps and insecticide impregnated screens for control purposes, using dark colours and host odour (carbon dioxide and acetone) to attract the tsetse flies. These methods of control are useful in small areas, such as riverine sections, ranches and feedlots and could be carried out by relatively unskilled personnel (Jordan, 1986).

Other methods of tsetse control include the use of the sterile insect technique which was so successful in the screw-worm eradication campaigns. However, the low reproductive potential and low rate of increase of *Glossina* make large scale rearing cumbersome, expensive and dependent on skilled manpower. Furthermore, the sterile insect technique is impractical for use against high density population of tsetse without prior reduction by some other means (Jordan, 1986).

Tsetse control as a strategy for trypanosomiasis control is only feasible under appropriate conditions. Controlled areas are generally not isolated from nearby infested areas and control measures, therefore, have to be repeated regularly which is expensive with methods based on insecticides. A careful assessment must be made, taking into

account technical, economic and social factors, before making any commitment to long-term tsetse control operations.

In recent years a new method of controlling flies has become available, namely, the pour-on. This form of control is environmentally more acceptable since the chemicals are sprayed directly onto the animals and also controls nuisance flies. However, again the conditions have to be appropriate. Pour-on is relatively expensive, labour intensive, and has to be applied regularly.

Factors influencing the pathogenesis and impact of the disease

Apart from the virulence of the trypanosome strain the most important factor influencing the pathogenesis and impact of the disease is the resistance of the host to the disease. Various breeds of livestock in West and Central Africa are able to survive and be productive in areas where other breeds of livestock succumb to trypanosomiasis. This quality of trypanotolerance is generally attributed to the N'Dama, and West African Shorthorn breeds of cattle, West African Dwarf goats and Djallonke sheep.

The resistance to trypanosomiasis exhibited by these breeds has developed by natural selection by constant exposure to infection over many generations over thousands of years. However, some individuals of supposedly trypanotolerant breeds are much more susceptible to infection than others (Roelants *et al.*, 1983).

Mechanisms of trypanotolerance

The mechanisms behind trypanotolerance are not clear yet and the search for a readily identifiable marker which is linked to the gene(s) regulating the responsiveness

of the host would be a useful aid in progress towards practical methods of selection. It is generally believed that the superior resistance of trypanotolerant animals lies in their capacity to control parasitaemia and resist anaemia. Research indicates that both processes although controlled genetically are not directly linked to each other (Murray and Dexter, 1988). Trail *et al.* (1992) found no relationship between mean optical density (OD) values in antigen ELISA and packed cell volume values.

Immune responses in trypanotolerant animals

Differences have been reported in the immune responses against the trypanosomes involved in African animal trypanosomiasis. Whereas antibody responses to *T.b.brucei* (Black *et al.*, 1983) and *T.congolense* (Mitchell and Pearson, 1986) occur after most organisms have differentiated into non-dividing forms when the parasite population expansion in the blood is no longer exponential, the antibody response to *T.vivax* (Mahan *et al.*, 1986) occurs during the exponential phase of the parasite population. Thus, the capacity to control *T.b.brucei* and *T.congolense* parasitaemia appears to lie in the ability to regulate parasite growth followed by the induction of the immune response, while in *T.vivax* infections it appears to be directly dependent on the ability to mount an immune response.

It appears that the superior capacity of trypanotolerant animals to control parasitaemia is mainly associated with the immune response. In a study comparing the immune response to *T.congolense* of trypanotolerant N'Dama cattle with trypanosusceptible Boran cattle it was shown that the N'Dama had significantly higher numbers of B cells and null cells than the Boran. The results suggested a superior capability of the trypanotolerant N'Dama to generate antibody responses that are

qualitatively and quantitatively better. More recent research suggested that, in contrast to N'Dama cattle, Boran cattle may have a dysfunction in the switch from IgM to IgG after trypanosome infections (Authie *et al.*, 1993). Superior antibody responses have also been reported in *T.congolense* infected Baoule cattle. The Baoule cattle mounted an earlier and much greater neutralising antibody response to the first peak bloodstream trypanosomes than Zebu cattle, possibly reflecting an inherent ability to produce a superior secondary response (Akol *et al.*, 1986; Pinder *et al.*, 1988).

Research with trypanosusceptible mice has shown that living trypanosomes or short-lived components of degenerating trypanosomes can impair the capacity of parasite-induced antibody-containing cells to secrete immunoglobulin.

There is also evidence to indicate that macrophages could play an important role in controlling parasitaemia. Wildebeest serum has been found to have a higher capacity than cattle to induce adherence of trypanosomes to their own peripheral blood leukocytes and that this difference could be attributed to the presence of IgM receptors in wildebeest peripheral blood leukocytes.

However, macrophages have also been found to produce cytokines, such as interferon gamma and cachectin/tumour necrosis factor which mediate the pathology of the disease (Tracey *et al*, 1988). These cytokines are also most probably involved in the regulation of trypanosome growth (Authie *et al.*, 1994). Nitric oxide produced by macrophages produces immunosuppression through the suppression of parasite-antigen-specific T-cell proliferative responses (Schleifer and Mansfield, 1993). Russo *et al.* (1989) reported that trypanosusceptible mice presented higher macrophage activation than resistant mice during an infection with a myotopic strain of *T.cruzi*.

Cachectin/tumour necrosis factor was only detected in the serum of the trypanosusceptible mice.

Plasma zinc levels have also been implicated in the susceptibility of trypanosomiasis. Traore-Leroux *et al.* (1985) reported significantly higher levels of plasma zinc in trypanosensitive cattle compared with trypanotolerant ones. Generally, infected animals characteristically exhibit, in addition to fever, a lowering of plasma zinc and iron concentrations (Van Miert, 1985). Researchers have suggested that the combination of fever and low plasma zinc and iron levels act together as a co-ordinated non-specific host defence mechanism (Zwart *et al.*, 1990).

Control of anaemia in trypanotolerant animals

Trail *et al.* (1992) reported possibilities for selection within the trypanotolerant N'Dama cattle breed on the basis of packed cell volume when antigenaemic since very significant effects were found of above or below average packed cell volume values on weight gain in animals that were antigen-positive a high number of times.

There is little doubt that the severity of anaemia is to a large extent dependent upon the intensity of parasitaemia, an observation that has led to the general belief that trypanotolerance has more to do with the ability to control parasitaemia than an inherent capacity to resist red cell destruction or mounting a more efficient erythropoietic response.

However, a number of researchers have reported that calves of less than one year are more resistant to the effects of trypanosome infections than adults. It was found that calves developed anaemia which was significantly less severe than in adults, despite similar intensities of parasitaemia, indicating the younger animals have a

superior erythropoietic response. Studies involving Red Maasai sheep suggest that its resistance to trypanosome infections is primarily a result of its ability to mount an effective erythropoietic response as opposed to the ability to control the level of parasitaemia (Murray and Dexter, 1988).

Esievo *et al.* (1986) reported that the sialic acid concentrations of erythrocytes were about seven fold greater in the trypanotolerant N'Dama than in the trypanosusceptible Zebu, suggesting that the red cell surfaces of the N'Dama are 'stronger' than the zebu. The N'Dama red cells would be protected more from trypanosome derived sialic acid-cleaving enzymes.

Other mechanisms of Trypanotolerance

The lipid-rich high density lipoprotein (HDL) of human serum has been found to be trypanolytic for animal strains of *T.b.brucei* (Rifkin, 1978). Hajduk *et al.* (1989) recently associated this trypanosomicidal with a minor subclass of high density lipoproteins containing at least 3 unique proteins. In contrast, Traore-Leroux *et al.* (1987) reported significantly higher levels of high density lipoprotein-cholesterol levels in trypanosensitive Zebu cattle compared with trypanotolerant Baoule cattle. They suggested that the higher levels of high density lipoprotein-cholesterol in the trypanosensitive zebu acts as a substrate supporting trypanosome growth and multiplication.

Factors influencing trypanotolerance

Although trypanotolerance has a genetic basis, it is not absolute as these animals can succumb to trypanosomiasis when exposed to high challenge, or when under stress caused by such factors as nutritional status of the host, intercurrent infection, pregnancy, lactation or a heavy workload (Murray *et al.*, 1979a).

In areas of Africa where livestock can be kept under low to medium tsetse challenge, with or without the help of trypanotolerant breeds of livestock, the other major constraint to animal production is malnutrition, especially during the prolonged dry season. N'Dama cattle in the Gambia have been shown to lose up to 15% of their body weight during the dry season (Dwinger, 1987; 1988). Herdsmen have, over centuries, weighted the increased risks of trypanosomiasis in the riverine areas against the better pastures in these areas during the dry season. Therefore, it is important to look at trypanotolerance in relation to the nutritional status of the animals. Research in The Gambia has already shown that there is a difference in the resistance to trypanosomiasis of N'Dama cattle between the wet and dry season which is most probably due to the difference in nutritional status of the animals (Agyemang *et al.*, 1990; 1992).

In the next chapter the interaction between nutrition and trypanosome infections will be reviewed. Results of work on other parasitic infections will also be included.

CHAPTER 2

Review of the relationship between nutrition and parasitic diseases in general and trypanosomiasis in particular

Introduction

Describing the relationship between nutrition and parasitic infections is not an easy task. On the one hand, it involves the direct effects of parasitic infections on feed intake, digestive function and metabolism of the host and, on the other hand, it involves the nutritional status of the host which will affect its' responses to the parasitic infection. Furthermore, these two factors are also linked to each other and interactive. The extent to which parasitic infections affect feed intake, digestive function and metabolism is determined by several factors including the virulence of the parasite, the stage of infection and the susceptibility of the host. The nutritional status and diet of the host may also directly affect feed intake, digestive function and metabolism during parasitic infections.

Nevertheless, an attempt will be made to discuss aspects of the parasite-nutrition relationship for parasitic diseases in general and for trypanosomiasis specifically.

Effects of parasitic infections on the host

Feed intake and diet selection during parasitic infections

One of the major consequences of parasitic infections on animal productivity is the occurrence of anorexia. Acute infections of *Babesia bovis* and *B.marginale* (Ristic, 1981) in cattle and *B.ovis* and *B.motasi* in sheep and goats (Hall, 1985) have been reported to cause anorexia. *Theileria* species, such as *Theileria parva* (Irvin and Cunningham, 1981) and *T.annulata* (Uileberg, 1981) also cause anorexia during acute infections in cattle as is the case with the rickettsial diseases, such as heartwater (*Cowdria ruminantium*) and anaplasmosis (Hall, 1985).

A reduction in feed intake has also been reported in many gastrointestinal nematode and trematode infections (Dargie *et al.*, 1979c; ; Sykes *et al.*, 1980; Abbott *et al.*, 1985b; Kimambo *et al.*, 1988; Dynes *et al.*, 1990). The degree of inappetence has been shown to vary with the level and duration of the parasitism. Blackburn *et al.* (1991) reported a depression of feed intake during the first three months in kids infected with *H.contortus* every two weeks, but feed intakes of the infected kids were higher than the controls during the fourth month of infection.

The control of feed intake is complex, involving both peripheral and central nervous system factors (Forbes, 1986). Little is known about the mechanism by which parasites induce a depression in feed intake but it appears to be dependent on the species of parasites involved. The reduced feed intake during *Ostertagia* infections has been associated with abdominal pain, altered abomasal pH, gastrointestinal motility, elevated circulating hormone concentrations, or direct neural effects on the central nervous system. Pain may be associated with local damage in the abomasum, although this is difficult to quantify. Elevated pH limits abomasal protein digestion and the consequent altered amino acid production could affect feed intake since some amino acids are known appetite stimulants (Leng, 1981). In this respect it is worth noting that the level of dietary protein intake has been found to affect total feed intake during *Haemonchus contortus* infections. The hormone gastrin becomes elevated during ostertagiasis (Fox *et al.*, 1989a; 1989b). Fox *et al.*, (1989c) reported a 40 % reduction in feed intake in calves during artificially elevated gastrin concentrations to levels similar to those observed in ostertagia infected calves.

Some evidence exists that *Trichostrongylus colubriformis* parasites cause elevated cholecystokinin levels (Symons and Hennessy, 1981c). Cholecystokinin has

been associated with feed intake depression (Forbes, 1986). However, Dynes *et al.* (1990) disputed the importance of cholecystokinin in the depression of feed intake during *Trichostrongylus colubriformis* infections and suggested the central satiety signals are involved.

In *Fasciola* infections appetite is generally not affected during fluke migration through the liver parenchyma, suggesting that liver damage *per se* is not important. There is, nevertheless, a good correlation between the onset and severity of feed intake depression and the development of anaemia and hypoproteinaemia in association with adult infections in the bile ducts (Berry and Dargie, 1976).

Generally, during acute infections in which the parasite burden is gradually lost there is generally a slow restoration of appetite, whilst in chronic infections which are not expelled the appetite may remain depressed (Parkins and Holmes, 1989).

Kyriazakis and Oldham (1993) reported that sheep are able to select a diet that meets their crude protein requirements. These results suggest that ruminants infected with parasites can ameliorate the effects of anorexia by selecting the more nutritious parts of a diet. Verstegen *et al.* (1989) suggested that the increased metabolisability of energy ingested in *Dictyocaulus viviparus* infected calves was due to a higher dietary concentrate to roughage ratio selected by the calves.

Feed intake and diet selection during trypanosome infections

A decrease in feed intake during a trypanosome infection has been reported in *T.vivax* and *T.congolense* infected West African Dwarf goats (Verstegen *et al.*, 1991). Wassink *et al.* (1993) found a large range in feed intake response during trypanosome infections with some goats refusing almost all the feed offered whereas others

continued to consume most of the feed offered. These results show that there is a large difference in productive response to a trypanosome infection within the trypanotolerant West African Dwarf goat breed. Reduced feed intakes have also been reported in *T.vivax* infected West African Dwarf sheep (Reynolds and Ekwuruke, 1988).

It is unlikely that the factors involved in feed intake depression during gastrointestinal parasite infections are the same during protozoal infections. Some researchers have implicated proteins produced by activated macrophages and endothelial cells during infections. Tracey *et al.* (1988) carried out some experiments with cachectin/tumor necrosis factor from macrophages and discovered rats injected with this factor showed a significant reduction in feed intake which was abolished after injections with anticachectin antibodies. Cachectin is also known to induce the release of interleukin-1 from endothelial cells and macrophages. Interleukin 1 has also been associated with feed intake depression (Moldawer *et al.*, 1987). Interleukins and interferons produced by activated phagocytic cells induce fever (McCarthry and Van der Kluger, 1984; 1985). The role of fever in trypanosome-induced anorexia remains unclear. Wassink *et al.* (1991) reported a significant correlation between rectal temperature and feed intake during a *T.congolense* infection (r=0.60) in West African Dwarf goats. Zwart *et al.* (1991) found a clear relation between rectal temperature and feed intake in four *T.vivax* infected West African Dwarf goats, but no such relationship was found in twelve others.

Reynolds and Ekwuruke (1988) found in their dwarf sheep that the proportions of *Panicum maximum*, *Leucaena leucocephala*, *Gliricidia* and cassava peel consumed remained constant after a *T.vivax* infection. However, results from *T.congolense* infected N'Dama cattle show a reduced intake of *Andropogon gayanus* while the

animals continued to consume the amount of groundnut hay and cake offered (Romney *et al.*, 1994). Diet selection is most probably a very important aspect in the savannah woodlands south of the Sahara desert where the variety of vegetation is greater.

Water intake and retention during parasitic infections

Increased water intake and water retention have been reported in calves (Parkins *et al.*, 1982a; 1982b) and sheep (Abbott *et al.*, 1986b) infected with helminths. Increases in water retention are associated with elevations in tissue water content, changes in body water turnover and expansion of the plasma volume (Parkins and Holmes, 1989). The causes in these alterations in fluid balance have not been examined in detail, however, these changes clearly demonstrate that tissue loss attributable to parasitic infections cannot be reliably determined from changes in body weight alone. Rowe *et al.* (1988) reported that *Haemonchus contortus* infected lambs lost less weight during the experimental period than their pair-fed controls which they attributed to the higher water retention and a change in body composition towards increased total body water in the infected animals. Oedema of the subcutaneous and intramuscular connective tissues has also been found in babesiosis (Hall, 1985) indicating changes in the body fluid balance.

Water intake and retention during trypanosome infections

Hardly any information on changes in water intake and water retention during trypanosome infections is available. However, increases in plasma volumes during trypanosome infections have been reported (Holmes 1976; Dargie *et al.*, 1979a; 1979b). It was suggested that the expansion of plasma volume is a normal homeostatic response for maintaining blood volume and pressure in the face of the drop in red cell mass.

It is known that N'Dama cattle are able, like camels, to withstand a limited rise in body temperature from 34.4 °C at dawn to 41.1 °C in the late afternoon (Greig and McIntyre, 1979). This phenomenon is probably an important means to reduce evaporative water loss.

Digestive function during parasitic infections

Dietary energy is often the first limiting factor on the level of production achieved in ruminants. Metabolisable energy intake in ruminants can be divided into fermentable metabolisable energy (FME), metabolisable energy from dietary oils and fats and metabolisable energy from fermentation acids present in forages such as silage, or pre-fermented feeds such as brewery and distillery by-products. Of these only FME can supply molecules of energy yielding ATP to the rumen microbes. The FME supply is normally the first limiting factor on microbial protein synthesis. The yield of microbial crude protein (y), expressed as g MCP per MJ of FME, is dependent upon the level of feeding and rumen outflow rates which can both be impaired during parasitic infections.

Occasionally, the nitrogen supply to microbes may be limiting the microbial protein synthesis. The effective rumen degradable protein (ERDP) is a measure of the total N x 6.25 supply captured by the microbes. By definition ERDP is used with an efficiency of 1.0 for microbial protein synthesis, so in ERDP limiting situations the amount of MCP is equal to the amount of ERDP.

About 25% of the MCP is present as nucleic acids, which cannot be used by the ruminant for synthesis. Furthermore, of the 75% MCP left only 85% is digestible in the intestines, so that the digestible microbial true protein is 0.6375 x MCP. The digestible undegraded feed protein (DUP) is the fraction of the feed which has not been degraded during its passage through the rumen, but which is sufficiently digestible to be absorbed in the lower intestines of the animal. Metabolisable protein is therefore defined as:

$$MP\ (g/d) = 0.6375MCP + DUP \qquad \text{(AFRC, 1993).}$$

Little is known about changes in microbial fermentation during a parasitic infection. Roseby (1977), working with *T.colubriformis* infections in sheep, has shown increases in the caecum-proximal colon volatile fatty acid and ammonia concentrations and pool sizes and speculated that increased microbial fermentation occurred. Rowe *et al.* (1988) reported a change in the pattern of fermentation in *Haemonchus contortus* infected sheep towards an increased value for propionate:acetate which was associated by the authors to either the increased reticulorumen motility or a more specific effect on the microbial population, or both. The finding of Steel (1972), of a 62% reduction in rumen acetate production without any change in rumen fluid outflow in sheep infected with *T.colubriformis*, indicate that a post rumen nematode infection may alter rumen fermentation without affecting fluid flow rate. Rowe *et al.* (1988) also found an

increase in ammonia and non-ammonium-N entering the duodenum in *Haemonchus contortus* infected sheep. This increase in the production of ammonia by the abomasum in parasitised lambs was probably due to the breakdown of blood protein by both the parasite and the host. There was more urea synthesis from ammonia, more urea excretion and increased recycling of urea to the gastrointestinal tract in parasitised lambs. Several authors had previously observed increases in plasma urea concentrations (Parkins *et al.*, 1973; Roseby, 1977; Dargie, 1980). However, part of this increased plasma urea concentration could have been caused by an increase in the catabolism of body protein stores. In situations of limited FME availability the increase in recycled urea to the rumen cannot be used by the microbes for protein synthesis. However, the work of Rowe *et al.* (1988) suggests a greater fermentation activity in the large intestine of *Haemonchus contortus* infected lambs indicated by a gain in non-ammonia-N between the ileum and the rectum.

No specific effect of fascioliasis on the digestibility of feed nitrogen was found (Sykes *et al.*, 1980). Nitrogen digestibilities were also unaffected in *Dictyocaulus viviparus* infected calves (Kroonen *et al.*, 1986; Verstegen *et al.*, 1989) despite the higher concentrate to roughage intake ratio found after infection. However, reduced apparent digestibility of N has been observed in lambs infected with the intestinal parasites *Trichostrongylus colubriformis*(Poppi *et al.*, 1986; Kimambo *et al.*, 1988) and a concurrent infection with *T.colubformis* and *Ostertagia circumcincta*(Bown *et al.*, 1991). This was not caused by malabsorption, as they proved by using ^{35}S labelled microbial protein, and the authors implicated increased plasma protein, epithelial cell desquamation and mucus secretion as the source of increased endogenous nitrogen. A trickle infection of *O.ostertagi* in calves has been shown to cause reduced nitrogen

digestibility which it was suggested may have been a response to hypergastrinaemia (Fox *et al.*, 1989a). Abbott *et al.* (1986b) reported no significant differences in crude protein digestibilities in *Haemonchus contortus* infected sheep.

Lungworm infections had no significant effect on energy digestibility (Kroonen *et al.*, 1986; Verstegen *et al.*, 1989). However, reduced apparent gross energy digestion during *Ostertagia circumcincta* infections has been reported by several authors in both cattle and sheep (Parkins *et al.*, 1973; 1990; Sykes and Coop, 1977). It has been suggested that much of the reduced digestible energy of food in sheep and calves parasitised with helminths may also be attributed to increased turnover of epithelial cells and plasma albumin (Parkins *et al.*, 1990).

Abbott *et al.* (1985) reported a greater absorption of dietary iron in *Haemonchus contortus* infected lambs than in the control lambs which resulted in abnormally high levels of serum iron during the latter stages of the infection.

Many studies have demonstrated that mechanical disturbances occur in the gastrointestinal tract during parasitic infections. However, the type and extent of these disturbances appear to depend on the type of parasites and/or host. A trickle infection of *O.ostertagi* in calves has been shown to cause a reduced rate of passage of digesta which the authors attributed to hypergastrinaemia (Fox *et al.*, 1989b). Ponies infected with strongyle nematodes showed significantly smaller motility responses in the large intestine than uninfected ponies, and it was concluded that reduced food intake in the infected ponies was the effect and not the cause of the changed motility pattern (Bueno *et al.*, 1979). Gregory *et al.* (1985) reported a reduced intestinal motility in the upper regions of the duodenum in *T.colubriformis* infected sheep. *Haemonchus contortus* infected sheep, however, were reported to have an increased rate of duodenal-

migrating myoelectric complexes and a shortened cycle of antral contractions of the abomasum. These disturbances were associated with alterations to abomasal acid secretions and to ionic permeability in the gastrointestinal mucosa (Bueno *et al.*, 1982). It is possible that these changes increased the motility of the reticulorumen.

Diarrhoea is a feature of babesiosis and occasionally accompanied by a spasm of the anal sphincter causing faeces to be expelled forcefully in a long thin stream (Hall, 1985). East coast fever (*Theileria parva*) also causes diarrhoea, but Mediterranean fever (*Theileria dispar*), a disease primarily of calves causes constipation rather than diarrhoea. In the latter disease haemorrhagic ulceration of the abomasum and sub-mucous haemorrhage of the small intestine can be found on necropsy. The recovery of cattle from heartwater (*Cowdria ruminantium*) is often accompanied by a severe diarrhoea and on necropsy haemorrhages can be found on the mucosa of the stomach and intestines (Hall, 1985).

Digestive function during trypanosome infections

Little is known about changes in digestibility of the diet components during trypanosomiasis. No significant differences in metabolisability of energy between *T.vivax* infected West African Dwarf goats and their controls were observed by Verstegen *et al.* (1991). Hamminga (1989) and Verstegen *et al.* (1991) also found no differences in nitrogen and dry matter digestibility between *T.vivax* infected West African Dwarf goats and their controls. However, the controls used in that experiment were not pair-fed which makes direct comparison difficult.

No differences in digestibility of nitrogen were found between *T.vivax* infected West African Dwarf goats and their controls, although variability within weeks appeared to be higher in the infected group (Hamminga, 1989; Verstegen *et al.*, 1991).

The reduced nitrogen digestibility due to endogenous nitrogen losses in the gastrointestinal tract demonstrated by work with gastrointestinal parasites are unlikely to occur in the majority of trypanosome infections, although in some *T.vivax* infections, which produce an acute syndrome resulting in death within 2 to 3 weeks of infection, massive haemorrhages into the alimentary tract have been found (Hudson, 1944; Mwongela *et al.*, 1981). These haemorrhages could possibly lead to the loss of endogenous nitrogen.

Hardly any studies have been conducted to examine changes in gastrointestinal motility during trypanosome infections. Van Miert *et al.* (1986) found inhibition of ruminal contractions during the acute phase response in *T.vivax* infected goats. In contrast, Veenendaal *et al.* (1976) did not find a significant inhibition of ruminal contractions also in *T.vivax* infected goats.

However, as reviewed in the paragraph on feed intake during trypanosome infections, feed intake is often reduced during infection and this may have a profound effect on both the rate of passage and the digestibility of the diet. The AFRC (1993) reported that the ratio of effective rumen degradable protein (ERDP) to digestible undegradable protein (DUP) is affected by rumen outflow rates and hence by the level of feeding. The decrease in feed intake often observed during trypanosome infections (see previous chapter) together with a possible reduced gastrointestinal motility may lead to a decrease in DUP and an increase in ERDP of the feed components. The decrease in DUP in favour of ERDP may lead to a change in nitrogen digestibility.

Energy and nitrogen metabolism during parasitic infections

A decrease in metabolisability of energy was found in *Dictyocaulus viviparus* infected calves (Kroonen *et al.*, 1986), but later research indicated this was likely to be due to a higher dietary concentrate to roughage intake ratio in the *Dictyocaulus viviparus* infected calves (Verstegen *et al.*, 1989). Energy utilisation was found to be compromised during *Ostertagia* infections. Sykes and Coop (1977) demonstrated a 30% reduction in the utilisation of metabolisable energy in lambs infected with *O.circumcincta*. Similar results were reported by the same authors in a *T.colubriformis* infection with a 37% reduction in the efficiency of utilisation of ME for growth (Sykes and Coop, 1976). Subsequent studies, however, indicated no differences in the relationship between energy expenditure *v.* ME intake between *T.colubriformis* infected sheep and their pair-fed controls (MacRae, 1993). Differences in results on energy utilisation may be attributed in part to high individual variability in energy utilisation between individuals and within individuals (Shetty, 1993).

Many balance studies have demonstrated that reduced nitrogen retention is often a characteristic feature of gastrointestinal parasitism. This is often the result of increased urinary nitrogen losses. Radioisotopic techniques have shown that high levels of blood protein loss into the gastrointestinal tract are a consistent finding in helminth infections (Holmes, 1985), but the increases in faecal nitrogen, as reported by Sykes and Coop (1977) and Symons *et al.* (1981a) may represent only a relatively small proportion of the total endogenous protein loss which has escaped re-absorption. Most losses occur via the urine as urea synthesised from ammonia absorbed in the gastrointestinal tract, but also as non-ammonia-N indicating poor re-utilisation of the reabsorbed nitrogen for protein synthesis at tissue level and deamination of amino acids

for glucose production. There is also evidence of a shift in protein synthesis of animals infected with gastrointestinal parasites away from muscle and bone and towards the repair, replacement and reaction to damage of the gut wall, to mucus production and to plasma and whole blood loss (MacRae, 1993).

Verstegen *et al.* (1989) reported that results from *Dictyocaulus viviparus* infected calves indicated that they needed more feed for body weight gain than uninfected calves. These researchers concluded that the extra feed requirements were due to an increased maintenance requirement and probably reduced protein retention from digested protein. The same increase in maintenance requirements is likely to occur in tickborne haemoparasites, like *Babesia*, *Theileria* and *Anaplasma* species, which are all known to cause fever (Hall, 1985).

Anaemia is a main characteristic of many parasitic diseases. Reductions in packed cell volume have been found in tickborne haemoparasitic diseases, such as *Anaplasma marginale* and *Babesia bigema* infections (Parker *et al.*, 1985). Gastrointestinal parasites, such as *Haemonchus contortus* (Abbott *et al.*, 1985; Rowe *et al.*, 1988) also cause anaemia, mainly through the loss of blood into the gastrointestinal tract. Severe anaemia leads to a decrease in the distribution of oxygen throughout the body necessary for the aerobic catabolism. The decrease in oxygen supply together with the increase in maintenance requirements will lead to a shift from aerobic catabolism to the less efficient anaerobic catabolism.

Energy and nitrogen metabolism during trypanosome infections

Changes in nitrogen and energy metabolism due to trypanosomiasis may be associated with a direct effect of the parasite and by the indirect effect of the decrease in feed intake.

Factors influencing the energy and nitrogen metabolism of the host during trypanosomiasis are fever, metabolism of the parasite, catabolism of immunological products (Nielsen *et al.*, 1978), repair of damaged tissue (Van den Ingh *et al.*, 1976b) and protein loss through damaged kidneys (Van den Ingh *et al.*, 1976a). An increase in maintenance energy requirements of 25% has been found in *T.vivax* infected West African Dwarf goats (Verstegen *et al.*, 1991) and Zwart *et al.* (1991) suggested that fever may be related to a higher metabolic rate. The efficiency of regulation of heat loss in relation to the environment is one of the factors affecting the amount of energy required to heat the body to a given temperature. When heat losses are not controlled properly, metabolic rate increases (Blatteis *et al.*, 1988). Temperature rises of 2.5 °C in 24 hours have been found in trypanosome-infected goats (Zwart *et al.*, 1991) indicating that these regulatory mechanisms are not well balanced during trypanosomiasis.

The increase in metabolic rate together with decrease in feed intake often reported during trypanosome infections results in the animal using its body protein and energy reserves. At the same time the anaemia, which is an inevitable consequence of trypanosome infections, will lead to reduced availability of oxygen in the tissues and lead to the inefficient anaerobic catabolism for energy production. The consequences will be especially severe in working animals.

The central factor in preventing disorders of energy metabolism is the regulation of homeostasis in the supply and demand for glucose. Even for ruminants glucose is an essential metabolite (Payne, 1989). They need an adequately high plasma glucose concentration for normal brain function. Normally this is effectively controlled by hormones (e.g. insulin, glucagon, and epinephrine). Glucose is not an end product of digestion in the ruminant. The fermentation of the carbohydrates (cellulose and hemicellulose) in the reticulo-rumen results in volatile fatty acids such as acetic, propionic, and butyric acids. Of these volatile fatty acids only propionic acid can serve as a precursor of glucose. Therefore, ruminants must synthesise their own supplies from glucogenic precursors such as propionic acid, glucogenic amino acids and from glycerol.

In a fasting animal plasma glucose levels are maintained by mobilising carbohydrates from anywhere they can be found. Normally hepatic glycogenolysis predominates, with some lipolysis and gluconeogenesis, but if starvation is prolonged lipolysis and even proteinolysis become important. Increased urinary urea and creatinine levels have been reported in *T.vivax* infected West African Dwarf goats (Hamminga, 1989) indicating that tissue protein catabolism occurs. Otesile *et al.* (1991) noted a rise in serum transaminase (serum aspartate and alanine aminotransferases) levels in *T.b.brucei* infected pigs, especially in the infected animals on a low plane of dietary energy.

Non-esterified fatty acids increase as a result of lipolysis. The glycerol resulting from lipolysis is used to synthesise glucose. When fasting proceeds, larger proportions of non-esterified fatty acids are converted into ketone bodies which can be used as fuel for tissues such as cardiac and skeletal muscles (Payne, 1989). Significant increases in

both non-esterified fatty acids and β-hydroxy butyrate have been reported in *T.congolense* infected West African Dwarf goats (Wassink *et al.*, 1993).

However, changes in the energy and nitrogen metabolism during trypanosomiasis cannot be explained solely by the increased demand on the body protein and energy reserves, especially, with the more pathogenic strains of trypanosomes.

In his review, Anosa (1988), concluded that hypoglycaemia only occurred in the presence of large numbers of parasites in the circulation presumably because of utilisation of glucose for trypanosome metabolism.

In infected animals the cells of the liver and lymphoid tissues rapidly increase their rates of synthesis of the proteins for the host defensive mechanisms (Beisel, 1985). These anabolic processes cost energy and may be limiting the synthesis of other proteins such as albumin.

Tracey *et al.* (1988) reported the isolation of a serum factor called cachectin/tumor necrosis factor produced by activated macrophages. This factor causes a syndrome termed cachexia and is characterised by loss of appetite, weight loss, a catabolic metabolism, and anaemia. Cachectin was found to suppress lipoprotein lipase and several other key lipogenic enzymes in adipocytes leading to hypertriglyceridaemia in mice and rabbits infected with *T.b.brucei*. Although a reduction in lipoprotein lipase activity has been reported in sheep infected with *T.vivax* and *T.congolense* this was not associated with hypertriglyceridaemia (Roberts, 1974; 1975). Katunguka-Rwakishaya (1992) also found no significant increase in plasma triglyceride concentrations in *T.congolense* infected sheep although he reported a

tendency for it to be higher in the infected groups. These results may be due to the fact that triglycerides have a rapid clearance from the blood plasma.

In contrast to increases in cholesterol concentrations found in *T.b.brucei* infected rabbits (Tracey *et al.*, 1988) decreased concentrations of cholesterol have been reported in humans (Huet *et al.*, 1990) and ruminants infected with trypanosomes (Roberts, 1974, 1975; Traore-Leroux *et al.*, 1987; Katunguka-Rwakishaya, 1992).

The reasons for the decrease in cholesterol during trypanosomiasis are not known. Direct uptake by trypanosomes has been implicated. The cholesterol is taken up by *T.b.brucei* via rapid receptor-mediated endocytotic uptake of low density lipoproteins followed by lysosomal digestion and the release of cholesterol into the cytosol (Vandeweerd and Black, 1989). Bloodstream forms of *T.b.brucei* has been found to use almost exclusively host sterols, but other stages may synthesise endogenous sterol (Coppens *et al.*, 1987).

The relationship between hypocholesterolaemia and anaemia has been investigated in man (Westerman, 1975) and it was concluded that hypocholesterolaemia was not related to anaemia. The author attributed the decrease in cholesterol to its redistribution or to plasma dilution.

Sheep infected with *T.vivax*, *T.congolense* or *T.b.brucei* show a significant reduction in blood phospholipids (Roberts, 1975; Katunguka-Rwakishaya, 1992). Roberts (1975) reported that this fall in phospholipids was mainly due to a fall in lysolecithin from 7 - 10% to 0 - 3% of the total phospholipid and suggested that this indicated a direct interference of trypanosomes in the host's metabolism in the liver, as the bulk of lysolecithin synthesis probably takes place in the liver. Another explanation may be the release of phospholipases and lysophospholipases by disrupted

trypanosomes which act on endogenous phospholipids leading to the release of fatty acids and the subsequent reduction in the concentration of phospholipids. The trypanosomes use these lipases for the metabolism of exogenous phospholipids taken up from the host. Mellors and Samad (1989) reported that bloodstream forms of trypanosomes are dependent on their hosts for fatty acids, choline and other components of membrane lipids. The bulk of the trypanosome's choline requirement is met by their ability to take up lysophospholipids from the host tissue fluids.

Mineral metabolism during parasitic infections

Sykes *et al.* (1977) reported that lambs infected with a continuous dose of *Ostertagia circumcincta* deposited bone minerals (calcium and phosphorus) at only 35% of that of non-infected pair-fed controls. In lambs infected with *T.colubriformis* the effect was even greater and almost complete cessation of skeletal growth has been reported (Sykes and Coop, 1976).

The possible causes of impaired skeletal growth in parasitised sheep appear to be dependent upon the level and site of infection. In lambs infected with the abomasal parasite *O.circumcincta* the skeletal changes have been attributed to deficiencies of protein and energy induced by the parasite leading to a matrix osteoporosis (Sykes *et al.*, 1977). In *T.colubriformis* infected lambs the matrix osteoporosis probably also occurs, but it is exacerbated by a reduction in the apparent absorption of phosphorus (Sykes and Coop, 1976; Poppi *et al.*, 1985) and the true absorption of phosphorus from the intestines (Wilson and Field, 1983; Bown *et al.*, 1989). There are also increased losses of endogenous phosphorus and calcium as a result of intestinal parasitism, but apparently not with abomasal infections (Wilson and Field, 1983).

The net effect of this is to induce a phosphorus deficiency, leading to a reduced flow of salivary phosphorus and a reduction in the plasma concentration of phosphorus (Coop and Field, 1983). The effect on calcium metabolism was found to be limited to an increase in endogenous faecal excretion. During ovine fascioliasis no significant changes in serum calcium, magnesium, phosphorus, urea, iron, bilirubin concentrations and total iron-binding capacity were observed (Sykes *et al.*, 1980).

Abbott *et al.* (1985) reported a fall in serum iron concentration for 8-10 weeks following a *Haemonchus contortus* infection but found a rise thereafter. The rapid rise to abnormally high levels was presumed to be due to increased absorption of dietary iron and not to the reabsorption of Hb-iron lost by haemorrhage.

Bang *et al.* (1990a) showed that infection of sheep with *T.circumcincta* affected copper metabolism primarily through the elevation of the pH of the abomasum. The results suggest that the reduced abomasal acidity decreases the solubility of administered copper oxide wire particles and hence lowers the uptake of copper by the liver.

Mineral metabolism during trypanosome infections

Little research has been conducted on the deposition of calcium and phosphorus in the skeleton of trypanosome-infected animals. Katunguka-Rwakishaya (1992) reported no significant differences in the concentration of phosphorus and calcium in the carcase and the whole body between *T.congolense* infected sheep and their controls.

French (1938a) reported increased excretions of body bases, chlorides and phosphates in trypanosome-infected sheep and cattle. The same author reported no

changes in the inorganic phosphorus content of the blood during trypanosomiasis, but increases in whole blood sodium and chlorine contents. The potassium contents of the blood fell during trypanosomiasis, the fall being attributable to the anaemic conditions of the blood reducing the proportion of blood cells to plasma (French, 1938b).

Although differences were found in plasma zinc levels between trypanotolerant and trypanosensitive cattle, no changes were found in plasma zinc levels after an infection with trypanosomes. Copper and magnesium levels were found to be unaffected by trypanosome infections in cattle (Traore-Leroux *et al.*, 1985).

Some research has been carried out on serum iron and iron-binding capacity. Tartour and Idris (1973) reported an early hypoferraemia during a *T.congolense* infection in Zebu cattle which was somewhat reduced during the later stages, while a hyperferraemia was observed in the terminal stages. The unsaturated iron-binding capacity was progressively reduced which together with the hypoferraemia led to a reduced total iron-binding capacity. Full saturation of transferrin appeared to be a constant feature of collapse before death, both in acute and chronic *T.congolense* infections. Hypoferraemia and reduced iron-binding capacities were also observed during a relatively mild infection of *T.congolense* in sheep (Katunguka-Rwakishaya, 1992).

Carcase composition during parasitic infections

As a result of the reduced retention of energy and nitrogen during parasitic infection discussed above less energy and nitrogen is available for growth. Entrocasso *et al.* (1986) clearly demonstrated that cattle infected with a mixed natural trichostrongyle infection over two grazing seasons had significantly poorer killing-out percentages and associated carcase measurements, and in particular the total masses of muscle and fat were depressed in affected animals. Significant carcase compositional differences were also observed in growing sheep infected with *Haemonchus contortus*, in which the total quantities of stored energy and protein were significantly lower in the infected animals (Parkins and Holmes, 1989).

Carcase composition during trypanosome infections

Little is known about changes in carcase composition in trypanosome-infected ruminants. Katunguka-Rwakishaya (1992) found lower total carcase protein and fat content after a relatively mild infection with *T.congolense* in sheep fed two levels of protein and this appeared to be due to differences in carcase weight and not to changes in the composition of the carcase. The different levels of dietary protein fed to the sheep appeared to have a greater influence on carcase composition than the trypanosome infection. As mentioned previously, the trypanosome infection did not have a significant effect on the calcium and phosphorus composition of the carcase.

Wool and milk production during parasitic infections

It has been reported that gastrointestinal parasites have an adverse effect on wool production. The threshold level of exposure to trichostrongyle infections in

weaner lambs for impairing wool growth was found to be between 950 and 3000 *T.colubriformis* larvae per week, with the maximum effect occurring during the first 12 weeks (Steel *et al.*, 1980). In the case of *O.circumcincta* an intake of more than 1200 larvae per week was required to produce significant effects (Symons *et al.*, 1981b). When lambs were concurrently infected with *T.colubriformis* and *O.circumcincta*, wool production was reduced by up to 66% (Steel *et al.*, 1982). Interestingly, Barger *et al.* (1973) observed a 17-18% reduction in wool production of sheep resistant to *T.colubriformis* when subjected to a larval challenge. This finding may be due to a shift of amino acids from wool production to the immune system .

Studies on the effects of trichostrogylosis on milk production in cattle have mainly concentrated on the effects of anthelmintic treatment around parturition but the results have been conflicting. Some workers have reported improvements in milk yield (McBeath *et al.*, 1979; Bliss *et al.*, 1982), whereas others found no beneficial effects of treatment (Michel *et al.*, 1982). In sheep, milk production was decreased by 17% during an infection with *O.circumcincta* (Leyva *et al.*, 1982) and by 23% during an infection with *H.contortus* (Thomas and Ali, 1983).

One of the symptoms of babesiosis in dairy cows is a fall in milk yield immediately after the first appearance of the parasites in the blood (Hall, 1985).

Wool and milk production during trypanosome infections

No information is available on changes in wool production during trypanosome infections, partly because wool production does not occur in areas infested with tsetse. However, milk production is important in the tsetse infested areas of Africa and some papers are available on milk production of trypanosome-infected ruminants. It was

demonstrated that trypanosome infections reduce the milk yield of N'Dama cows kept under village management conditions in The Gambia (Agyemang *et al.*, 1990). In a recent experiment Little *et al.* (in press) observed a slightly lower rate of decline in milk production of trypanosome-infected N'Dama cows receiving cotton seed as a supplement than trypanosome-infected unsupplemented cows, but differences were not statistically significant. Milk production in sheep was found to be significantly affected by a *T.vivax* infection during the second half of lactation but not during early lactation. No effects of infection on the different milk components were found during this infection (Akinbamijo, personal communication).

There have been some reports that the stress of lactation adversely affects the resistance to trypanosome infection. Agyemang *et al.* (1992) concluded that the physiological states of pregnancy and lactation tended to predispose N'Dama cows to trypanosome infections, and that the interaction between physiological status and trypanosome infections appeared to affect their ability to maintain PCV levels and body weights.

Effects of nutrition on the hosts' responses to parasitic infections

Effect of nutritional status and diet on the hosts' responses to parasitic infections

Most research on the effects of nutrition on the resistance to parasitic infections has concentrated on supplementation with dietary protein. Berry and Dargie (1976) conducted two experiments with *Fasciola hepatica* infected sheep. In the first experiment *F.hepatica* infected sheep were divided into a group fed a diet containing 6% crude protein and a group fed a diet containing 13% crude protein. However, the

diets did not appear to be completely isocaloric. It was found that the group on the lower protein ration experienced more rapid anaemia, hypoalbuminaemia and weight loss, and died earlier than their better fed counterparts. Since the fluke burdens were comparable between the two groups it was concluded that the advantages of the high protein diet were the greater capacity of the sheep to withstand the parasites' pathogenic effects rather than a superior ability to limit infection. The results of the first experiment were supported by the findings in the second experiment in which chronically infected sheep were switched from a high to a low protein diet. These sheep also developed the disease faster.

Dargie *et al.* (1979c) demonstrated that weight loss was higher in *Fasciola hepatica* infected sheep fed a diet consisting of only hay compared to a diet consisting of hay and concentrate. The authors concluded that blood loss and inappetance led to catabolism of muscle protein, particularly in low protein diets.

The effects of dietary protein on the pathogenesis and pathophysiology of acute ovine haemonchosis was investigated by Abbott *et al.* (1986a, b). The diets used were isocaloric. The results showed that the lambs on the low protein (88 g cp/kg DM) were less able to withstand the pathogenic effects of infection with 350 *H.contortus* larvae kg^{-1} body weight than lambs given a higher protein diet (170 g cp/kg DM). Mortality was greater in the low protein group and adverse clinical signs such as anorexia, weight loss and oedema were observed more frequently in this group than in lambs given the higher protein diet. Haematological studies indicated more severe anaemia, hypoalbuminaemia and hypoproteinaemia in the low protein group, despite similar levels of gastric blood loss in both dietary groups. Circulating red cell volumes were similar in both groups of infected lambs and plasma volumes were expanded in both

groups to a similar degree. The majority of lambs in both dietary groups responded equally well to the gastric haemorrhage by increasing the rate of red cell production and, although serum iron levels fell after infection, absorption of dietary iron was greater in the infected lambs than in the controls. Faecal egg counts, total daily faecal egg output and worm burdens were similar in both dietary groups, and therefore, diet did not appear to have had any effect on parasite establishment.

In a more recent experiment in which goats were infected every two weeks with *H.contortus* larvae it was also found that nutrition was more important to counteract the consequences of infection than to counteract the establishment of that same infection (Blackburn *et al.*, 1991). In this experiment the PCV of the kids with the highest worm burdens (2000 larvae) and the lowest level of nutrition (growth + maintenance) did not recover in month 4 of infection and remained below 20%, whereas the PCV of the other 3 groups (growth + maintenance + 500 larvae; twice growth + maintenance + 2000 or 500 larvae) did recover.

Wilson and Trueman (1978) investigated the effects of reduced energy intake on the development of anaplasmosis in *Bos indicus* cross steers. Surprisingly, anaplasmosis was found to be less severe in the animals on the low energy diet. The animals on the low energy diet had a significantly lower parasitaemia and a lower decrease in packed cell volume. The anaplasmosis caused losses in body weight in the animals on the high energy diet but not in the ones on the low energy diet compared with their controls. The only advantage of the higher energy diet was found in the quicker recovery from anaemia in the recovery phase of the experiment. However, it is unlikely that the low energy diet was only low in energy. The authors acknowledge

that the lower parasitaemia in the low energy group could be due to a amino acid deficiency essential to *A.marginale* parasite growth.

Although it is not surprising that dietary protein affects the development of immunity, as many immune components such as immunoglobulin and lymphokines are largely proteinaceous in nature, there is little information about the effect of dietary protein on the development of immunological competence against parasites. Recently, Kambara *et al.* (1993) demonstrated that dietary protein level (110 g CP or 200 g CP/kg DM) had an effect on the resistance to *T.colubriformis* in young lambs, but this was not apparent in older animals. Similarly, Abbott and Holmes (1990) showed that protein supplementation of 7-month old lambs which were immunised with irradiated *H.contortus* larvae had no effect on the response to vaccination whereas a high protein diet (169 g CP/kg DM) has been shown to increase host resistance to infection with *H.contortus* in 4-month old lambs (Abbott *et al.*, 1988). Wagland *et al.* (1984) also found a positive effect of the level of dietary protein on resistance to infection with *T.colubriformis* and a significant correlation between the serum complement-fixing antibodies and resistance in young lambs which were 17 to 21 weeks of age at challenge. However, Gregg *et al.* (1978) reported that resistance in older animals vaccinated with *T.colubrifomis* was not antibody dependent and hence dietary protein may not be that important in this age group. Kambara *et al.* (1993) found that lymphocyte responsiveness to L3 larval antigen was negatively correlated with faecal egg counts, and with the number of eggs present in the nematode uterus, but was not associated with worm burdens. They concluded that lymphocyte responsiveness to L3 antigen and non-specific mitogens is affected by age, and may be involved in the resistance to endoparasites exhibited by older vaccinated lambs. Interestingly, a higher

resistance to anaplasmosis has been found in younger animals than in older ones. Contradictory results on the lower susceptibility to *Babesia bigemina* infections in animals previously infected with *Anaplasma marginale* have been blamed on age effects (Parker *et al.*, 1985).

Of major importance is research to investigate whether genetic resistance is lost when nutrition is sub-optimal. Abbott *et al.* (1985a, b) investigated the effects of different levels of dietary protein (170 g CP or 88 g CP/kg DM) in two breeds of sheep (Finn-Dorset and Scottish Blackface) infected with *Haemonchus contortus* known to differ in their susceptibility to the disease. It was found that both breed and diet influenced the severity of the pathophysiological changes associated with the infection. Finn-Dorset lambs had higher faecal egg counts and more pronounced anaemia than the Blackface lambs. Diet influenced the degree of anaemia in both breeds but not the faecal egg count. The results indicated that the benefits of a superior genetic background are not lost on a low protein diet whilst a high protein diet can overcome the disadvantages of an inferior genetic background, at least in sheep exposed to moderate parasite challenge.

Although macro mineral and trace element deficiency and nematode infections occur concurrently in many grazing situations hardly any research has been carried out on supplementation with macro-minerals and trace elements and its relationship with nematode infections.

Coop and Field (1983) were able to show that an increase in the phosphorus content of the diet (from 1.88 to 2.75 g P/kg DM) increased the weight gain of lambs receiving 2500 *Trichostrongylus vitrinus* L_3/day. The faecal egg counts and total *Trichostrongylus vitrinus* burdens were higher (10,950 worms) in lambs receiving the

low phosphorus diet compared to those on the high phosphorus ration (1240 worms) suggesting that low phosphorus levels may impair the development of resistance to continuous infection.

Bang *et al.* (1990b) showed that administration of copper oxide needles, which release soluble copper in the abomasum, 5 days prior to infection with gastrointestinal nematodes reduced the establishment of *H.contortus* and *T.circumcincta* in treated lambs by 96 and 56 % respectively. There was no effect of the copper oxide wire particles on the establishment of *T.colubriformis*.

The addition of molybdenum to the diet in sheep either trickle-infected with *T.vitrinus* (Suttle *et al.*, 1992a) or with *H.contortus* (Suttle *et al.*, 1992b) reduced worm populations by 23 and 78 % respectively and higher numbers of intraepithelial mast cells occurred in the supplemented *H.contortus* infected lambs. Indirect evidence suggested that molybdenum may enhance the inflammatory process in addition to having a direct effect on the parasite.

Cobalt deficiency has been reported to induce higher faecal egg counts and increased pepsinogen levels in *T.circumcincta* infected lambs (Ferguson *et al.*, 1989), whereas selenium deficiency does not appear to affect resistance to nematode infection (McDonald *et al.*, 1989).

Effect of nutritional status and diet on hosts' responses to trypanosome infections

What little is known about the relationship between nutrition and the hosts' responses to trypanosome infection is mainly based on protein supplementation. (Holmes, 1993). These responses are mostly measured as anaemia and haematopoietic responses and the changes in productive performance.

Ageymang *et al.* (1990) reported that the effects of trypanosomiasis on packed cell volume were more severe during the late dry season than during the early dry season. Similar results on packed cell volumes were found by the same authors in cattle monitored for three years (Ageymang *et al.* 1992). Ageymang *et al.* (1990) also found that *T.congolense* infected grazing N'Dama cows supplemented with a mixture of rice bran, groundnut cake, milled andropogon hay and common salt showed a fall in packed cell volume to a similar extend as unsupplemented grazing animals. However, the packed cell volume of the supplemented N'Damas recovered during the second month after infection to pre-infection levels, whereas the packed cell volume of the unsupplemented infected animals continued to fall. Little *et al.* (in press) found similar results in that cotton seed supplemented lactating cows recovered from a decline in packed cell volume due to trypanosomiasis, whereas unsupplemented infected cows did not recover. Little *et al.* (1990) showed a beneficial effect towards packed cell volume of a higher plane of nutrition in *T.congolense* infected N'Dama bulls. However, the syndrome appeared to be more severe in animals in better body condition at the time of infection.

No significant differences were found in packed cell volume changes after a *T.congolense* infections between sheep on different dietary protein levels. However,

the anaemia resulted in a haematopoietic response, as measured by the increase in mean corpuscular volume, in the sheep on a high protein diet but not in the ones on a low protein diet (Katunguka-Rwakishaya, 1992). The infected sheep on a low protein diet experienced greater retardation of growth than their control group, while infected and control animals on a high protein diet grew at the same rate. Similar results were found by Agyemang *et al.* (1990) in N'Dama cattle exposed to natural fly challenge. These results indicate that diets supplemented with protein can increase the response of trypanosome-infected animals to anaemia. Abdullahi *et al.* (1986) reported low PCV concentrations in protein deprived sheep indicating that dietary protein may be of great importance in preventing anaemia. The question whether the impaired erythropoiesis is due to a direct effect of protein deprivation or a lack of the haemopoiesis stimulating agent, erythropoietin, remains unsolved.

The conclusion that dietary protein can enhance the response to anaemia was further supported by the more rapid recovery of the packed cell volume after treatment with isometamidium chloride in the sheep fed a diet high in protein (Katunguka-Rwakishaya, 1992).

There appeared to be a greater decline in packed cell volume, red blood cell count and haemoglobin levels in *T.congolense* infected sheep fed a diet low in energy compared with those on a higher plane of energy (Katunguka-Rwakishaya, 1992). Similar results were reported in *T.b.brucei* infected pigs which showed a decline in packed cell volume of 11.0, 35.1 and 37.5% on a high, medium and low energy ration (Fagbemi *et al.*, 1990).

In contrast to sheep fed a low protein diet, infected sheep on a low energy diet did not show an impairment of the haematopoietic responses (Katunguka-Rwakishaya, 1992).

Trail *et al.* (1992) reported a strong relationship between packed cell volume values and weight gain during trypanosome infections. The authors concluded that this may be a basis for selection of the most resistant animals.

The results of the research reviewed above indicate that level of protein in the diet may affect the genetic responses of the host to trypanosome infections and may therefore influence selection on the basis of anaemia during infection.

Katunguka-Rwakishaya (1992) observed higher levels of serum total lipids, cholesterol and non-esterified fatty acids in sheep fed a high protein diet compared with those on a low protein diet. The author could not explain these results but indicated that the higher intensity of parasitaemia observed in the sheep fed the high protein diet may be the result of better substrate conditions for trypanosome growth in these sheep.

Introduction to experimental work

The experimental work presented in this thesis was conducted to study the relationship between the resistance to trypanosomiasis and diet in ruminants. The interaction between nutrition and trypanosomiasis on digestive function was investigated in particular. The experiments described were part of a wider project funded by the EEC DGXII programme. The institutes involved were the International Trypanotolerance Centre in The Gambia, the Centre de Recherches sur les Trypanosomoses Animales in Burkina Fasso, the Centre de Cooperation International de Recherche Agronomique pour le Developpement in France, the Natural Resources Institute in Chatham and the University of Glasgow Veterinary School. The title of the project was: "The Interaction between Nutrition and Genetic Resistance to Trypanosomiasis in Trypanotolerant Livestock".

The purpose of the experiments conducted at Glasgow University Veterinary School was to examine some of the effects of the disease in more detail and in a more controlled environment. Scottish Blackface wether lambs were used as experimental animals in the experiments, since they have been found to be relatively resistant to trypanosomiasis. It has to be stressed that unlike the trypanotolerant breeds of cattle, sheep and goats in West Africa these sheep have not acquired an innate resistance over generations. They are, however, like the N'Dama very hardy animals which can survive under difficult circumstances. Pair-fed control animals were used in the experiments which were brothers of the infected animals. With the use of pair-feeding the infected animals can be fed *ad libitum* and this allows the effects of nutrient utilisation to be separated from effects on appetite. This is especially important when conducting experiments on diet digestibility and rate of passage.

Chapter 3 describes the General Materials and Methods used to conduct the experiments.

The work described in Chapter 4 investigated the changes in feed intake, diet digestibility and rate of passage, the blood haematology and biochemistry in *T.congolense* infected lambs fed two differing types of roughage diets.

Chapter 5 describes the changes in feed intake, nitrogen balance diet digestibility and rate of passage, the blood haematology and biochemistry in *T.congolense* infected lambs fed two levels of roughage fibre. Chapter 6 describes a similar experiment as the one in Chapter 5 but this time a *T.vivax* infection is used.

Chapter 7 discusses the influence of urea-supplementation on the pathophysiology of a *T.congolense* infection in lambs.

In Chapter 8 an experiment is reported in which the effect of dietary arginine restriction on parasitaemia levels in *Trypanosoma congolense* infected mice is investigated.

A general discussion of the results, its implications and conclusions end the thesis in chapter 9.

CHAPTER 3

General Materials and Methods

Experimental animals

The Scottish Blackface lambs used in the experiments were selected from a local hill farm flock in the West of Scotland. The lambs were male castrates, aged between 5 and 6 months of age and had not been weaned prior to the arrival at Glasgow University Veterinary School. With the exception of the lambs used in the experiment of Chapter 5, pairs of twin lambs were selected. On the farm the lambs had been vaccinated against orf and pasteurella and had been wormed twice. An hour before transport the animals were ear tagged. Prior to transport, the lambs were also walked through a footbath containing Hoof Phast (Net-Tex Agricultural Ltd.) against footrot and treated with Crovevect (pour on) (Crown Veterinary Pharmaceuticals Ltd.) against tics, headflies, lice and blow flies.

Upon arrival at the Veterinary School the animals were immunised against pasteurella and clostridial infections using Ovivac-P (Hoechst Animal Health) and dewormed with Systamex 2265. Faecal samples were checked at the time of deworming and two to three weeks later to confirm their worm-free status. The feet of the animals were trimmed and dipped in a 10% solution of zinc sulphate to prevent foot rot. The animals were housed and weaned onto high quality hay. After about two week they were also offered small amounts of barley grain. All lambs were used in the experiments within a few weeks of weaning, with the exception of the lambs in the experiment of Chapter 5 which were kept at grass for 7 months.

Three weeks before each experiment started the animals were introduced to the experimental feeds. Blood samples were taken 3 weeks and 1 week before each experiment started and analysed for packed cell volume (PCV) to check for underlying problems.

Experimental housing

In the first two experiments the lambs were housed in individual pens on a concrete floor bedded with wood shavings. In the last two sheep experiments the sheep were kept in metabolic stalls for the collection of faeces and urine. The animals were fed twice a day at 0900 h and 1500 h. The lambs were given the roughage in wooden boxes to reduce the amount of wastage. They had free access to fresh water at all times.

Experimental infections

Approximately two weeks after the experiments started one animal of each pair was infected with trypanosomes. The stocks of trypanosomes were kept in microhaematocrit tubes stored in liquid nitrogen. The trypanosomes were cultured in irradiated mice and harvested during the first rising parasitaemia. Each lamb was inoculated intravenously with 5×10^5 trypanosomes in 2 to 4 ml phosphate buffered saline (PBS) (containing 1.5% glucose).

Digestibility and nitrogen balance measurements

Rumen degradability measurements

Samples of all feeds were analysed for rumen degradability in 2 fistulated cows. The samples were incubated in the rumen in dacron bags. Duplicate samples of the concentrates were taken from the rumen after 8, 16, 24, and 48 hours and roughage samples 16, 24, 48 and 72 hours of incubation. Dry matter (DM) and crude protein (CP) were determined using standard methods.

The results were expressed as the loss of CP from the bag over the period (t, hours) of incubation:

CP loss = 1 - (Residual CP in bag)/(Original CP in bag(t=0)).

DM Degradation curves were determined using the NEWAY programme (Rowett Research Institute, Bucksburn, Aberdeen; McDonald, 1981).

Collection of feed, faecal and urine samples

Apparent digestibility of the proximate feed fractions and nitrogen balances (feed N - (faecal N + Urinary N)) for four animals in each of the infected and pair-fed control groups were measured during two different balance periods. During each of the 7 day collection periods composite samples of roughage and concentrate feeds offered were collected. Where applicable, refusals for each animal were collected before the morning feed and stored. After the 7 day collection period a composite sub-sample of the feeds were taken. Total daily faecal output was collected using plastic bags connected to harnesses (Fishwick, 1973). The bags were emptied at 0800 h and 1700 h and the weights recorded. During each period cumulative daily faeces from each animal was stored in sealed plastic bins. At the end of each period 2 duplicate sub-samples of the fresh faeces were taken. One of the samples was slurried (using an atomix mixer) with approximately 5 ml of toluene and 20 ml of water to prevent nitrogen losses due to bacterial fermentation. The other sample was dried.

Urine was collected into a plastic vessel previously acidified with 100 ml 5 mol/l hydrochloric acid. The weight of the daily urine production was recorded and 10 % bulked for each animal. At the end of each period 2 duplicate sub-samples of the urine were taken.

Analysis of feed, faecal and urine samples

The slurried samples were subsequently analysed for nitrogen (N) and DM.

The composite samples of the roughage and concentrates offered, the dried composite faecal samples and feed refusals were analysed for DM, ash, N, neutral-detergent fibre (NDF), acid-detergent fibre (ADF) and gross energy (GE) using methods described below.

DM was determined by heating known quantities in a forced air-draft oven at 80^{o}C for 48 - 72 h until constant weight. Ash content was measured after heating a feed sample at 500^{o}C in a muffle furnace overnight.

Nitrogen content was determined using an automated Kjeldahl method. Neutral and acid detergent fibre were analysed using methods described by MAFF/ADAS (1981).

Gross energy (GE) content of dried feed samples was measured in an automatic adiabatic bomb calorimeter.

Organic matter (OM) was calculated as the DM minus Ash. The digestibilities of OM, N, NDF, ADF and GE were calculated from the difference in these values between intake and faeces.

The intakes of metabolisable energy, fermentable metabolisable energy, effective rumen degradable protein, digestible undegraded protein and metabolisable protein were estimated using values and calculations from AFRC (1993) and the *in sacco* rumen degradability measurements described previously. Examples of how these intake data were estimated are given in the Appendix.

Creatinine and urea concentrations of the urine sub-samples were measured at the end of each balance period.

Rate of passage measurements

General

The rate of passage of the roughage through the digestive tract was measured in the infected and pair-fed control animals over 5 day periods. Approximately 30 - 50 g of chromium (Cr) mordanted roughage (approx. 120g Cr/kg roughage DM) was offered at approximately 0830 h and before the daily feed. The mordanted roughage was thoroughly mixed with concentrate to increase palatability. Faecal samples were taken at 8, 11, 17, 23, 30, 38, 48, 72, 96 and 120 h after feeding the mordanted roughage.

Preparation of Cr mordant

The roughage was washed thoroughly using a laundry detergent and then rinsed thoroughly with water and acetone to remove all dirt and green material. The fibre that was left after rinsing was then dried at 65^{o}C.

A solution of $Na_2Cr_2O_7$ was prepared containing an amount of chromium equivalent to 12-14% of the weight of the fibre in 2-3 litres of water. The molecular weight of $Na_2Cr_2O_7.2H_2O$ is 298 therefore for 1 kg fibre 401 g $Na_2Cr_2O_7$ is required. The fibre was place in an ovenproof container and the solution added. The fibre was compressed and just enough water added to cover the fibre. The fibre was mixed thoroughly with the solution. The container was covered with aluminium foil and baked in the oven at 100^{o}C for 24 hours. The fibre was then thoroughly washed with tap water.

The fibre was suspended in tap water and ascorbic acid added as 1/2 weight of fibre (i.e. 500 g ascorbic acid for 1 kg of fibre) and left to stand for 1 hour. The fibre was washed thoroughly until free of soluble green matter and dried at $65^{o}C$.

To achieve a rough estimate of the Cr_2O_3 content of the roughage, samples of roughage before and after treatment were dried and ashed and the difference in ash content assumed to be due to the chromium. Quantities administered were calculated using the estimated value. (Uden *et al.*, 1980, 1982; Aitchison *et al.*, 1985; Gasa and Sutton, 1991).

Analysis of the chromium in the faeces

The amount of Cr excreted was determined using Atomic Absorption Spectroscopy after wet digestion according to the method by Christian and Coup (1954).

The model of Dhanoa *et al.* (1985) was used to analyse the chromium excretion data, which contains a double exponential term derived by considering digesta flow as a multi-component exponential process.

The formula is:

$$y = Ae^{-k_1 t} e^{-Be^{-(k_2 - k_1)t}}$$

Where y is Cr concentration, A and B are constants, k_1 and k_2 are rate constants and t is the time of sampling. The rate constants k_1 and k_2 are outflow rate constants for the two largest compartments in the digestive tract likely to be the rumen and possibly the caecum. Mean retention time (MRT) is the mean time between

chromium administration and the appearance of chromium in the faeces. The transit time (TT) or lag time is the time between chromium administration and the first appearance of chromium in the faeces (Dhanoa *et al.*, 1985).

Haematological measurements

Blood was collected from the jugular vein into 5 ml tubes containing ethylene tetra acetic acid (EDTA) for estimation of parasitaemia, packed cell volume (PCV) and a range of blood haematological indices, including red blood cell count (RBC), haemoglobin concentration (Hb), mean corpuscular volumes (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (PLT) and white blood cell count (WBC). The haematological indices were determined by an automated blood cell counter (ABX Minos, Roche Diagnostica). The blood samples were collected between 0800 h and 0900 h, just before the morning feed.

Two microhaematocrit tubes were filled with blood from each sample and the mean PCV determined by spinning the tubes in a microhaematocrit centrifuge for 5 minutes. The buffy coat of one of the haematocrit tubes per sample was examined using the dark ground/buffy coat method (Murray *et al.*, 1977; Paris *et al.*, 1982). The intensities of parasitaemia was graded 1 to 6 (Table 3.1).

Biochemical measurements

Blood was collected from the jugular vein for biochemical analysis in 10 ml tubes containing lithium heparin. In all the experiments the concentration of plasma cholesterol, urea and albumin were measured. Plasma cholesterol and urea

concentrations were determined using commercial kits and plasma albumin concentration by the standard biuret method using an autoanalyser (Technicon, UK). In the first experiment the non-esterified fatty acids were also measured using a commercial kit, but in these case, the analysis was done on blood collected in EDTA tubes. Again the samples were collected between 0800 h and 0900 h, just before the morning feed.

Table 3.1 Parasitaemia scoring

Score	Trypanosomes per field	Estimated parasitaemia (trypanosomes per ml)
1	1-3 per film	10^2 - 10^3
2	4-10 per film	10^3 - 10^4
3	1 per field	5×10^3 - 5×10^4
4	2-10 per field	10^4 - 5×10^5
5	10-100 per field	$> 5 \times 10^5$
6	more than 100 per field	$> 6 \times 10^6$

* magnification = x 400

Statistical analysis

All parameters, except intensity of parasitaemia, were subjected to statistical analysis using a randomised block design with each block consisting of a pair of lambs (one I, one PC). Mean effect over time was calculated and subjected to split plot analysis of variance with consideration of variation between treatments, between animals within treatments and interaction between treatments.

Differences in intakes of the diet components in the infected lambs pre- and post-infection were also tested using the block design mentioned above with each block consisting of a pair of pre- and post-infection measurements.

Intensities of parasitaemia were evaluated by the non-parametric Mann-Whitney test. Growth rates were determined using linear regression analysis.

CHAPTER 4

The Pathophysiology of *Trypanosoma congolense* in Scottish Blackface Sheep. Influence of Type of Diet on Digestive Function

Introduction

Long-term monitoring studies in trypanotolerant N'Dama cattle kept under village conditions in The Gambia have revealed that the effects of trypanosome infections are more severe during the dry season when the quality of the feed available is lower (Agyemang *et al.*, 1990, 1992). In a recent study, at the University of Glasgow Veterinary School, it was found that high protein diets can ameliorate the effects of *T.congolense* infections in Scottish Blackface sheep (Katunguka-Rwakishaya *et al.*, 1993). Similar results had previously been demonstrated in ovine fascioliasis (Berry and Dargie, 1976) and haemonchosis (Abbott *et al.*, 1986).

Whereas a number of experiments have been conducted on the effects of protein supplementation during parasite infections, no studies have been reported on the effects of different types of roughage. In a recent study in The Gambia it was found that N'Dama heifers reduced their intake of poor quality *Andropogon guyanus* hay but consumed all the groundnut hay and cake offered after an infection with *T.congolense* (Romney *et al.*, 1994).

In the present study Scottish Blackface lambs infected with *T.congolense* were offered diets based on barley straw and lucerne hay. Barley straw, like *Andropogon guyanus* hay, is high in fibre but low in nitrogen, whereas lucerne hay is high in both fibre and nitrogen. The differences in quality of the two roughages resulted in different levels of energy being consumed on the two treatments. To reduce the difference in level of crude protein between the diets extra protein was added to the concentrate offered to the lambs receiving barley straw. Scottish Blackface lambs were used in this experiment since they are hardy animals and have been shown, like trypanotolerant breeds, to resist the effects of trypanosomiasis well. The infected and control animals

were pair-fed to avoid the confounding direct effects on nutrient utilisation with decreased intake due to infection.

Feed intake, meal patterns over 24 hour periods and rate of passage of the roughage through the digestive tract were determined. Crude protein, energy and fibre digestibilities of the diets were also measured. Several plasma metabolite and blood haematology parameters were measured. Carcase and fleece fat and protein compositions were determined at the end of the experiment.

Materials and methods

Experimental diets

Four pairs of Scottish Blackface lambs were offered chopped barley straw plus 366 g dry matter of a pelleted barley/soyabean meal concentrate mixture (Diet BS) and the other 4 pairs chopped lucerne hay plus 366 g dry matter of pelleted barley concentrate (Diet LH) (Table 4.1). The concentrates were offered in order to provide the lambs on Diet BS a diet slightly above their maintenance requirements. The roughage was offered *ad libitum* (20% greater than previous day's intake) to one animal of each pair (I). The other animal of each pair was used as a pair-fed control (PC), being offered the same amount of feed the infected counterpart had consumed on the previous day.

The chemical composition of the diets is shown in Table 4.2. Prior to infection, intake of Diet BS resulted in metabolisable protein (MP) and metabolisable energy (ME) intakes of approximately 70 g/day and 8.3 MJ/day, respectively, and for Diet LH intakes of MP and ME were approximately 140 g/day and 13.3 MJ/day, respectively.

Table 4.1 Composition (g DM/day) of the experimental diets offered to both dietary groups

	Diet BS	Diet LH
Barley Concentrate	366	-
Barley/Soyabean Meal Concentrate	-	366
Barley Straw	*Ad libitum*	-
Lucerne Hay	-	*Ad libitum*

Table 4.2 Dry matter (DM; g/kg), organic matter (OM; g/kg DM), metabolisable energy (ME; MJ/kg DM), fermentable metabolisable energy (FME; MJ/kg DM), neutral detergent fibre (NDF; g/kg DM), acid detergent fibre (ADF; g/kg DM), ether extract (EE; g/kg DM), crude protein (CP; g/kg DM), effective rumen degradable dietary protein (ERDP; g/kg DM) and digestible undegraded protein (DUP; g/kg DM) of the diet components

	Diet BS		Diet LH	
Diet Composition	Concentrate	Barley Straw	Concentrate	Lucerne Hay
DM	862	873	862	894
OM	944	953	956	901
ME#	13.3	6.5	13.3	8.8
FME#	12.7	5.9	12.7	7.8
NDF	229	781	256	343
ADF	52	490	46	265
EE	14	9	12	15
CP	209	46	109	206
ERDP*	138	27	84	116
DUP*	71	8	18	47

#: AFRC (1993) values
*: Values derived from in-sacco degradation and AFRC (1993) calculations

Four extra lambs were slaughtered at the start of the experiment to use as baseline control animals.

Experimental infection

Three weeks after the experiment started the lambs were infected with *T.congolense* 1180 (GRVPS 57/6) isolated in Serengeti, Tanzania (Nantulya *et al.*, 1984). Each animal was inoculated intravenously with 5 x 10^5 trypanosomes in 3 to 4 ml phosphate buffered saline (PBS) (containing 1.5% glucose).

Measurements

General measurements

Roughage, concentrate and water intake were measured daily by collecting refusals between 0800 h and 0900 h. Rectal temperatures (RT) were recorded Mondays to Fridays between 0830 h and 0900 h during 3 weeks before infection and the first 4 weeks after infection in the infected groups only. Clinical observations were made daily for any abnormal behaviour. The animals were weighed once a week.

Digestive function measurements

Meal patterns were investigated in the infected groups on day 42 after infection and on day 43 in the pair-fed control groups by measuring the amount of feed eaten at 1200, 1500, 1800 and 2100 h and comparing the infected and pair-fed control groups consuming approximately the same amount of feed.

Digestibility of the diet components were measured during three digestibility (Dig) block periods of 1 week each, one before and 2 after infection. Digestibility

period 1 lasted from day -10 to -3 pre-infection, period 2 from day 19 to 26 post-infection and period 3 from day 50 to 57 post-infection.

The rate of passage of the roughage through the digestive tract was measured on day 37 by feeding chromium mordanted straw fibre to the animals on Diet BS and chromium mordanted lucerne hay fibre to the animals on Diet LH. Details of the procedures were explained in the General Materials and Methods (Chapter 3).

Blood haematological and biochemical measurements

On Mondays, Wednesdays and Fridays blood was collected into tubes containing ethylene tetra acetic acid (EDTA) for the estimation of the level of parasitaemia and blood haematological measurements.

Once a week during the last 3 weeks pre-infection and the first 4 weeks post-infection, and fortnightly during the rest of the trial period, extra blood was taken for biochemical analysis into tubes containing lithium heparin for plasma cholesterol, triglyceride, albumin and urea measurements.

Carcase composition

Method

The carcase composition of the animals was investigated according to the method of Katunguka-Rwakishaya (1992). The animals were killed by stunning with a captive bolt pistol and exsanguinated. After death they were skinned and eviscerated. A sub-sample of the fleece including the skin was then taken and frozen. The carcase was split down the midline with a saw. The right half of the empty carcase was

weighed minus head and stored at -20°C until maceration. The 7th to 10th ribs of the left half of the carcase were removed for dissection.

Preparation of the macerated samples

The right half of the carcase was sawn into small portions of about 15x10x10 cm and minced in an industrial mincer. The samples were minced twice, first through a plate with 15 mm perforations and then through one with 5 mm perforations. The minced samples were mixed thoroughly and 2 sub-samples of about 500 g taken and stored at -20°C.

Analysis of the macerated samples

About 200 g of each sub-sample from each sheep was freeze dried to a constant weight in a high vacuum freeze drier. The dried samples were finely chopped in a liquidiser and subsamples analysed for crude protein (Kjeldahl), crude fat (Ether Extract), ash, calcium and phosphorus. The dry matter, crude protein, crude fat, ash, calcium and phosphorus gain of the animals during the experimental period was determined by subtracting the values of the 4 baseline control animals.

Dissection of the indicator joint

The best end neck (7th to the 10th rib) joint of the left half of the carcase was removed for dissection. The joint was dissected into muscle, fat and bone. The dissected parts were then weighed and the composition expressed as relative percentages.

Analysis of the fleece sub-sample

A fleece sub-sample from each sheep was freeze dried to a constant weight in a high vacuum freeze drier and subsamples analysed for nitrogen (Kjeldahl), fat (Ether Extract), ash (in muffle furnace at 500^{o}C overnight) and gross energy (adiabatic bomb calorimeter).

Results

Rumen degradability of the diet components

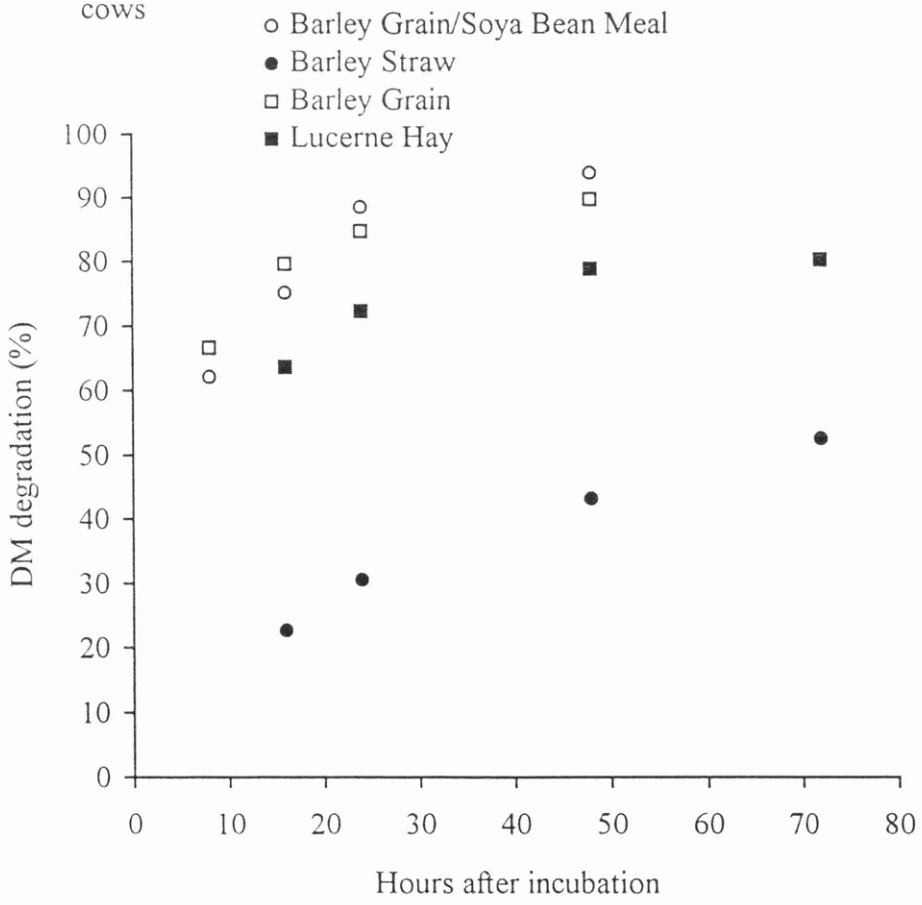
Hardly any difference was found in rumen degradability of the concentrates, both being around 90%, but the degradation of the barley straw was much slower and lower than that of the lucerne hay (Figure 4.1). The results were used to calculate the metabolisable protein (MP) intakes presented in the next chapter on the feed intake of the diet components.

The crude protein losses from the dacron bags in Table 4.3 show that the crude protein loss of the high protein concentrate was slower than that of the low protein concentrate during the first 16 hours after incubation but faster between 16 and 48 hours. Both concentrates ended up at similar level of crude protein loss from the dacron bags. The barley straw crude protein loss was very low compared with that of the lucerne hay.

Table 4.3 Crude protein loss of the different diet ingredients from dacron bags incubated in the rumen of two cows

Incubation time (h)	Diet BS		Diet LH	
	High Protein Concentrate	Barley Straw	Low Protein concentrate	Lucerne Hay
8	0.38		0.57	
16	0.59	0.10	0.76	0.60
24	0.86	0.14	0.89	0.77
48	0.98	0.27	0.95	0.86
72		0.35		0.89

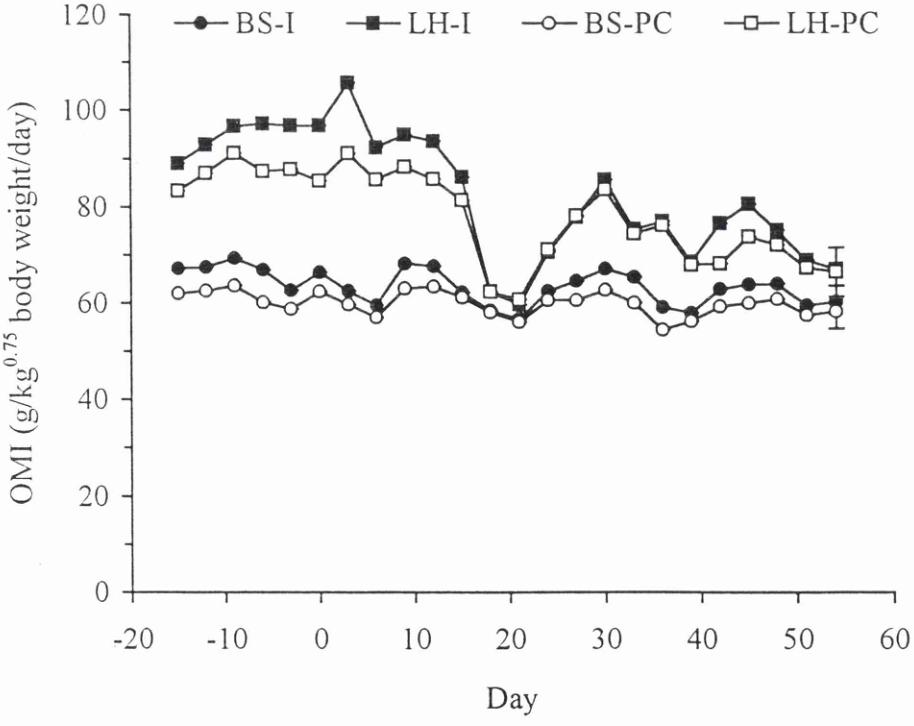
Figure 4.1 Dry matter degradation (%) of the components of Diet BS (barley straw and barley grain/soya bean meal) and Diet LH (lucerne hay and barley grain) incubated in the rumen of fistulated cows



Feed Intake

The organic matter intake (OMI) was slightly higher in the infected animals compared to their pair-fed controls, but this was mainly the case during the pre-infection period (Figure 4.2). Between day 12 and 21 the organic matter intake (OMI) of the infected group fed Diet LH fell, resulting in the OMI being significantly different pre- and post-infection ($p<0.01$; Table 4.4). The organic matter intake (OMI) of the infected group fed Diet BS also decreased significantly ($p<0.01$) but not as much as the infected Diet LH group in absolute terms ($p<0.01$). Despite the difference in the level of feed intake between the two diets the neutral and acid detergent fibre intakes were remarkably similar (Table 4.4).

Figure 4.2 Mean organic matter intake (OMI; $g/kg^{0.75}$ body weight/day) of *T.congolense* infected sheep fed Diet BS (BS-I) or Diet LH (LH-I) and their respective pair-fed controls BS-PC and LH-PC



Due to the decrease in organic matter intake after infection the individual energy and protein components of the diet also decreased. Except for fermentable metabolisable energy (FME; $p<0.05$) these decreases were not statistically significant (Table 4.4).

When calculating the microbial crude protein (MCP) supply from both the fermentable metabolisable energy (FME) and effective rumen degradable dietary protein (ERDP) supplies (AFRC, 1993), it was revealed that in Diet BS the effective rumen degradable dietary protein (ERDP) was limiting the microbial crude protein (MCP) supply whereas in Diet LH the fermentable metabolisable energy (FME) was limiting the microbial crude protein supply (MCP). These results were hardly affected by the *T.congolense* infection.

Diet selection

One of the aims of this trial was to look at changes in diet selection after a *T.congolense* infection. However, the lambs used in this trial showed only a moderate depression in intake and no measurable changes were found in diet selection after infection, although the percentage of concentrate in the diet increased slightly.

Table 4.4 Mean organic matter (OM), metabolisable energy (ME), fermentable metabolisable energy (FME), neutral detergent fibre (NDF), acid detergent fibre (ADF), crude protein (CP), effective rumen degradable dietary protein (ERDP), digestible undegraded protein (DUP) and metabolisable protein (MP) intake of *T.congolense* infected sheep (n=4) fed either Diet BS (M/D = 9.5, y = 9.5, L = 1.4 - 1.7) or Diet LH (M/D = 10.1, y = 10.5, L = 2.2 - 2.7) during the pre- (day -14 - 0) and post- (day 1 - 56) infection periods

Diet	OM ($g/kg^{0.75}$/day)	ME# (MJ/day)	FME# (MJ/day)	NDF (g/day)	ADF (g/day)	CP (g/day)	ERDP# (g/day)	DUP# (g/day)	MP# (g/day)
Pre-BS	66.7	8.1	7.6	461	255	99.7	64.2	30.2	71.2
Post-BS	62.5	7.9	7.4	455	252	98.2	63.2	29.7	70.1
Pooled SE	1.63	0.15	0.14	18.0	11.3	1.10	0.65	0.20	0.62
Pre-LH	94.9	13.2	12.0	417	266	233.9	140.1	50.8	140.2
Post-LH	79.4	12.9	11.8	410	262	229.9	137.6	50.0	137.7
Pooled SE	3.86	0.63	0.55	24.3	18.7	14.59	8.22	3.33	8.57
Diet Effect	**	**	**	ns	ns	**	**	**	**
Period Effect	**	ns	*	ns	ns	ns	ns	ns	ns
Interaction	**	ns	ns	ns	ns	ns	ns	ns	ns

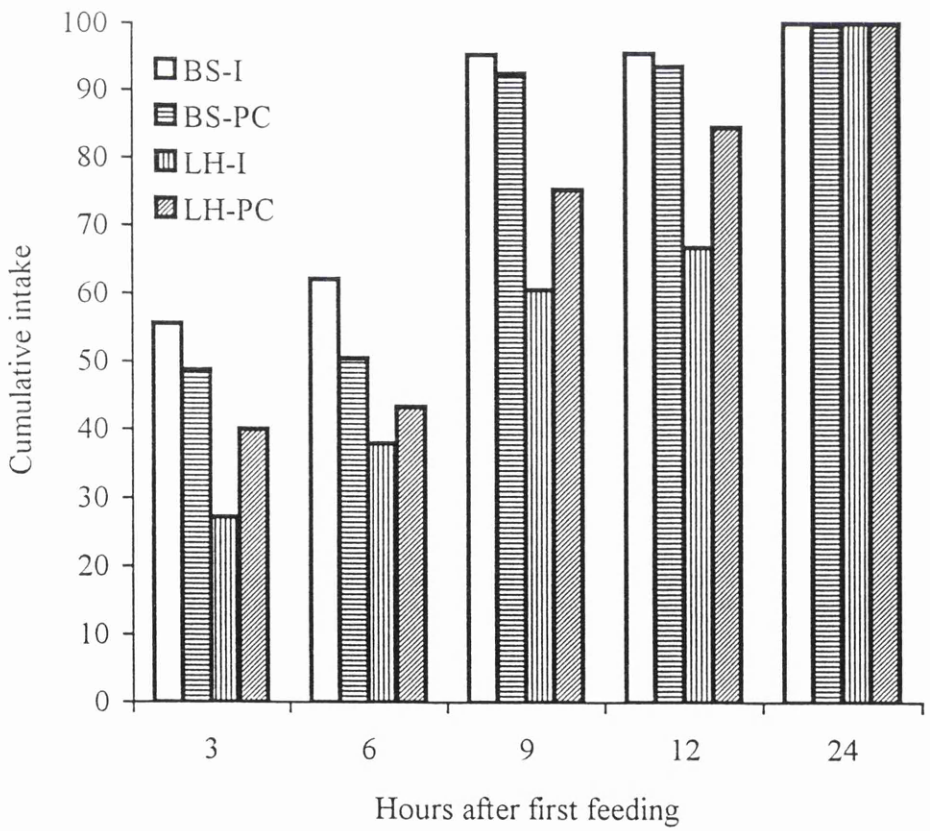
* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means
: Calculated using the AFRC (1993) methods

Feed intake pattern

It was observed that the lambs on the barley straw (Diet BS) consumed most of their daily intake just after the morning and afternoon feeding. In contrast, the intake of the lambs on the lucerne hay (Diet LH) was more spread over the 24 hour period.

There was no apparent difference in barley straw (Diet BS) intake pattern between infected and pair-fed control animals. The infected group fed Diet LH tended to consume a larger part of their total daily lucerne hay intake during the night compared to their uninfected counterparts (Figure 4.3).

Figure 4.3 Mean roughage intake pattern of *T.congolense* infected (I) lambs and the respective pair-fed controls (PC) fed Diet BS or Diet LH over 24 hours on day 42 post-infection. The sheep were fed half the amount at hour 1 and half at hour 6.



Digestibility coefficients of the diets

The samples were dried at 80°C which made the neutral (NDF) and acid detergent fibre (ADF) analysis not very reliable. The neutral and acid detergent fibre digestibility coefficients were at similar levels in the lambs on both diets. There was a high fluctuation of neutral and acid detergent fibre digestibility coefficients between lambs within a group and between periods and no definite conclusions could be obtained from the data (Table 4.5) even though a significant statistical difference was found in acid detergent fibre digestibility coefficients between the infected and pair-fed control lambs on Diet LH ($p<0.01$).

The apparent organic matter (OM) digestibility coefficients were approximately 10 units higher in Diet LH than in Diet BS ($p<0.01$; Table 4.6). The organic matter digestibility coefficients remained stable in the infected groups on both diets throughout the trial period. However, the apparent organic matter digestibility coefficients increased in the pair-fed control lambs as the experiment progressed ($p<0.01$). The concentrate to roughage ratios hardly changed in the lambs fed Diet BS but increased in the lambs fed Diet LH from 0.43 in digestibility period 1 to 0.63 in digestibility period 2. In digestibility period 3 the ratio returned to 0.51.

As for organic matter digestibility coefficients the apparent digestibility coefficients of gross energy (GE) in the Diet LH fed lambs were approximately 10 units higher than those of the Diet BS fed lambs ($p<0.01$). Digestibility coefficients of gross energy remained stable throughout the trial period for both the infected groups. Digestibility coefficients in the pair-fed controls went slightly up towards the end of the trial and were significantly different between the infected and pair-fed control groups ($p<0.01$; Table 4.6). However, since the gross energy digestibility coefficients also

tended to be significantly different between infected and pair-fed control lambs before infection ($p<0.05$) no firm conclusions can be drawn from these results.

The apparent crude protein (CP) digestibility coefficients were approximately 8 units higher in the lambs fed Diet LH compared to the ones on Diet BS ($p<0.01$; Table 4.6). Whereas the crude protein digestibility coefficients remained stable in the pair-fed control lambs throughout the experimental period the digestibility coefficients of the crude protein in the infected lambs decreased ($p<0.01$). No significant interaction effects of diet and infection were observed on these digestibility coefficients.

Table 4.5 Mean digestibility coefficients of neutral (NDF) and acid detergent fibre (ADF) in *T.congolense* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BS or Diet LH during period 1 (day -10 - -3), period 2 (day 19 - 26) and period 3 (day 50 - 57)

	NDF digestibility coefficients			ADF digestibility coefficients		
Period	1	2	3	1	2	3
BS-I	0.49	0.46	0.50	0.42	0.34	0.43
BS-PC	0.44	0.52	0.57	0.36	0.42	0.50
Pooled SE	0.015	0.013	0.020	0.017	0.017	0.017
LH-I	0.51	0.53	0.56	0.41	0.42	0.49
LH-PC	0.50	0.60	0.63	0.40	0.47	0.51
Pooled SE	0.010	0.018	0.018	0.011	0.019	0.022
Diet effect	ns	**	**	ns	*	ns
Infection effect	**	*	ns	ns	**	ns
Interaction	*	ns	ns	ns	ns	ns

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Table 4.6 Mean digestibility coefficients of organic matter (OM), gross energy (GE) and crude protein (CP) in *T.congolense* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BS (barley straw) or Diet LH (lucerne hay) during period 1 (day -10 - -3), period 2 (day 19 - 26) and period 3 (day 50 - 57)

	OM digestibility coefficients			GE digestibility coefficients			CP digestibility coefficients		
Period	1	2	3	1	2	3	1	2	3
BS-I	0.62	0.60	0.61	0.60	0.57	0.59	0.66	0.64	0.59
BS-PC	0.60	0.64	0.67	0.57	0.61	0.64	0.68	0.68	0.67
Pooled SE	0.008	0.010	0.014	0.007	0.012	0.014	0.008	0.012	0.019
LH-I	0.73	0.72	0.72	0.68	0.68	0.69	0.74	0.70	0.71
LH-PC	0.72	0.76	0.76	0.67	0.73	0.73	0.72	0.74	0.75
Pooled SE	0.006	0.009	0.010	0.006	0.010	0.011	0.007	0.010	0.015
Diet effect	**	**	**	**	**	**	**	**	**
Infection effect	ns	**	**	*	**	**	ns	**	**
Interaction	ns	ns	ns	ns	ns	ns	ns	ns	ns

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Mean retention time of the roughage through the digestive tract

Figure 4.4 shows the average excretion of chromium in the faeces of each group of sheep after administration of the chromium mordanted roughage fibre.

The mean retention time (MRT) of the lambs fed Diet BS was significantly longer than those of the lambs fed Diet LH ($p<0.05$) which appeared to be caused by a lower outflow rate constant k_1 ($p<0.01$) in the animals fed Diet BS.

Both the mean retention time ($p<0.01$) and transit time (TT) ($p<0.05$) were significantly longer in the infected lambs compared with the pair-fed control lambs. The rate constants k_1 and k_2 did not appear to have been affected significantly by the infection. No significant interaction effects of diet and infection on any of the mean retention time parameters were found (Table 4.7).

Figure 4.4 Mean excretion of chromium (µg/g)in the faeces after administration of chromium mordanted roughage to *T.congolense* infected sheep fed Diet BS (BS-I) or Diet LH (LH-I) and their respective pair-fed controls BS-PC and LH-PC

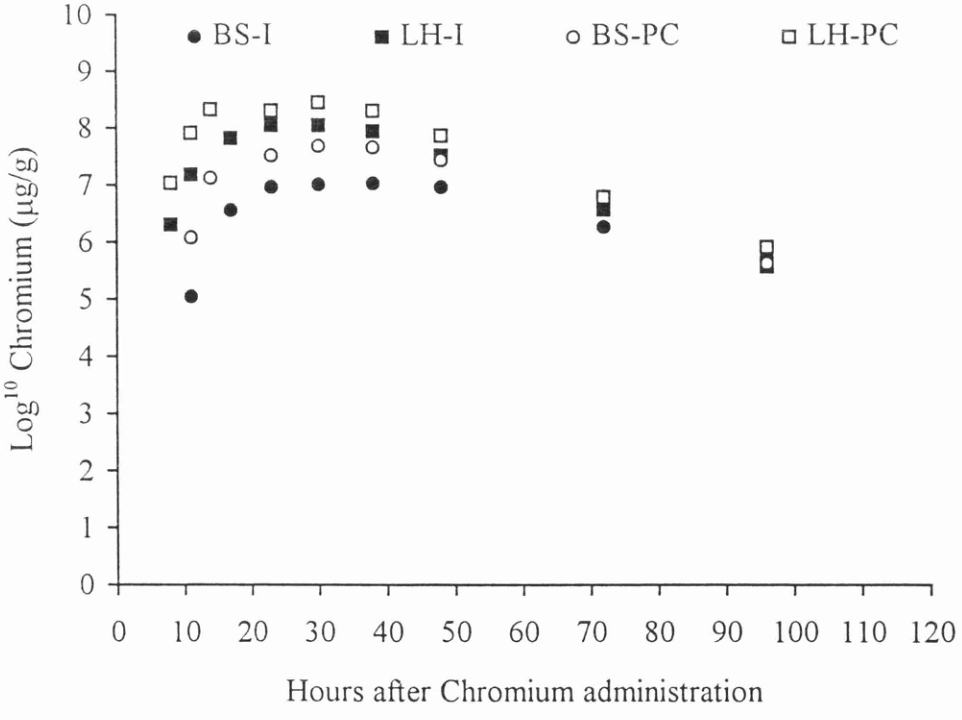


Table 4.7 Mean retention time (MRT; h), transit time (TT; h) and rate constants (k_1, k_2; h^{-1}) of chromium mordanted roughage offered on day 37 post-infection to *T.congolense* infected (I) sheep (n=4) fed either Diet BS (barley straw) or Diet LH (lucerne hay) and their respective pair-fed controls (PC)

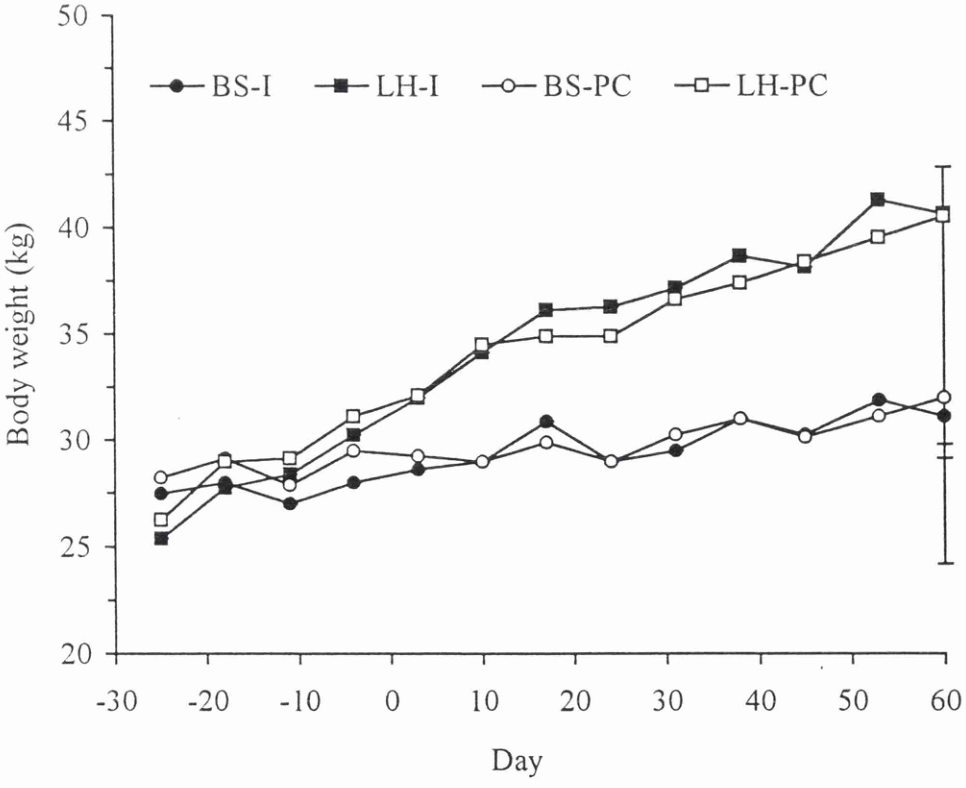
Group	MRT	TT	k_1	k_2
BS-I	62.7	14.5	0.024	0.18
BS-PC	55.4	13.0	0.028	0.21
Pooled SE	3.33	0.49	0.002	0.017
LH-I	43.9	13.1	0.040	0.21
LH-PC	40.7	10.2	0.040	0.21
Pooled SE	1.33	0.85	0.001	0.027
Diet effect	*	ns	**	ns
Infection effect	**	*	ns	ns
Interaction	ns	ns	ns	ns

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Body weight

The body weight changes of the pair-fed control lambs followed the changes of their infected counterparts closely (Figure 4.5). The body weights of the Diet BS fed groups did not change much over the weeks, whereas the Diet LH fed groups showed a body weight gain of approximately 10 kg on average over 10 weeks. After week 3 growth of the Diet LH groups decreased slightly possibly due to the decrease in roughage intake. The growth rates for the Diet BS fed infected and pair-fed control lambs were 51 ± 3.0 and 38 ± 3.8 grams per day, respectively. The growth rates for the Diet LH fed infected and pair-fed control lambs were 185 ± 14.7 and 159 ± 20.0 grams per day, respectively. Growth was significantly affected by nutrition ($p<0.01$) but not by the *T.congolense* infection.

Figure 4.5 Mean body weight (kg) of *T.congolense* infected sheep fed Diet BS (BS-I) or Diet LH (LH-I) and their respective pair-fed controls BS-PC and LH-PC



Killing-out percentage

The final body weight was significantly higher in the lambs on Diet LH than in the ones on Diet BS ($p<0.01$). Infection had no effect on the final body weight (Table 4.8). The killing-out percentage of the Diet BS fed lambs was about 6% lower than the percentage of the Diet LH fed lambs ($p<0.01$). No infection effect on the killing-out percentage was found.

Table 4.8 Body weight (kg), carcase weight (kg) and killing-out percentage (KO %) of *T.congolense* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BS (barley straw) or Diet LH (lucerne hay)

Group	Body weight	Carcase weight	KO %
BS-I	31	13	41
BS-PC	33	13	39
Pooled SE	.8	.4	.6
LH-I	41	19	46
LH-PC	42	19	45
Pooled SE	1.3	.5	.5
Diet effect	**	**	**
Infection effect	ns	ns	ns
Interaction	ns	ns	ns

** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Carcase composition

The carcase of the Diet BS group had a significantly lower dry matter (DM) content than the Diet LH group ($p<0.01$; Table 4.9). The type of diet affected both the fat and protein content ($p<0.01$) of the carcase, with the Diet LH fed lambs showing the higher ether extract (EE), but the lower protein (CP) content per kg dry matter. Total carcase dry matter, ether extract and crude protein gain were significantly higher in the lambs fed Diet LH ($p<0.01$; Table 4.10).

Infection resulted in significantly lower dry matter content and total carcase dry matter gain ($p<0.05$). The total carcase dry matter gain was about 500 grams lower in the infected compared to the pair-fed control lambs. Ether extract and protein contents of the carcase were not significantly different between the infected and pair-fed control lambs.

Total carcase ether extract and crude protein gains were lower in the infected groups compared to their pair-fed control counterparts, however, differences were not statistically significant (Table 4.10).

The percentage of ash, calcium and phosphorus in the carcase was significantly higher in the lambs fed Diet BS compared with those on Diet LH (Table 4.9), but the total gain of these inorganic materials was significantly higher in the lambs on Diet LH (Table 4.10).

No interaction was found between the diet and infection on any of the carcase parameters measured.

Table 4.9 Mean carcase dry matter (DM (g/kg)), ether extract (EE (g/kg DM)), crude protein (CP (g/kg DM)), ash (g/kg DM), calcium (Ca (g/kg DM)) and phosphorus (P (g/kg DM)) composition of *T.congolense* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BS (barley straw) or Diet LH (lucerne hay)

Group	DM (g/kg FM)	EE (g/kg DM)	CP (g/kg DM)	ash (g/kg DM)	Ca (g/kg DM)	P (g/kg DM)
BS-I	342	375	457	131	37	23
BS-PC	376	405	439	114	32	20
Pooled SE	8.8	16.4	13.8	5.1	1.6	0.8
LH-I	433	536	326	97	29	18
LH-PC	452	543	327	97	28	17
Pooled SE	7.0	7.8	6.6	2.2	0.8	0.4
Diet effect	**	**	**	**	**	**
Infection effect	*	ns	ns	ns	ns	ns
Interaction	ns	ns	ns	ns	ns	ns

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Table 4.10 Mean total carcase dry matter (DM (g)), ether extract (EE (g)) crude protein (CP (g)), ash (g), calcium (Ca (g)) and phosphorus (P (g)) gain# of *T.congolense* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BS (barley straw) or Diet LH (lucerne hay)

Group	DM (g)	EE (g)	CP (g)	ash (g)	Ca (g)	P (g)
BS-I	1415	738	456	186	48	25
BS-PC	1966	1091	616	180	46	27
Pooled SE	234.4	160.0	71.9	20.6	6.7	3.6
LH-I	5252	3484	1147	417	125	73
LH-PC	5727	3796	1308	459	132	77
Pooled SE	320.6	219.2	92.3	32.5	9.9	6.2
Diet effect	**	**	**	**	**	**
Infection effect	*	ns	ns	ns	ns	ns
Interaction	ns	ns	ns	ns	ns	ns

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means
: Gain compared to the baseline control animals

Indicator joint dissection

The percentage bone in the best end neck joint was slightly higher in the Diet BS fed lambs but the differences were not statistically significant. Statistically significant differences between the diets were found in fat and muscle percentages ($p<0.01$). The *T.congolense* infection had no significant effect on indicator joint composition (Table 4.11). The muscle:bone ratios were very similar in all groups.

Fleece composition

In contrast to the carcase dry matter the fleece dry matter percentage (Table 4.12) was significantly higher in the infected lambs ($p<0.01$) in both dietary groups. Fleece crude protein percentage was significantly higher in the lambs fed Diet BS than in the lambs fed Diet LH. However, the ether extract of the lambs fed Diet BS was lower, though not significantly. No significant infection effects were found on fleece crude protein and ether extract percentages. The fleece ash and gross energy contents were unaffected by both the diet and infection.

Total fleece dry matter, ether extract, crude protein and gross energy gain during the experimental period were significantly higher in the lambs fed Diet LH ($p<0.01$). The ash content also appeared to be higher in the Diet LH groups but differences were not significant. The total fleece dry matter ($p<0.05$) and crude protein ($p<0.01$) were higher in the infected lambs in both dietary groups. The fleece ether extract and gross energy gains also appeared higher in the infected groups but differences were not significant (Table 4.13).

Table 4.11 Mean composition of the best end neck joint of *T.congolense* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BS (barley straw) or Diet LH (lucerne hay)

Group	Bone %	Fat %	Muscle %	Muscle/ Bone Ratio
BS-I	24	7	69	3.1
BS-PC	21	9	69	3.4
Pooled SE	1.8	1.8	1.2	0.28
LH-I	16	31	54	3.5
LH-PC	20	31	49	2.6
Pooled SE	1.3	1.5	1.9	0.28
Diet effect	ns	**	**	ns
Inf. effect	ns	ns	ns	ns
Interaction	ns	ns	ns	ns

** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Table 4.12 Mean fleece dry matter (DM (g/kg)), ether extract (EE (g/kg DM)), crude protein (CP (g/kg DM)), ash (g/kg DM) and gross energy (GE (MJ/kg DM)) composition of *T.congolense* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BS (barley straw) or Diet LH (lucerne hay)

Group	DM (g/kg FM)	EE (g/kg DM)	CP (g/kg DM)	ash (g/kg DM)	GE (MJ/kg DM)
BS-I	545	76	790	56	20.9
BS-PC	494	80	789	70	21.1
Pooled SE	10.8	6.9	8.8	9.6	0.08
LH-I	503	113	743	80	20.9
LH-PC	485	104	707	75	21.2
Pooled SE	11.1	8.8	8.7	6.6	0.20
Diet effect	ns	ns	**	ns	ns
Infection effect	**	ns	ns	ns	ns
Interaction	ns	ns	ns	ns	ns

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Table 4.13 Mean total Fleece dry matter (DM (g)), ether extract (EE (g)), crude protein (CP (g)), ash (g) and gross energy (GE (MJ)) gain# of *T.congolense* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BS (barley straw) or Diet LH (lucerne hay)

Group	DM (g)	EE (g)	CP (g)	ash (g)	GE (MJ)
BS-I	906	78	683	63	19
BS-PC	789	74	596	84	17
Pooled SE	71.1	11.1	53.3	20.6	1.5
LH-I	1790	246	1253	178	38
LH-PC	1718	215	1109	159	37
Pooled SE	76.2	26.9	51.9	21.9	1.6
Diet effect	**	**	**	ns	**
Infection effect	*	ns	**	ns	ns
Interaction	ns	ns	ns	ns	ns

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means
: Gain compared to the baseline control animals

Rectal temperature

A significant difference in the average rectal temperature (RT) was found before infection between the Diet BS and Diet LH groups with the Diet BS group having the lower rectal temperature (Table 4.14; $p<0.01$).

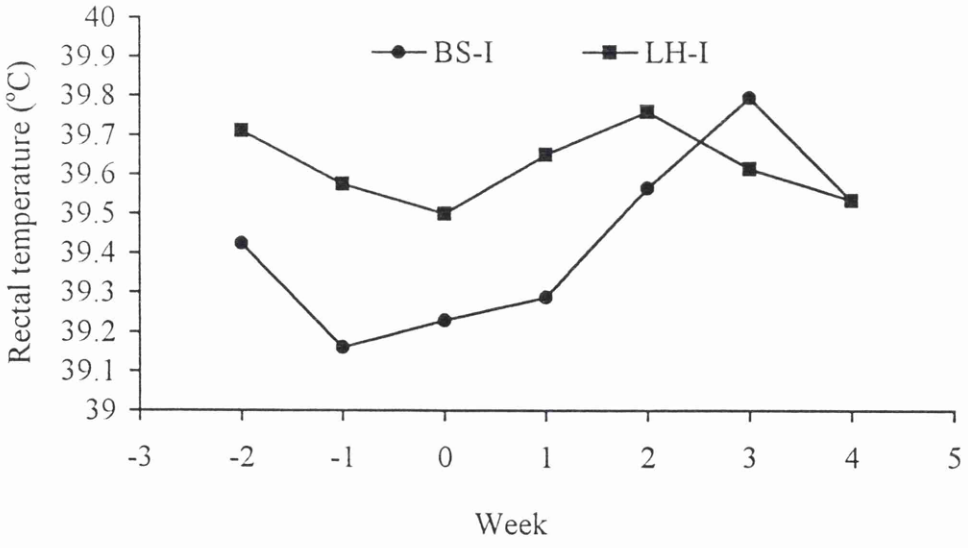
After infection the Diet BS fed lambs showed a higher pyrexic effect than the Diet LH fed lambs (Figure 4.6).

Table 4.14 Mean rectal temperature (^{o}C) of *T.congolense* infected (I) sheep (n=4) fed either Diet BS (barley straw) or Diet LH (lucerne hay) during period 1 (day -15 - -3) and period 2 (day 14 - 32)

Group	Period 1	Period 2
BS-I	39.3	39.6
SE	0.02	0.02
LH-I	39.6	39.6
SE	0.05	0.05
Diet effect	**	ns

** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Figure 4.6 Mean rectal temperature (^{o}C) of *T.congolense* infected sheep fed Diet BS (BS-I) or Diet LH (LH-I)

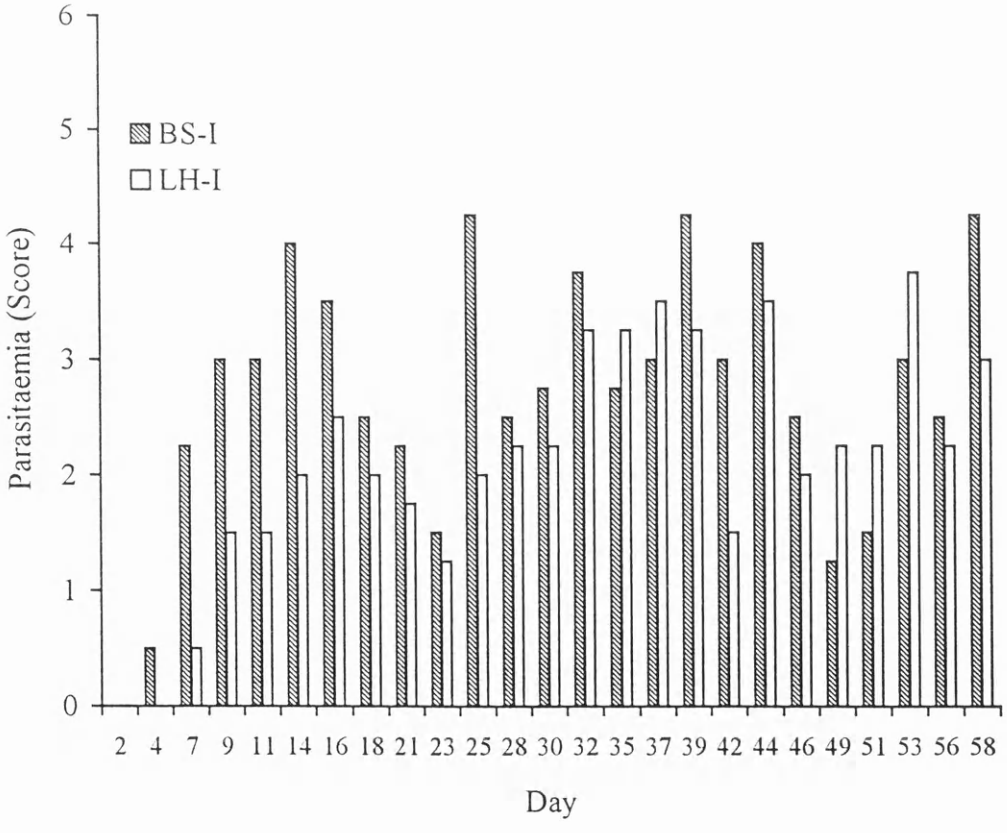


Haematology

Level of parasitaemia

Although the first parasitaemic wave occurred at a similar time in both groups, the number of trypanosomes found appeared to be lower in the infected lambs fed Diet LH than in the lambs fed Diet BS (Figure 4.7). The second peak parasitaemia in the lambs on Diet BS started earlier and was higher than the parasitaemia in the lambs fed Diet LH which gradually increased to its second peak. After that, the intensities of parasitaemia fluctuated in both dietary groups to a similar extent. None of the differences between the dietary groups were found to be statistically significant.

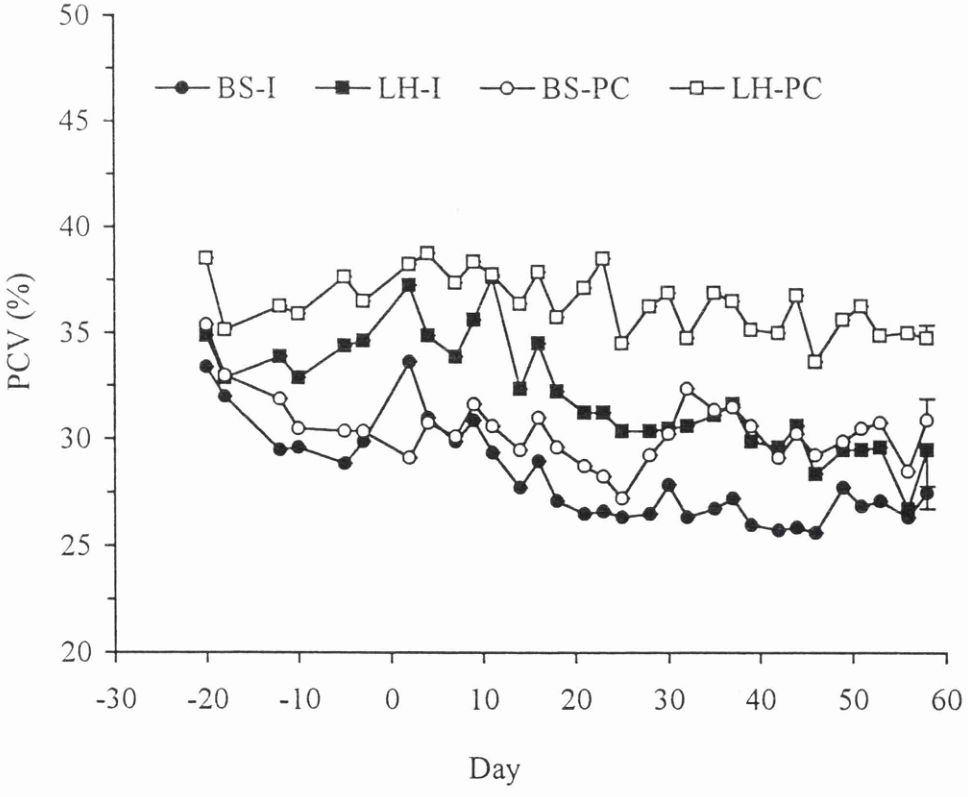
Figure 4.7 Mean parasitaemia (Score) of *T.congolense* infected Scottish Blackface sheep fed Diet BS (BS-I) or Diet LH (LH-I)



Packed cell volume

The packed cell volume (PCV) before infection was found to be significantly lower in the lambs fed Diet BS ($p<0.01$) and tended to be lower in the lambs to be infected compared with the pair-fed controls ($p<0.05$; Table 4.15). After infection the packed cell volume of the lambs on both diets showed a gradual decrease from approximately day 10 to day 20 after which the packed cell volume stabilised at around 30% for the lambs on Diet LH and 27% for the lambs on Diet BS (Figure 4.8). The packed cell volume was affected by both infection ($p<.01$) and diet ($p<0.01$). The packed cell volume in the lambs on Diet LH appeared to be more affected by the infection than the lambs on Diet BS but no interactive effect between infection and diet was found.

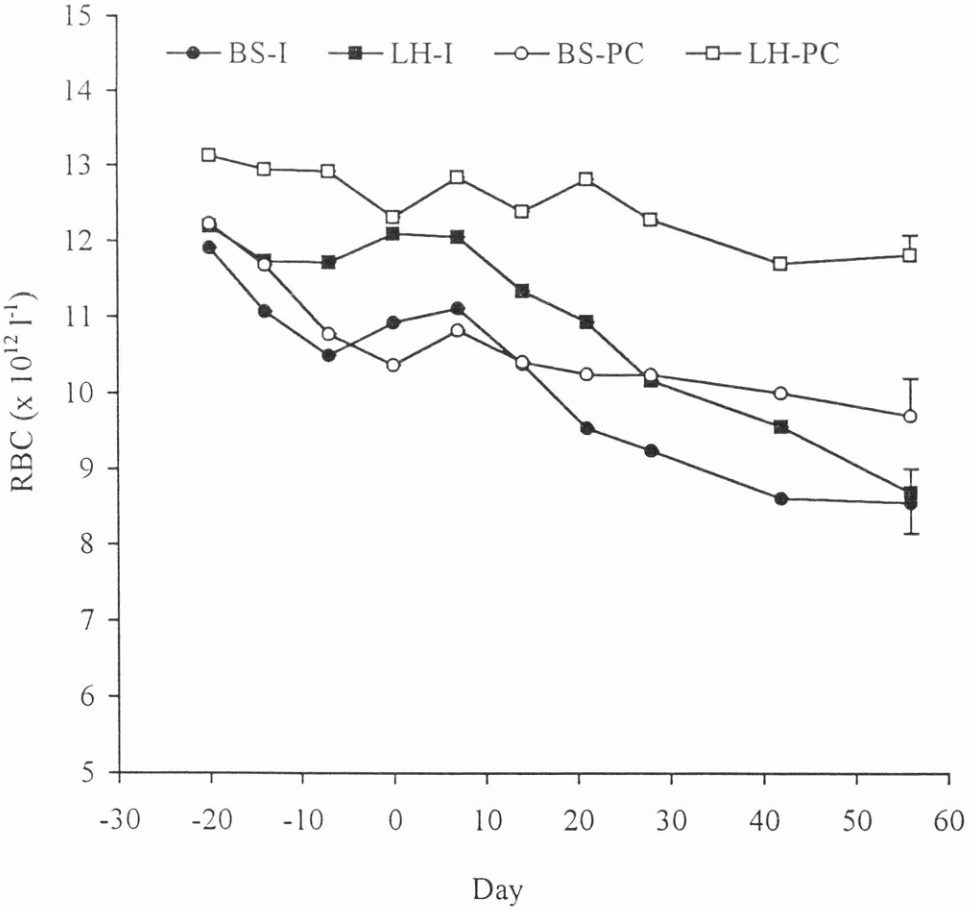
Figure 4.8 Mean packed cell volume (PCV; %) of *T.congolense* infected sheep fed Diet BS (BS-I) or Diet LH (LH-I) and their respective pair-fed controls BS-PC and LH-PC



Red blood cell count

Before the *T.congolense* infection the red blood cell (RBC) count was slightly lower ($p<0.05$) in the animals fed Diet BS (Table 4.15). After infection the red blood cell count was affected by both the diet ($p<0.01$) and infection ($p<0.01$). However, a slight difference between the infected and pair-fed control groups was also found before infection ($p<0.05$). The decrease in red blood cell count seemed to be greater for the infected animals fed Diet LH (Figure 4.9), but no interaction between diet and infection on red blood cell was found.

Figure 4.9 Mean red blood cells (RBC; x 10^{12} l^{-1}) of *T.congolense* infected sheep fed Diet BS (BS-I) or Diet LH (LH-I) and their respective pair-fed controls BS-PC and LH-PC



Haemoglobin concentration

The haemoglobin (Hb) concentration appeared to be slightly affected by nutrition ($p<0.05$) before infection with the animals fed Diet BS showing the lower haemoglobin concentration (Table 4.15). A significant difference was also found between the lambs to be infected and pair-fed control groups before infection ($p<0.01$). After infection the haemoglobin concentration was affected by both diet ($p<0.01$) and infection ($p<0.01$). The haemoglobin concentration appeared to be more affected by infection in the lambs fed Diet LH than in the lambs fed Diet BS (Figure 4.10), however, again no interaction between diet and infection was found. Whereas the haemoglobin concentration of the animals on Diet BS appeared to have stabilised after about 21 days the haemoglobin concentration in animals on Diet LH was decreasing over the entire post-infection period.

Figure 4.10 Mean haemoglobin (Hb; g dl^{-1}) of *T.congolense* infected sheep fed Diet BS (BS-I) or Diet LH (LH-I) and their respective pair-fed controls BS-PC and LH-PC

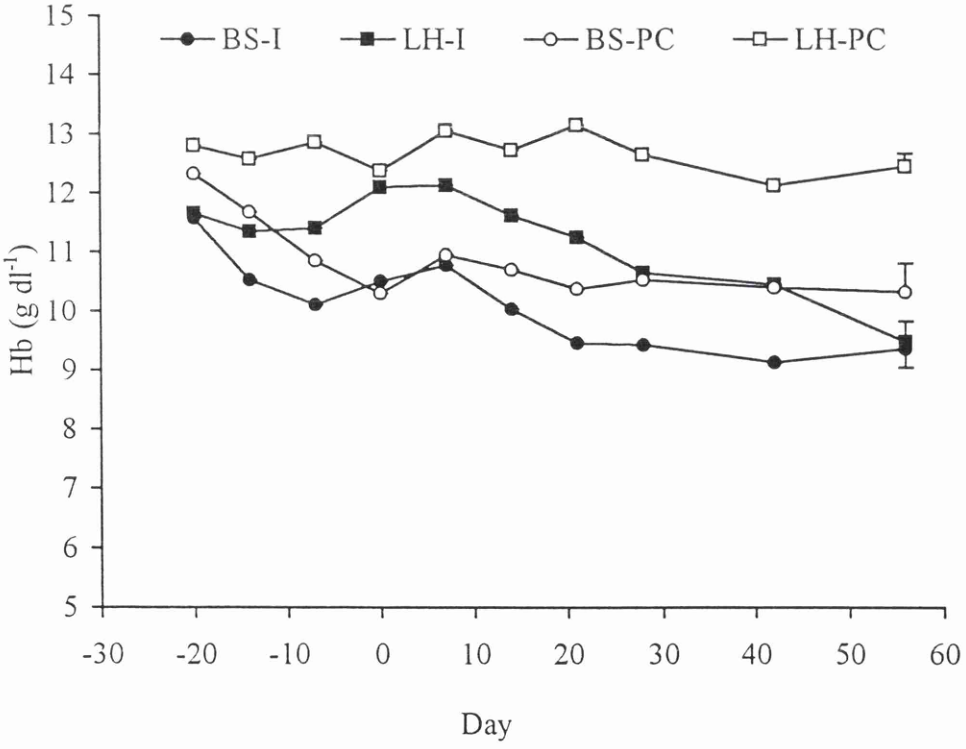


Table 4.15 Mean packed cell volume (PCV; %), red blood cell count (RBC; x 10^{12} l^{-1}) and haemoglobin concentration (Hb; g dl^{-1}) of *T.congolense* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BS (barley straw) or Diet LH (lucerne hay) during pre- (day -20 - -3) and post-infection (day 14 - 56)

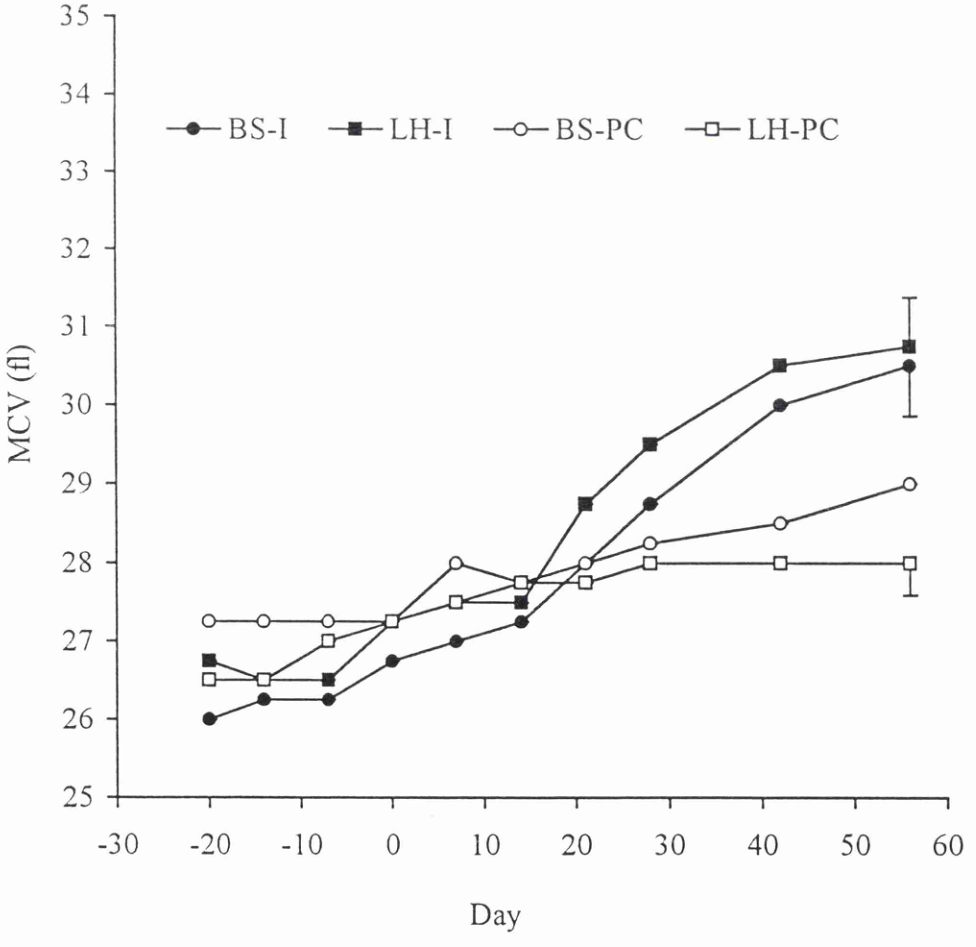
	PCV		RBC		Hb	
Group	Pre-	Post-	Pre-	Post-	Pre-	Post-
BS-I	30.5	26.9	11.1	9.3	10.7	9.5
BS-PC	31.9	29.9	11.3	10.1	11.3	10.5
Pooled SE	0.77	0.83	0.25	0.28	0.24	0.26
LH-I	33.9	30.5	11.9	10.1	11.6	10.7
LH-PC	36.6	35.9	12.8	12.2	12.7	12.6
Pooled SE	0.71	1.11	0.22	0.40	0.25	0.40
Diet effect	**	**	*	**	*	**
Infection effect	*	**	*	**	**	**
Interaction	ns	ns	ns	ns	ns	ns

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Mean corpuscular volume

The mean corpuscular volume (MCV) did not appear to be significantly affected by nutrition (Table 4.16). The *T.congolense* infection resulted in a slight rise of the mean corpuscular volume in the infected groups on both diets ($p<0.05$). The increase in mean corpuscular volume was greatest during the latter stages of the *T.congolense* infection (Figure 4.11). The mean corpuscular volume in the pair-fed control groups also showed a slight increase during the experimental period.

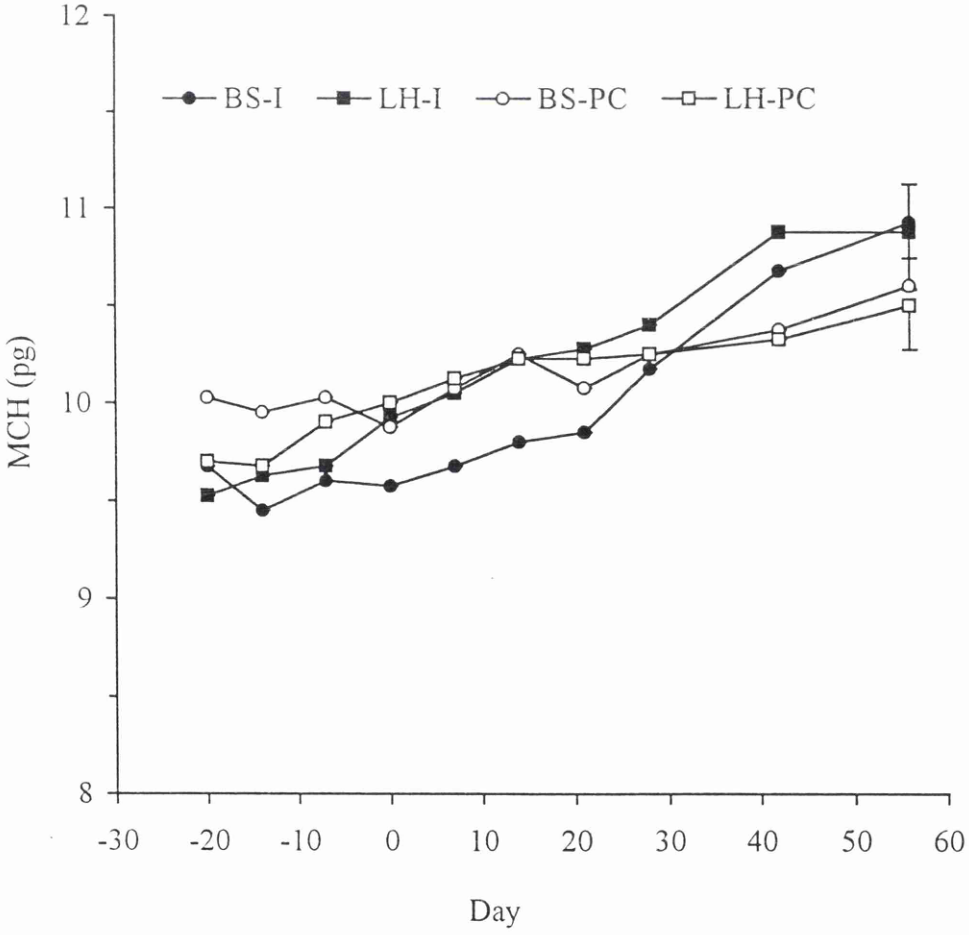
Figure 4.11 Mean corpuscular volume (MCV; fl) of *T.congolense* infected sheep fed Diet BS (BS-I) or Diet LH (LH-I) and their respective pair-fed controls BS-PC and LH-PC



Mean corpuscular haemoglobin

No significant differences in mean corpuscular haemoglobin (MCH) were found between the Diet BS and Diet LH groups. Infection did appear to result in a slight increase in mean corpuscular haemoglobin about 28 days after infection, but the mean corpuscular haemoglobin in the pair-fed control groups also rose slightly during the experimental period resulting in statistically non-significant differences (Figure 4.12; Table 4.16). A significant difference was found between the groups to be infected and the pair-fed control groups during the pre-infection period ($p<0.01$).

Figure 4.12 Mean corpuscular haemoglobin (MCH; pg) of *T.congolense* infected sheep fed Diet BS (BS-I) or Diet LH (LH-I) and their respective pair-fed controls BS-PC and LH-PC



Mean corpuscular haemoglobin concentration

No significant differences in mean corpuscular haemoglobin concentration (MCHC) were found due to nutrition before and after infection. Infection resulted in a significant decrease in mean corpuscular haemoglobin concentration in the infected lambs on both diets compared with their pair-fed controls ($p<0.01$; Table 4.16). The mean corpuscular haemoglobin concentration appeared to stabilise in both groups about 28 days after infection (Figure 4.13)

Figure 4.13 Mean corpuscular haemoglobin concentration (MCHC; g dl^{-1}) of *T.congolense* infected sheep fed Diet BS (BS-I) or Diet LH (LH-I) and their respective pair-fed controls BS-PC and LH-PC

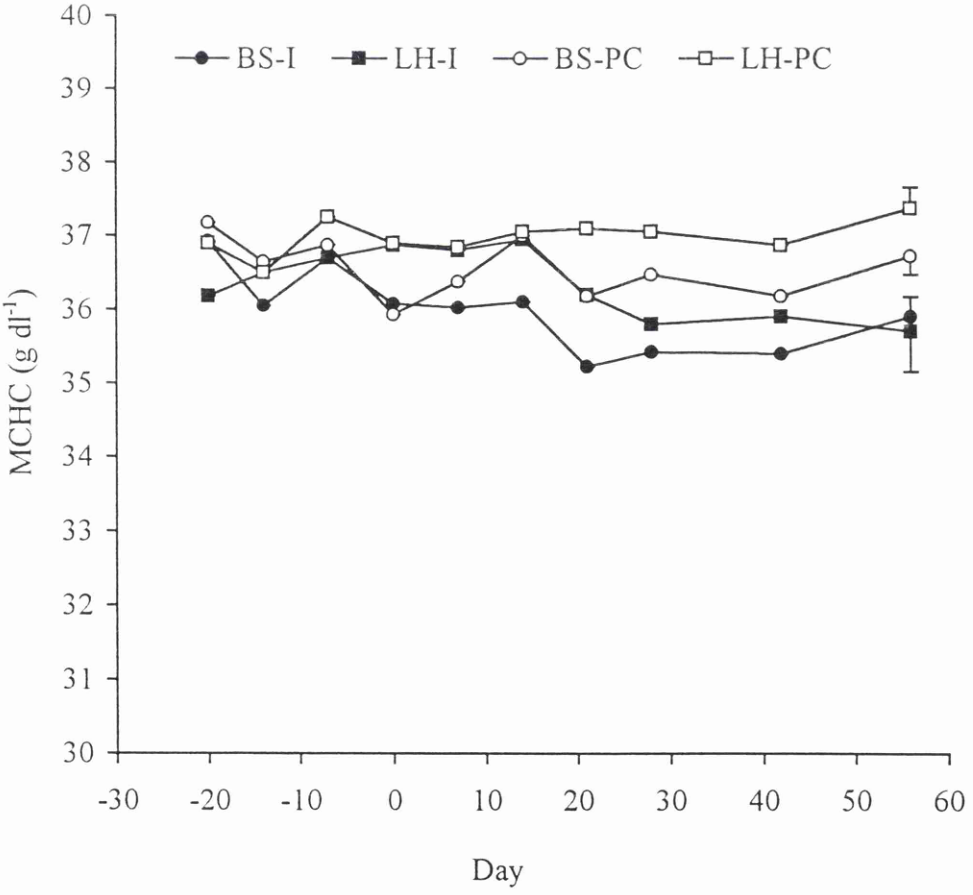


Table 4.16 Mean corpuscular volume (MCV; fl), mean corpuscular haemoglobin (MCH; pg) and mean corpuscular haemoglobin concentration (MCHC; g dl^{-1}) of *T.congolense* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BS (barley straw) or Diet LH (lucerne hay) during pre- (day -20 - -3) and post-infection (day 14 - 56)

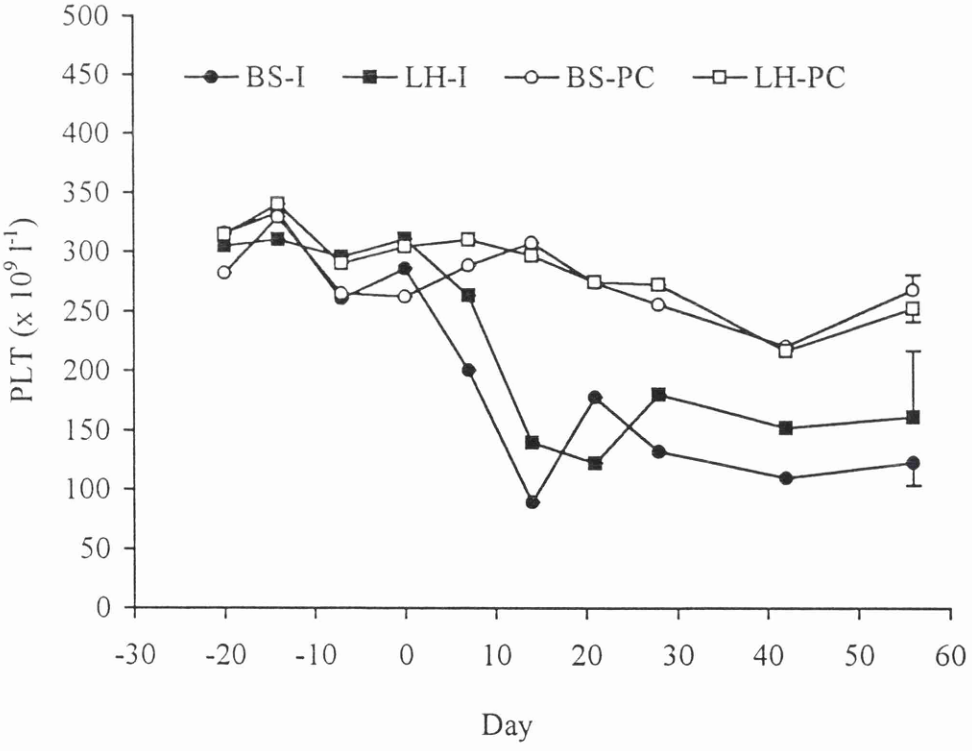
	MCV		MCH		MCHC	
Group	Pre-	Post-	Pre-	Post-	Pre-	Post-
BS-I	26.3	28.9	9.6	10.3	36.4	35.6
BS-PC	27.3	28.3	10.0	10.3	36.7	36.5
Pooled SE	0.30	0.23	0.13	0.11	0.21	0.24
LH-I	26.8	29.4	9.7	10.5	36.6	36.1
LH-PC	26.8	27.9	9.8	10.3	36.9	37.1
Pooled SE	0.30	0.36	0.13	0.16	0.24	0.29
Diet effect	ns	ns	ns	ns	ns	ns
Infection effect	ns	*	**	ns	ns	**
Interaction	ns	ns	ns	ns	ns	ns

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Platelet count

No significant nutritional effect was found on the number of platelets (PLT) in the blood. A sharp fall in numbers of platelets was found in the infected groups between day 0 and 14 after the *T.congolense* infection. About 21 days after the infection the platelet counts started to stabilise at a platelet count of about 120 for the infected lambs on Diet BS and at about 140 for infected lambs on Diet LH (Figure 4.14). Aggregation of platelets was found in all animals and occasionally the number of platelets could not be counted. Platelet counts also showed a slight decline in the pair-fed control groups on both diets. Table 4.17 shows highly significant differences between infected and pair-fed control lambs ($p<0.01$) after infection. No interaction between diet and infection was found on platelet counts.

Figure 4.14 Mean platelet count (PLT; x 10^9 l^{-1}) of *T.congolense* infected sheep fed Diet BS (BS-I) or Diet LH (LH-I) and their respective pair-fed controls BS-PC and LH-PC



White blood cell count

The animals on Diet BS appeared to have a lower white blood cell (WBC) count than the animals on Diet LH, however, the differences were not statistically significant. Infection did appear to increase the white blood cell count in both infected groups at about day 7 following infection followed by a decrease back to pre-infection values (Figure 4.15). However, no significant differences were found after infection between *T.congolense* infected and pair-fed control lambs due to the high standard error of mean in the infected groups (Table 4.17). There appeared to be a considerable difference in white blood cell response between individual animals.

Figure 4.15 Mean white blood cell count (WBC; x 10^9 l^{-1}) of *T.congolense* infected sheep fed Diet BS (BS-I) or Diet LH (LH-I) and their respective pair-fed controls BS-PC and LH-PC

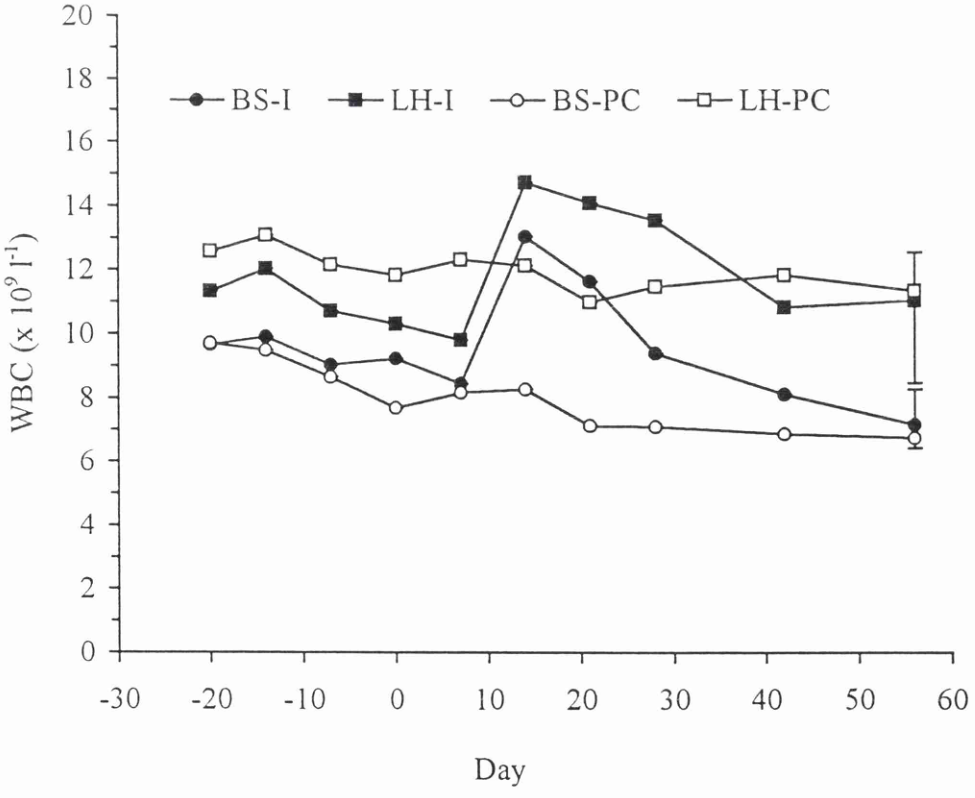


Table 4.17 Mean platelet (PLT; x 10^9 l^{-1}) and white blood cell count (WBC; x 10^9 l^{-1}) of *T.congolense* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BS (barley straw) or Diet LH (lucerne hay) during pre- (day -20 - -3) and post-infection (day 14 - 56)

	PLT		WBC	
Group	Pre-	Post-	Pre-	Post-
BS-I	299	126	9.5	9.9
BS-PC	285	265	8.9	7.2
Pooled SE	15.3	29.0	0.86	0.94
LH-I	306	139	11.1	12.6
LH-PC	312	263	12.4	11.6
Pooled SE	22.4	27.7	1.00	0.86
Diet effect	ns	ns	ns	ns
Infection effect	ns	**	ns	ns
Interaction	ns	ns	ns	ns

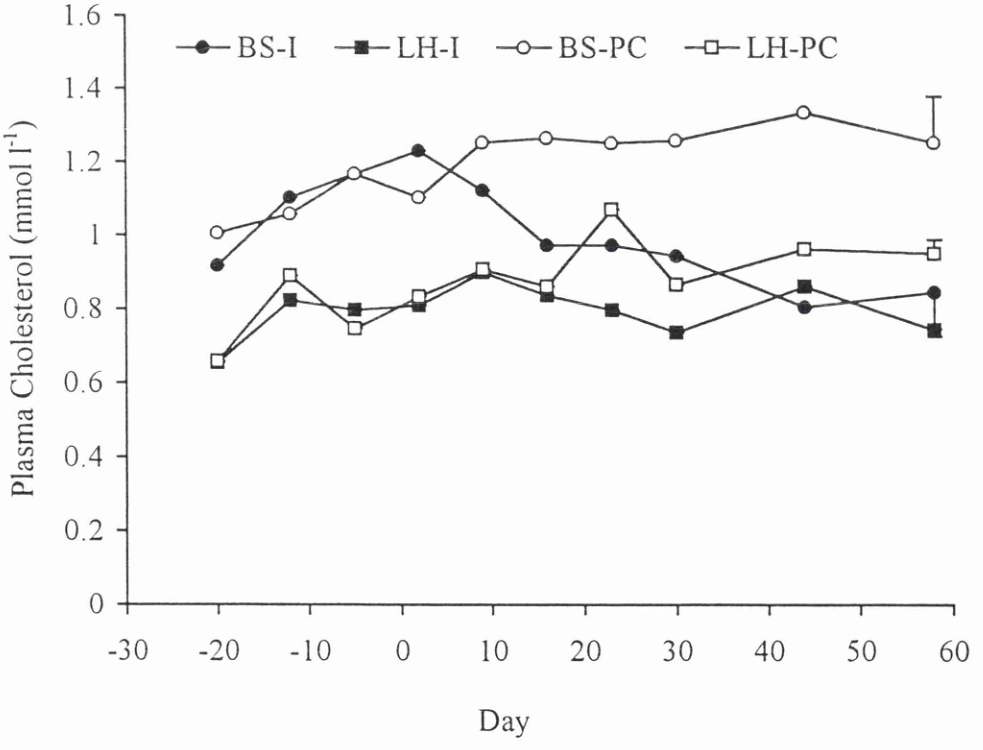
* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Blood biochemistry

Plasma cholesterol

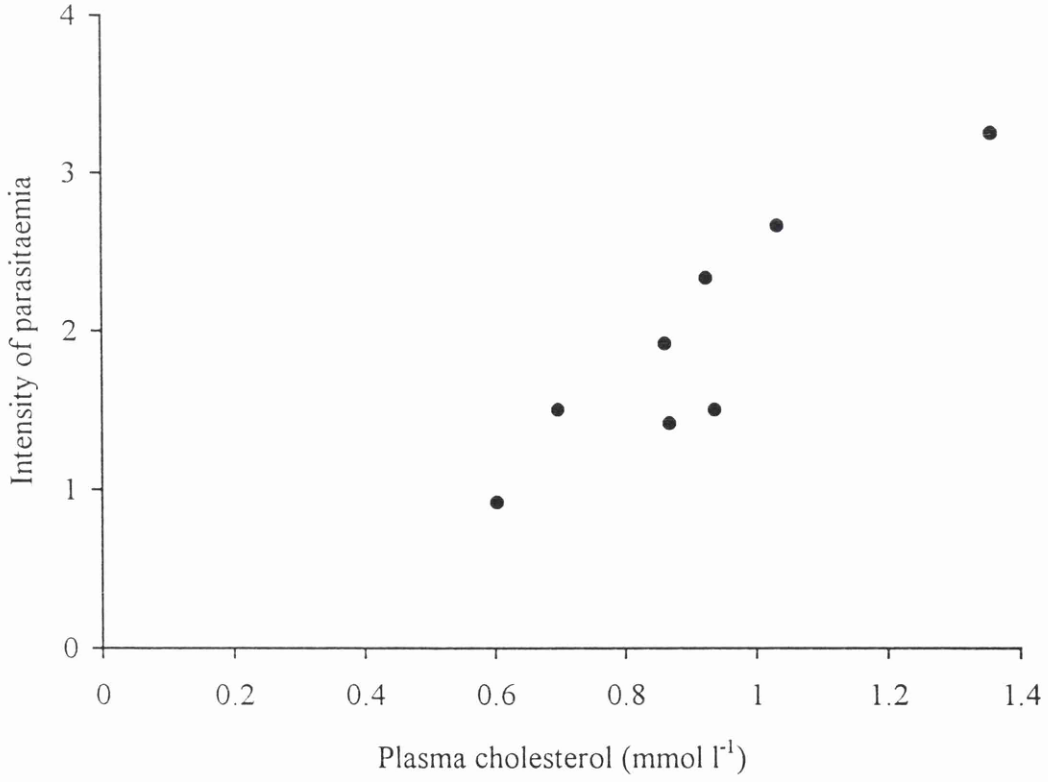
Plasma cholesterol concentration was lower in lambs on the lucerne hay than those on the barley straw diet ($p<0.05$; Table 4.18). The plasma cholesterol levels in the pair-fed control and infected groups on Diet BS were still rising during the pre-infection period. Infection significantly lowered plasma cholesterol concentrations ($p<0.01$; Table 4.18). Figure 4.16 shows that there was a sharp decrease in plasma cholesterol immediately after infection in the Diet BS fed lambs which appeared to stabilise at the same level as the pair-fed control animals fed Diet LH. However, no significant interaction was found between diet and infection on plasma cholesterol concentration.

Figure 4.16 Mean plasma cholesterol (mmol l^{-1}) of *T.congolense* infected sheep fed Diet BS (BS-I) or Diet LH (LH-I) and their respective pair-fed controls BS-PC and LH-PC



A relationship was found between the average plasma cholesterol concentration before infection and the average intensity of parasitaemia during the first month after infection ($r=0.90$; $p<0.01$) (Figure 4.17). However, one has to take into account that the intensity of parasitaemia is not normally distributed.

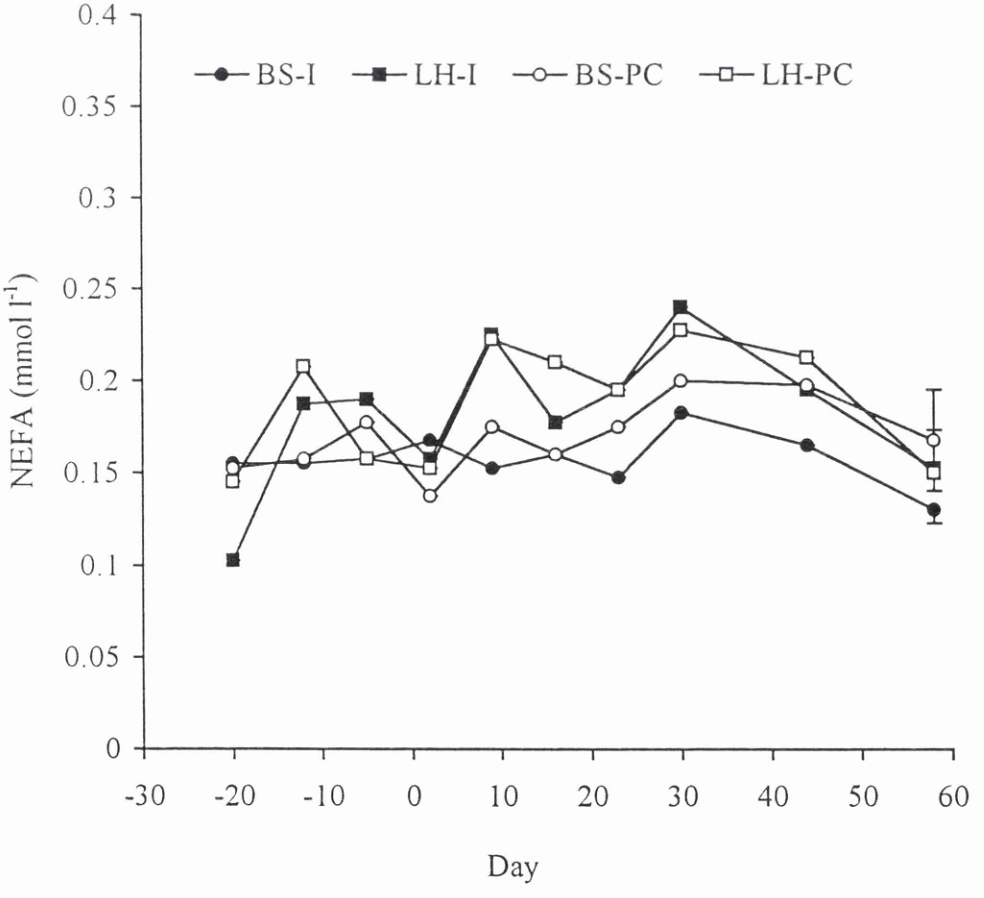
Figure 4.17 The relationship between the average plasma cholesterol concentration (mmol l^{-1}) before the *T.congolense* infection and the average intensity of parasitaemia during the first month after infection in sheep



Plasma triglyceride

Plasma triglyceride level fluctuated greatly between weeks. No significant effects on plasma triglyceride concentration were found of either infection or nutrition, although levels for the animals fed Diet LH appeared to be slightly higher (Figure 4.18; Table 4.18).

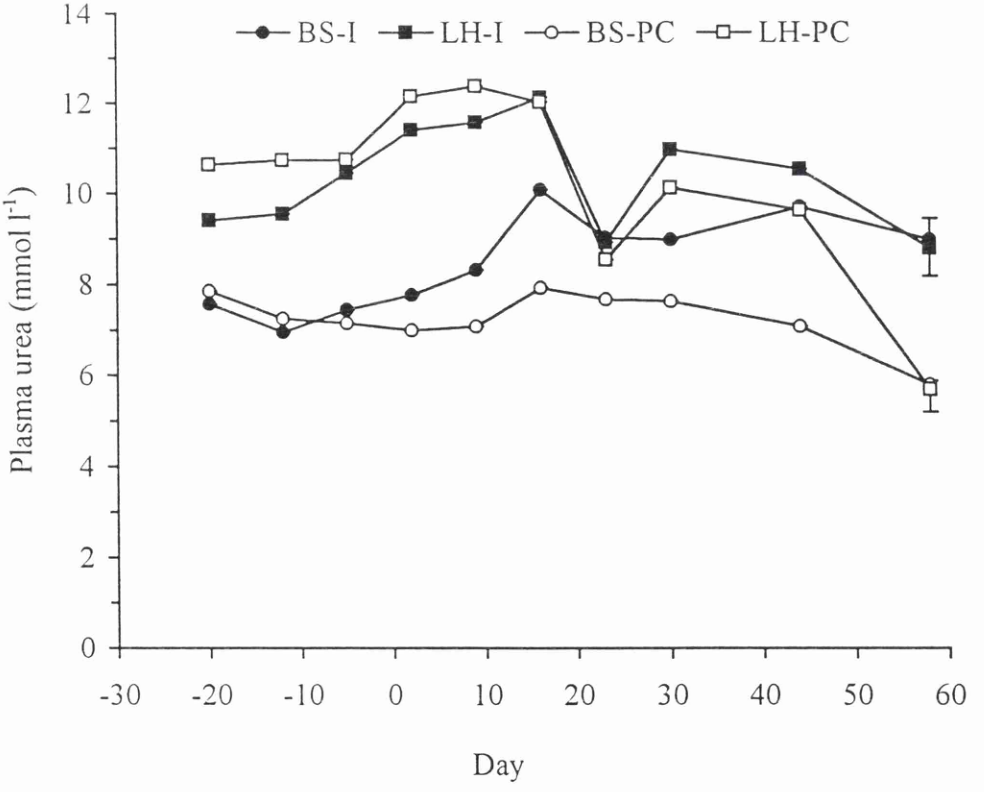
Figure 4.18 Mean plasma non-esterified fatty acids (NEFA; mmol l^{-1}) of *T.congolense* infected sheep fed Diet BS (BS-I) or Diet LH (LH-I) and their respective pair-fed controls BS-PC and LH-PC



Plasma urea

A strong nutritional effect was found before infection on plasma urea concentration ($p<0.01$; Table 4.18). A sharp decrease in plasma urea concentration in both the infected and pair-fed control lambs on Diet LH was detected around day 23 (Figure 4.19) possibly due to the feed intake depression at that time. Plasma urea concentration was significantly higher in the infected lambs compared with their pair-fed control partners, particularly in the ones fed Diet BS ($p<0.01$). However, no statistically significant interaction effects were observed between diet and infection on plasma urea concentrations (Table 4.18).

Figure 4.19 Mean plasma urea (mmol l^{-1}) of *T.congolense* infected sheep fed Diet BS (BS-I) or Diet LH (LH-I) and their respective pair-fed controls BS-PC and LH-PC



Plasma albumin

A significant difference in plasma albumin concentration was detected between lambs on Diet BS and Diet LH ($p<0.01$; Table 4.18). Infection resulted in a decrease in plasma albumin concentration in the lambs on both diets ($p<0.05$) but the nutritional effect was still greater ($p<0.01$; Figure 4.20). The effects of nutrition and infection on plasma albumin concentration were additive.

Figure 4.20 Mean plasma albumin (g l^{-1}) of *T.congolense* infected sheep fed Diet BS (BS-I) or Diet LH (LH-I) and their respective pair-fed controls BS-PC and LH-PC

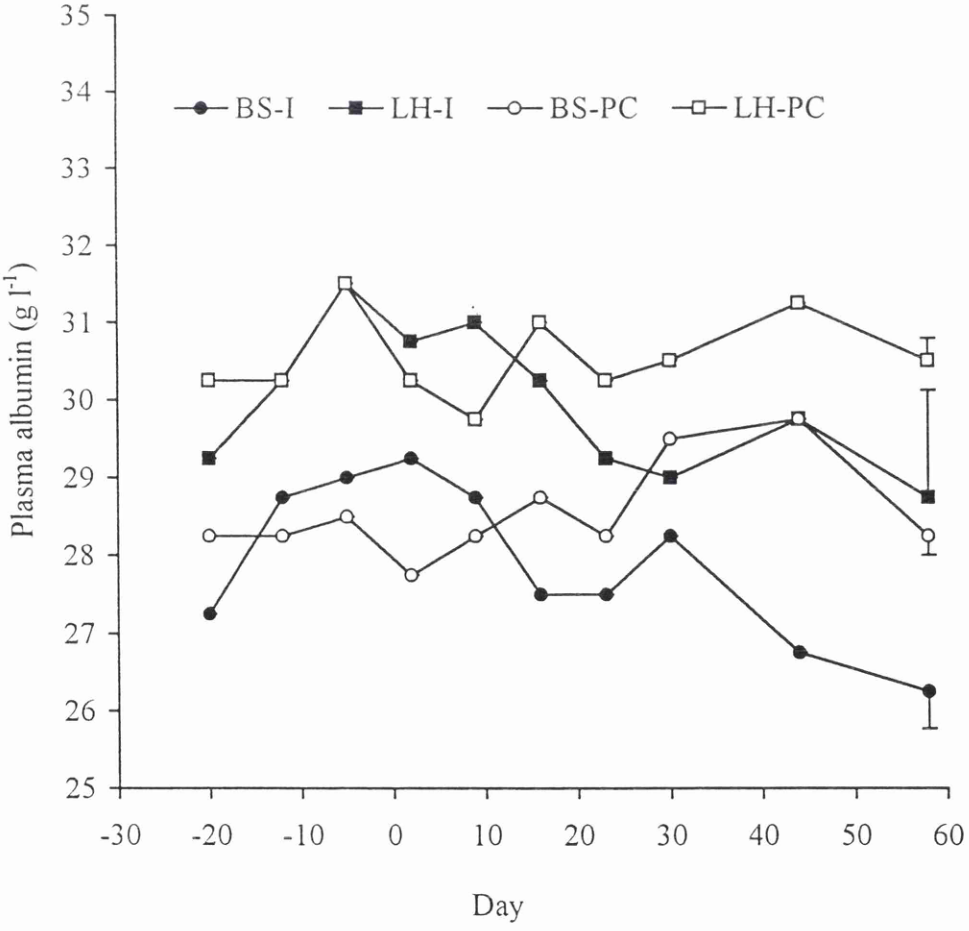


Table 4.18 Mean plasma cholesterol (mmol l^{-1}), triglyceride (mmol l^{-1}), urea (mmol l^{-1}) and albumin (g l^{-1}) concentration of *T.congolense* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BS (barley straw) or Diet LH (lucerne hay) during pre- (day -20 - -3) and post-infection (day 14 - 58)

	Cholesterol		Triglyceride		Urea		Albumin	
Group	Pre-	Post-	Pre-	Post-	Pre-	Post-	Pre-	Post-
BS-I	1.06	.91	0.16	0.16	7.3	9.4	28.3	27.3
BS-PC	1.08	1.27	0.16	0.18	7.4	7.2	28.3	28.9
Pooled SE	0.071	0.095	0.016	0.011	0.27	0.51	0.28	0.33
LH-I	0.76	0.79	0.16	0.19	9.8	10.3	30.3	29.4
LH-PC	0.76	0.94	0.17	0.20	10.7	9.2	30.7	30.7
Pooled SE	0.041	0.055	0.006	0.008	0.27	0.42	.27	0.43
Diet effect	*	ns	ns	ns	**	ns	**	**
Infection effect	ns	**	ns	ns	ns	**	ns	*
Interaction	ns	ns	ns	ns	ns	ns	ns	ns

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Discussion

In this experiment the influence of the type of roughage on digestive function was studied in sheep infected with *Trypanosoma congolense*. The results showed that digestive function was altered by the *T.congolense* infection. The mean retention time of the roughage through the digestive tract was longer in the infected animals but this did not result in an expected increase of the organic matter and crude protein digestibility. However, no differences could be observed between body weight changes of infected lambs and their pair-fed controls. The type of diet significantly affected packed cell volume, cholesterol, urea and albumin levels but possibly these were due to differences in the plane of nutrition. Despite these differences no interaction effects between infection and diet were observed on any of the parameters measured.

Although there was a large difference in dry matter intake of the lambs between the two diets the total intakes of neutral and acid detergent fibre were very similar. This is in accordance with the review of Van Soest (1982) who reported a relationship between roughage intake and the amount of fibre in the roughage.

The organic matter intake of both infected groups decreased significantly after infection ($p<0.01$) but seemed to be more depressed in the lambs fed Diet LH than the organic matter intake of the infected group fed Diet BS. The severe depression in organic matter intake between day 12 and 21 in lambs on Diet LH was probably due to the combination of a slight fall in the quality of lucerne hay and the stress of putting a harness on the animals for the collection of faeces. In contrast, the additional stress did not appear to affect organic matter intake in the infected lambs on Diet BS. These results might have been caused by the difference in type of roughage. The first limiting factor on the roughage intake in the lambs on the barley straw is likely to be rumen

size, whereas the intake limiting factors in the lucerne hay fed animals are likely to be the metabolites of digestion e.g. propionate (Aitchison *et al.*, 1986; Forbes, 1986; Farningham and Whyte, 1993).

Depressions of voluntary intake were reported by Reynolds and Ekwuruke (1988) in *T.vivax* infected West African dwarf sheep fed *Panicum maximum* and cassava peel with or without a 1:1 mixture of *Leucaena leucocephala* and *Glyricidia sepium*. Depression of voluntary intake of alfalfa pellet were also found during *T.vivax* (Akinbamijo, 1992; Zwart *et al.*, 1991; Wassink *et al.*, 1993) and *T.congolense* (Wassink *et al.*, 1993) infections in WAD goats fed alfalfa pellets.

The *T.congolense* infected lambs fed barley straw showed relatively less intake depression than *T.congolense* infected N'dama heifers fed *Andropogon guyanus* hay. The N'dama heifers were also fed groundnut cake and groundnut hay which might have affected the *Andropogon guyanus* hay intake (Romney *et al.*, 1994).

Since the intake depression due to infection in this experiment was only marginal no changes in feed selection were observed. Reynolds and Ekwuruke (1988) found in their *T.vivax* infected West African Dwarf sheep that the proportions of panicum, leucaena, gliricidia and cassava peel consumed remained constant. However, results from *T.congolense* infected N'dama cattle showed a reduced intake of *Andropogon gayanus* while the animals consumed the amount of groundnut hay and cake offered (Romney *et al.*, 1994).

The organic matter digestibility results indicated no direct effect of the infection on organic matter digestibility but an increase in organic matter digestibility in the pair-fed control lambs ($p<0.01$), despite the shorter mean retention time of the roughage through the digestive tract. One would expect a higher digestibility in animals having a

longer mean retention time. Verstegen *et al.* (1991) did not find any differences in dry matter digestibility between *T.vivax* infected West African Dwarf goats and their controls. However, the controls used in that experiment were not pair-fed.

The *T.congolense* infection resulted in a decrease in crude protein digestibility ($p<0.01$). Reduced apparent digestibility of nitrogen has been observed in lambs infected with the intestinal parasites *Trichostrogylus colubformis* (Poppi *et al.*, 1986; Kimambo *et al.*, 1988) and a concurrent infection with *T.colubformis* and *Ostertagia circumcincta* (Bown *et al.*, 1991). These authors implicated increased plasma protein, epithelial cell desquamation and mucus secretion as the source of increased endogenous nitrogen. In certain *T.vivax* isolates which produce an acute syndrome resulting in death within 2 to 3 weeks of infection, massive haemorrhages into the alimentary tract have been found (Hudson, 1944; Mwongela *et al.*, 1981). These haemorrhages might cause loss of endogenous nitrogen. However, in contrast to *T.vivax*, *T.congolense* does not have the capacity to invade tissues of domestic ruminants (Murray and Dexter, 1988). No differences in digestibility of nitrogen between *T.vivax* infected West African Dwarf goats and their controls were found, although variability within weeks seemed to be higher in the infected group (Verstegen *et al.*, 1991). Similar results were found by Hamminga (1989) also in *T.vivax* infected West African Dwarf goats. Nitrogen digestibilities were unaffected in *Dictiocaulus viviparus* infected calves (Kroonen *et al.*, 1986; Verstegen *et al.*, 1989).

The increase in plasma urea concentration in the infected animals during the present experiment may have caused an increase in faecal nitrogen excretion. However, one would expect more urea being recycled when the mean retention time of the roughage is longer.

Verstegen *et al.* (1989) suggested that the increased metabolisability of energy ingested in *Dictyocaulus viviparus* infected calves was due to a higher dietary concentrate to roughage ratio.

Digestibilities of neutral and acid detergent fibre do not show any consistency and were not very reliable due to the fact that the samples were dried at 80 ^{o}C. It would have been better to dry the samples at 65 ^{o}C (M. Gill, Personal communication).

As expected, the animals on Diet BS had a longer mean retention time of roughage in the gastrointestinal tract than the animals fed Diet LH which was due to a significant difference in the outflow rate constant k_1 ($p<0.01$). This result supports the conclusion by Aitchison *et al.* (1986) that the constant k_1 is the rumen outflow rate constant. The mean retention time of the roughage was significantly longer in the infected animals than their respective pair-fed control counterparts ($p<0.01$). The results indicate that the longer mean retention time was due to a slower rate of passage throughout the entire digestive tract. In previous experiments, Van Miert *et al.* (1986) found inhibition of ruminal contractions during the acute phase response in *T.vivax* infected goats, whereas Veenendaal *et al.* (1976) did not find a significant inhibition of the forestomach contractions in *T.vivax* infected goats.

The body weight gains of the lambs were similar to those predicted by the requirements of housed, castrate lambs published by the AFRC (1993), around 50 g/day for the lambs on Diet BS and around 200 g/day for those on Diet LH. The body weight changes of the infected lambs in both dietary groups were not significantly different from the body weight changes of their pair-fed control counterparts.

However, carcase dry matter content and total carcase dry matter were significantly lower in the infected animals compared to their pair-fed controls ($p<0.05$). Increases in water retention during parasitic infections have been associated with elevations in tissue water content, changes in body water turnover and expansion of the plasma volume (Parkins and Holmes, 1989). Rowe *et al.* (1988) reported that *Haemonchus contortus* infected lambs lost less weight during the experimental period than their pair-fed controls which they attributed to the higher water retention and a change in body composition towards increased total body water in the infected animals. Fever may play a role in the increased water retention because it leads to cutaneous vasoconstriction (Ruckebusch *et al.*, 1991).

The lower carcase dry matter led to lower total carcase crude protein and ether extract in the infected lambs compared with the pair-fed controls, although this was not statistically significant due to the variation in differences within the pairs. Katunguka-Rwakishaya (1992) found lower total carcase protein and fat contents in *T.congolense* infected sheep fed two levels of protein but this appeared to be mainly due to differences in carcase weight.

Verstegen *et al.* (1991) found an increase in heat production of around 15% and an increase in maintenance requirements of approximately 25% in West African Dwarf goats between day 10 and 40 after infection with *T.vivax*. Kroonen *et al.* (1986) associated the increase in heat production of *Dictiocaulus viviparus* infected calves with either increased maintenance requirements or reduced partial efficiency of feed conversion above maintenance. If these findings are true a smaller part of the nutrients taken up would be available for growth. The differences found in this experiment do not appear to be large enough to support a 25% increase in maintenance requirements.

In contrast to the carcase dry matter the fleece dry matter content was significantly higher in the infected lambs ($p<0.01$). The reason for this finding is not known. Further research is necessary to investigate the water retention in infected animals.

Rectal temperatures of the infected lambs on Diet BS were significantly lower before infection than the rectal temperatures of the infected lambs on Diet LH ($p<0.05$) possibly due to a lower metabolic rate in the lambs on Diet BS. Hamminga (1989) and Wassink *et al.* (1993) found a lower rectal temperatures in West African Dwarf goats fed below maintenance. However, after infection pyrexia was higher in the infected lambs fed Diet BS and the rectal temperatures of these lambs reached the same levels as the lambs fed Diet LH. This higher pyrexia was possibly due to the slightly higher parasitaemia levels observed during this period in the lambs on Diet BS. The higher energy cost of pyrexia in the lambs on Diet BS was not reflected in their body weight changes.

The first two peak parasitaemias tended to be higher in the infected lambs fed Diet BS than in the infected lambs fed Diet LH. Otesile *et al.* (1991) found that pigs infected with *Trypanosoma brucei* on a low energy diet developed significantly higher intensities of parasitaemia than those on the high energy diet. Similar tendencies, though not significantly, were found by Katunguka-Rwakishaya (1992) in *T.congolense* infected sheep. The theory that the parasites are more affected by malnutrition than the host is not supported by the results of these experiments. In contrast, no difference in intensity of parasitaemia could be attributed to the plane of nutrition, maintenance and sub-maintenance, in *T.vivax* infected West African Dwarf sheep (Reynolds and Ekwuruke, 1988).

Diet significantly affected packed cell volume levels ($p<0.01$). Agyemang *et al.* (1990, 1992) found lower packed cell volume levels in N'dama cattle kept under field conditions as the dry season progressed due to poorer nutrition. Abdullahi *et al.* (1986) revealed low packed cell volume concentrations in protein deprived sheep.

The packed cell volume was affected by both the *T.congolense* infection ($p<0.01$) and nutrition ($p<0.01$). The anaemia was only moderate. Although the effect of the trypanosome infection appeared to be higher in the Diet LH fed animals no interaction was found between nutrition and infection on packed cell volume. Katunguka-Rwakishaya (1991) found that the packed cell volume in the *T.congolense* infected Scottish Blackface lambs fed a low energy diet was more affected than in the animals on a high energy diet. In ovine fascioliasis animals on a lower level of protein showed a higher decrease in packed cell volume than animals on a higher level of protein (Berry and Dargie, 1976). In contrast, no differences were observed in packed cell volume of *T.congolense* infected sheep on two levels of protein (Katunguka-Rwakishaya *et al.*, 1993). However, in all these experiment no dietary effects on packed cell volume before infection were observed.

The increase in mean corpuscular volume ($p<0.05$) and decrease in mean corpuscular haemoglobin concentration ($p<0.01$) in the *T.congolense* infected animals shows that the anaemia was both mildly macrocytic and hypochromic. The macrocytic and hypochromic responses were similar in both dietary groups. The low digestible crude protein intake in the infected lambs on Diet BS did not result in a lower erythropoietic response compared with the infected lambs on Diet LH. Reissman (1964) found that erythropoiesis was markedly reduced in the presence of low protein intake. Katunguka-Rwakishaya *et al.* (1993) reported a dietary effect in that the

increase in mean corpuscular volume was much higher in *T.congolense* infected Scottish Blackface sheep fed a diet high in protein than in those fed a low protein diet. Berry and Dargie (1976) also found a positive response to protein supplementation of the mean corpuscular volume in ovine fascioliasis. Taking the moderate anaemia into consideration, it is possible that in the present experiment the protein offered in the concentrate to the lambs fed Diet BS was enough to support an increase in erythropoiesis.

White blood cell counts were highly variable between animals before and after infection. The white blood cell counts of the Diet LH fed animals appear to be higher than those fed Diet BS, though differences were not significant. Katunguka-Rwakishaya (1991) found significantly higher white blood cells, lymphocytes and neutrophils in Scottish Blackface sheep fed a high energy diet compared with those fed a low energy diet. The higher white blood cell counts may have influenced the ability of the animals on the higher energy intake to control the number parasites in these experiments.

After infection the number of white blood cells in both the infected groups rose sharply, but no statistical differences could be found, indicating large individual differences in response to infection. The values returned to normal towards the end of the experiment. Katunguka-Rwakishaya (1991) also reported leucocytosis in *T.congolense* infected Scottish Blackface sheep. Murray and Dexter (1988) reported in their review that most bovine trypanosome infections result in leukopaenia followed by leukocytosis which seems to be different from the response in sheep.

Platelet counts were significantly affected by the *T.congolense* infection irrespective of diet. However, the electronic cell counter (Cobus Minor) was often

unable to count the platelets due to platelet aggregation which made the counts unreliable. Thrombocytopaenia has been observed in goats (Van Den Ingh *et al.*, 1976), cattle (Wellde *et al.*, 1983) and sheep (Katunguka-Rwakishaya, 1991). Thrombocytopaenia precedes the other coagulation abnormalities, like platelet aggregation, defective function, reduced platelet half-life and activation of the coagulation pathway (Murray and Dexter, 1988). A relationship has been found between the onset severity and persistence of the thrombocytopaenia and the onset, intensity and prevalence of parasitaemia (Wellde *et al.*, 1983). This relation cannot be found in the data presented in this chapter, however, as indicated before, the data may not be reliable. There are reasons to believe that thrombocytopaenia leads to red cell damage with resultant phagocytosis.

The cholesterol concentration was higher in the animals on Diet BS ($p<.05$). Katunguka-Rwakishaya (1992) reported significantly higher plasma cholesterol concentrations in Scottish Blackface sheep on a low energy diet compared with those on a high energy diet. As reported by Katunguka-Rwakishaya (1992), plasma cholesterol levels decreased markedly ($p<0.01$), immediately after infection, especially in the infected lambs on Diet LH suggesting direct uptake of cholesterol by trypanosomes. Trypanosomes cannot synthesise cholesterol (Carroll *et al.*, 1986) but are able to take up low and high density lipoproteins (Black and Vanderweerd, 1989; Vanderweerd and Black, 1989) which are used by the trypanosomes for growth and multiplication. Carroll *et al.* (1986) found that in *T.b.rhodesiense* and *T.b.brucei* the only membrane sterol is cholesterol.

This is supported by the finding of a relationship between the plasma cholesterol concentration before infection and the average intensity of parasitaemia

during the first month after infection ($r=0.90$; $p<0.01$). A tendency for higher intensities of parasitaemia with higher plasma cholesterol concentrations was also found in Katunguka-Rwakishaya's (1992) experiment in Scottish Blackface sheep. It is likely that a higher host plasma cholesterol concentration is beneficial to parasite growth and multiplication. Traore-Leroux *et al.* (1987) found significantly higher HDL-cholesterol levels in trypanosensitive Zebu cattle than in trypanotolerant Baoule cattle. Since cholesterol levels in the blood are partly heritable (Arave *et al.*, 1974) it might play an important role in trypanotolerance.

Plasma triglyceride concentrations were not affected by either nutrition or trypanosomiasis as was the case in the experiments carried out by Katunguka-Rwakishaya (1991). These results are in contrast to the findings in *T.b.brucei* infected rabbits in which hypertriglyceridemia was reported (Rouser and Cerami, 1980). The authors concluded the hypertriglyceridemia resulted from a defect in triglyceride degradation.

Plasma urea concentrations followed the protein intake levels closely ($p<0.01$). Infection resulted in an increase in plasma urea concentration especially in the lambs on Diet BS possibly indicating catabolism of body protein in this group. These results are comparable to the findings of Abbott *et al.* (1986) in ovine haemonchosis at two levels of protein intake. Increased plasma urea concentrations were also reported by Wassink *et al.* (1993) in *T.congolense* infected West African Dwarf goat. Hamminga (1989) found significantly higher urinary urea levels in West African Dwarf goats compared with the controls.

Plasma albumin concentrations were also significantly affected by nutrition ($p<0.01$). Katunguka-Rwakishaya (1993) found higher albumin concentration in

Scottish Blackface sheep fed higher levels of protein. The plasma albumin concentration was more affected by nutrition than by trypanosomiasis and the effects were additive. Plasma albumin concentrations were found to be significantly lower in the *T.congolense* infected Scottish Blackface sheep fed a diet low in protein (Katunguka-Rwakishaya *et al.*, 1993). A greater fall in serum albumin concentration was also recorded during ovine haemonchosis (Abbott *et al.*, 1986) and ovine fascioliasis (Berry and Dargie, 1976) fed a diet low in protein compared with those on a high protein diet.

The reasons for the lower albumin levels in *T.congolense* infected animals are not clear. Direct uptake of albumin by trypanosomes has been reported (Coppens *et al.*, 1987). Another possibility is that during infection the liver shifts from albumin to globulin production. Katunguka Rwakishaya (1992) suggested hypoalbuminaemia may be largely due to haemodilution.

Conclusions

In conclusion, there is strong evidence that digestive function was altered by the *T.congolense* infection. While the mean retention time was longer in the infected animals their organic matter and crude protein digestibilities were lower than in their pair-fed counterparts. The effects of diet and infection were additive on these parameters. Despite these results no difference could be observed between body weight changes of infected lambs and their pair-fed controls.. The effects of the *T.congolense* infection on PCV, cholesterol, urea and albumin, which were relatively mild in this experiment, were affected by the type of roughage and possibly these were due to differences in plane of nutrition. These effects were additive rather than interactive.

CHAPTER 5

The Pathophysiology of *Trypanosoma congolense* in Scottish Blackface Sheep. Influence of Type of Diet on Digestive Function and Nitrogen Balance

Introduction

In a previous experiment in The Gambia it was found that *Trypanosoma congolense* infected N'dama heifers reduced their intake of *andropogon guyanus* hay but consumed all the groundnut cake and groundnut hay offered (Romney *et al.*, 1994). These results indicate that trypanosome infected animals will select a higher quality diet when a decrease in feed intake occurs due to infection. Kyriazakis *et al.* (1994) demonstrated that nematode infected sheep can compensate for the reduced feed intake due to infection by selecting a diet which meets their protein requirements. Results reported in chapter 4 on sheep fed either lucerne hay or barley straw *ad libitum* indicated that although the intake of the straw was much lower, the amount of fibre consumed, as measured by neutral and acid detergent fibre, was approximately the same. The digestibility of the diet was about 10 points higher in the lucerne hay than in the barley straw fed animals, but diet digestibility was found to be affected by a trypanosome infection in both groups to a similar extend. Differences were also found in the mean retention time of the roughage between trypanosome-infected sheep and their pair-fed controls.

In order to investigate whether the dietary source of the nutrients required for maintenance and growth influences the pathophysiology of digestive function during a trypanosome infection an experiment was set up in which *T.congolense* infected Scottish Blackface wethers were fed different levels of roughage and concentrate. One group of sheep were fed higher levels of barley grain concentrate but lower levels of grass hay than the other group. All sheep were offered barley straw *ad libitum*. An effort was made to have equal amount of energy and protein in the two diets. However, as the source of protein was different a difference in the proportions of

effective rumen degradable protein (ERDP) and digestible undegraded protein (DUP) intake was expected. To investigate whether the different protein sources had an effect on the efficiency of nitrogen retention after infection the nitrogen balance was measured. Research in The Netherlands has shown that the nitrogen balance is altered after a trypanosome infection in West African dwarf goats, indicating a change in utilisation of the nitrogen (Akinbamijo *et al.*, 1992).

Feed and water intake were measured. The mean retention time of the roughage through the digestive tract was determined. The nitrogen retention and diet digestibility were measured during two balance periods. Several plasma metabolite and blood haematology parameters were also measured.

Materials and methods

Experimental animals

Sixteen, one year old, healthy, castrated Scottish Blackface sheep were selected and divided into two groups of 8 infected (I) and 8 pair-fed control (PC) animals. Each pair-fed control animal was offered the amount of ration eaten by its infected partner on the previous day. Each infected and pair-fed control were matched on body weight before the experiment started but, unlike the other experiments, were not related to each other. Four weeks before the experiment started the animals were introduced to the experimental feeds and the animals were put in the metabolic stalls two weeks prior to the start of the experiment.

Experimental diet

One group of 4 trypanosome infected Scottish Blackface sheep and their pair-fed controls received 200 g DM grass hay and 425 g DM crushed barley grain (plus minerals) in the morning and barley straw in the afternoon (Diet BG). The other group of 4 trypanosome infected animals and their pair-fed controls were fed 400 g DM grass hay and 315 g DM crushed barley grain (plus minerals) in the morning and barley straw in the afternoon (Diet GH) (Table 5.1). The roughage had a fibre length of approximately 5 cm. The barley straw was offered *ad libitum* (20% more than previous days' intake) to the infected sheep but was restricted in the pair-fed controls to the amount eaten by their infected partner the day before. The grass hay and barley grain were also given on a pair-feeding basis to the controls.

Table 5.1 Composition (g DM/day) of the experimental diets offered to both dietary groups

	Diet BG	Diet GH
Barley Concentrate	425	315
Grass Hay	200	400
Barley Straw	*Ad libitum*	*Ad libitum*

The nutritional values of the different diet components are given in Table 5.2. Based on the intake of barley straw in the experiment discussed in chapter 4 it was estimated that the daily intake of metabolisable energy and protein on both diets would be 10 MJ and 60 grams, respectively.

Table 5.2 Dry matter (DM; g/kg), organic matter (OM; g/kg DM), metabolisable energy (ME; MJ/kg DM), fermentable metabolisable energy (FME; MJ/kg DM), neutral detergent fibre (NDF; g/kg DM), acid detergent fibre (ADF; g/kg DM), ether extract (EE; g/kg DM), crude protein (CP; g/kg DM), effective rumen degradable dietary protein (ERDP; g/kg DM) and digestible undegraded protein (DUP; g/kg DM) of the diet components

Diet Composition	Barley Concentrate	Grass Hay	Barley Straw
DM	886.8	878.3	876.4
OM	964.2	921.6	949.6
ME[#]	13.3	9.2	6.5
FME[#]	12.7	8.6	5.9
NDF	256.3	633.5	795.1
ADF	62.9	350.4	477.1
EE	12.9	14.0	13.1
CP	124.6	116.0	37.8
ERDP[*]	96	55	22
DUP[*]	18	43	6

#: AFRC (1993) values

*: Values derived from in-sacco degradation and AFRC (1993) calculations

Experimental infection

Two weeks after the experiment started the sheep were infected with *T.congolense* 1180 (GRVPS 57/6) (Nantulya *et al.*, 1984) following the procedure explained in the General Materials and Methods (Chapter 3).

Measurements

Feed and water intake were measured daily by collecting refusals between 8.00 and 9.00 h. Clinical observations were made daily for any abnormal behaviour. The animals were weighed once a week.

Feed digestibilities and nitrogen balance of the infected and pair-fed control groups were measured during two balance periods of 1 week. Balance Period I lasted from day 26 to 32 after infection and Balance Period II from day 47 to 53 after infection. The rate of passage of the roughage through the digestive tract was measured on days 21 and 42 after infection using chromium as a marker.

On Mondays, Wednesdays and Fridays 5 ml of blood was collected into tubes containing ethylene tetra acetic acid (EDTA) and lithium heparin for the measurement of blood haematological and biochemical parameters.

All the procedures and statistical analysis were explained in the General Materials and Methods of Chapter 3.

Results

Feed intake

The organic matter intakes of the animals were very similar in both dietary groups (Table 5.3). The voluntary straw dry matter intake appeared to be higher in the sheep fed Diet BG but differences were not statistically significant due to high individual variation between sheep (Figure 5.1). Organic matter intake was depressed significantly ($p<0.01$) after infection in both dietary groups with no differences in response to infection between the two dietary groups. The feed intake depression was mostly due to reduced intakes of the *ad libitum* fed barley straw. The infected animals on both diets continued to consume most of the grass hay and barley concentrate offered.

The fermentable metabolisable energy intakes were similar in the two dietary groups as were the neutral and acid detergent fibre intakes despite coming from different dietary sources. The reduced levels of barley straw intake of the infected sheep led to significantly lower intakes of fibre and thus energy ($p<0.01$).

Although the crude protein levels in the two diet were very similar in both diets the different sources of the dietary protein led to significant differences ($p<0.01$) in digestible undegraded protein (DUP) between the two diets. The diet higher in barley concentrate (Diet BG) provided lower levels of digestible undegraded protein (DUP) compared to the diet higher in grass hay (Diet GH). As a result the metabolisable protein provided by the diets was slightly different ($p<0.05$). The microbial crude protein (MCP) supply was limited by the intake of effective rumen degradable protein (ERDP) in both diets and not by the intake of fermentable

metabolisable energy (FME). The infection led to significantly lower metabolisable protein intakes ($p<0.01$) compared to pre-infection levels.

Figure 5.1 Mean straw dry matter (DM) intake (g/day) of *T.congolense* infected Scottish Blackface sheep fed Diet BG (BG-I) or Diet GH (GH-I)

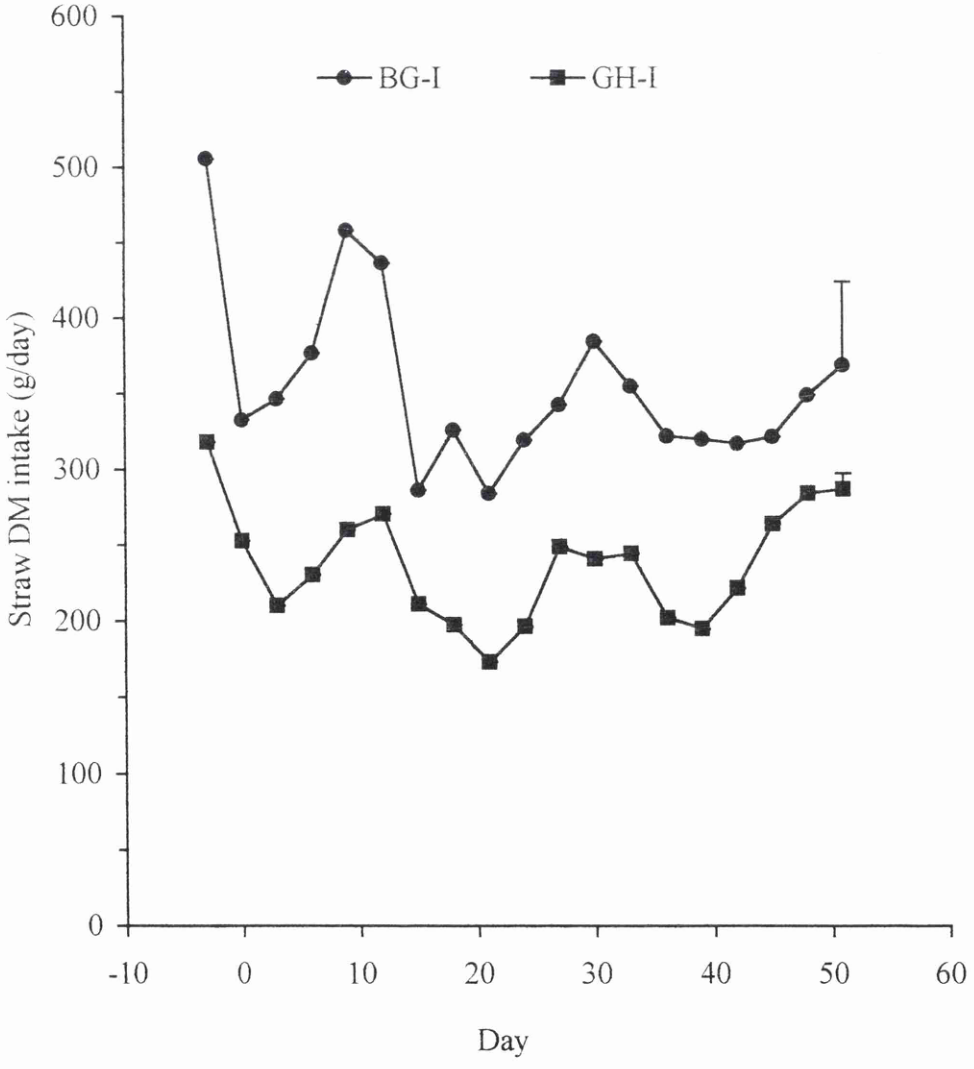


Table 5.3 Mean organic matter (OM), metabolisable energy (ME), fermentable metabolisable energy (FME), neutral detergent fibre (NDF), acid detergent fibre (ADF), crude protein (CP), effective rumen degradable dietary protein (ERDP), digestible undegraded protein (DUP) and metabolisable protein (MP) intake of *T.congolense* infected sheep (n=4) fed either Diet BG or Diet GH during the pre- (day -3 - 0) and post- (day 1 - 53) infection periods (M/D = 10, y = 9.5, L = 1.5)

Diet	OM ($g/kg^{0.75}$/day)	ME# (MJ/day)	FME# (MJ/day)	NDF (g/day)	ADF (g/day)	CP (g/day)	ERDP# (g/day)	DUP# (g/day)	MP# (g/day)
Pre-BG	66.0	10.5	9.9	574	293	96.4	64.0	20.1	60.9
Post-BG	57.4	10.0	9.4	515	261	92.1	61.4	19.2	58.3
Pooled SE	1.81	0.29	0.26	33.6	19.9	1.81	1.09	0.33	1.02
Pre-GH	61.9	10.0	9.4	570	296	100.4	61.0	26.0	64.9
Post-GH	56.1	9.6	9.0	526	273	97.1	59.1	25.3	62.9
Pooled SE	1.82	0.19	0.17	22.6	13.5	1.17	0.69	0.22	0.66
Diet Effect	ns	ns	ns	ns	ns	ns	ns	**	*
Period Effect	**	**	**	**	**	**	**	**	**
Interaction	ns	ns	ns	ns	ns	ns	ns	ns	ns

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means
: Values derived from in-sacco degradation and using AFRC (1993) methods

Water Intake

The water intakes fluctuated between 150 and 220 ml per kg metabolic weight. The intakes were not significantly affected by the type of diet offered to the animals. The water intakes of the sheep appear to slightly higher in the infected groups during both pre- and post-infection but this was only statistically significant during the pre-infection period (Table 5.4).

Table 5.4 Mean water intake (ml/kg$^{0.75}$ metabolic weight/day) of *T.congolense* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BG or Diet GH during the pre (day -3 - 0), post 1 (day 1 - 28) and post 2 (day 29 - 53) infection periods

	Water Intake (ml/kg$^{0.75}$ metabolic weight)		
	period		
Group	pre	post 1	post 2
BG-I	204	215	188
BG-PC	152	165	167
Pooled SE	12.1	22.9	13.8
GH-I	176	175	159
GH-PC	164	169	156
Pooled SE	9.5	9.4	8.2
Diet effect	ns	ns	ns
Infection effect	**	ns	ns
Interaction	ns	ns	ns

** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Body weight

The control sheep on both diets were growing at approximately 100 grams per day which is in accordance with the growth reported in AFRC (1993) for the levels of intake of metabolisable energy and protein of the sheep. Body weight changes were not significantly affected by diet (Table 5.5) but were significantly lower in the *T.congolense* infected sheep compared with their pair-fed controls ($p<0.05$).

Table 5.5 Mean body weight gain (g/day) of *T.congolense* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BG or Diet GH during the post-infection period (day 10-53)

Group	Growth (g)
BG-I	85.5
BG-PC	96.3
Pooled SE	7.3
GH-I	70.8
GH-PC	105.2
Pooled SE	8.2
Diet effect	ns
Infection effect	*
Interaction	ns

* : There is a significant difference between means ($p<0.05$)
ns : No significant difference between means

Digestive function

Diet digestibility coefficients

Apparent neutral detergent fibre digestibility coefficients were slightly lower in the sheep on Diet BG and this was significant during Balance Period I ($p<0.05$). The differences in apparent acid detergent fibre digestibility coefficients were more apparent between the two diets with Diet GH having an approximately 5% higher digestibility coefficient ($p<0.01$). Although the apparent neutral and acid detergent fibre digestibility coefficients appeared to be slightly lower in the infected groups none of the differences were statistically significant (Table 5.6).

Table 5.6 Mean apparent digestibility coefficients of neutral (NDF) and acid detergent fibre (ADF) in *T.congolense* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BG or Diet GH during Balance Period I (day 26 - 32) and II (day 47 - 53)

	NDF digestibility coefficients		ADF digestibility coefficients	
	Balance Period		Balance Period	
Group	I	II	I	II
BG-I	0.57	0.56	0.47	0.49
BG-PC	0.57	0.57	0.47	0.52
Pooled SE	0.010	0.016	0.011	0.015
GH-I	0.61	0.59	0.51	0.54
GH-PC	0.62	0.63	0.54	0.58
Pooled SE	0.008	0.011	0.007	0.010
Diet effect	*	ns	*	**
Infection effect	ns	ns	ns	ns
Interaction	ns	ns	ns	ns

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Apparent organic matter and gross energy digestibility coefficients were very similar in the sheep in both dietary groups and no significant differences were observed (Table 5.7). Apparent organic matter and gross energy digestibility coefficients were slightly lower in the infected sheep and this was significant during Balance Period II ($p<0.05$).

Table 5.7 Mean apparent digestibility coefficients of organic matter (OM) and gross energy (GE) in *T.congolense* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BG or Diet GH during Balance Period I (day 26 - 32) and II (day 47 - 53)

	OM digestibility coefficients		GE digestibility coefficients	
	Balance Period		Balance Period	
Group	I	II	I	II
BG-I	0.65	0.65	0.63	0.61
BG-PC	0.66	0.68	0.64	0.64
Pooled SE	0.011	0.014	0.010	0.014
GH-I	0.66	0.65	0.63	0.60
GH-PC	0.67	0.67	0.65	0.64
Pooled SE	0.006	0.007	0.007	0.011
Diet effect	ns	ns	ns	ns
Infection effect	ns	*	ns	*
Interaction	ns	ns	ns	ns

* : There is a tendency of difference between means ($p<.05$)
ns : No significant difference between means

Mean retention time of the roughage through the digestive tract

The mean retention time of the roughage through the digestive tract on day 21 post-infection was not significantly affected by the type of diet although the rate constant k_2 tended to be slightly higher in the groups fed Diet BG (Table 5.8; $p<0.05$).

Table 5.8 Mean retention time (MRT; h), transit time (TT; h) and rate constants (k_1, k_2; h^{-1}) of chromium mordanted roughage offered on day 21 post-infection to *T.congolense* infected (I) sheep (n=4) fed either Diet BG or Diet GH and their respective pair-fed controls (PC)

Group	MRT	TT	k_1	k_2
BG-I	64.3	20.5	0.029	0.114
BG-PC	46.2	15.5	0.040	0.265
Pooled SE	3.5	1.6	0.003	0.040
GH-I	59.0	21.5	0.036	0.126
GH-PC	55.1	23.7	0.047	0.122
Pooled SE	2.5	2.2	0.005	0.014
Diet effect	ns	ns	ns	*
Infection effect	**	ns	ns	ns
Interaction	*	ns	ns	ns

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

The *T.congolense* infection significantly affected ($p<0.01$) the mean retention time but more so in the animals fed Diet BG compared with those on Diet GH ($p<0.05$). The slower mean retention time in the sheep on Diet BG appears to be mainly caused by slower outflow rate constants k_1 and k_2. As a result the transit time was also slower in the infected animals on Diet BG than in their pair-fed controls. However, none of the differences in these parameters were statistically significant. The difference in mean retention time caused by the infection in the sheep fed Diet GH was small and appears to be mainly caused by differences in the outflow rate constant k_1 but differences were not great enough to be of statistical significance (Table 5.8).

Table 5.9 gives the results of the mean retention time measured on day 42 post-infection. The results for one of the infected sheep on Diet BG did not make sense. Accordingly, the result for this animal and its pair-fed control were eliminated from the statistical analysis.

Again diet had no significant effect on mean retention time. However, as found on day 21 post-infection, both groups of *T.congolense* infected sheep showed a longer mean retention time ($p<0.01$), the effect being greater in the animals fed Diet BG ($p<0.05$). On day 42 post-infection (but not on day 21) differences in transit time were also statistically significant and longer in the infected sheep compared with their pair-fed controls ($p<0.01$). A small interaction effect was also found in the rate constant k_1, the infected sheep on Diet BG showing a lower ($p<0.05$) outflow rate constant k_1 than their pair-fed controls. The outflow rate constant k_2 appeared to be slower in the infected sheep but differences were not statistically significant.

Table 5.9 Mean retention time (MRT; h), transit time (TT; h) and rate constants (k_1, k_2; h^{-1}) of chromium mordanted roughage offered on day 42 post-infection to *T.congolense* infected (I) sheep (n=4) fed either Diet BG or Diet GH and their respective pair-fed controls (PC)

Group	MRT	TT	k_1	k_2
BG-I	63.1	20.0	0.028	0.151
BG-PC	47.0	16.5	0.040	0.190
Pooled SE	3.9	1.4	0.003	0.017
GH-I	60.6	21.9	0.033	0.139
GH-PC	56.8	18.1	0.031	0.179
Pooled SE	2.2	2.2	0.002	0.018
Diet effect	ns	ns	ns	ns
Infection effect	**	**	ns	ns
Interaction	*	ns	*	ns

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Urinary urea and creatinine

Urinary urea excretion was higher in the sheep fed Diet GH, but this was only significant during Balance Period I ($p<0.01$). Urinary creatinine excretion was also slightly higher in the sheep fed Diet GH during Balance Period I. Both the urinary urea and creatinine levels were not affected by the *T.congolense* infection in either Balance Period (Table 5.10).

Table 5.10 Mean urinary urea and creatinine excretion (g/day) of *T.congolense* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BG or Diet GH during Balance Period I (day 26 - 32) and II (day 47 - 53)

	Urinary urea (g/day)		Urinary creatinine (g/day)	
	Balance Period		Balance Period	
Group	I	II	I	II
BG-I	8.0	6.2	0.87	0.89
BG-PC	6.6	6.3	0.90	0.88
Pooled SE	0.39	0.48	0.05	0.05
GH-I	10.9	8.2	0.87	0.85
GH-PC	11.9	7.7	1.01	0.89
Pooled SE	0.48	0.54	0.05	0.05
Diet effect	**	ns	*	ns
Infection effect	ns	ns	ns	ns
Interaction	ns	ns	ns	ns

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Nitrogen balance

The intake of dietary nitrogen in Balance Period I appeared to be slightly higher in the sheep fed Diet GH but the differences were not statistically significant (Table 5.11). No differences in faecal nitrogen excretion were found between the diets, but urinary nitrogen excretion was found to be greater in the sheep fed Diet GH in Balance Period I ($p<0.01$). This significant difference did not carry through to the overall nitrogen retention. Average nitrogen digestibility was slightly higher in the sheep on Diet GH but differences were not statistically significant.

None of the parameters measured were significantly affected by *the T.congolense* infection in Balance Period I, although the nitrogen retention, nitrogen digestibility and the ratio nitrogen retention/nitrogen intake appear to be slightly lower in the infected sheep.

The nitrogen balance results of Balance Period II were very similar to those of Balance Period I (Table 5.12). Again the urinary nitrogen excretions were significantly higher in the sheep on Diet GH but differences were not as pronounced ($p<0.05$) as during Balance Period I. Urinary nitrogen excretion decreased in both the infected and pair-fed control groups in Balance Period II compared with Balance Period I, possibly due to the lower dietary nitrogen intake in Balance Period II ($p<0.01$). However, the ratio nitrogen retention/nitrogen intake increased significantly in Balance Period II in all four groups compared to Balance Period I ($p<0.05$). Again, nitrogen retention, digestibility and the ratio nitrogen retention/nitrogen intake were slightly lower in the infected sheep compared to their pair-fed controls in both dietary groups during both Balance Periods but none of the differences were statistically significant.

Table 5.11 Mean feed nitrogen intake (N_I; g/kg$^{0.75}$), faecal nitrogen (N_F; g/kg$^{0.75}$), urinary nitrogen (N_U; g/kg$^{0.75}$), digestible nitrogen intake (N_{DI}; g/kg$^{0.75}$), nitrogen digestibility (N_{DIG}; %), nitrogen retention/nitrogen intake ratio (N_R/N_I; %) and nitrogen retention (N_R; g/kg$^{0.75}$) of *T.congolense* infected (I) sheep and their respective pair-fed controls (PC) fed either Diet BG or Diet GH during Balance Period I (day 26 - 32)

Group	N_I	N_F	N_U	N_{DI}	N_{DIG}	N_R/N_I	N_R
BG-I	0.91	0.42	0.31	0.49	53.63	19.47	0.18
BG-PC	0.92	0.41	0.27	0.51	55.17	25.43	0.24
Pooled SE	0.02	0.006	0.010	0.027	1.58	2.08	0.024
GH-I	0.97	0.42	0.39	0.55	57.05	16.75	0.16
GH-PC	0.97	0.40	0.39	0.57	58.67	18.64	0.18
Pooled SE	0.02	0.015	0.018	0.012	0.85	1.72	0.016
Diet effect	ns	ns	**	ns	ns	ns	ns
Infection effect	ns	ns	ns	ns	ns	ns	ns
Interaction	ns	ns	ns	ns	ns	ns	ns

** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Table 5.12 Mean feed nitrogen intake (N_I; $g/kg^{0.75}$), faecal nitrogen (N_F; $g/kg^{0.75}$), urinary nitrogen (N_U; $g/kg^{0.75}$), digestible nitrogen intake (N_{DI}; $g/kg^{0.75}$), nitrogen digestibility (N_{DIG}; %), nitrogen retention/nitrogen intake ratio (N_R/N_I; %) and nitrogen retention (N_R; $g/kg^{0.75}$) of *T.congolense* infected (I) sheep and their respective pair-fed controls (PC) fed either Diet BG or Diet GH during Balance Period II (day 47 - 53)

Group	N_I	N_F	N_U	N_{DI}	N_{DIG}	N_R/N_I	N_R
BG-I	0.88	0.38	0.28	0.49	56.07	23.67	0.21
BG-PC	0.90	0.39	0.26	051	56.54	27.53	0.25
Pooled SE	0.019	0.007	0.012	0.020	1.17	2.27	0.025
GH-I	0.94	0.43	0.32	0.51	54.68	20.77	0.20
GH-PC	0.93	0.39	0.31	0.54	58.03	24.64	0.23
Pooled SE	0.018	0.011	0.011	0.015	1.01	1.60	0.017
Diet effect	ns	ns	*	ns	ns	ns	ns
Infection effect	ns	ns	ns	ns	ns	ns	ns
Interaction	*	ns	ns	ns	ns	ns	ns

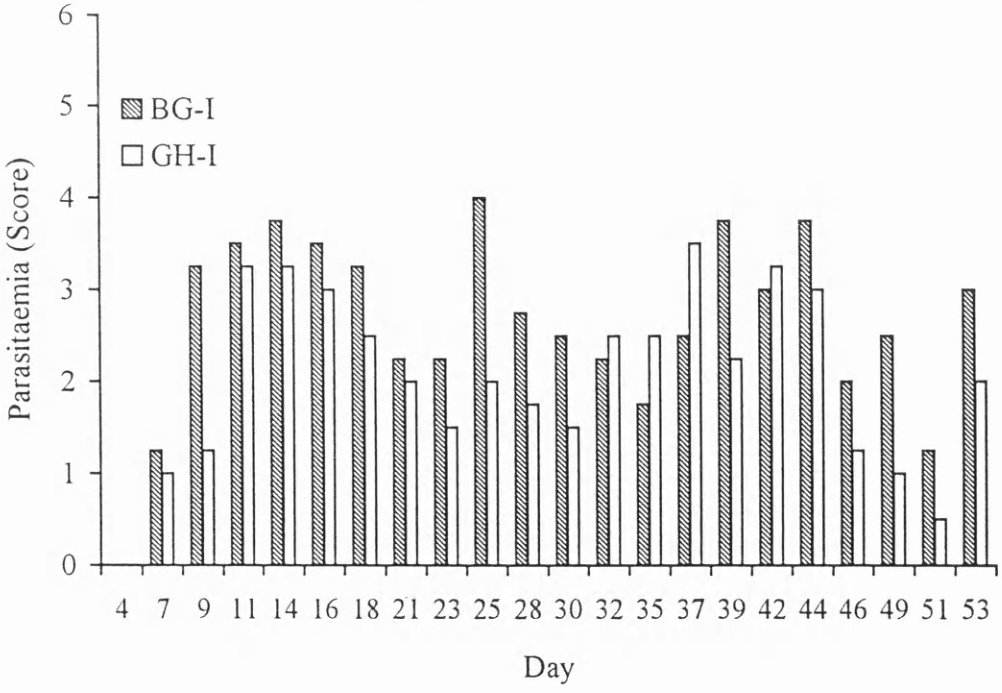
* : There is a significant difference between means ($p<0.05$)
ns : No significant difference between means

Haematology

Parasitaemia

The parasitaemia levels were very similar between the two groups and appeared to be relatively low (Figure 5.2). The first parasites were detected six days after infection. The first peak parasitaemia occurred around day 14 in both infected dietary groups the average parasitaemia score being approximately 3.5 ^{10}log trypanosomes/ml. After day 14 the parasitaemia decreased and started to fluctuate. The infected lambs on Diet BG appears to have a large second peak at around day 25. The second peak in the infected lambs on Diet GH was much lower on day 25 than the peak parasitaemia of the lambs on Diet BG. The third peak parasitaemia was more obvious and appeared to be around day 40 post-infection after which the number of parasites dropped sharply. None of the differences in parasitaemia scores between the two groups were statistically significant.

Figure 5.2 Mean parasitaemia (Score) of *T.congolense* infected sheep fed Diet BG (BG-I) or Diet GH (GH-I)

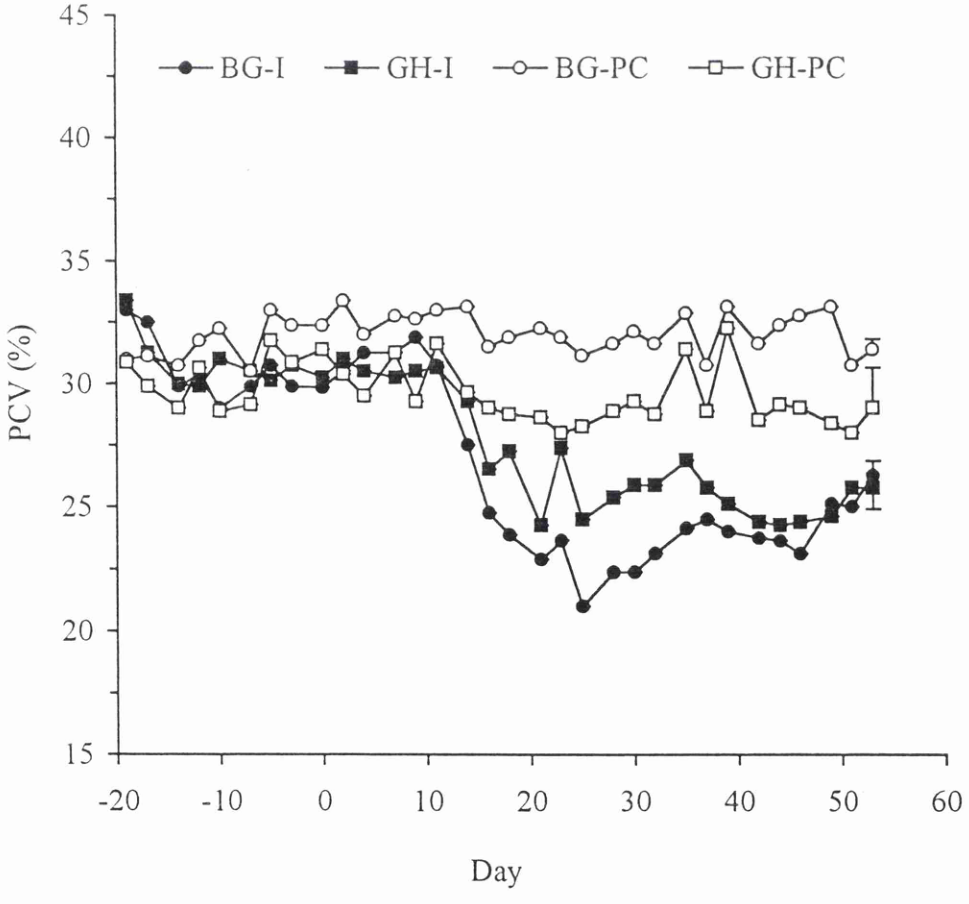


Packed Cell Volume

No significant dietary effects on packed cell volume were found although the pair-fed control animals on Diet GH appeared to have a slightly lower packed cell volume than the sheep on Diet BG (Figure 5.3; Table 5.13).

Packed cell volume decreased significantly between day 14 and 28 after infection ($p<0.01$), the decrease being greater in the infected animals fed Diet BG especially between day 14 and 28 after infection ($p<0.01$). After day 28 the values stabilised at similar levels in both infected groups.

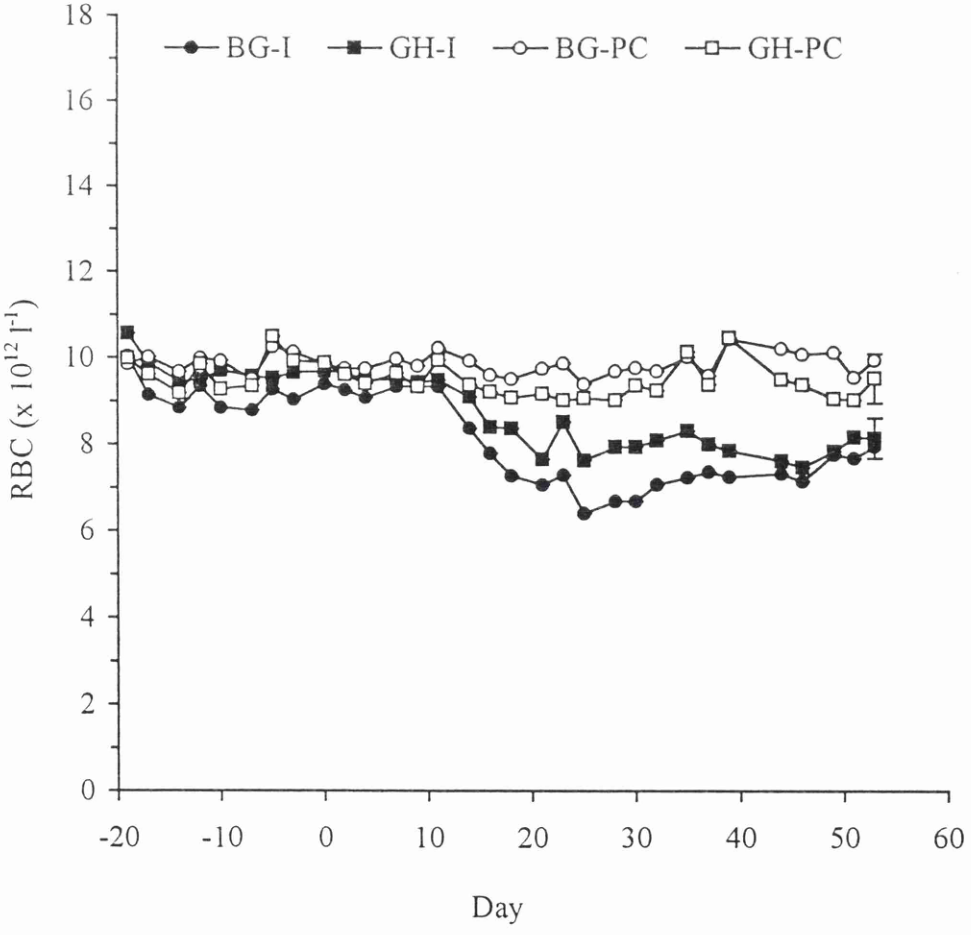
Figure 5.3 Mean packed cell volume (PCV; %) of *T.congolense* infected sheep fed Diet BG (BG-I) or Diet GH (GH-I) and their respective pair-fed controls BG-PC and GH-PC



Red Blood Cells

The number of red blood cells decreased significantly 10 days after infection ($p<0.01$) but stabilised around 20 days after infection (Figure 5.4; Table 5.13). The decrease in the number of red blood cells was initially slightly higher in the sheep fed Diet BG ($p<0.05$).

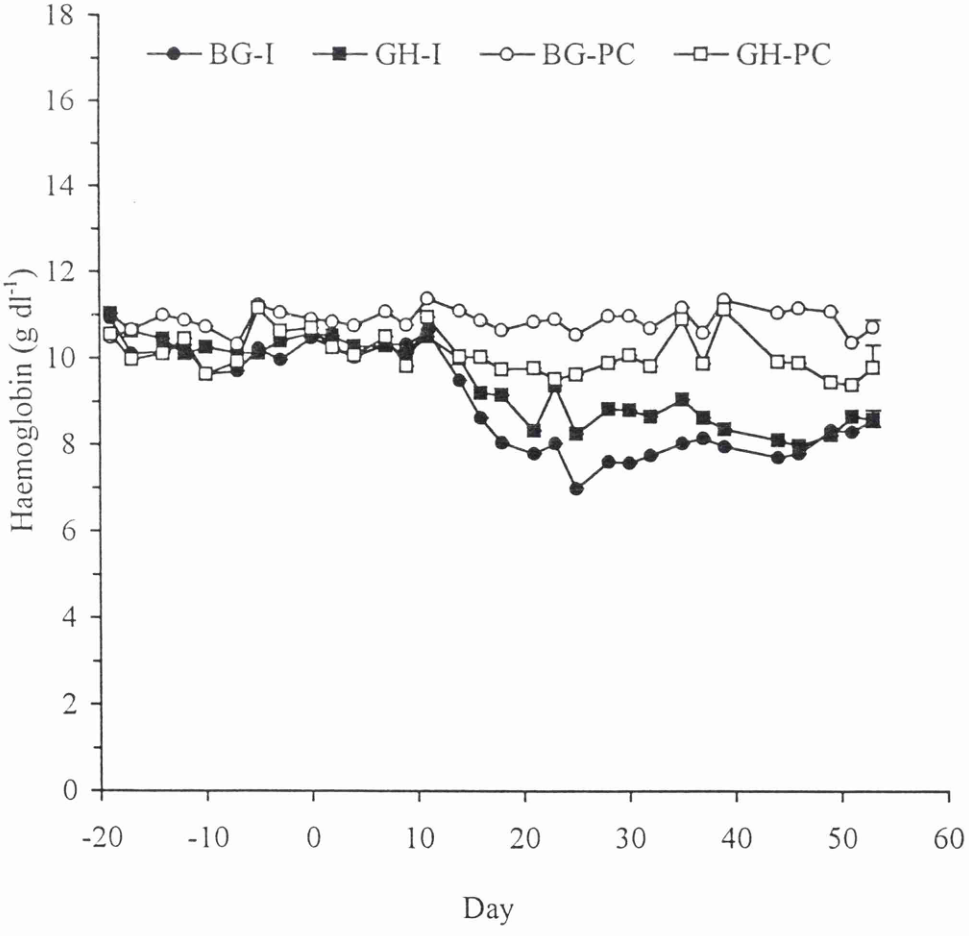
Figure 5.4 Mean red blood cells (RBC; x 10^{12} l^{-1}) of *T.congolense* infected sheep fed Diet BG (AI) or Diet GH (GH-I) and their respective pair-fed controls BG-PC and GH-PC



Haemoglobin concentration

The haemoglobin concentration also decreased 10 days after infection and stabilised after 20 days. The decrease was significantly higher in the sheep fed Diet BG ($p<0.01$) especially from day 14 to 28 (Figure 5.5; Table 5.13).

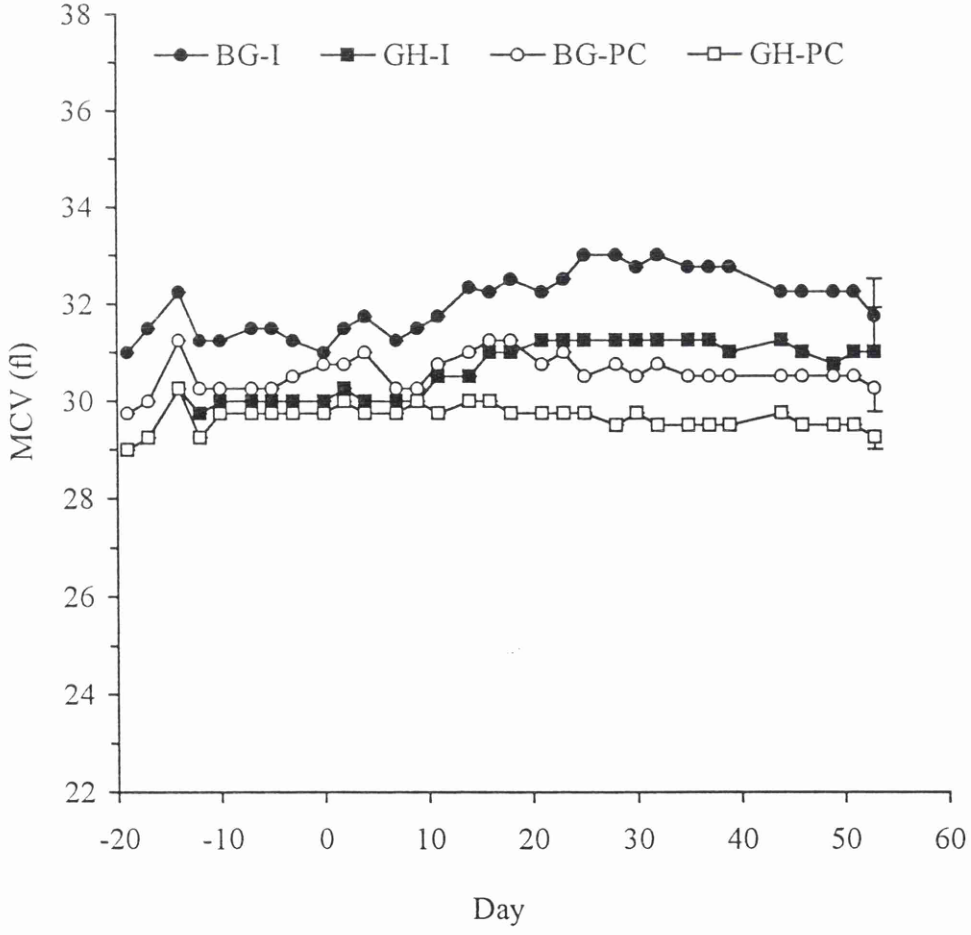
Figure 5.5 Mean haemoglobin (g dl^{-1}) of *T.congolense* infected sheep fed Diet BG (BG-I) or Diet GH (GH-I) and their respective pair-fed controls BG-PC and GH-PC



Mean corpuscular volume

The mean corpuscular volume of the sheep fed Diet BG appeared to be slightly higher than the sheep fed Diet GH during the pre-infection period but differences were not significant(Figure 5.6; Table 5.14). Mean corpuscular volume levels of the infected sheep gradually increased between day 10 and 20 after infection and then stabilised. A significant infection effect was found during the latter part of the infection period ($p<0.05$).

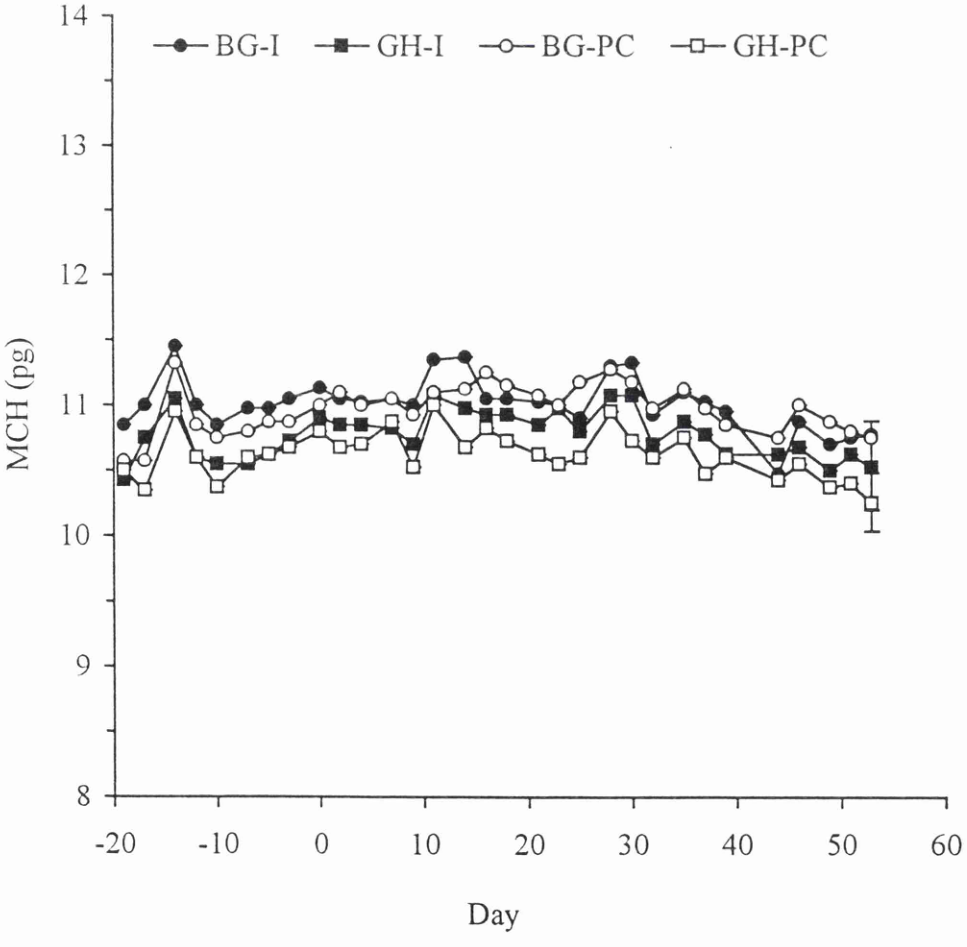
Figure 5.6 Mean corpuscular volume (MCV; fl) of *T.congolense* infected sheep fed Diet BG (BG-I) or Diet GH (GH-I) and their respective pair-fed controls BG-PC and GH-PC



Mean corpuscular haemoglobin

No significant differences were found between any of the groups although the mean corpuscular haemoglobin levels appeared to be slightly higher in the sheep fed Diet BG (Figure 5.7; Table 5.14).

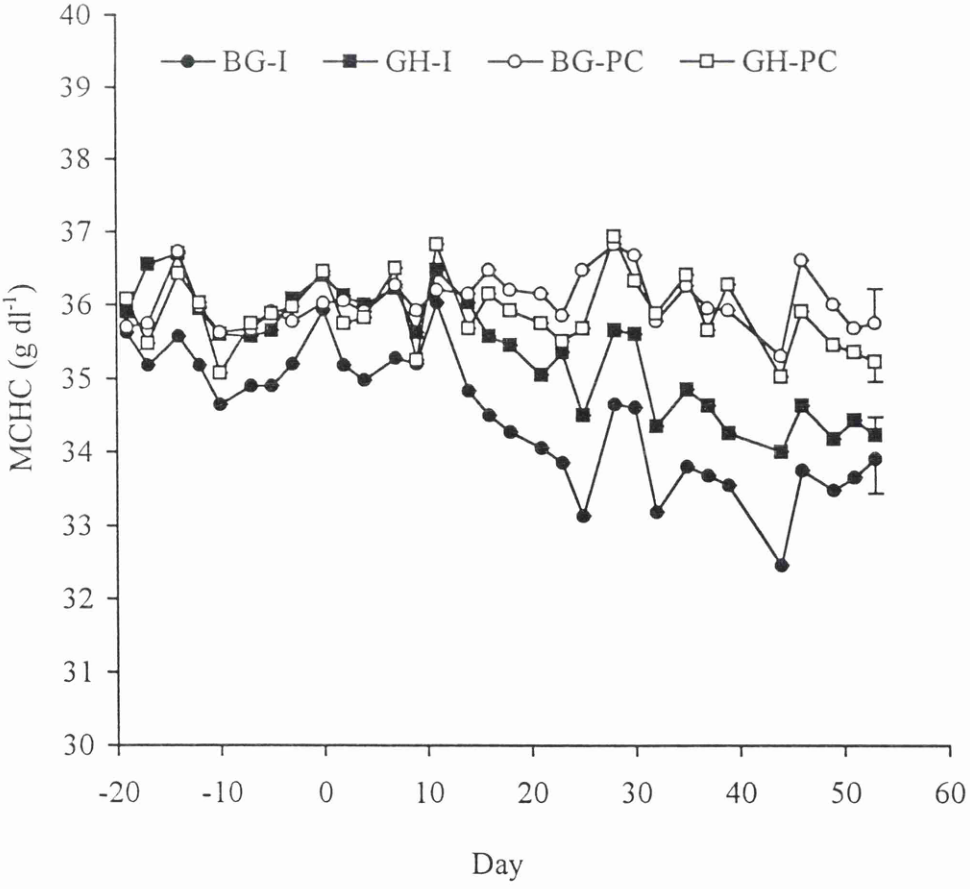
Figure 5.7 Mean corpuscular haemoglobin (MCH; pg) of *T.congolense* infected sheep fed Diet BG (BG-I) or Diet GH (GH-I) and their respective pair-fed controls BG-PC and GH-PC



Mean corpuscular haemoglobin concentration

The mean corpuscular haemoglobin concentration were unaffected by the type of diet fed to the sheep (Figure 5.8; Table 5.14). Mean corpuscular haemoglobin concentrations decreased significantly ($p<0.01$) from day 14 after infection and were more affected in the sheep fed Diet BG ($p<0.05$).

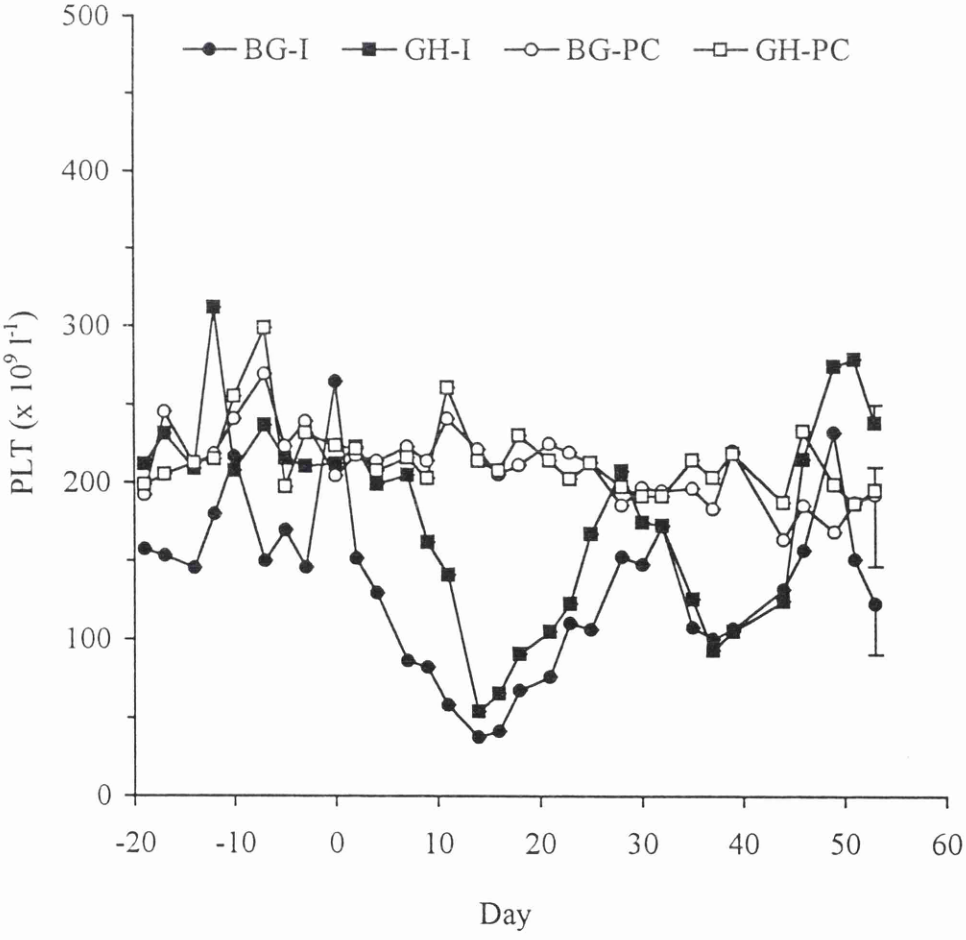
Figure 5.8 Mean corpuscular haemoglobin concentration (MCHC; g dl^{-1}) of *T.congolense* infected sheep fed Diet BG (BG-I) or Diet GH (GH-I) and their respective pair-fed controls BG-PC and GH-PC



Platelet count

The number of platelets decreased rapidly after infection in both infected groups ($p<0.01$) and reached its lowest level on day 14 coinciding with the first peak parasitaemia and then recovered to near normal levels (Figure 5.9; Table 5.15). However, the number of platelets dropped again after day 32 to reach the second trough on day 37 coinciding with the second peak parasitaemia. Platelet aggregation was frequently observed. After day 37 of infection the number of platelets recovered to normal values.

Figure 5.9 Mean platelet count (PLT; x 10^9 l^{-1}) of *T.congolense* infected sheep fed Diet BG (BG-I) or Diet GH (GH-I) and their respective pair-fed controls BG-PC and GH-PC



White blood cells

The number of white blood cells were unaffected by the type of diet fed to the sheep. Although differences were not significant the number of white blood cells started to fluctuate more after infection in the infected groups than in the pair-fed control groups. The number of white blood cells appear to increase in the infected sheep during the latter stages of the infection (Figure 5.10; Table 5.15).

Figure 5.10 Mean white blood cell count (WBC; x 10^9 l^{-1}) of *T.congolense* infected sheep fed Diet BG (BG-I) or Diet GH (GH-I) and their respective pair-fed controls BG-PC and GH-PC

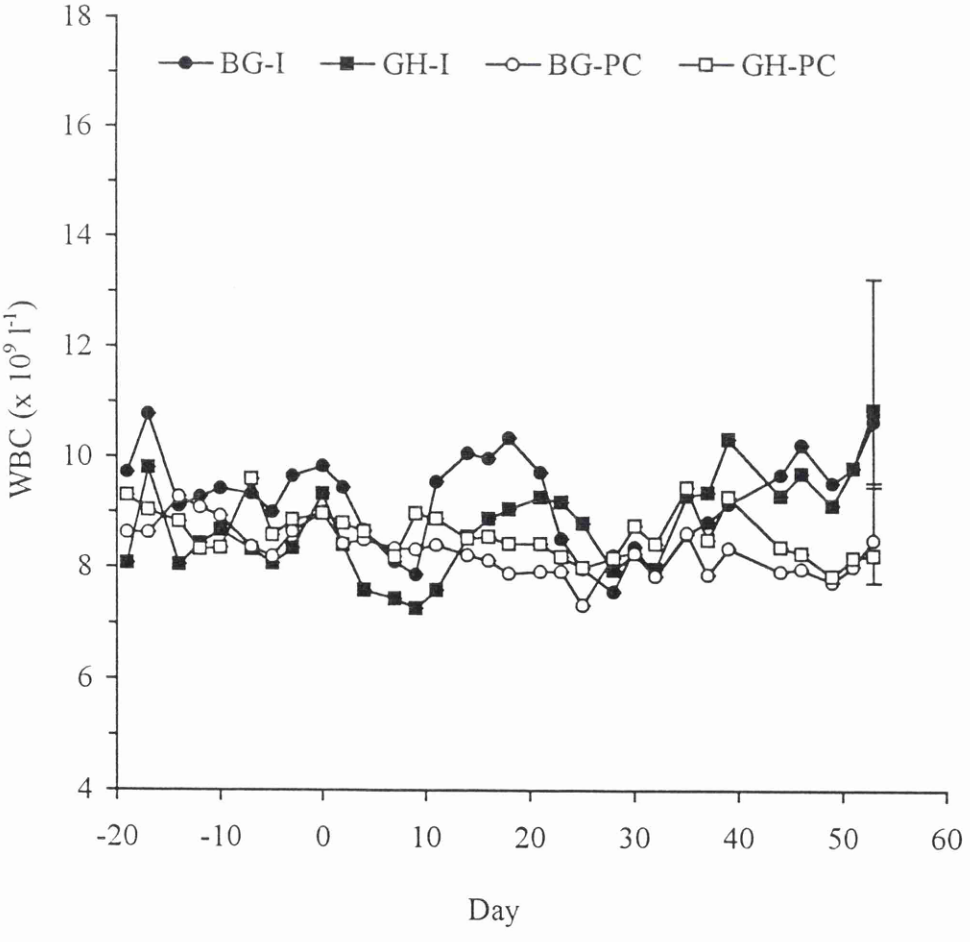


Table 5.13 Mean packed cell volume (%), red blood cell count (x 10^{12} l^{-1}) and haemoglobin concentration (g dl^{-1}) of *T.congolense* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BG or Diet GH during the pre (day -19 - 0), post 1 (day 14 - 28) and post 2 (day 30 - 53) infection periods

	Packed Cell Volume (%)			Red Blood Cells (x 10^{12} l^{-1})			Haemoglobin Concentration (g dl^{-1})		
	period			period			period		
Group	pre	post 1	post 2	pre	post 1	post 2	pre	post 1	post 2
BG-I	30.6	23.6	24.2	9.17	7.23	7.35	10.1	8.1	8.0
BG-PC	31.7	31.9	32.1	9.91	9.70	9.95	10.8	10.9	10.9
Pooled SE	0.39	1.65	1.62	0.18	0.48	0.52	0.20	0.55	0.58
GH-I	30.8	26.4	25.5	9.73	8.25	7.93	10.4	9.0	8.5
GH-PC	30.3	28.7	29.4	9.73	9.13	9.48	10.3	9.8	10.0
Pooled SE	0.61	0.75	1.07	0.28	0.32	0.43	0.16	0.23	0.37
Diet effect	ns	ns	ns	ns	ns	ns	ns	ns	ns
Infection effect	ns	**	**	ns	**	**	ns	**	**
Interaction	ns	**	*	ns	*	ns	ns	**	*

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Table 5.14 Mean corpuscular volume (fl), mean corpuscular haemoglobin (pg) and mean corpuscular haemoglobin concentration (g dl^{-1}) of *T.congolense* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BG or Diet GH during the pre (day -19 - 0), post 1 (day 14 - 28) and post 2 (day 30 - 53) infection periods

	Mean Corpuscular Volume (fl)			Mean Corpuscular Haemoglobin (pg			Mean Corpuscular Haemoglobin Concentration (g dl^{-1})		
	period			period			period		
Group	pre	post 1	post 2	pre	post 1	post 2	pre	post 1	post 2
BG-I	31.4	32.5	32.5	11.0	11.1	10.9	35.2	34.2	33.6
BG-PC	30.4	30.9	30.5	10.8	11.2	10.9	35.9	36.3	36.0
Pooled SE	0.56	0.58	0.55	0.19	0.19	0.15	0.32	0.47	0.49
GH-I	29.8	31.1	31.1	10.7	11.0	10.7	36.0	35.4	34.5
GH-PC	29.6	29.8	29.5	10.6	10.7	10.6	35.9	35.9	35.8
Pooled SE	0.39	0.48	0.55	0.17	0.17	0.16	0.17	0.27	0.32
Diet effect	ns	ns	ns	ns	ns	ns	ns	ns	ns
Infection effect	ns	ns	*	ns	ns	ns	ns	**	**
Interaction	ns	ns	ns	ns	ns	ns	ns	*	ns

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Table 5.15 Mean platelet (x 10^9 l^{-1}) and white blood cell count (x 10^9 l^{-1}) of *T.congolense* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BG or Diet GH during the pre (day -19 - 0), post 1 (day 14 - 28) and post 2 (day 30 - 53) infection periods

	Platelet Count (x 10^9 l^{-1})			White Blood Cells x 10^9 l^{-1})		
	period			period		
Group	pre	post 1	post 2	pre	post 1	post 2
BG-I	173	86	143	9.6	9.1	9.3
BG-PC	227	212	189	8.7	8.0	8.1
Pooled SE	19	31	22	0.40	0.69	0.73
GH-I	227	116	180	8.6	8.8	9.4
GH-PC	227	211	202	8.9	8.3	8.5
Pooled SE	12	22	9	0.82	0.66	0.82
Diet effect	ns	ns	ns	ns	ns	ns
Infection effect	ns	**	ns	ns	ns	ns
Interaction	ns	ns	ns	ns	ns	ns

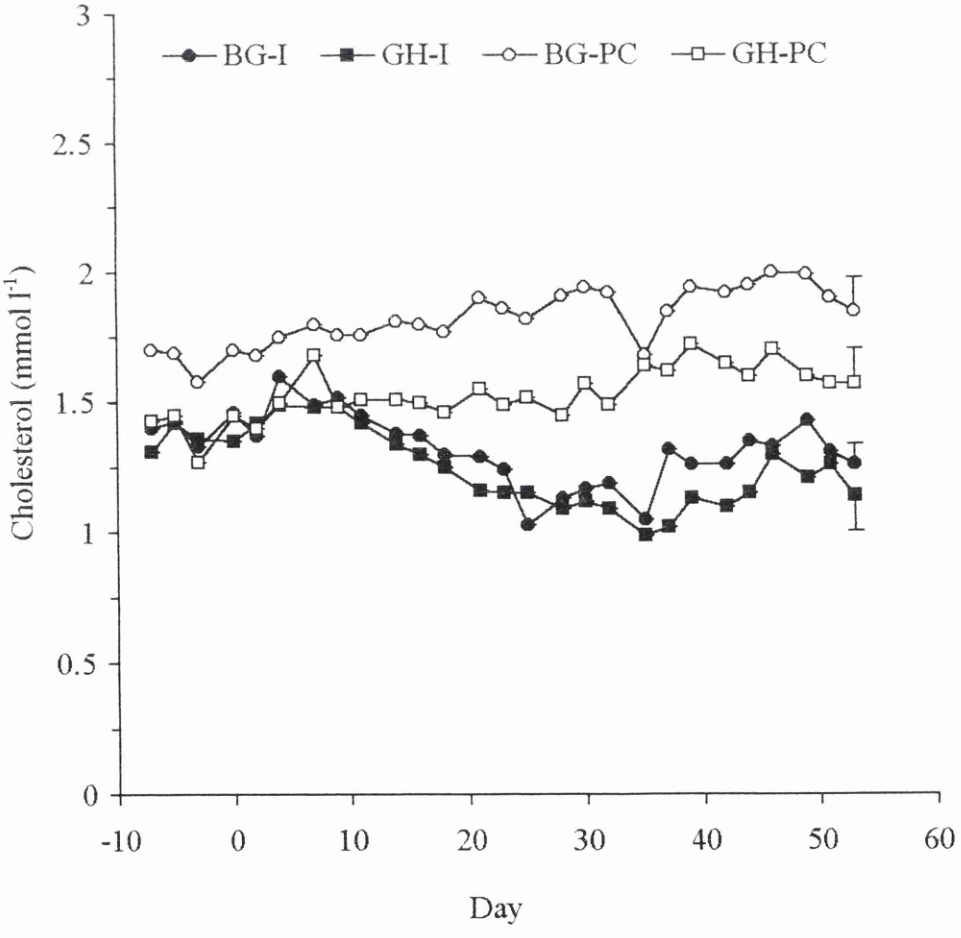
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Blood biochemistry

Plasma cholesterol

The type of diet had no effect on plasma cholesterol levels (Figure 5.11; Table 5.16). Plasma cholesterol concentrations decreased significantly 10 days after infection in both infected groups ($p<0.01$) but appeared to recover after approximately 25 days. In contrast, the plasma cholesterol levels of the pair-fed controls increased as the experiment progressed.

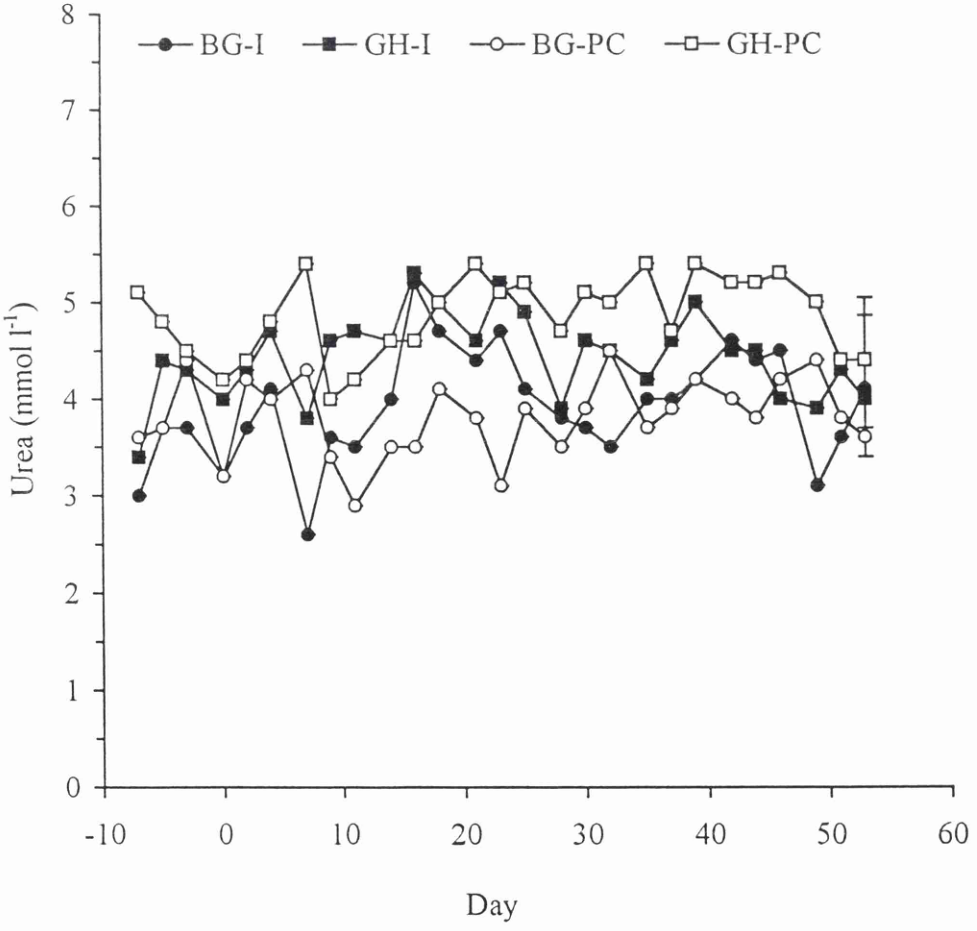
Figure 5.11 Mean plasma cholesterol (mmol l^{-1}) of *T.congolense* infected sheep fed Diet BG (BG-I) or Diet GH (GH-I) and their respective pair-fed controls BG-PC and GH-PC



Plasma urea

Plasma urea levels were not significantly affected by the type of diet although the levels of the sheep on Diet GH appeared to be slightly higher (Figure 5.12; Table 5.16). The *T.congolense* infection also had no significant effect on plasma urea concentrations.

Figure 5.12 Mean plasma urea (mmol l^{-1}) of *T.congolense* infected sheep fed Diet BG (BG-I) or Diet GH (GH-I) and their respective pair-fed controls BG-PC and GH-PC



Plasma albumin

Plasma albumin concentrations were not significantly affected by the type of diet offered to the sheep. Plasma albumin concentrations decreased after infection ($p<0.01$) (Figure 5.13; Table 5.16). The decrease in plasma albumin was initially higher in the infected sheep on Diet BG ($p<0.05$). The plasma albumin concentration appeared to recover in the infected groups after day 28 but decreased again on day 35. After day 35 the plasma albumin levels increased but levels of the infected sheep were still lower than the pair-fed controls at the end of the experiment.

Figure 5.13 Mean plasma albumin (g l^{-1}) of *T.congolense* infected sheep fed Diet BG (BG-I) or Diet GH (GH-I) and their respective pair-fed controls BG-PC and GH-PC

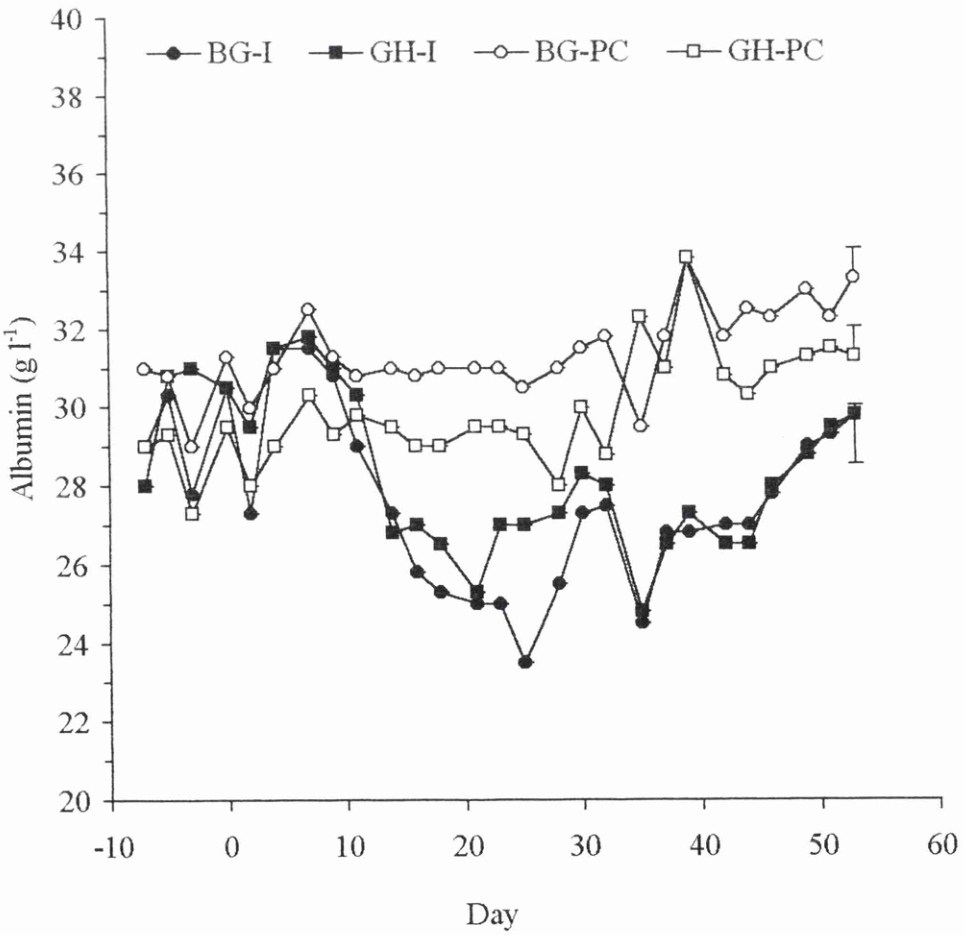


Table 5.16 Mean plasma cholesterol (mmol l^{-1}), urea (mmol l^{-1}) and albumin (g l^{-1}) concentration of *T.congolense* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BG or Diet GH during the pre (day -19 - 0), post 1 (day 14 - 28) and post 2 (day 30 - 53) infection periods

	Cholesterol (mmol l^{-1})			Urea (mmol l^{-1})			Albumin (g l^{-1})		
	period			period			period		
Group	pre	post 1	post 2	pre	post 1	post 2	pre	post 1	post 2
BG-I	1.40	1.25	1.27	3.4	4.4	4.0	29.4	25.3	27.5
BG-PC	1.67	1.84	1.90	3.7	3.6	4.0	30.5	30.9	32.1
Pooled SE	0.08	0.14	0.14	0.2	0.4	0.3	0.5	1.1	1.0
GH-I	1.36	1.20	1.14	4.0	4.8	4.4	30.1	26.7	27.6
GH-PC	1.40	1.50	1.61	4.6	4.9	5.0	28.8	29.1	31.1
Pooled SE	0.07	0.11	0.13	0.3	0.2	0.2	0.5	0.6	0.8
Diet effect	ns	ns	ns	ns	ns	ns	ns	ns	ns
Infection effect	ns	**	**	ns	ns	ns	ns	**	**
Interaction	ns	ns	ns	ns	ns	ns	ns	*	ns

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Discussion

This experiment was set up to investigate changes in digestive function and nitrogen balance in *T.congolense* infected Scottish Blackface sheep fed different amounts of roughage and concentrate. The infection significantly decreased organic matter intake in the sheep on both diets. The body weight gains were also slightly decreased in the infected sheep. The organic matter and gross energy digestibilities decreased slightly after infection. Fibre digestibility was affected by the type of diet but not by infection. The *T.congolense* infection significantly increased the mean retention time of the roughage through the digestive tract especially in the sheep fed Diet BG. The nitrogen balance appeared to be slightly lower in the infected animals but none of the differences were significant. Packed cell volume decreased significantly after infection and the decrease was greater in the sheep fed Diet BG. The mean corpuscular volume increased toward the end of the experiment and the mean corpuscular haemoglobin concentration decreased significantly after infection. Plasma cholesterol and albumin levels decreased after infection, but plasma urea were unaffected. It was concluded that the effects of the *T.congolense* infection on digestive function and nitrogen balance were very mild.

The composition of the diets were only different in the amount of digestible undegraded protein (DUP), the level being higher in Diet GH ($p<0.01$). As a result the metabolisable protein intake was slightly higher in the animals fed Diet GH ($p<0.05$).

The organic matter intake of the sheep decreased to a similar extend in both dietary groups after the *T.congolense* infection ($p<0.01$). The sheep continued to eat all the grass hay and barley concentrate offered and the decrease was due to a reduction in the intake of barley straw. In a previous experiment in which the same

strain of *T.congolense* was used the sheep also reduced their straw dry matter intake (Chapter 4). Reduced intakes of poor quality *Andropogon guyanus* hay has been reported in *T.congolense* infected cattle in The Gambia. These cattle also consumed all the groundnut hay and cake (Romney *et al.*, 1994). Van Dam (1996), reported that the percentage decrease of digestible organic matter intake due to a *T.vivax* infection was not different for lucerne and grass straw fed West African Dwarf goats. However, the goats had no choice between high and low quality feeds.

The water intakes appeared slightly higher in the infected animals but since this effect was greater pre-infection than post-infection it is more likely to have been caused by the restricted levels of feeding in the pair-fed controls than by the infection.

No dietary effect was observed on body weight gains indicating that there were no or insignificant benefits from the higher digestible undegraded protein in Diet GH. The *T.congolense* infected sheep had a significantly lower body weight gain than their pair-fed controls ($p<0.05$) but differences were only marginal. The infection appears to have been very mild compared with the *T.vivax* infection of the West African Dwarf goats in which an increase in heat production of 16.7 % in lying animals was reported (Van Dam, 1996). Similarly, increases of 25 and 22% in the energy maintenance requirements of *T.vivax* infected West African Dwarf goats have been reported by Verstegen *et al.* (1991) and Van Dam (1996), respectively. As a result the West African Dwarf goats lost more body weight than their controls. It should be stressed that the West African Dwarf control goats were not pair-fed in contrast to the control sheep in the experiment presented here. Nevertheless, the feed intake reduction in the infected sheep in this experiment was only marginal and it is likely that the changes in

heat production and maintenance requirements are related to pathogenicity of the disease.

The organic matter and gross energy digestibilities were slightly lower in the infected sheep compared with their pair-fed controls and this became significant during Balance Period II ($p<0.05$). Similar results were found in a previous experiment using the same strain of *T.congolense* in Scottish Blackface sheep but different types of diet (Chapter 4). Van Dam (1996) did not find any evidence of a decrease in organic matter digestibility due to a *T.vivax* infection in animals fed lucerne pellets or grass straw. Akinbamijo *et al.* (1994a, 1994 b) also found no changes in the digestibility coefficients of organic matter in *T.vivax* infected West African Dwarf ewes fed different levels of a mixture of *Panicum maximum*, dried cassava peels, *Gliricidia sepium* and *leucaena leucocephala*.

Fibre digestibilities were found to be lower in the sheep fed Diet BG possibly due to the higher intake of barley straw in these animals. No effects of infection on fibre digestibility was found in this experiment.

The mean retention time of the roughage through the digestive tract were similar in both dietary groups on day 21 and 42 after infection. The mean retention time was significantly affected by the *T.congolense* infection ($p<0.01$) and this effect was greater in the sheep fed Diet BG ($p<0.05$). The longer mean retention time in the infected sheep on Diet BG seems to be caused by a longer transit time and a lower rumen outflow rate constant k_1 than in the pair-fed controls. A slightly lower digestibility of the organic matter in the infected sheep may cause a longer retention time. However, this lower organic matter digestibility did not appear to be diet

dependent. The results of Chapter 4 also indicated a longer mean retention time of the roughage in the digestive tract.

Urinary urea was found to be higher in the sheep fed Diet GH especially during Balance Period I, most probably due to the slightly higher metabolisable protein level of Diet GH. Infection had no significant effect on the urinary urea excretion. Haminga (1989) found only a small difference in urinary urea excretion in one of three balance periods after an infection with *T.vivax*. In contrast to the findings of Van Dam (1996) the urinary creatinine measured in this experiment was found to be unaffected by the infection. Variation in creatinine excretion indicates differences in muscle mass and since the differences in body weight were relatively small after infection this may explain the failure to show significant changes in creatinine excretion.

The total urinary nitrogen excretion in Balance Period I was significantly higher ($p<0.01$) in the sheep fed Diet GH due to the higher urea excretion. The nitrogen digestibility, nitrogen retention and the ratio nitrogen retention/nitrogen intake were all slightly, though not significantly, lower in the infected groups compared with their pair-fed controls.

Nitrogen intake in Balance Period II was found to be significantly lower ($p<0.01$) than in Balance Period I. However, nitrogen retention appeared to be more efficient in Balance Period II in all 4 groups possibly as a result of the slight decrease in nitrogen intake (0.05). Akinbamijo *et al.*, (1992) showed that nitrogen retention became more efficient in animals receiving a restricted feed ration. Again the nitrogen digestibility, nitrogen retention and the ratio nitrogen retention/nitrogen intake were slightly, though not significantly, lower in the infected groups compared with their pair-fed controls. Since the effects of the *T.congolense* infection on the nitrogen

balance were only marginal in this experiment no beneficial effect of the higher digestible undegraded protein in Diet GH was measured.

A lower nitrogen digestibility was found in Scottish Blackface sheep fed barley straw or lucerne hay after an infection with the same strain of *T.congolense* (Chapter 4). In contrast, Akinbamijo *et al.* (1992) reported no changes in nitrogen digestibility *T.vivax* infected West African Dwarf goats. However, an increase in nitrogen digestibility was found of approximately 10% after the goats were infected possibly due to the large reduction in feed intake. Van Dam (1996) also found no evidence of a decrease in nitrogen digestibility in *T.vivax* infected West African Dwarf goats.

Verstegen *et al.* (1991), Akinbamijo *et al.* (1992) and Van Dam (1996) all found a lower nitrogen retention in *T.vivax* infected West African dwarf goats compared with their controls. However, no evidence was found of a change in the relationship due to the *T.vivax* infection between digestible organic matter intake of any of the diets used and nitrogen retention. Since at the same time a large increase in heat production and maintenance requirements was found it was concluded that during trypanosome infections fat mobilisation is of greater importance than protein breakdown. As Akinbamijo *et al.* (1994a; 1994b) also found that *T.vivax* infected West African dwarf ewes retained less nitrogen than the uninfected controls, but also concluded that a decrease in feed intake was largely responsible.

The parasitaemia scores of the two infected groups followed the same pattern as in previous experiments using the same strain of *T.congolense* (Chapter 4 and 5; Katunguka-Rwakishaya, 1992). The first peak parasitaemia occurred around day 14 after which the parasitaemia started to fluctuate. The second clear peak occurred around day 40. Although the parasitaemia scores in the group BG sheep appeared to

be slightly higher throughout the experimental period the differences were never statistically significant. No significant differences in parasitaemia scores in ruminants due to nutrition have been reported in the past.

The packed cell volume, red blood cell count and haemoglobin concentration were all significantly decreased in both dietary groups by the infection ($p<0.01$). Compared with previous experimental infections using the same strain (Katunguka-Rwakishaya, 1992), however, the changes were relatively small. The decrease was significantly smaller in the infected Diet GH fed sheep compared with their pair-fed controls than in the infected sheep fed Diet BG ($p<0.01$). The slightly higher protein levels in Diet GH may have played a role in this difference in response to the infection.

The increase in mean corpuscular volume was relatively small and was only significant towards the end of the experiment ($p<0.05$). This low response may have been due to the limited anaemia found in this experiment. As in previous experiment (Chapter 4) the mean corpuscular haemoglobin concentration decreased significantly after infection. The decrease was slightly higher in the Diet BG fed sheep ($p<0.05$).

The number of platelets fell sharply ($p<0.01$) due to aggregation after infection but unlike previous experiments the number appeared to recover and follow the parasitaemia pattern. Wellde *et al.* (1983) found a relationship between the onset, severity and persistence of the thrombocytopaenia and the onset, intensity and prevalence of parasitaemia. Thrombocytopaenia precedes the other coagulation abnormalities, like platelet aggregation, defective function, reduced platelet half-life and activation of the coagulation pathway (Murray and Dexter, 1988). In a previous experiment it was shown that high parasitaemias are related to high plasma nitric oxide

produced by activated macrophages. One of the products of nitric oxide, peroxynitrite, has been found to cause aggregation of human platelets (Moro *et al.*, 1994).

Like in previous experiments plasma cholesterol and albumin concentrations were significantly decreased after infection ($p<0.01$). The plasma albumin levels were slightly less affected in the Diet GH infected sheep during the early stages of infection. Both the plasma cholesterol and albumin concentrations recovered slightly towards the end of the experiment. Plasma albumin (Coppens *et al.*, 1987) and cholesterol (Black and Vanderweerd, 1989; Vanderweerd and Black, 1989) can both be taken up by the trypanosomes for growth and the recovery may have been due to a decrease in the number of parasites. Plasma albumin may also have been lowered due to a shift from albumin production to globulin production in the liver. Katunguka Rwakishaya (1992) suggested that the lowered albumin and cholesterol levels may be largely due to haemodilution.

Conclusions

The relatively mild *T.congolense* infection of the Scottish Blackface sheep in this experiment caused relatively minor changes in digestive function and nitrogen balance. These small but cumulative changes in digestive function and nitrogen balance may have contributed to the lower body weight gain of the infected sheep. The higher digestible undegraded protein (DUP) in Diet GH did not appear to have any beneficial effect on the patho-physiology of the infection in this experiment.

The *T.congolense* infection caused a slight anaemia in the Scottish Blackface sheep, especially in the animals on Diet BG. The infection also resulted in lower platelet counts, and lower plasma cholesterol and albumin levels.

CHAPTER 6

The Pathophysiology of *Trypanosoma vivax* in Scottish Blackface Sheep. Influence of Type of Diet on Digestive Function and Nitrogen Balance

Introduction

The pathophysiological effects of the *T.congolense* infection used in the experiment reported in chapter 5 turned out to be relatively mild. Research in the Netherlands (Hamminga, 1989, Verstegen *et al.*, 1991) indicated greater effects of trypanosomiasis on the nitrogen balance using a more pathogenic strain of *Trypanosoma vivax*. In the experiment reported in this chapter a stabilate of the same strain of *T.vivax* as used in The Netherlands was used to investigate changes in the nitrogen balance and digestive function in Scottish Blackface lambs and to compare those changes with the result of the *T.congolense* infection reported in chapter 5. The diets were basically the same as in the previous experiment but the level of feeding was slightly lower and kept around maintenance levels. One group of lambs were fed higher levels of barley grain concentrate but lower levels of grass hay than the other group. All sheep were offered barley straw *ad libitum*. Again a difference in the proportions of effective rumen degradable protein (ERDP) and digestible undegraded protein (DUP) intake between the two groups was expected. The same measurements were taken as during the previous experiment.

Materials and methods

Experimental animals

Eight pairs of twin, castrated Scottish Blackface lambs were selected. The animals were healthy and six months old at the start of the experiment. Four weeks before the experiment started the animals were introduced to the experimental feeds and the animals were put in the metabolic stalls two weeks prior to the start of the experiment. One lamb of each pair was infected with *Trypanosoma vivax* while the

other was used as a pair-fed control. Each pair-fed control lamb was offered the amount of ration eaten by its infected partner on the previous day.

Experimental diet

One group of four trypanosome-infected Scottish Blackface sheep and their pair-fed controls received 150 g DM grass hay and 319 g DM crushed barley grain (plus mineral mix) in the morning and barley straw in the afternoon (Diet BG). The other group of 4 trypanosome infected animals and their pair-fed controls were fed 300 g DM grass hay and 236 g DM crushed barley grain (plus mineral mix) in the morning and barley straw in the afternoon (Diet GH; Table 6.1). The roughage had a fibre length of approximately 5 cm. The composition of the diet components are given in Table 6.2. The barley straw was offered *ad libitum* (20% more than previous days' intake) to the infected sheep but was restricted in the pair-fed controls to the amount eaten by their infected partner the day before. The grass hay and barley grain was also given on a pair-feeding basis to the controls.

The expected metabolisable energy (ME) intake was approximately 7 MJ per day and the metabolisable protein (MP) intake was estimated to be approximately 45 grams per day by the animals on both diets. However, the digestible undegraded protein intake levels were expected to be higher in the lambs fed Diet GH.

Table 6.1 Composition (g DM/day) of the experimental diets offered to both dietary groups

	Diet BG	Diet GH
Barley Concentrate	319	236
Grass Hay	150	300
Barley Straw	*Ad libitum*	*Ad libitum*

Table 6.2 Dry matter (DM; g/kg), organic matter (OM; g/kg DM), metabolisable energy (ME; MJ/kg DM), fermentable metabolisable energy (FME; MJ/kg DM), neutral detergent fibre (NDF; g/kg DM), acid detergent fibre (ADF; g/kg DM), ether extract (EE; g/kg DM), crude protein (CP; g/kg DM), effective rumen degradable dietary protein (ERDP; g/kg DM) and digestible undegraded protein (DUP; g/kg DM) of the diet components

Diet Composition	Barley Concentrate	Grass Hay	Barley Straw
DM	867.4	848.1	862.8
OM	950.2	936.6	937.8
ME[#]	13.3	9.2	6.5
FME[#]	12.7	8.6	5.9
NDF	221.4	583.7	756.1
ADF	55.5	310.8	467.7
EE	8.3	9.9	11.3
CP	129.2	131.8	26.5
ERDP[*]	99	63	16
DUP[*]	19	50	3

#: AFRC (1993) values

*: Values derived from in-sacco degradation and AFRC (1993) calculations

Experimental infection

Two weeks after the experiment started the lambs were infected with *Trypanosoma vivax* Leeflang (Leeflang *et al.*, 1976). The procedure was the same as with the *Trypanosoma congolense* infection and explained in the General Materials and Methods (Chapter 3). Each animal was inoculated intravenously with 5 x 10^5 trypanosomes in 3 to 4 ml phosphate buffered saline (PBS) (containing 1.5% glucose).

Measurements

Feed and water intake were measured daily by collecting refusals between 8.00 and 9.00 h. Clinical observations were made daily for any abnormal behaviour. The animals were weighed once a week on Wednesday.

Feed digestibilities and nitrogen balance of the infected and pair-fed control groups were measured during two balance periods of 1 week after the *T.vivax* infection. Balance Period I lasted from day 26 to 32 and Balance Period II from day 47 to 53 post-infection. The rate of passage of the roughage through the digestive tract was measured on day 21 and 42 after infection using chromium as a marker.

On Mondays, Wednesdays and Fridays 5 ml of blood was collected into tubes containing ethylene tetra acetic acid (EDTA) and lithium heparin for the measurement of blood haematological and biochemical parameters.

All the procedures and statistical analysis were explained in the general materials and methods of Chapter 3.

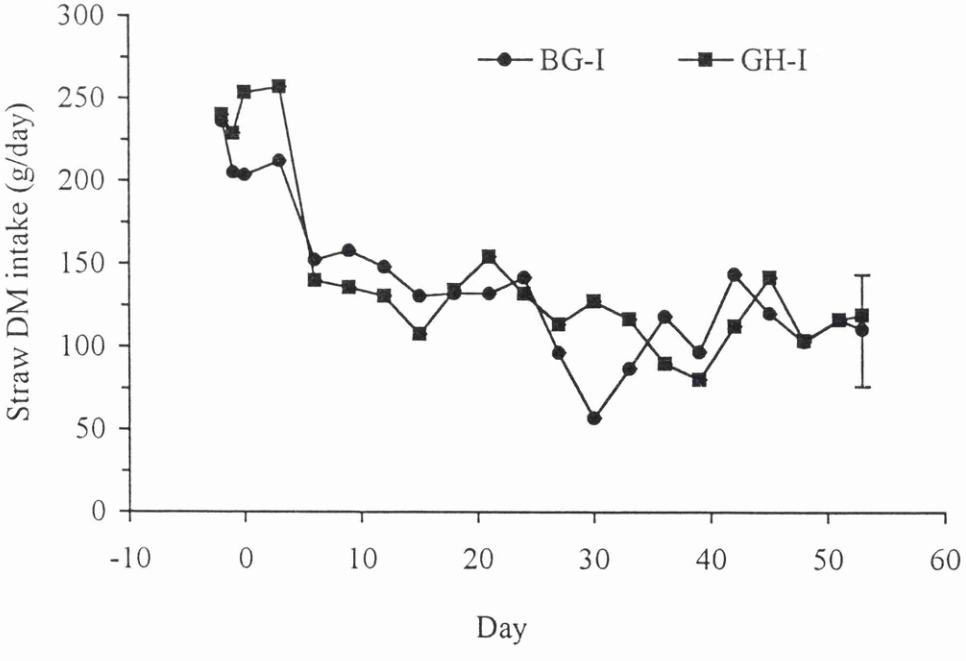
Results

One of the infected animals on Diet GH had to be withdrawn from the experiment on day 35 after infection due to recumbency as a result of the infection. Its pair-fed control counterpart was withdrawn at the same time. On day 44 one of the control lambs on Diet BG had to be withdrawn due to digestive problems. Its infected counterpart was also withdrawn.

Feed intake

The straw intake was very similar in both dietary groups (Figure 6.1). However, the organic matter intake and the intakes of neutral and acid detergent fibre were all slightly higher in the lambs on Diet GH than in the lambs on Diet BG (Table 6.3; $p<0.05$).

Figure 6.1 Mean straw dry matter (DM) intake (g/day) of *T.vivax* infected Scottish Blackface sheep fed Diet BG (BG-I) or Diet GH (GH-I)



The metabolisable protein (MP) intakes of the lambs on Diet GH were significantly higher than those on Diet BG ($p<0.01$). This was mainly due to the higher intake of digestible undegraded protein (DUP) in the lambs on Diet GH, whereas the effective rumen degradable protein (ERDP) intakes were very similar in both dietary groups. The *T.vivax* infection reduced the organic matter intake significantly and thus the intakes of dietary energy and protein (Table 6.3; $p<0.01$). The depression in feed intake was mostly due to a decrease in barley straw intake but, occasionally, the infected lambs stopped eating almost completely and left their hay and concentrate. The microbial crude protein (MCP) supply was limited by the intake of effective rumen degradable protein (ERDP) in both diets and not by the intake of fermentable metabolisable energy (FME) intake.

Table 6.3 Mean organic matter (OM), metabolisable energy (ME), fermentable metabolisable energy (FME), neutral detergent fibre (NDF), acid detergent fibre (ADF), crude protein (CP), effective rumen degradable dietary protein (ERDP), digestible undegraded protein (DUP) and metabolisable protein (MP) intake of *T.vivax* infected sheep (n=4) fed either Diet BG or Diet GH during the pre- (day -6 - 0) and post-(day 1 - 53) infection periods (M/D = 9.5, y = 9.0, L = 1.0)

Diet	OM ($g/kg^{0.75}$/day)	ME# (MJ/day)	FME# (MJ/day)	NDF (g/day)	ADF (g/day)	CP (g/day)	ERDP# (g/day)	DUP# (g/day)	MP# (g/day)
Pre-BG	49.5	7.0	6.6	318	164	66.9	44.8	14.1	42.6
Post-BG	42.3	6.3	5.9	250	122	62.8	42.0	13.5	40.3
Pooled SE	1.77	0.20	0.18	18.2	11.0	1.15	0.76	0.17	0.65
Pre-GH	55.9	7.3	6.9	396	211	75.3	45.5	19.8	48.8
Post-GH	48.3	6.6	6.3	322	166	71.7	43.5	19.1	46.8
Pooled SE	1.96	0.20	0.18	22.6	13.9	0.92	0.53	0.16	0.48
Diet Effect	*	ns	ns	*	*	**	ns	**	**
Period Effect	**	**	**	**	**	**	**	**	**
Interaction	ns	ns	ns	ns	ns	ns	ns	ns	ns

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means
: Values derived from in-sacco degradation and using AFRC (1993) methods

Water Intake

The water intakes of the lambs did not appear to be affected by either diet or infection (Table 6.4). The values fluctuated around a 100 ml per kg metabolic weight.

Table 6.4 Mean water intake (ml/kg$^{0.75}$ metabolic weight/day) of *T.vivax* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BG or Diet GH during the pre (day -6 - 0), post 1 (day 1 - 28) and post 2 (day 29 - 53) infection periods

	Water Intake (ml/kg$^{0.75}$ metabolic weight)		
	period		
Group	pre	post 1	post 2
BG-I	98	107	97
BG-PC	96	91	101
Pooled SE	11.7	9.2	11.1
GH-I	103	128	89
GH-PC	87	108	93
Pooled SE	6.2	8.6	9.7
Diet effect	ns	ns	ns
Infection effect	ns	ns	ns
Interaction	ns	ns	ns

ns : No significant difference between means

Body weight

Body weight gains were significantly affected by the *T.vivax* infection ($p<0.01$). While the pair-fed control lambs maintained a positive growth rate, the infected lambs in both dietary groups lost weight after infection (Table 6.5). The lambs on Diet BG lost more weight than the lambs on Diet GH but this was not statistically significant.

Table 6.5 Mean body weight gain (g/day) of *T.vivax* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BG or Diet GH during the post infection period (day 10-53)

Group	Growth (g)
BG-I	-12.9
BG-PC	11.6
Pooled SE	6.7
GH-I	-7.7
GH-PC	13.3
Pooled SE	9.8
Diet effect	ns
Infection effect	**
Interaction	ns

** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Digestive function

Diet digestibility

The apparent digestibility coefficients of neutral (NDF) and acid detergent fibre (ADF) were very similar in both dietary groups although the acid detergent fibre digestibility appeared to be slightly higher in the lambs on Diet GH than in the lambs on Diet BG during Balance Period I ($p<0.05$; Table 6.6). The difference may have been caused by the slightly higher fibre intakes in the lambs on Diet GH (Table 6.3). There appears to be a tendency for the digestibility coefficients to be lower in the *T.vivax* infected lambs compared with their pair-fed controls but none of the differences were statistically significant.

Table 6.6 Mean apparent digestibility coefficients of neutral (NDF) and acid detergent fibre (ADF) in *T.vivax* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BG or Diet GH during Balance Period I (day 26 - 32) and II (day 47 - 53)

	NDF digestibility coefficients		ADF digestibility coefficients	
	Balance Period		Balance Period	
Group	I	II	I	II
BG-I	0.59	0.56	0.47	0.48
BG-PC	0.59	0.58	0.48	0.53
Pooled SE	0.008	0.014	0.020	0.020
GH-I	0.58	0.56	0.50	0.49
GH-PC	0.62	0.59	0.56	0.54
Pooled SE	0.011	0.015	0.016	0.019
Diet effect	ns	ns	*	ns
Infection effect	ns	ns	ns	ns
Interaction	ns	ns	ns	ns

* : There is a significant difference between means ($p<0.05$)
ns : No significant difference between means

The apparent organic matter digestibility coefficients were significantly higher in the lambs on Diet BG than in the lambs on Diet GH ($p<0.05$). The apparent gross energy digestibility coefficients were also higher in the lambs on Diet BG but this was only significant during Balance Period II ($p<0.05$; Table 6.7). The organic matter and gross energy digestibility coefficients appear to be slightly lower in the *T.vivax* infected lambs, especially in the ones fed Diet GH, compared with their pair-fed controls. However, none of the differences were statistically significant.

Table 6.7 Mean apparent digestibility coefficients of organic matter (OM) and gross energy (GE) in *T.vivax* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BG or Diet GH during Balance Period I (day 26 - 32) and II (day 47 - 53)

	OM digestibility coefficients		GE digestibility coefficients	
	Balance Period		Balance Period	
Group	I	II	I	II
BG-I	0.72	0.73	0.68	0.70
BG-PC	0.72	0.74	0.68	0.71
Pooled SE	0.006	0.010	0.008	0.011
GH-I	0.68	0.67	0.64	0.63
GH-PC	0.70	0.69	0.67	0.66
Pooled SE	0.009	0.010	0.010	0.011
Diet effect	*	*	ns	*
Infection effect	ns	ns	ns	ns
Interaction	ns	ns	ns	ns

* : There is a significant difference between means ($p<0.05$)
ns : No significant difference between means

Mean retention time of the roughage through the digestive tract

The mean retention time of the roughage through the digestive tract on day 21 after infection was similar in the two dietary groups (Table 6.8). The mean retention time was significantly longer in the *T.vivax* infected lambs compared with their pair-fed controls ($p<0.05$), but the difference between the infected and the pair-fed controls was very similar in both dietary groups and approximately 9 hours. The transit time was also longer in the infected animals but differences were not statistically significant. The outflow rate constants k_1 and k_2 were not significantly affected by the infection.

Table 6.8 Mean retention time (MRT; h), transit time (TT; h) and rate constants (k_1, k_2; h^{-1}) of chromium mordanted roughage offered on day 21 post-infection to *T.vivax* infected (I) sheep fed either Diet BG or Diet GH and their respective pair-fed controls (PC)

Group	MRT	TT	k_1	k_2
BG-I	77.2	30.1	0.031	0.100
BG-PC	68.4	19.4	0.026	0.156
Pooled SE	4.8	3.2	0.003	0.021
GH-I	64.4	22.1	0.030	0.143
GH-PC	55.5	19.4	0.035	0.146
Pooled SE	3.7	2.3	0.002	0.014
Diet effect	ns	ns	ns	ns
Infection effect	*	ns	ns	ns
Interaction	ns	ns	ns	ns

* : There is a significant difference between means ($p<0.05$)
ns : No significant difference between means

The results on the mean retention time of the roughage through the digestive tract on day 42 after infection were very similar to those on day 21 after infection (Table 6.9). Again a significant infection effect was found on the mean retention time ($p<0.05$). The longer mean retention time found in the infected lambs appears to be mainly due to a slower outflow rate constant k_2. However differences in outflow rate constant k_2 between infected lambs and pair-fed controls were not significant.

Table 6.9 Mean retention time (MRT; h), transit time (TT; h) and rate constants (k_1, k_2; h^{-1}) of chromium mordanted roughage offered on day 42 post-infection to *T.vivax* infected (I) sheep fed either Diet BG or Diet GH and their respective pair-fed controls (PC)

Group	MRT	TT	k_1	k_2
BG-I	71.8	25.1	0.031	0.123
BG-PC	63.6	22.4	0.032	0.134
Pooled SE	2.5	4.7	0.005	0.017
GH-I	61.2	22.7	0.035	0.126
GH-PC	55.0	19.0	0.035	0.147
Pooled SE	3.0	1.6	0.003	0.010
Diet effect	ns	ns	ns	ns
Infection effect	*	ns	ns	ns
Interaction	ns	ns	ns	ns

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Nitrogen balance

The faecal nitrogen excretion during Balance Period I was slightly higher in the lambs fed Diet GH ($p<0.05$; Table 6.10). The infection significantly increased the faecal nitrogen excretion in both dietary groups to a similar extend ($p<0.05$). As a result, the nitrogen digestibility coefficient was slightly, though not significantly, lower in the infected animals. Urinary nitrogen excretion was also significantly higher in the infected groups ($p<0.05$) and this effect appears to be greater in the lambs fed Diet BG, although no interaction effect was observed. The nitrogen retention was significantly lower in the *T.vivax* infected lambs ($p<0.01$), the effect being greater in the dietary group BG ($p<0.01$).

The results of Balance Period II were very similar to those observed in Balance Period I (Table 6.11). Though not statistically significant, the faecal nitrogen excretion was slightly higher in the infected lambs leading to a significantly lower nitrogen digestibility coefficient in the infected lambs compared with their pair-fed controls ($p<0.05$). The urinary nitrogen excretion was significantly higher in the infected animals ($p<0.01$) and this effect was much greater in the lambs fed Diet BG ($p<0.01$). The resultant nitrogen retention was lower in the infected lambs ($p<0.01$). Whereas the nitrogen retention of the lambs on Diet GH was only slightly lower than that of their pair-fed controls, the nitrogen retention of the infected lambs on Diet BG was significantly lower than the nitrogen retention of their pair-fed controls ($p<0.05$). However, the infected lambs on Diet BG seem to have recovered slightly compared with the results of Balance Period I.

Table 6.10 Mean feed nitrogen intake (N_I; g/kg$^{0.75}$), faecal nitrogen (N_F; g/kg$^{0.75}$), urinary nitrogen (N_U; g/kg$^{0.75}$), digestible nitrogen intake (N_{DI}; g/kg$^{0.75}$), nitrogen digestibility (N_{DIG}; %), nitrogen retention/nitrogen intake ratio (N_R/ N_I; %) and nitrogen retention (N_R; g/kg$^{0.75}$) of *T.vivax* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BG or Diet GH during Balance Period I (day 26 - 32)

Group	N_I	N_F	N_U	N_{DI}	N_{DIG}	N_R/ N_I	N_R
BG-I	0.74	0.28	0.41	0.46	62.66	6.32	0.05
BG-PC	0.74	0.24	0.30	0.50	66.85	26.73	0.20
Pooled SE	0.016	0.012	0.029	0.019	1.65	4.01	0.030
GH-I	0.88	0.33	0.38	0.55	62.48	18.96	0.17
GH-PC	0.86	0.29	0.37	0.57	66.47	23.29	0.20
Pooled SE	0.027	0.012	0.013	0.023	1.24	1.86	0.018
Diet effect	*	*	ns	ns	ns	ns	*
Infection effect	ns	*	*	ns	ns	**	**
Interaction	ns	ns	ns	ns	ns	**	**

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Table 6.11 Mean feed nitrogen intake (N_I; g/kg$^{0.75}$), faecal nitrogen (N_F; g/kg$^{0.75}$), urinary nitrogen (N_U; g/kg$^{0.75}$), digestible nitrogen intake (N_{DI}; g/kg$^{0.75}$), nitrogen digestibility (N_{DIG}; %), nitrogen retention/nitrogen intake ratio (N_R/ N_I; %) and nitrogen retention (N_R; g/kg$^{0.75}$) of *T.vivax* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BG or Diet GH during Balance Period II (day 47 - 53)

Group	N_I	N_F	N_U	N_{DI}	N_{DIG}	N_R/ N_I	N_R
BG-I	0.78	0.30	0.37	0.48	61.73	13.77	0.11
BG-PC	0.75	0.25	0.27	0.51	67.40	31.32	0.24
Pooled SE	0.021	0.019	0.026	0.018	1.94	4.81	0.036
GH-I	0.91	0.35	0.40	0.57	62.10	18.04	0.17
GH-PC	0.88	0.32	0.38	0.56	63.75	20.71	0.18
Pooled SE	0.039	0.018	0.006	0.022	0.79	2.03	0.023
Diet effect	ns	ns	ns	ns	ns	ns	ns
Infection effect	ns	ns	**	ns	*	**	**
Interaction	ns	ns	**	ns	ns	**	*

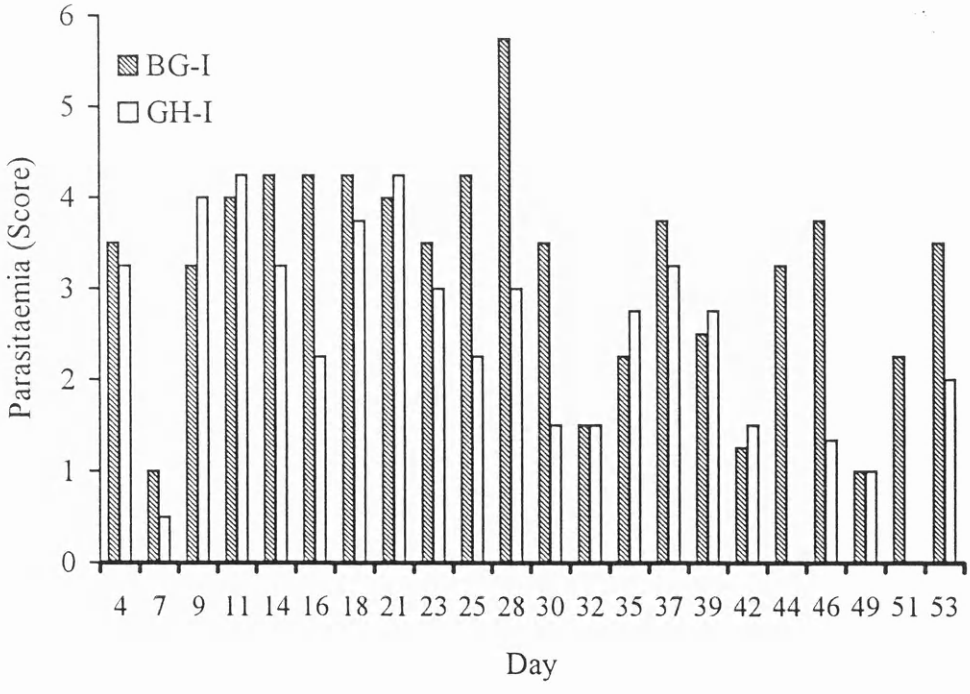
* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Haematology

Parasitaemia

Levels of parasitaemias were relatively high in both infected dietary groups compared with the *T.congolense* infection (Figure 6.2). The pre-patent period was extremely short. When the parasitaemia was checked on day 4 after infection the animals were already in the middle of the first peak parasitaemia. After the first trough on day 7 after infection the infected lambs on Diet GH showed a fluctuating parasitaemia with decreasing values towards the end of the experiment. The lambs on Diet BG reacted somewhat different in that their parasitaemia remained high after the trough on day 7 until around day 30 when it started to fluctuate. However, one of the animals on Diet BG had a parasitaemia score of 5+ for about 3 weeks. No significant differences in parasitaemia between the two dietary groups were observed at any day post-infection.

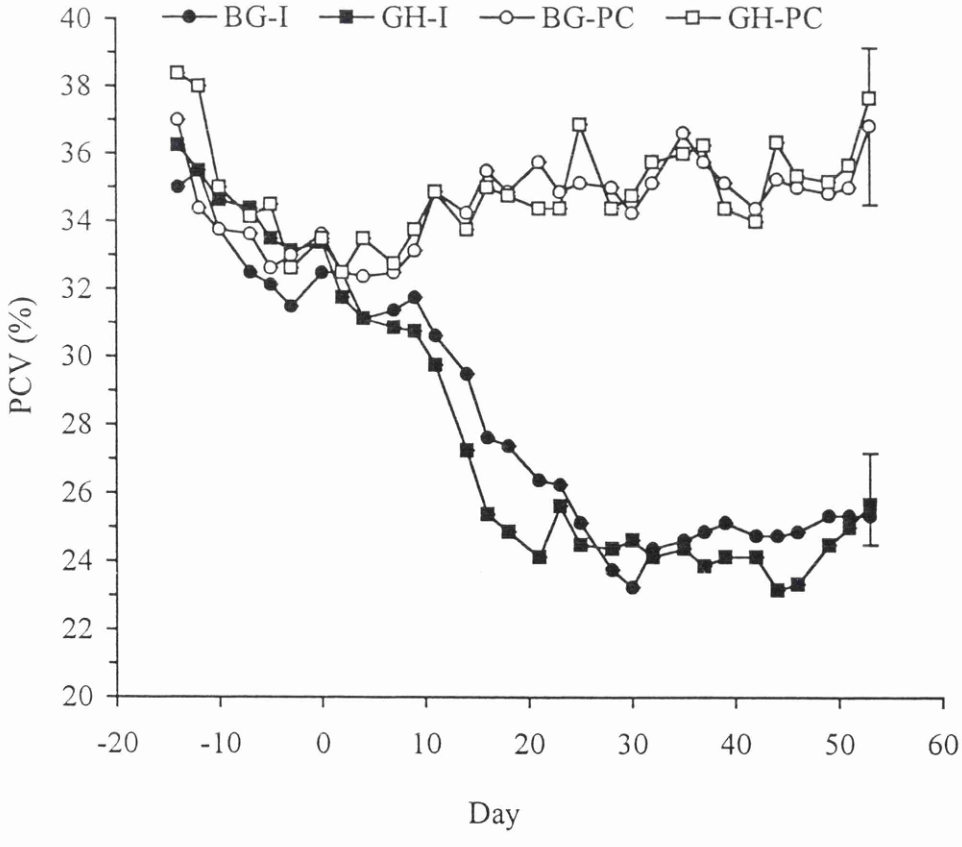
Figure 6.2 Mean parasitaemia (Score) of *T.vivax* infected Scottish Blackface sheep fed Diet BG (BG-I) or Diet GH (GH-I)



Packed cell volume

No dietary effects on packed cell volume were found before and after infection (Figure 6.3, Table 6.12). Packed cell volume decreased significantly ($p<0.01$) in both infected groups between day 10 and 25 after infection. The decrease in packed cell volume was very similar in both infected dietary groups and no significant interaction was found between diet and infection. After day 25 the packed cell volume values stabilised at around 10 percentage points below the packed cell volume levels of the pair-fed controls.

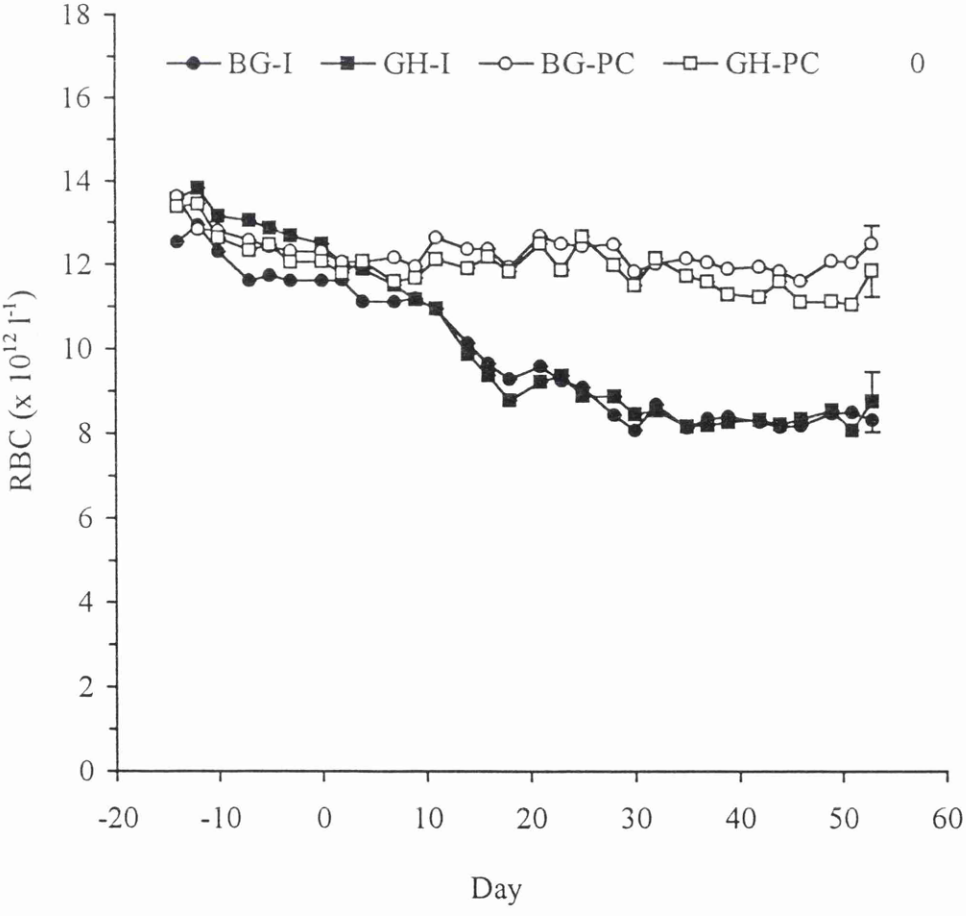
Figure 6.3 Mean packed cell volume (PCV; %) of *T.vivax* infected sheep fed Diet BG (BG-I) or Diet GH (GH-I) and their respective pair-fed controls BG-PC and GH-PC



Red blood cell count

The number of red blood cells fell significantly in both infected groups ($p<0.01$). The decrease followed the same pattern in both dietary groups (Figure 6.4; Table 6.12). The number of red blood cells also fell slightly in both pair-fed control groups.

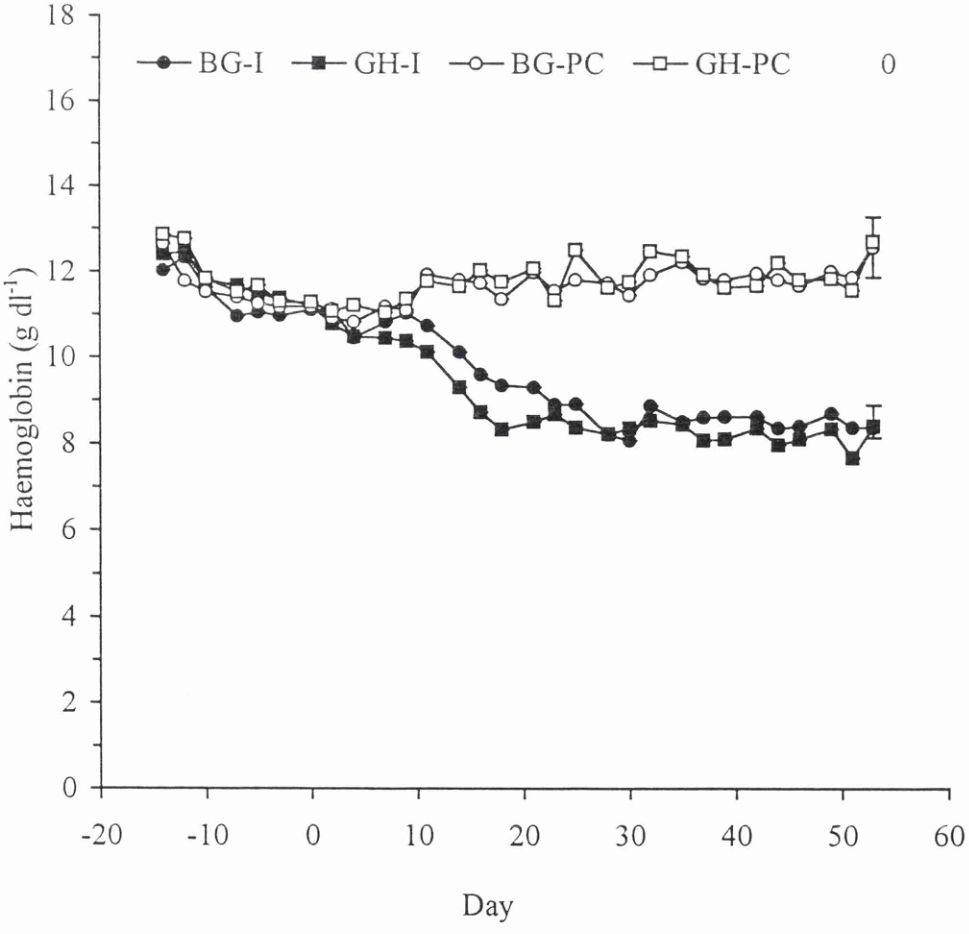
Figure 6.4 Mean red blood cells (RBC; x 10^{12} l^{-1}) of *T.vivax* infected sheep fed Diet BG (BG-I) or Diet GH (GH-I) and their respective pair-fed controls BG-PC and GH-PC



Haemoglobin concentration

As expected, the haemoglobin concentration also decreased significantly ($p<0.01$) in both dietary groups. However, in contrast to the number of red blood cells the haemoglobin concentration appeared to increase slightly in the pair-fed control lambs (Figure 6.5; Table 6.12).

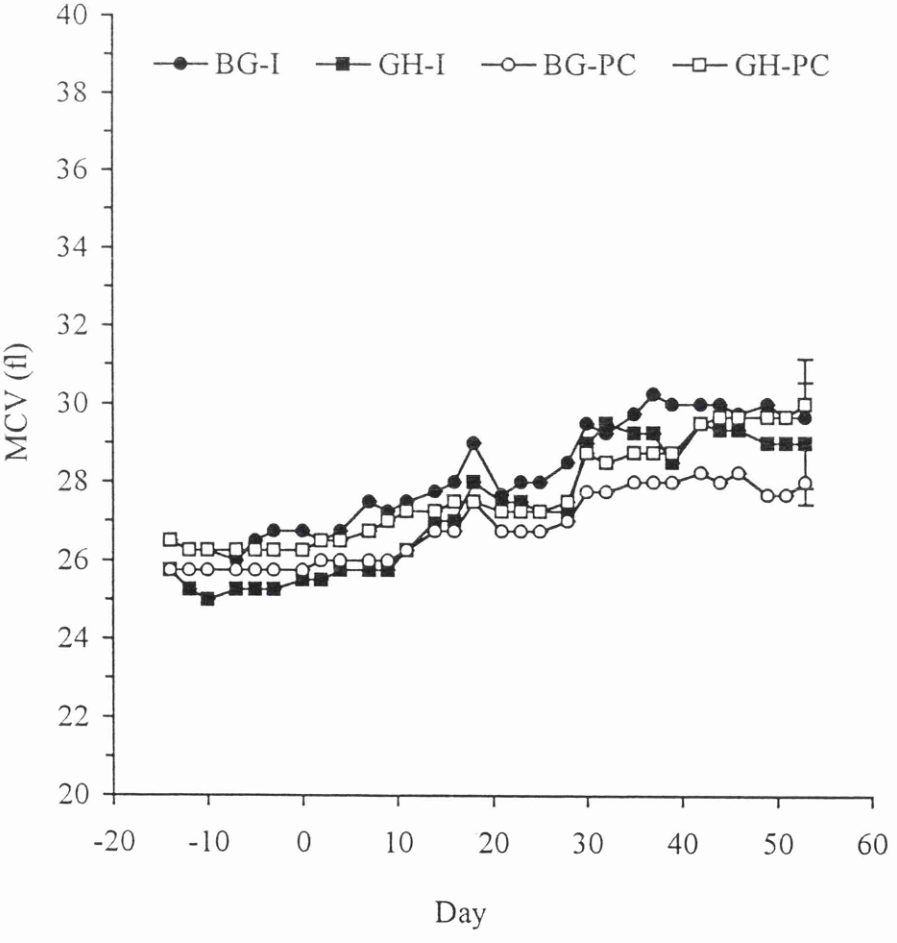
Figure 6.5 Mean haemoglobin (g dl^{-1}) of *T.vivax* infected sheep fed Diet BG (BG-I) or Diet GH (GH-I) and their respective pair-fed controls BG-PC and GH-PC



Mean corpuscular volume

Mean corpuscular volume levels increased in all 4 groups during the experiment and no significant differences were observed between the groups. The mean corpuscular volume was unaffected by the type of diet offered to the lambs (Figure 6.6; Table 6.13).

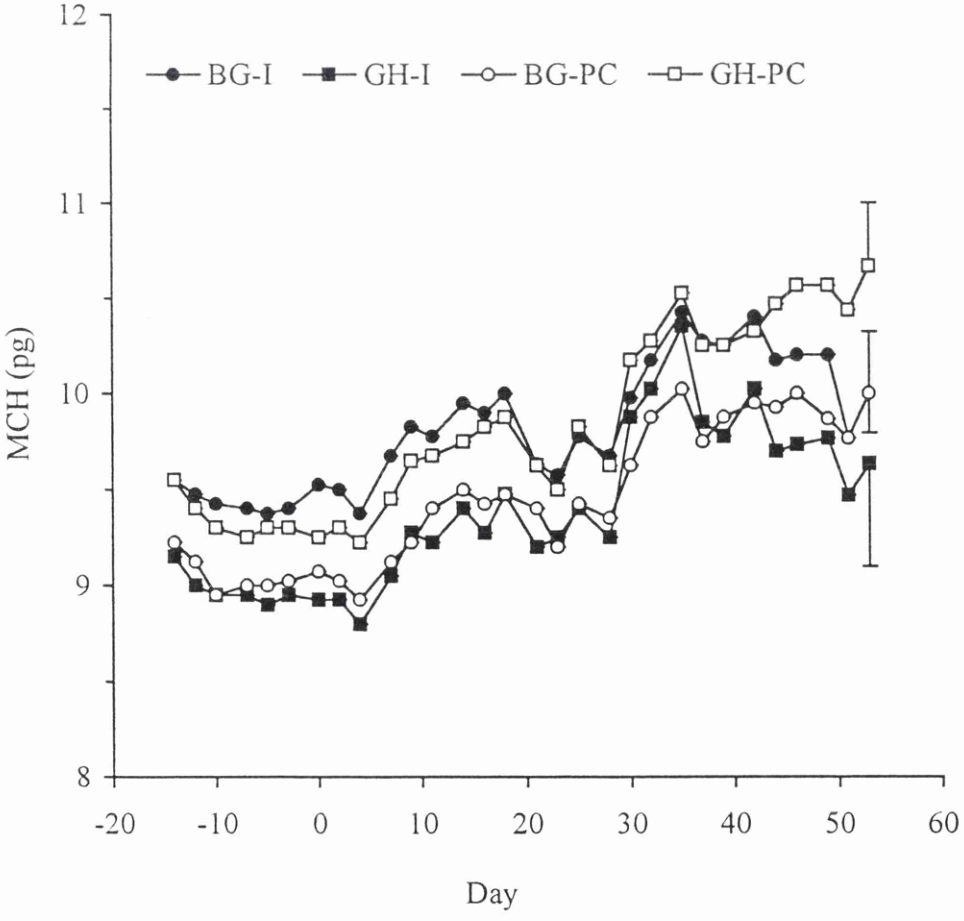
Figure 6.6 Mean corpuscular volume (MCV; fl) of *T.vivax* infected sheep fed Diet BG (BG-I) or Diet GH (GH-I) and their respective pair-fed controls BG-PC and GH-PC



Mean corpuscular haemoglobin

The mean corpuscular haemoglobin also increased in all four groups during the experiment. No significant differences were observed between the groups (Figure 6.7; Table 6.13). However, whereas the mean corpuscular haemoglobin values continued to rise in the pair-fed control lambs towards the end of the experiment the values of the infected animals decreased from day 35 after infection.

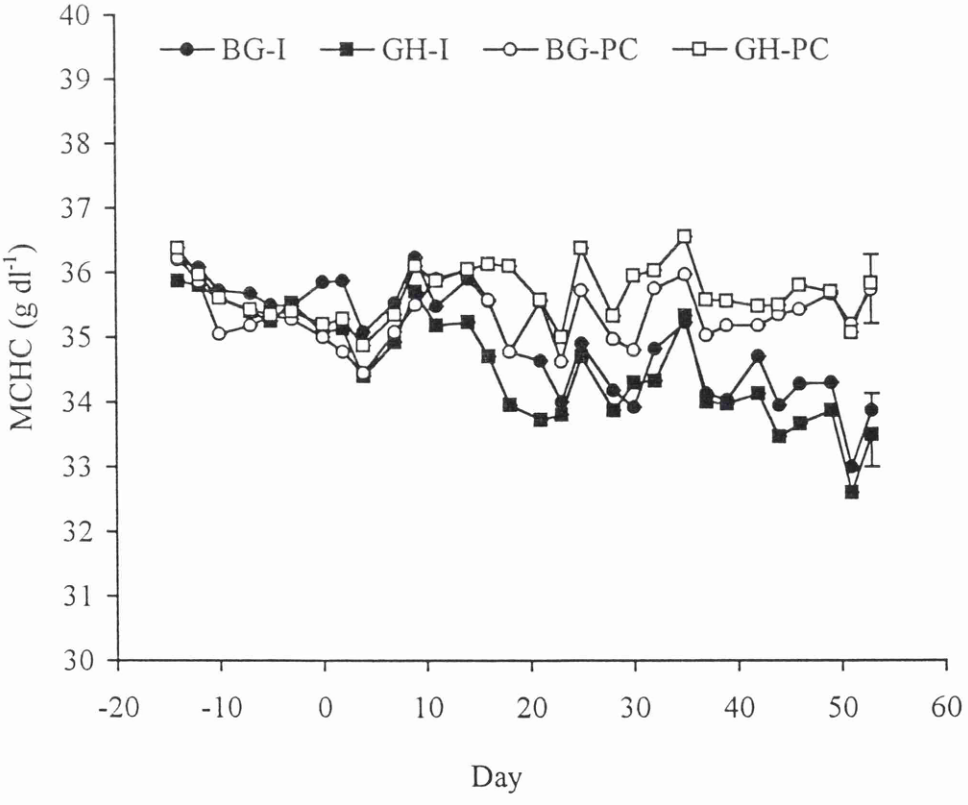
Figure 6.7 Mean corpuscular haemoglobin (MCH; pg) of *T.vivax* infected sheep fed Diet BG (BG-I) or Diet GH (GH-I) and their respective pair-fed controls BG-PC and GH-PC



Mean corpuscular haemoglobin concentration

Mean corpuscular haemoglobin concentration decreased in the infected groups from around day 14 but the decrease was relatively small and no significant infection effects were found during the early stages of the experiment (Figure 6.8; Table 6.13). The finding that the mean corpuscular haemoglobin decreased in the infected groups towards the end of the experiment together with the increase in mean corpuscular volume resulted in a significant decrease in mean corpuscular haemoglobin concentration ($p<0.05$) during the last part of the experiment. The mean corpuscular haemoglobin concentration of both pair-fed control groups remained relatively stable throughout the experiment.

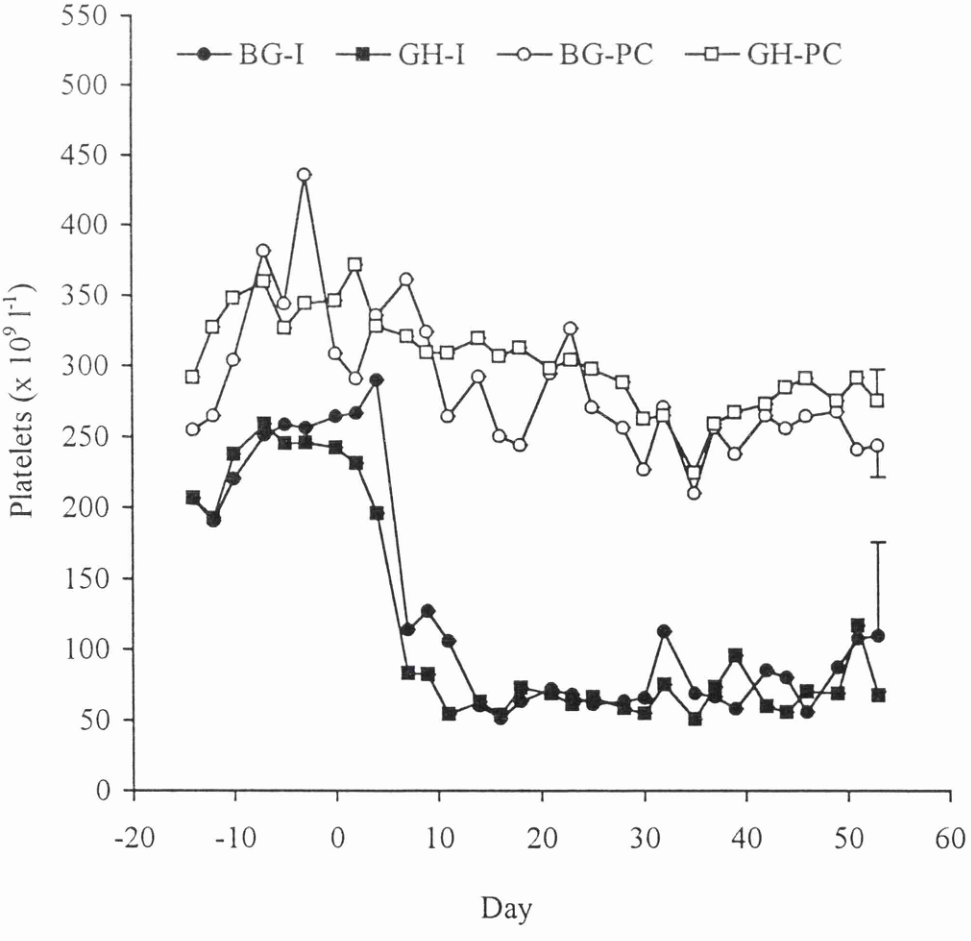
Figure 6.8 Mean corpuscular haemoglobin concentration (MCHC; g dl^{-1}) of *T.vivax* infected sheep fed Diet BG (BG-I) or Diet GH (GH-I) and their respective pair-fed controls BG-PC and GH-PC



Platelet counts

A marked decrease in platelet counts occurred in both infected groups following patency ($p<0.01$) although there was a slight difference before infection between the infected lambs and their pair-fed controls (Figure 6.9; Table 6.14). Platelet aggregation was frequently observed. The number of platelets in the pair-fed control lambs also fell during the experiment but not as much as in the infected groups.

Figure 6.9 Mean platelet count (PLT; x 10^9 l^{-1}) of *T.vivax* infected sheep fed Diet BG (BG-I) or Diet GH (GH-I) and their respective pair-fed controls BG-PC and GH-PC



White blood cells

The number of white blood cells appeared to be slightly higher in the lambs fed Diet GH but differences were not significant (Figure 6.10; Table 6.14). White blood cell counts decreased during the pre-infection period in all 4 groups. Infection had no significant effect on white blood cell count in the lambs on both diets although the values started to fluctuate after infection.

Figure 6.10 Mean white blood cell count (WBC; x 10^9 l^{-1}) of *T.vivax* infected sheep fed Diet BG (BG-I) or Diet GH (GH-I) and their respective pair-fed controls BG-PC and GH-PC

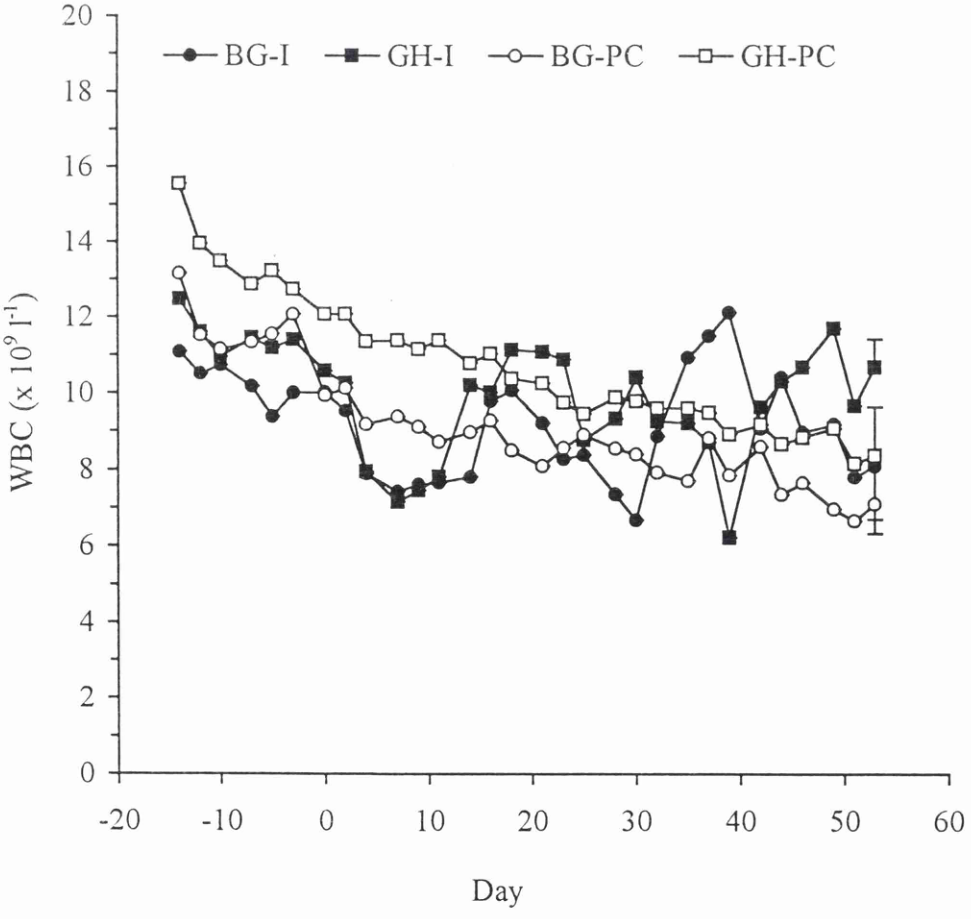


Table 6.12 Mean packed cell volume (%), red blood cell count (x 10^{12} l^{-1}) and haemoglobin concentration (g dl^{-1}) of *T.vivax* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BG or Diet GH during the pre (day -19 - 0), post 1 (day 14 - 28) and post 2 (day 30 - 53) infection periods

	Packed Cell Volume (%)			Red Blood Cells (x 10^{12} l^{-1})			Haemoglobin Concentration (g dl^{-1})		
	period			period			period		
Group	pre	post 1	post 2	pre	post 1	post 2	pre	post 1	post 2
BG-I	33.3	26.6	24.7	12.06	9.31	8.33	11.4	9.2	8.5
BG-PC	34.0	35.1	35.1	12.71	12.39	11.90	11.6	11.7	11.8
Pooled SE	0.92	1.98	2.23	0.41	0.74	0.73	0.33	0.64	0.71
GH-I	34.4	25.2	24.3	13.09	9.20	8.38	11.8	8.6	8.3
GH-PC	35.2	34.8	35.4	12.64	12.13	11.56	11.9	11.9	12.0
Pooled SE	1.21	1.99	2.20	0.40	0.60	0.64	0.39	0.64	0.73
Diet effect	ns	ns	ns	ns	ns	ns	ns	ns	ns
Infection effect	ns	**	**	ns	**	**	ns	**	**
Interaction	ns	ns	ns	ns	ns	ns	ns	ns	ns

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Table 6.13 Mean corpuscular volume (fl), mean corpuscular haemoglobin (pg) and mean corpuscular haemoglobin concentration (g dl^{-1}) of *T.vivax* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BG or Diet GH during the pre (day -19 - 0), post 1 (day 14 - 28) and post 2 (day 30 - 53) infection periods

	Mean Corpuscular Volume (fl)			Mean Corpuscular Haemoglobin (pg)			Mean Corpuscular Haemoglobin Concentration (g dl^{-1})		
	period			period			period		
Group	pre	post 1	post 2	pre	post 1	post 2	pre	post 1	post 2
BG-I	26.4	28.2	29.7	9.5	9.8	10.2	35.8	34.9	34.2
BG-PC	25.8	26.9	28.1	9.1	9.4	9.9	35.4	35.3	35.3
Pooled SE	0.44	0.50	0.53	0.12	0.12	0.13	0.33	0.42	0.35
GH-I	25.3	27.4	29.1	9.0	9.3	9.8	35.5	34.3	34.0
GH-PC	26.3	27.4	29.0	9.3	9.7	10.3	35.6	35.8	35.8
Pooled SE	0.55	0.50	0.61	0.21	0.20	0.24	0.28	0.36	0.42
Diet effect	ns	ns	ns	ns	ns	ns	ns	ns	ns
Infection effect	ns	ns	ns	ns	ns	ns	ns	*	*
Interaction	ns	ns	ns	ns	ns	ns	ns	ns	ns

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Table 6.14 Mean platelet (x 10^9 l^{-1}) and white blood cell count (x 10^9 l^{-1}) of *T.vivax* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BG or Diet GH during the pre (day -19 - 0), post 1 (day 14 - 28) and post 2 (day 30 - 53) infection periods

	Platelet Count (x 10^9 l^{-1})			White Blood Cells x 10^9 l^{-1})		
	period			period		
Group	pre	post 1	post 2	pre	post 1	post 2
BG-I	235	63	79	10.3	8.7	9.4
BG-PC	328	276	251	11.5	8.7	8.0
Pooled SE	21	42	34	0.57	0.48	0.84
GH-I	233	64	71	11.4	10.2	9.3
GH-PC	335	304	262	13.4	10.2	9.2
Pooled SE	30	49	37	0.79	0.52	0.57
Diet effect	ns	ns	ns	ns	ns	ns
Infection effect	*	**	**	ns	ns	ns
Interaction	ns	ns	ns	ns	ns	ns

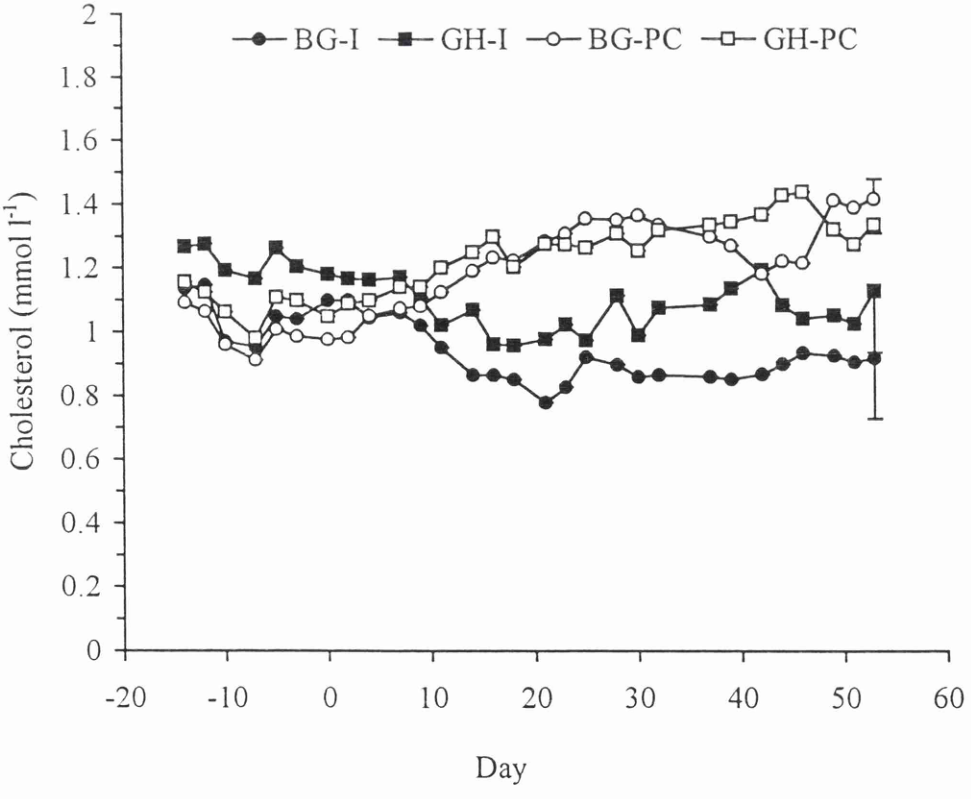
* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Blood biochemistry

Plasma cholesterol

No significant differences in plasma cholesterol levels were observed before infection due to the diets (Figure 6.11; Table 6.15). The plasma cholesterol levels of the pair-fed control animals increased during the experiment. Plasma cholesterol levels of the infected groups decreased approximately 7 days after infection and then stabilised but remained below pair-fed control levels ($p<0.01$). Standard error of mean increased in the infected groups indicating an increased variance between the lambs within the infected groups (Figure 6.11). No differences in plasma cholesterol levels were observed between the diets in response to the disease.

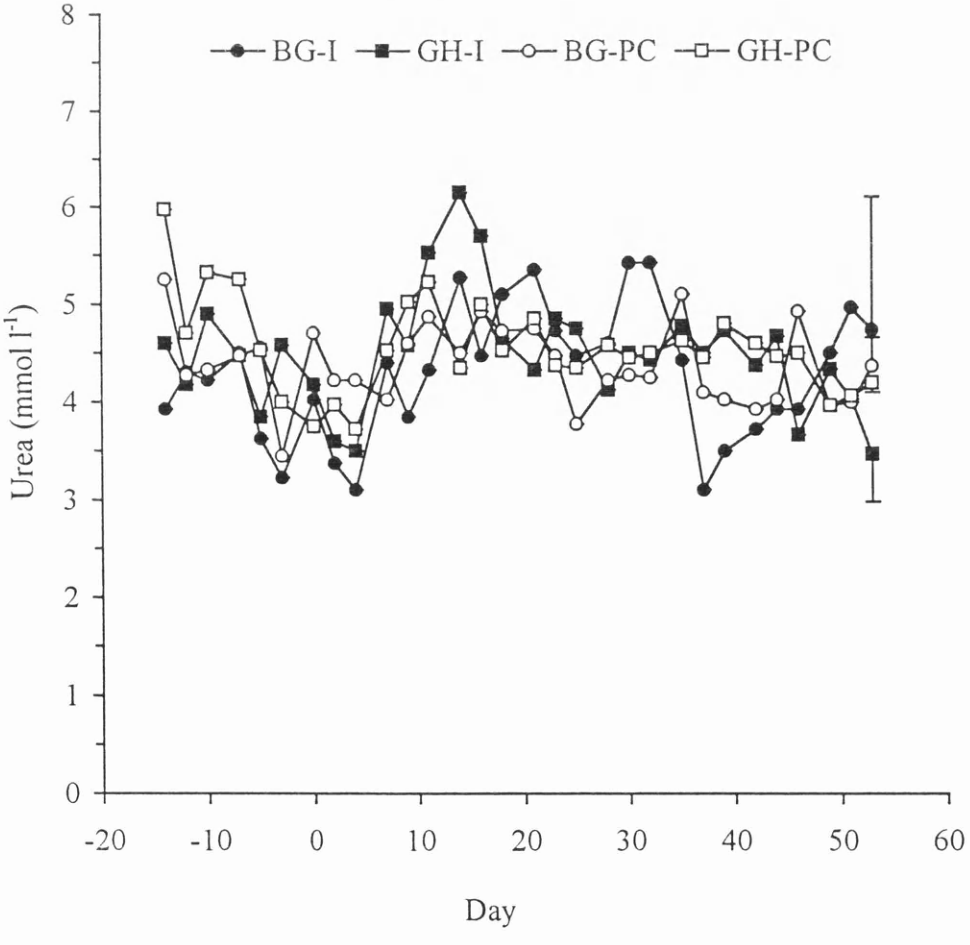
Figure 6.11 Mean plasma cholesterol (mmol l^{-1}) of *T.vivax* infected sheep fed Diet BG (BG-I) or Diet GH (GH-I) and their respective pair-fed controls BG-PC and GH-PC



Plasma urea

Plasma urea levels were not significantly affected by the type of diet. Infection did not affect the plasma urea levels although values fluctuated more and the standard errors were higher in the infected groups compared with their pair-fed controls (Figure 6.12; Table 6.15).

Figure 6.12 Mean plasma urea (mmol l^{-1}) of *T.vivax* infected sheep fed Diet BG (BG-I) or Diet GH (GH-I) and their respective pair-fed controls BG-PC and GH-PC



Plasma albumin

Plasma albumin levels were unaffected by the type of diet fed to the lambs. Plasma albumin levels of the infected sheep decreased significantly 10 days after infection ($p<0.01$) in both dietary groups and were still decreasing at the end of the experimental period (Figure 6.13; Table 6.15).

Figure 6.13 Mean plasma albumin (g l^{-1}) of *T.vivax* infected sheep fed Diet BG (BG-I) or Diet GH (GH-I) and their respective pair-fed controls BG-PC and GH-PC

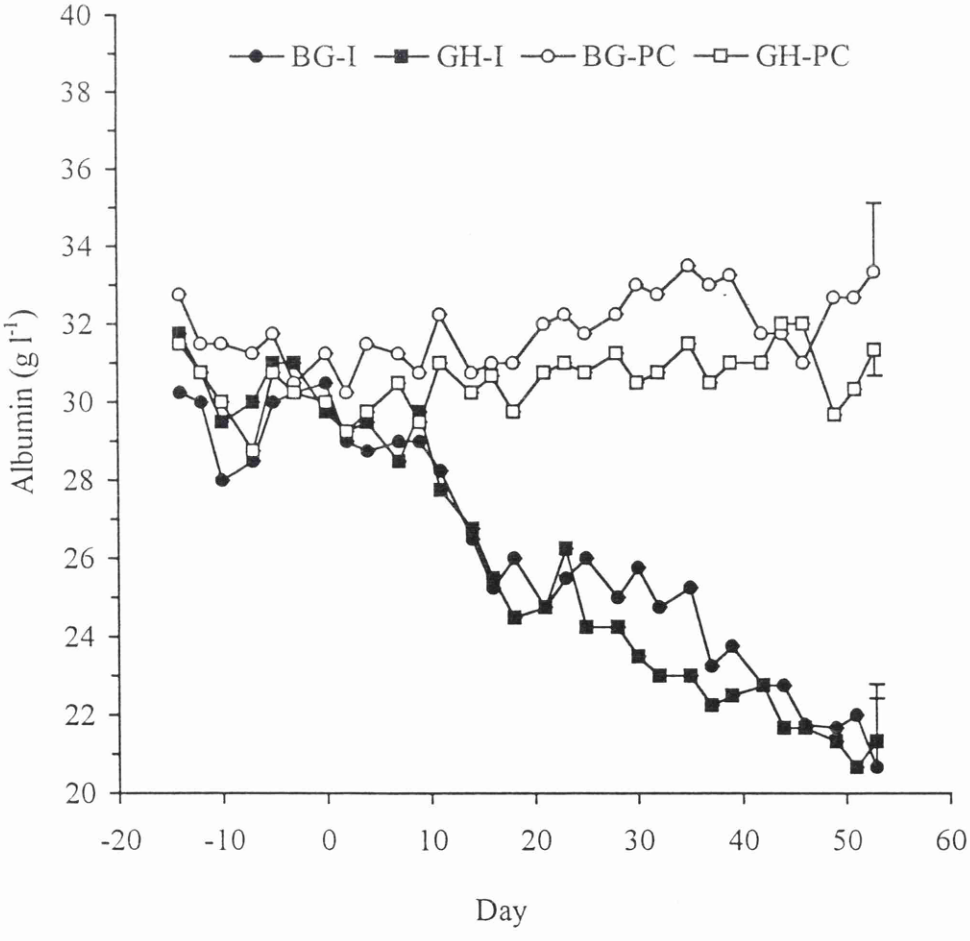


Table 6.15 Mean plasma cholesterol (mmol l^{-1}), urea (mmol l^{-1}) and albumin (g l^{-1}) concentration of *T.vivax* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either Diet BG or Diet GH during the pre (day -19 - 0), post 1 (day 14 - 28) and post 2 (day 30 - 53) infection periods

	Cholesterol (mmol l^{-1})			Urea (mmol l^{-1})			Albumin (g l^{-1})		
	period			period			period		
Group	pre	post 1	post 2	pre	post 1	post 2	pre	post 1	post 2
BG-I	1.06	0.86	0.88	4.0	4.9	4.2	29.6	25.6	23.3
BG-PC	1.00	1.28	1.27	4.4	4.5	4.2	31.5	31.6	32.4
Pooled SE	0.08	0.11	0.10	0.2	0.3	0.4	0.9	1.4	1.9
GH-I	1.22	1.01	1.09	4.4	4.9	4.4	30.5	25.2	22.3
GH-PC	1.08	1.27	1.34	4.8	4.6	4.5	30.3	30.7	31.0
Pooled SE	0.07	0.08	0.06	0.2	0.2	0.2	0.3	1.2	1.7
Diet effect	ns	ns	ns	ns	ns	ns	ns	ns	ns
Infection effect	ns	**	**	ns	ns	ns	ns	**	**
Interaction	ns	ns	ns	ns	ns	ns	ns	ns	ns

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Discussion

The pathophysiological effects of the *T.vivax* infection on the Scottish Blackface lambs in this experiment were relatively high compared with the *T.congolense* infection. The intake of dietary metabolisable protein (MP) was significantly higher in the dietary group GH than in the lambs on Diet BG due to a higher intake of digestible undegraded protein (DUP). The *T.vivax* infection significantly decreased the organic matter intake. The infected lambs on both diets lost weight after infection whereas their uninfected pair-fed controls gained weight. The digestibility coefficients were slightly lower in the infected animals on both diets but the differences were not statistically significant. The mean retention time of the roughage through the digestive tract was significantly longer in the infected lambs on both diets. The faecal nitrogen excretion was slightly higher in the infected lambs resulting in a lower nitrogen digestibility coefficient compared with their pair-fed controls. The urinary nitrogen excretion was significantly higher in the infected animals and this effect was greater in the lambs fed Diet BG. The resulting nitrogen retention was significantly lower in the infected lambs, the effect being greater in the ones fed Diet BG.

In contrast to the results from the previous experiment the voluntary straw intake was very similar in both dietary groups. Whereas the sheep on Diet BG in the previous experiment (Chapter 5) compensated for the lower intake of hay fibre by increasing the voluntary intake of straw, the lambs on Diet BG in this experiment did not increase the voluntary intake of straw. As a result, the intake of dietary fibre was significantly lower in the lambs on Diet BG in this experiment. The difference in age between the animals in the two experiments may have played a role in this difference.

The lambs in this experiment were 6 months of age, whereas the animals in the previous experiment were 1 year old (Chapter 5). Different mechanisms could have limited the feed intake in the two experiments.

As in the previous experiment (Chapter 5), the intake of digestible undegraded protein (DUP) was significantly higher in the lambs fed Diet GH compared to the intake of the lambs on Diet BG. Similarly, the intake of effective rumen degradable protein was limiting the microbial crude protein production.

Organic matter intake was significantly decreased after the infection with *T.vivax* and this was mainly due to a lower intake of barley straw. However, some infected animals left their hay and concentrate on several days. The *T.congolense* infected sheep in the experiment reported in Chapter 5 also reduced their intake of barley straw, but continued to consume the hay and concentrate offered. The impact of the *T.vivax* infection on feed intake appears to have been greater than the impact of the milder *T.congolense* infection on feed intake.

The results of the water intake of the lambs supplied no evidence of an increase in water retention. A lower dry matter content of the whole body was found in the *T.congolense* infected lambs of chapter 4. Increased water intake and water retention have been reported in calves (Parkins *et al.*, 1982a; 1982b) and sheep (Abbott *et al.*, 1986b) infected with helminths.

The data on body weight gain shows that the animals were fed close to maintenance level. According to the AFRC (1993) tables the fermentable metabolisable energy (FME) intakes of the lambs supported a higher growth rate but the metabolisable protein intake levels were only sufficient for maintenance level. Whereas the infected lambs were losing weight, their pair-fed controls were gaining

weight. However in absolute terms differences were small between the infected and their pair-fed controls and no interaction effect between diet and infection was observed. There was an indication that the lambs on Diet GH with the higher digestible undegraded protein (DUP) lost less weight than the lambs on Diet BG but differences were not statistically significant.

The fibre digestibility results were very similar to those found in the previous experiment (Chapter 5). The fibre digestibility coefficients were slightly higher in the lambs fed Diet GH. As found in the *T.congolense* infected sheep, the fibre digestibility coefficients were slightly, though not significantly, lower in the infected lambs compared with their pair-fed controls. The organic matter and gross energy digestibility coefficients were slightly higher in this experiment compared with the previous experiment possibly due to the lower barley straw intake of the younger lambs in this experiment. The organic matter and gross energy digestibility coefficient were slightly higher in the lambs on Diet BG compared with those on Diet GH ($p<0.05$) since there was no compensatory intake of barley straw in the lambs on Diet BG. As found in previous experiments (Chapter 4 and 6) the organic matter digestibility coefficients were marginally lower in the infected lambs compared with their pair-fed controls.

The effects of the *T.vivax* infection on the mean retention time of the roughage through the digestive tract were relatively small compared with the results found during the relatively mild *T.congolense* infection. The mean retention times were slightly longer in the infected lambs ($p<0.05$) but the interaction effect between diet and infection found in the *T.congolense* experiment was not observed in the *T.vivax* infected lambs. This difference may be due to the finding that the voluntary

barley straw intake was similar between the two dietary groups in the present experiment but higher in the dietary group BG than in group GH of the previous experiment. The results confirm the findings of Chapter 4 that the mean retention time of the roughage through the digestive tract is significantly longer in trypanosome infected sheep.

The urinary nitrogen excretion was significantly higher in the infected lambs, especially in the second Balance Period, and this effect was greater in the lambs on Diet BG. The faecal nitrogen excretion was also slightly higher in the infected animals on both diets and led to a slightly lower nitrogen digestibility coefficient in the infected lambs. Due to the higher urinary nitrogen excretion the nitrogen retention of the infected lambs on Diet BG was significantly lower than the nitrogen retention of their pair-fed controls and was close to zero in the first Balance Period but increased slightly in the second Balance Period. These results imply that a higher digestible undegraded protein (DUP) intake is very useful to the infected lamb, as it reduces urinary nitrogen losses. However, since no such effect was observed in the *T.congolense* infected animals the usefulness of the digestible undegraded protein (DUP) appears to be related to the pathogenicity of the disease and/or level of nutrition. Van dam (1996) found a different relationship between nitrogen retention and nitrogen intake below and above maintenance level in *T.vivax* infected West African Dwarf goats and their controls. It was not clear whether this was due to a difference in efficiency below and above maintenance or due to the *T.vivax* infection. Alterations in protein dynamics have been observed between fasted and *ad libitum* fed healthy ruminants (Lobley, 1992).

The beneficial effect of protein supplementation found during previous trypanosome infections of sheep (Katunguka-Rwakishaya, 1992) may have been caused by a higher intake of digestible undegraded protein (DUP) leading to an improved nitrogen retention during infection.

The nitrogen retention per kg metabolic weight was remarkably similar to the nitrogen retention found during the *T.congolense* infection. Although the nitrogen intake was slightly higher in the *T.congolense* experiment the nitrogen digestibility coefficient was slightly lower than in the *T.vivax* experiment. However, in contrast to the sheep in the *T.congolense* experiment, the lambs in this *T.vivax* experiment were growing slowly or losing weight. The amount of nitrogen retained calculated from the nitrogen balance did not equate with the actual body weight loss or gain of the lambs. There appears to have been an overestimation of the amount of nitrogen retained.

The lambs were parasitaemic less than three days after infection in both dietary groups. As in the *T.congolense* infection the animals on Diet BG appeared to have a slightly higher parasitaemia score but again this was not statistically significant. The higher digestible undegraded protein (DUP) in the lambs on Diet GH does not appear to have enhanced the immune response in these animals. Van Houtert *et al.* (1995a) found a more pronounced eosinophilia in *T.colubriformis* infected sheep given fishmeal than in those not given fishmeal. The authors reported a high correlation during the latter stages of the experiment between eosinophilia and rates of expulsion of nematodes from the gastrointestinal tract and between eosinophilia and geometric mean egg counts.

A large decrease in packed cell volume, red blood cell counts and haemoglobin concentration was found in the infected animals. The mean corpuscular

volume increased slightly in all 4 groups but no significant infection effect was found indicating the animals were not able to respond well to the anaemia. This was supported by the finding that the mean corpuscular haemoglobin initially increased and then decreased towards the end of the experiment in the infected lambs. This was probably due to the low protein intake of the animals. Katunguka-Rwakishaya (1993) showed that the increase in mean corpuscular volume was greater in sheep on a higher level of protein in the diet.

Platelet counts decreased rapidly after infection and stabilised at very low levels. In contrast to the *T.congolense* infection the platelet counts of the animals infected with *T.vivax* were not able to recover during the experimental period.

Plasma cholesterol levels decreased significantly after the *T.vivax* but the decrease was not greater than in the *T.congolense* infection. Trypanosomes can take up cholesterol from the bloodstream for their growth and multiplication. Since the level of parasitaemia was much higher during the *T.vivax* infection than during the *T.congolense* it is unlikely that trypanosomes are able to affect the plasma cholesterol concentrations through the uptake of cholesterol. This puts into doubt the previous finding of a relationship between pre-infection cholesterol levels and trypanosome counts during the first month after infection (Chapter 4), although uptake by different species of trypanosomes may vary.

Plasma urea concentrations were unaffected by both diet and infection. These findings are similar to the findings of the previous experiment. The plasma urea concentrations are usually related to the dietary nitrogen intake (Parkins, personal communication).

Plasma albumin decreased dramatically after infection and in contrast to previous infections with *T.congolense* the plasma albumin levels did not stabilise during the latter part of the *T.vivax* infection and continued to fall. As discussed in Chapter 5, the reason for this decrease in plasma albumin is not clear.

Conclusions

The pathophysiological effects of the *T.vivax* infection in the Scottish Blackface lambs were relatively high compared with previous infections using *T.congolense*. Packed cell volume, platelet counts, plasma cholesterol and albumin levels all decreased dramatically. However, changes in organic matter and fibre digestibility coefficients and the mean retention time of the roughage through the digestive tract were not greater than during the *T.congolense* infection. The greatest effects were found in the nitrogen balance. The *T.vivax* infected lambs with a lower intake of digestible undegraded protein (DUP) had a higher excretion of faecal nitrogen resulting in a lower nitrogen retention than the *T.vivax* infected lambs with a higher digestible undegraded protein (DUP) intake. It was not clear where the extra retained protein was used by the lambs. A better immune response was not obvious from parasite numbers and although the infected lambs with the higher nitrogen retention lost slightly less weight, differences were not significant. The overall intake of protein was low in both dietary groups and, as a result, the infected animals were apparently not able to respond to the anaemia as judged by the mean corpuscular volume values.

CHAPTER 7

The Effect of Urea Supplementation on the Pathophysiology of trypanosomiasis in Scottish Blackface Sheep infected with *Trypanosoma congolense*

Introduction

Abbott *et al.* (1985a; 1985b; 1986) concluded from a series of experiments that lambs on a low protein diet were less able to withstand the pathogenic and pathophysiological effects of *Haemonchus contortus* infection than lambs that received a high protein diet, despite having similar worm burdens. Katunguka-Rwakishaya *et al.* (1993) investigated whether similar results could be obtained during trypanosome infection and found that high protein diets can ameliorate the effects of *T.congolense* infections. Positive results of dietary protein on the immunity and resistance development in lambs to vaccination with *Trichostrongylus colubiformis* have also been reported (Kambara *et al.*, 1993).

Recent experiments have been undertaken to see whether urea supplementation can produce the same beneficial effects as occur with dietary protein. Wallace *et al.* (1994) reported that the pathophysiology of *H.contortus* infection in a genetically susceptible breed (Hampshire Down sheep) can be reduced by the addition of urea (≡ 60 g CP/kg DM) to the basal ration containing 88 g crude protein per kg dry matter but only if the feed intake is sufficient for both growth and maintenance.

Macrophages have been found to produce nitric oxide during trypanosome infections. Nitric oxide has been related to immunosuppression through the suppression of parasite-antigen-specific T-cell proliferative responses (Schleifer and Mansfield, 1993) and to cytostatic effects on trypanosomes *in vitro* (Vincendeau *et al.*, 1991, 1992). No information is available on whether nitric oxide levels in sheep are raised during trypanosome infections and whether these nitric oxide levels are related to the intake of nitrogen.

This experiment was conducted to investigate the effects urea supplementation on the pathophysiology of *Trypanosoma congolense* in Scottish Blackface lambs. Twin pairs of Scottish Blackface wether lambs were used. One lamb of each pair was infected with *Trypanosoma congolense* and the other used as a pair-fed control. Feed intake and body weight gains were measured as well as several blood haematological and biochemical parameters. Extra blood samples were taken to investigate whether nitrate levels, one of the stable end-products of nitric oxide, were raised during the *T.congolense* infection and whether these nitrate levels were related to the levels of parasitaemia and nitrogen intake.

Materials and methods

Experimental design

Twelve pairs of male, twin Scottish Blackface lambs were selected and divided into two groups of six pairs. All animals received a basal diet of 428 grams grass hay dry matter (DM) per day and 395 grams dry matter of pelleted barley grain. One group of six pairs received an extra amount of 12.9 grams per day of urea added to the pelleted barley grain (Table 7.1). The composition of the diet components is shown in Table 7.2. Metabolisable protein levels supplied by the basal and urea-supplemented diet were estimated at 56 and 72 grams per day respectively. Metabolisable energy levels supplied by both diets were similar at around 9 MJ per day. Two weeks after the start of the experiment one animal of each twin pair was infected (I) with *T.congolense* and the other animal used as a pair-fed control (PC).

Table 7.1 Composition (g DM/day) of the two experimental diets offered to both dietary groups

	Basal Diet (BD)	Urea-supplemented Diet (UD)
Grass Hay	428	428
Barley Concentrate	395	395
Urea	0	12.9

Table 7.2 Dry matter (DM; g/kg), organic matter (OM; g/kg DM), metabolisable energy (ME; MJ/kg DM), fermentable metabolisable energy (FME; MJ/kg DM), neutral detergent fibre (NDF; g/kg DM), acid detergent fibre (ADF; g/kg DM), ether extract (EE; g/kg DM), nitrogen (N; g/kg DM), effective rumen degradable dietary protein (ERDP; g/kg DM) and digestible undegraded protein (DUP; g/kg DM) of the diet components

Diet Composition	BD Concentrate	UD Concentrate	Grass Hay
DM	849.6	832.5	856.0
OM	959.6	955.1	939.0
ME#	13.3	12.9	9.2
FME#	12.7	12.3	8.6
NDF	309.8	319.3	654.4
ADF	55.6	61.6	320.9
EE	7.4	8.7	5.2
N	18.4	32.7	15.4
ERDP*	88	148	46
DUP*	16	16	35

#: AFRC (1993) values
*: Values derived from AFRC (1993) calculations

Infection

Two weeks after the experiment started the lambs were infected with *T.congolense* 1180 (GRVPS 57/6) (Nantulya *et al.*, 1984) following the procedure explained in the general materials and methods. Each lamb was inoculated intravenously with 5 x 10^5 trypanosomes in 3 to 4 ml phosphate buffered saline (PBS) (containing 1.5% glucose).

Measurements

Feed intake was measured daily and feed samples analysed using the methods described in the General Materials and Methods (Chapter 3). AFRC (1993) tables were used to calculate metabolisable energy and protein levels in the diets. Clinical observations were made daily for any abnormal behaviour. Body weight was measured weekly.

On Mondays and Thursdays 5 ml of blood was collected into tubes containing ethylene tetra acetic acid (EDTA) for the determination of a range of haematological indices as described in the General Materials and Methods (Chapter 3).

Blood in which trypanosomes could be detected in the buffy coat was used to make Giemsa-stained thick blood smears. The ratio of white blood cells to trypanosomes was determined using a phase contrast microscope. The number of parasites per ml of blood could then be estimated by using the equation:

$$\text{WBC/ml} \times \text{Trypanosomes/WBC} = \text{Trypanosomes/ml}$$

Parasitaemias were also measured using the buffy coat method (Murray *et al.*, 1977; Paris *et al.*, 1982) and the results used to determine the relationship between parasitaemia and plasma nitrate levels.

On Mondays and Thursdays 5 ml of blood was also collected into tubes containing lithium heparin for plasma cholesterol pre- and post-infection, and urea and albumin post-infection.

Extra blood was taken for plasma nitrate level determination. Nitrate was stoichiometrically reduced to nitrite by the method described by Mabbutt *et al.* (1994). The nitrite concentration in the plasma sample was then assayed by a standard Griess reaction (Ding *et al.*, 1988).

Statistical analysis

The parameters were subjected to statistical analysis as described in the general materials and methods. Differences in the number of parasites calculated from the thick blood smear were checked for statistical significance using the Students' t-Test. The relationship between parasitaemia levels and plasma nitrate levels was tested using the Kruskal Wallis test.

Results

Feed intake

Feed intake was unaffected by the *T.congolense* infection. None of the animals refused any of the feed offered to them at any stage of the experiment. Table 7.3 shows that the lambs on the urea-supplemented diet received approximately 16 grams per day more metabolisable protein.

When deriving the microbial crude protein (MCP) supply from both the fermentable metabolisable energy (FME) supply and the effective rumen degradable protein (ERDP) it appeared that the effective rumen degradable protein was limiting microbial crude protein (MCP) in the basal diet. The amount of microbial crude protein (MCP) which could be supplied by fermentable metabolisable energy (FME) was approximately 80 grams per day on both diets and the microbial crude protein (MCP) supplied by effective rumen degradable protein (ERDP) was 55 grams per day for the basal diet and 80 grams per day for the urea-supplemented diet. The added urea appears to have balanced the diet by increasing the effective rumen degradable protein (ERDP) and thus the microbial crude protein (MCP) supply of the diet.

Table 7.3 Mean dry matter (DM), metabolisable energy (ME), fermentable metabolisable energy (FME), nitrogen (N), effective rumen degradable dietary protein (ERDP), digestible undegraded protein (DUP) and metabolisable protein (MP) intake of Scottish Blackface lambs on a basal (BD) or urea-supplemented (UD) diet

Diet	DM (kg/day)	ME# (MJ/day)	FME# (MJ/day)	N (g/day)	ERDP# (g/day)	DUP# (g/day)	MP# (g/day)
BD	0.823	9.2	8.7	13.8	54.5	21.4	56
UD	0.828	9.1	8.6	19.7	78.9	21.4	72

#: Calculated using the AFRC (1993) methods (M/D = 11.0, y = 9.5, L = 1.5)

Body weight gain

The average body weight of the pair-fed control lambs on the urea-supplemented diet was higher from the start of the experiment than that of the other three groups (Figure 7.1). The body weight gains of the pair-fed control lambs on both diets were close to 100 grams per day. A significant infection effect were found with the infected lambs having the lower body weight gains. The body weight gains of the infected lambs on the urea-supplemented diet appeared more affected by the infection than the lambs on the basal diets. However, no interaction effect was found between diet and infection (Table 7.4).

Figure 7.1 Mean body weight (kg) of *T.congolense* infected sheep fed a basal (BD-I) or a urea-supplemented (UD-I) diet and their respective pair-fed controls BD-PC and UD-PC

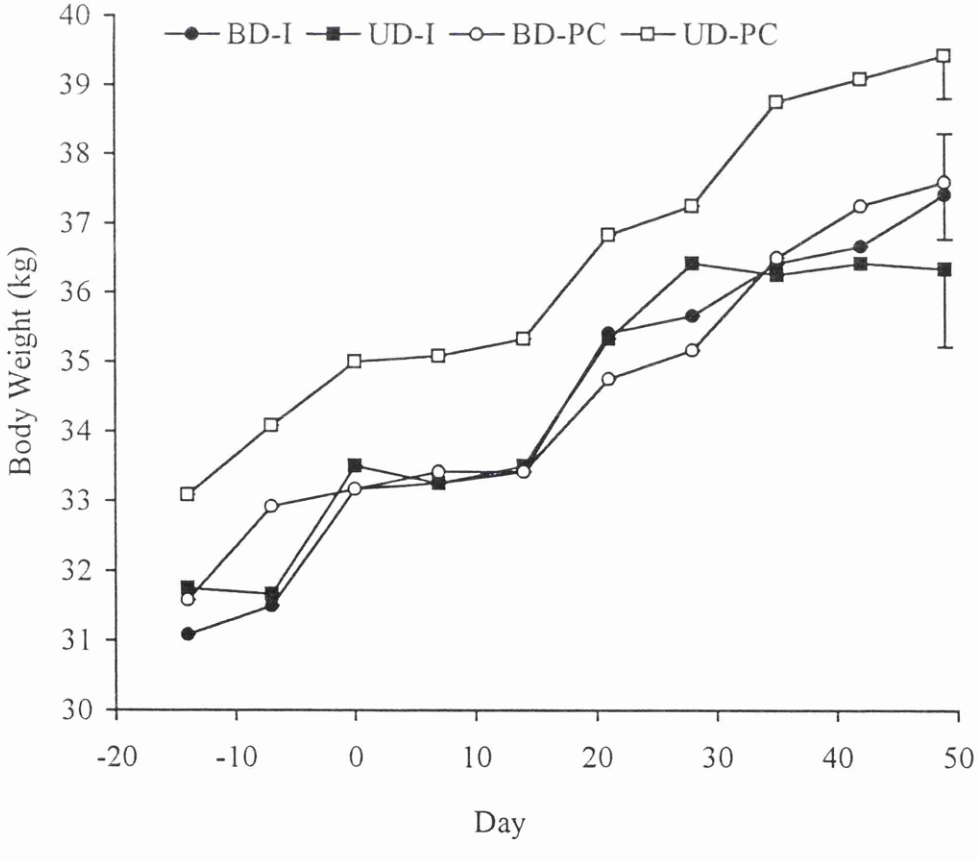


Table 7.4 Mean body weight gain (g/day) of *T.congolense* infected (I) sheep and their respective pair-fed controls (PC) fed a basal (BD) or a urea-supplemented (UD) diet (Day 0 - 50)

Group	Growth (g/day)
BD-I	95
BD-PC	102
Pooled SE	5.6
UD-I	77
UD-PC	105
Pooled SE	6.4
Diet effect	ns
Infection effect	**
Interaction	ns

** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Haematology

Parasitaemia

No significant differences in parasitaemia could be detected between the basal and urea-supplemented diet using the thick blood smear method (Table 7.5). At very low parasitaemias the ratio trypanosomes to white blood cells in the thick blood smear was difficult to assess accurately. The level at which there are not enough trypanosomes in the thick blood smear to estimate accurately the ratio trypanosomes to white blood cells was previously estimated to be 100,000 or less trypanosomes per ml of blood ($^{10}\log 5$) (Hamminga, 1989).

The first peak parasitaemia, around day 10 post-infection, appeared to be slightly higher in the urea-supplemented group than in the infected group on the basal diet. The second peak parasitaemia appeared to be slightly earlier in the lambs on the basal diet (day 30) compared with the lambs on the urea-supplemented diet (day 47; Figure 7.2). The levels of parasitaemia were found to be relatively low. The results of the parasitaemia levels found using the buffy coat method show a very similar pattern (Figure 7.3).

Table 7.5 Mean intensity of parasitaemia using the thick blood smear method of *T.congolense* infected (I) sheep (n=6) fed either a basal (BD) or a urea-supplemented (UD) diet

Group	Thick Blood Smear (^{10}log tryps/ml)
BD-I	5.35
SE	0.075
UD-I	5.40
SE	0.087
Significance	ns

ns: No Significant Difference

Figure 7.2 Mean parasitaemia (x 10^4 Tryps/ml) using the thick blood smear method of *T.congolense* infected sheep fed a basal (BD-I) or a urea-supplemented (UD-I) diet

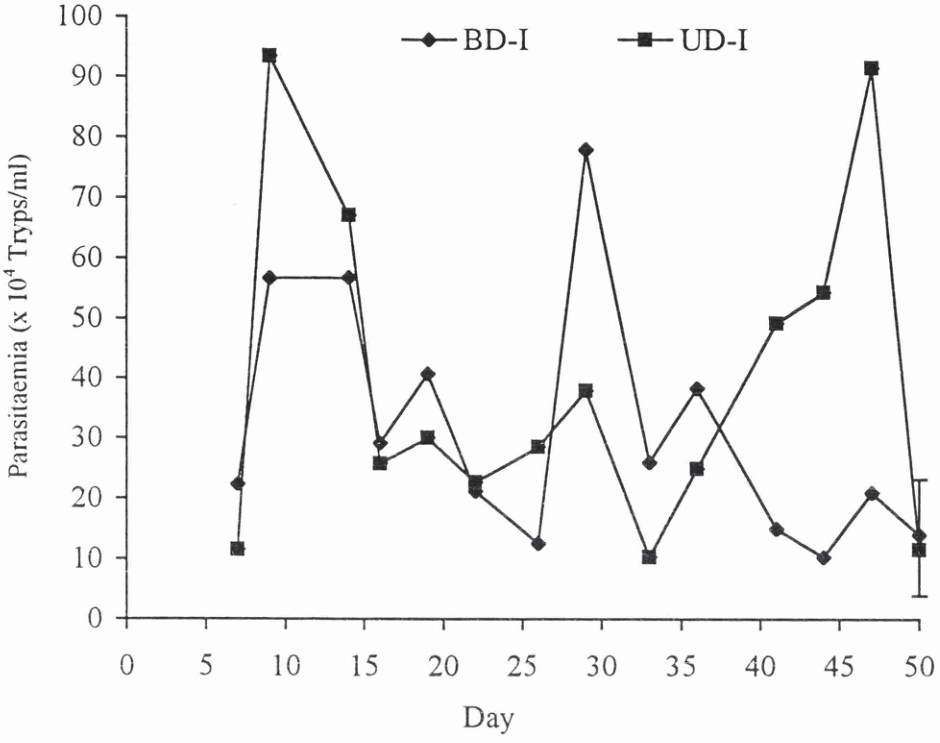
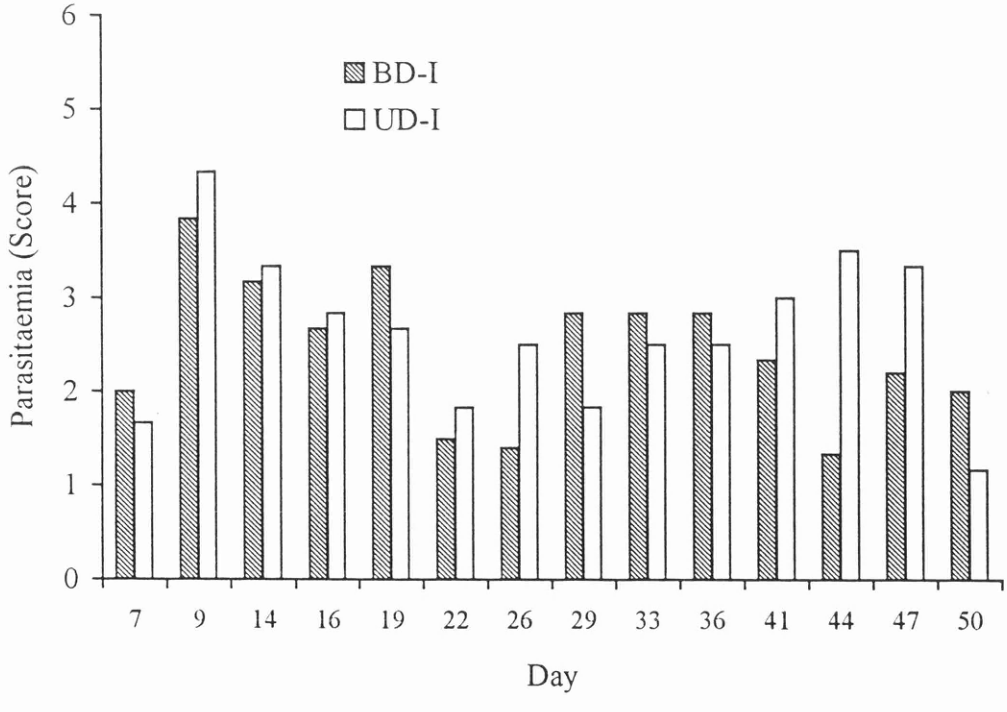


Figure 7.3 Mean parasitaemia (Score) using the buffy coat method of *T.congolense* infected sheep fed a basal (BD-I) or a urea-supplemented (UD-I) diet



Packed cell volume

Packed cell volume (PCV) followed the normal pattern during a trypanosome infection and decreased after infection (Figure 7.4). However, the anaemia was relatively mild and approximately 20 days after infection the packed cell volume was stabilising. Average packed cell volume was significantly ($p<0.01$) lower in the infected than in the pair-fed control animals (Table 7.6). No dietary effect could be observed and the decrease in packed cell volume after *T.congolense* infection was very similar in the two dietary groups.

Figure 7.4 Mean packed cell volume (PCV; %) of *T.congolense* infected sheep fed a basal (BD-I) or a urea-supplemented (UD-I) diet and their respective pair-fed controls BD-PC and UD-PC

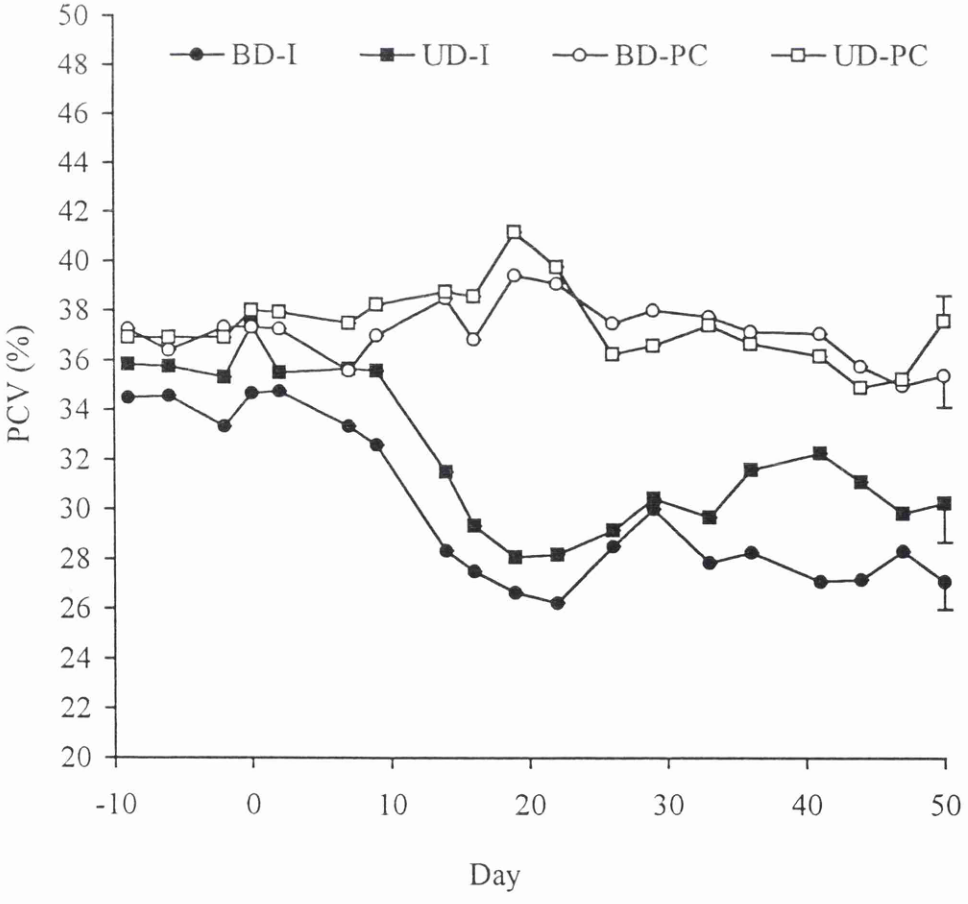


Table 7.6 Mean packed cell volume (%) of *T.congolense* infected (I) sheep and their respective pair-fed controls (PC) fed a basal (BD) or urea-supplemented (UD) diet during pre- (day -9 - 0) and post-infection (day 14 - 49)

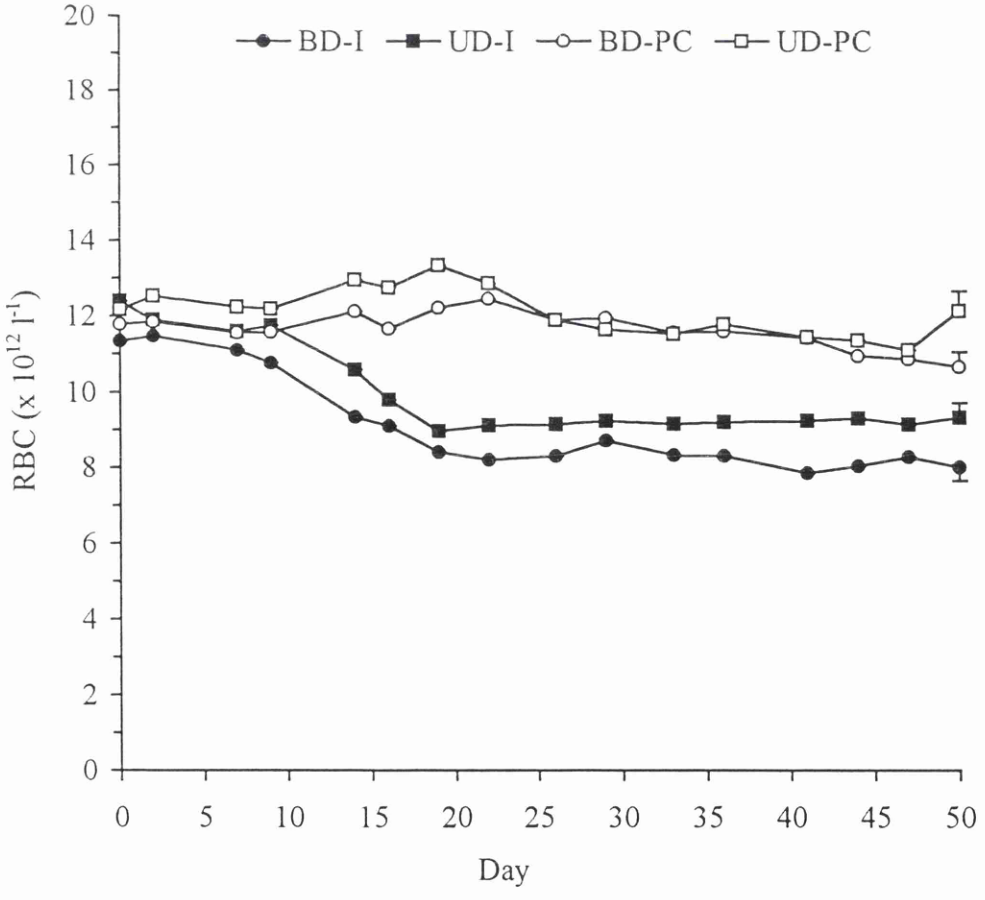
	Packed Cell Volume (%)	
	Period	
Group	pre	post
BD-I	34.1	27.8
BD-PC	37.0	37.4
Pooled SE	1.0	1.6
UD-I	35.6	30.1
UD-PC	36.9	37.4
Pooled SE	.7	1.3
Diet effect	ns	ns
Infection effect	ns	**
Interaction	ns	ns

** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Red Blood Cell Count

The red blood cell (RBC) counts were also decreased significantly between day 7 and 20 after infection and then stabilised at around 3 units under the red blood cell counts of the pair-fed controls (Figure 7.5; $p<0.01$). No significant dietary effect was found on the level of decrease in red blood cell counts (Table 7.7).

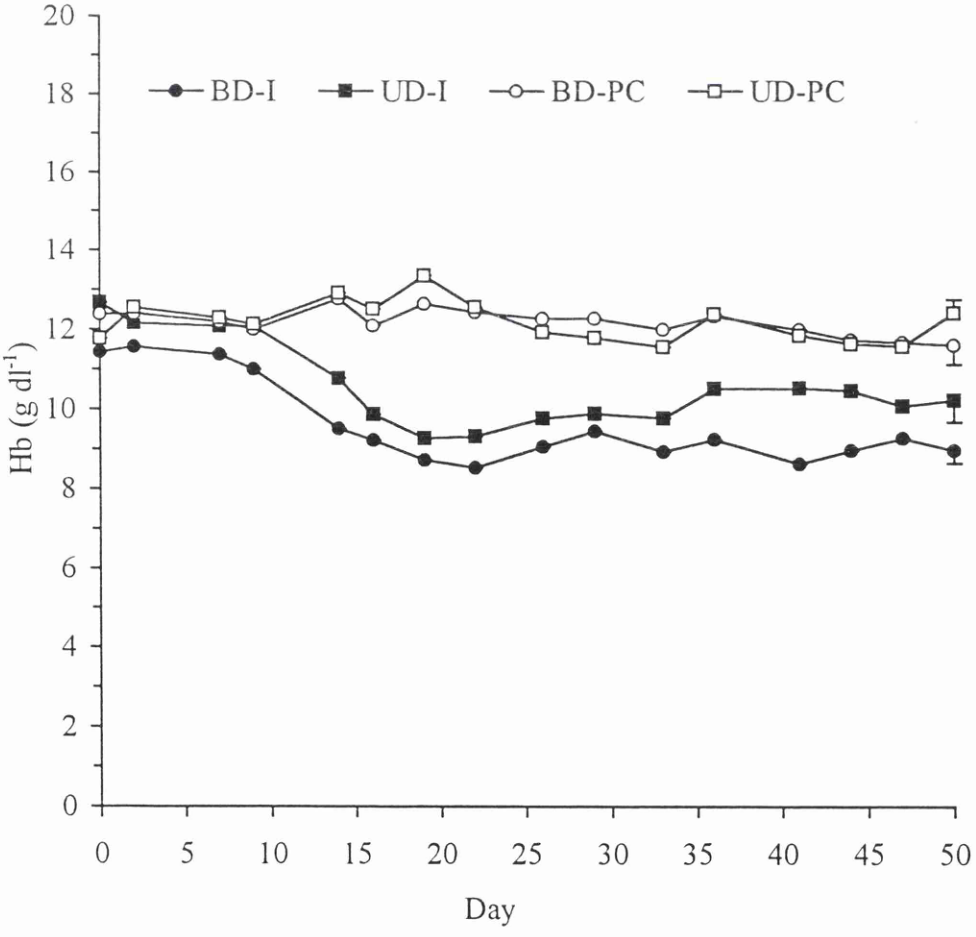
Figure 7.5 Mean red blood cells (RBC; x 10^{12} l^{-1}) of *T.congolense* infected sheep fed a basal (BD-I) or a urea-supplemented (UD-I) diet and their respective pair-fed controls BD-PC and UD-PC



Haemoglobin concentration

The decrease in haemoglobin concentration (Hb) (Figure 7.6) during the *T.congolense* infection developed in a similar manner as the red blood cell counts. The haemoglobin concentration was significantly lower in the infected groups compared with their pair-fed control partners ($p<0.01$) (Table 7.7). No significant dietary effect or interaction effect between diet and infection was found on the haemoglobin concentration.

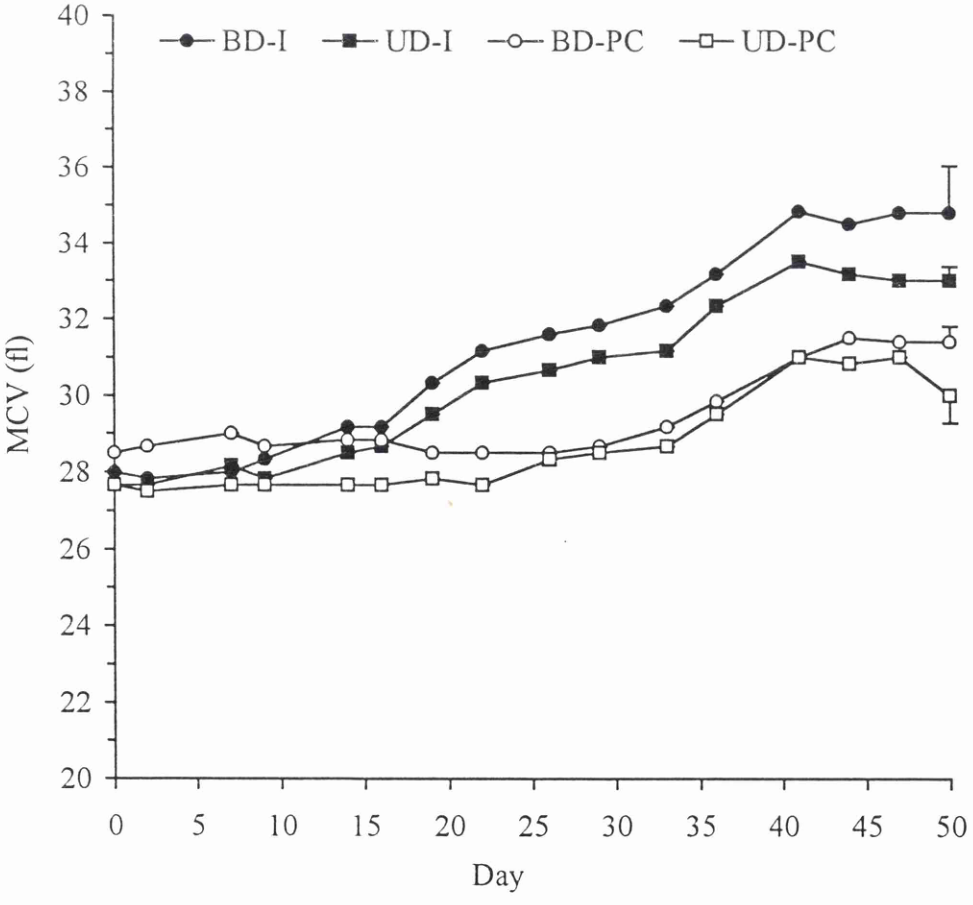
Figure 7.6 Mean haemoglobin (Hb; g dl^{-1}) of *T.congolense* infected sheep fed a basal (BD-I) or a urea-supplemented (UD-I) diet and their respective pair-fed controls BD-PC and UD-PC



Mean corpuscular volume

The mean corpuscular volume (MCV) increased significantly during infection ($p<0.01$) and appeared to be slightly higher in the infected group fed the basal diet (Figure 7.7). However, no statistically significant interaction effect between diet and infection was found (Table 7.7). The mean corpuscular volume in the pair-fed control lambs also increased but not as much as in the infected groups. The mean corpuscular volume appeared to stabilise at around day 40 after infection in all four groups.

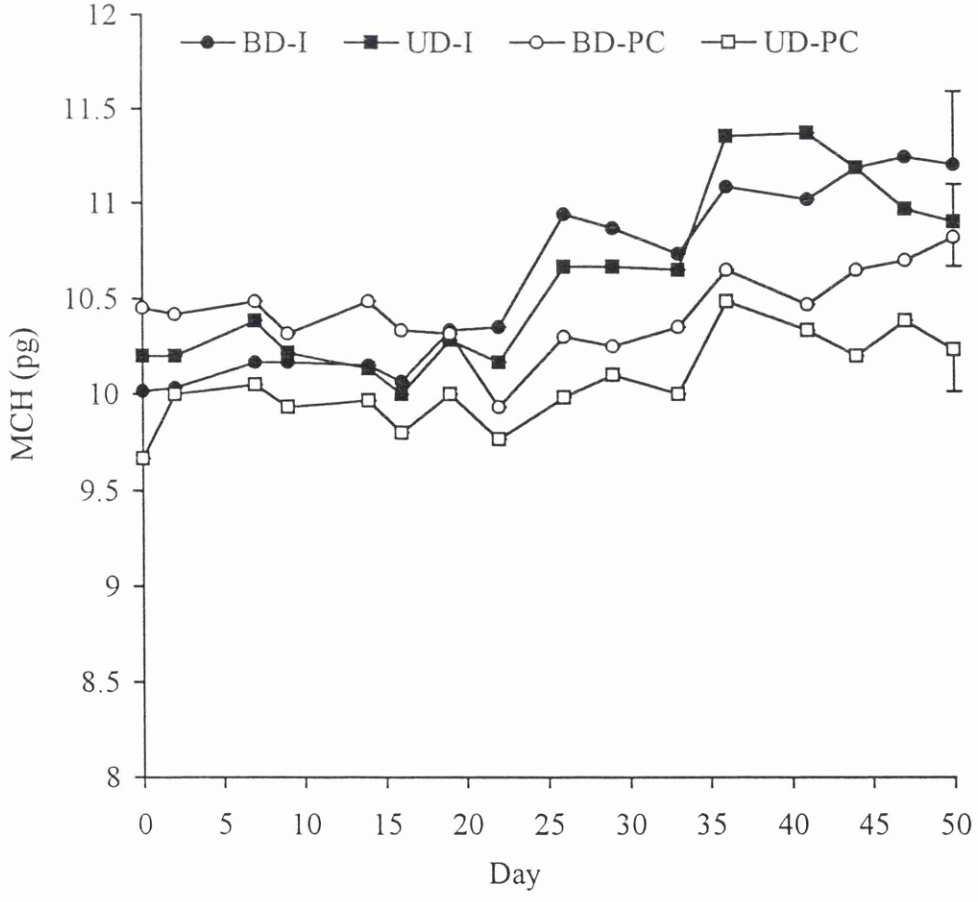
Figure 7.7 Mean corpuscular volume (MCV; fl) of *T.congolense* infected sheep fed a basal (BD-I) or a urea-supplemented (UD-I) diet and their respective pair-fed controls BD-PC and UD-PC



Mean corpuscular haemoglobin

The mean corpuscular haemoglobin (MCH; Figure 7.8) increased slightly due to infection in both dietary groups from approximately day 20 after infection. Differences in mean corpuscular haemoglobin were statistically slightly significant between the infected lambs and their pair-fed controls ($p<0.05$). No differences in mean corpuscular haemoglobin between the dietary groups was observed (Table 7.7).

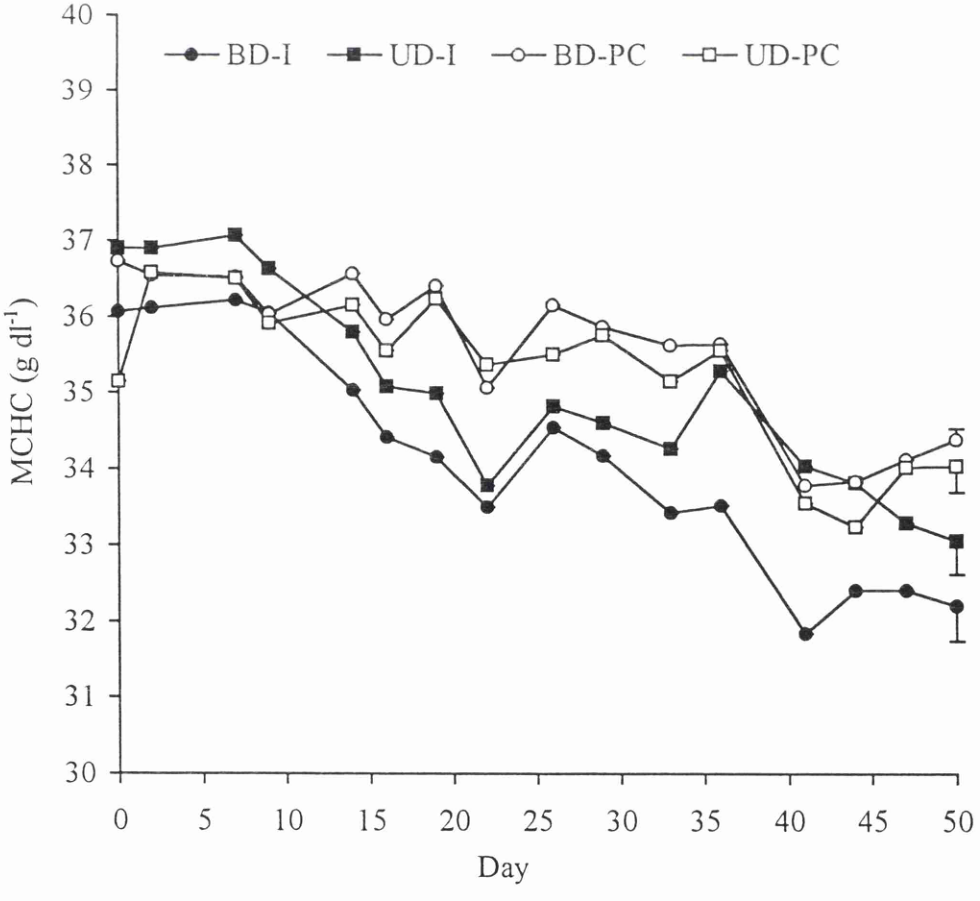
Figure 7.8 Mean corpuscular haemoglobin (MCH; pg) of *T.congolense* infected sheep fed a basal (BD-I) or a urea-supplemented (UD-I) diet and their respective pair-fed controls BD-PC and UD-PC



Mean corpuscular haemoglobin concentration

The mean corpuscular haemoglobin concentration (Figure 7.9) decreased after infection in the infected group fed the basal diet, but not in the infected lambs fed the urea-supplemented diet ($p<0.01$) (Table 7.7) which could be partly explained by the slightly higher mean corpuscular volume in the infected, basal diet fed group.

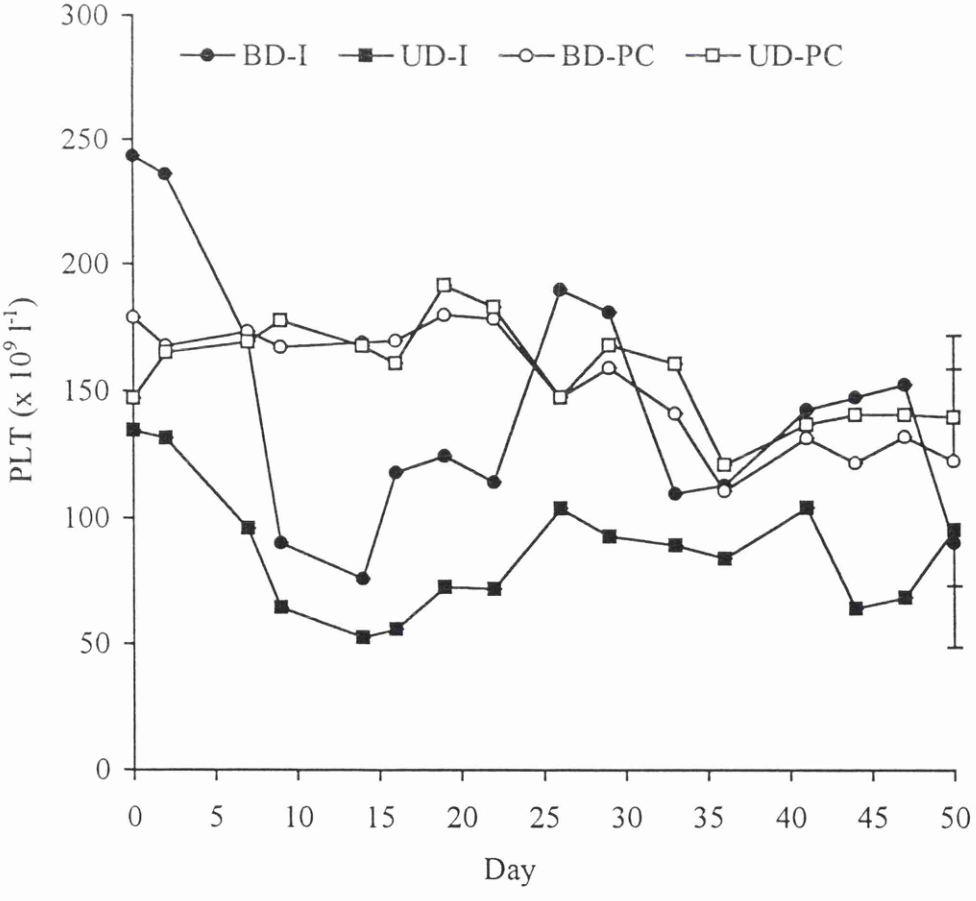
Figure 7.9 Mean corpuscular haemoglobin concentration (MCHC; g dl^{-1}) of *T.congolense* infected sheep fed a basal (BD-I) or a urea-supplemented (UD-I) diet and their respective pair-fed controls BD-PC and UD-PC



Platelet counts

Platelet counts decreased rapidly after infection (Figure 7.10), but tended to recover after about day 14 after infection, especially in the lambs on urea-supplemented diet. However, the variance in platelet counts between animals was high and no significant differences were found between the groups (Table 7.7).

Figure 7.10 Mean platelet count (PLT; x 10^9 l^{-1}) of *T.congolense* infected sheep fed a basal (BD-I) or a urea-supplemented (UD-I) diet and their respective pair-fed controls BD-PC and UD-PC



White blood cell count

There was no clear pattern to the levels of white blood cells after the *T.congolense* infection. The variance in white blood cell counts between animals within treatment groups was very high, especially in the infected groups, and none of the differences between the groups were statistically significant (Table 7.7; Figure 7.11).

Figure 7.11 Mean white blood cell count (WBC; x 10^9 l^{-1}) of *T.congolense* infected sheep fed a basal (BD-I) or a urea-supplemented (UD-I) diet and their respective pair-fed controls BD-PC and UD-PC

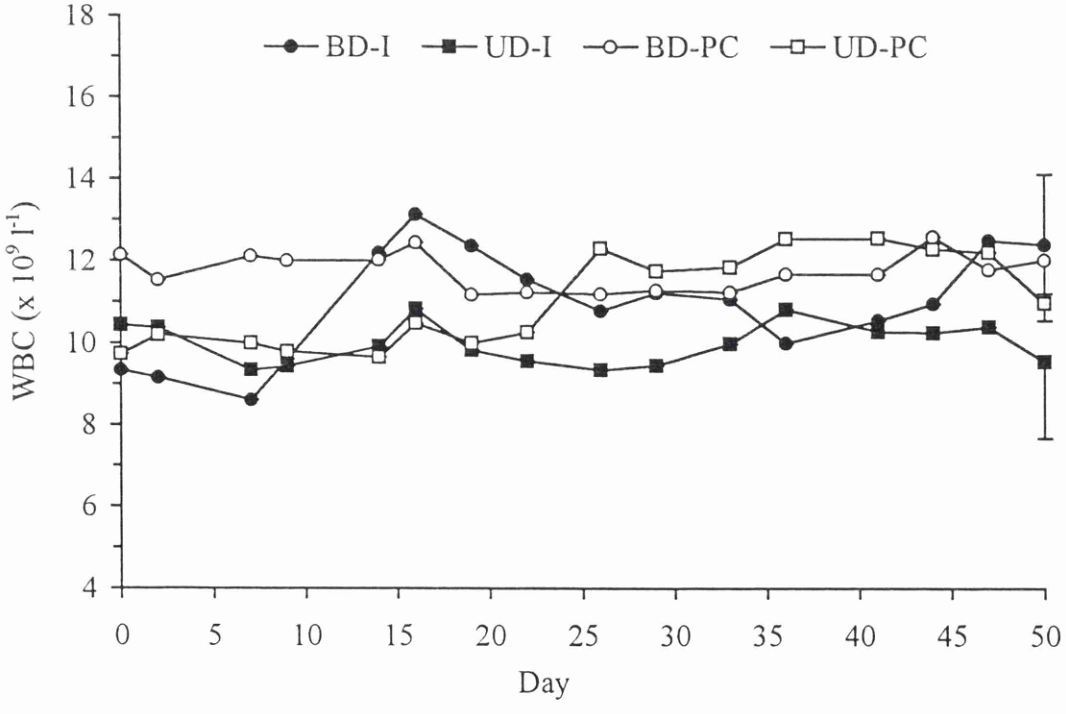


Table 7.7 Mean red blood cell count (RBC; x 10^{12} l^{-1}), haemoglobin concentration (Hb; g dl^{-1}), mean corpuscular volume (MCV; fl), mean corpuscular haemoglobin (MCH; pg), mean corpuscular haemoglobin concentration (MCHC; g dl^{-1}), platelet count (PLT; x 10^9 l^{-1}) and white blood cell count (WBC; x 10^9 l^{-1}) of *T.congolense* infected (I) sheep and their respective pair-fed controls (PC) fed a basal (BD) or a urea-supplemented (UD) diet during post-infection (day 14 - 49)

Group	RBC	Hb	MCV	MCH	MCHC	PLT	WBC
BD-I	8.42	9.1	32.2	10.7	33.5	138	11.5
BD-PC	11.68	12.2	29.6	10.4	35.3	117	11.7
Pooled SE	.54	.5	.6	.1	.3	16	.7
UD-I	9.34	10.0	31.2	10.7	34.4	79	10.0
UD-PC	12.07	12.2	29.1	10.1	35.0	155	11.4
Pooled SE	.48	.4	.4	.1	.2	20	.9
Diet effect	ns	ns	ns	ns	ns	ns	ns
Infection effect	**	**	**	*	**	ns	ns
Interaction	ns	ns	ns	ns	**	ns	ns

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Blood biochemistry

Plasma cholesterol

No dietary effect on the plasma cholesterol concentration was found during the pre-infection period (Table 7.8). The variation in plasma cholesterol levels between animals within groups was also low and no statistically significant relationship between cholesterol and parasite levels, as was found in Chapter 4, was observed. Plasma cholesterol concentration of the urea-supplemented pair-fed control animals was lower pre-infection, though not significantly, than in the other three groups. The plasma cholesterol concentration decreased rapidly after infection in both dietary groups ($p<0.01$) (Figure7.12).

Figure 7.12 Mean plasma cholesterol (mmol l^{-1}) of *T.congolense* infected sheep fed a basal (BD-I) or a urea-supplemented (UD-I) diet and their respective pair-fed controls BD-PC and UD-PC

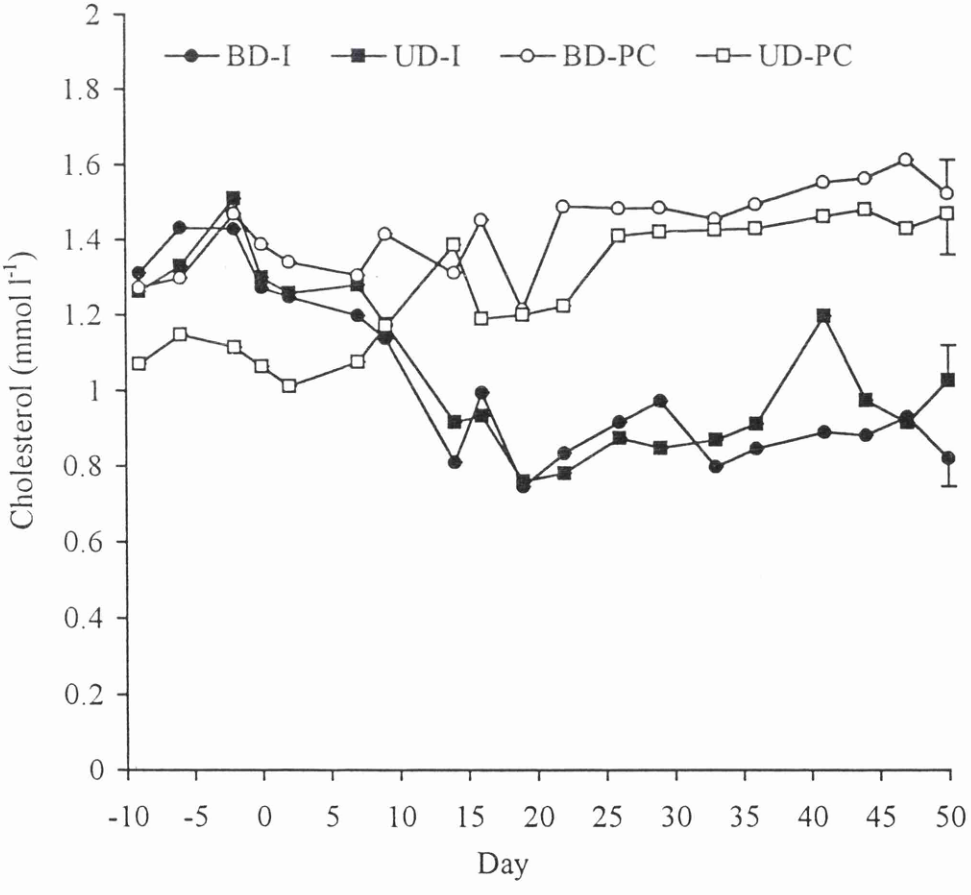


Table 7.8 Mean plasma cholesterol (mmol l^{-1}) during pre- (day -9 - 0) and post- (day 14 - 49) infection of *T.congolense* infected (I) sheep and their respective pair-fed controls (PC) fed a basal (BD) or a urea-supplemented (UD) diet

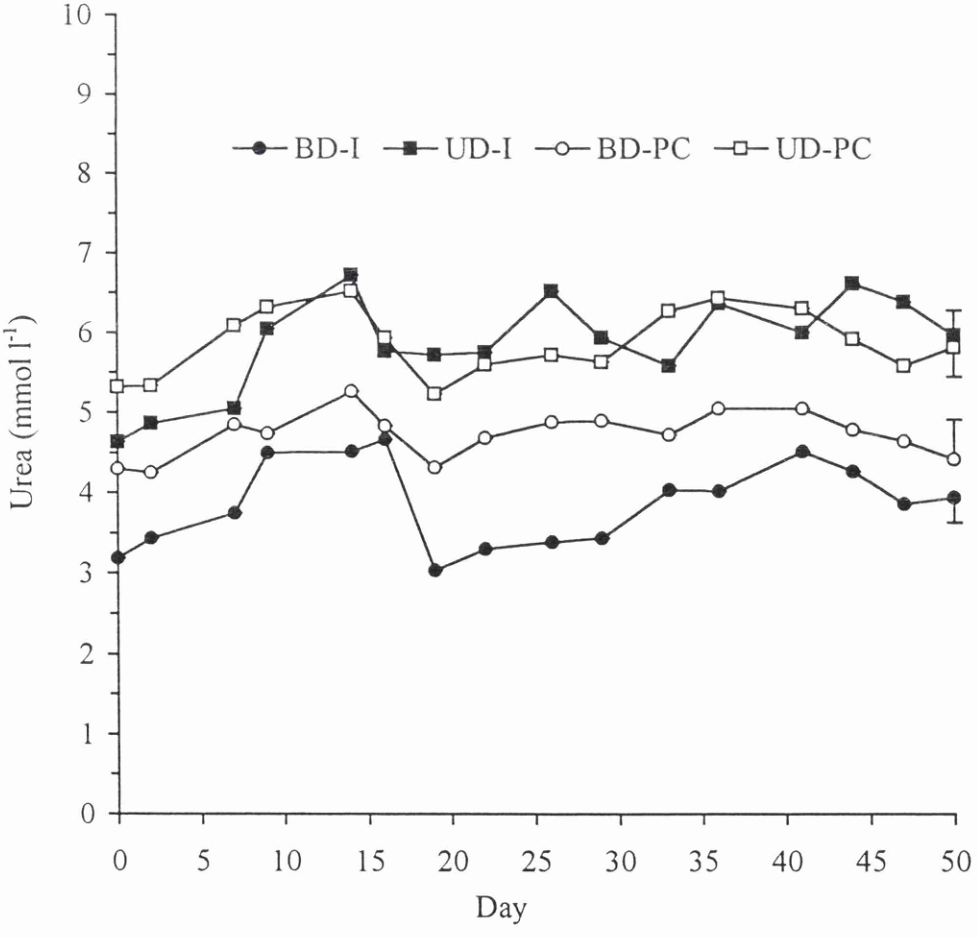
	Cholesterol (mmol l^{-1})	
	Period	
Group	Pre	Post
BD-I	1.36	0.87
BD-PC	1.36	1.47
Pooled SE	.04	.10
UD-I	1.35	0.92
UD-PC	1.10	1.38
Pooled SE	.07	.09
Diet effect	ns	ns
Infection effect	ns	**
Interaction	ns	ns

** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Plasma urea

The plasma urea concentration was significantly higher in the urea-supplemented groups ($p<0.01$). A significant interaction effect between diet and infection was found on plasma urea concentrations, as the infection significantly decreased the plasma urea concentration in the basal diet fed lambs but not in the urea-supplemented lambs ($p<0.01$) (Table 7.9; Figure 7.13).

Figure 7.13 Mean plasma urea (mmol l^{-1}) of *T.congolense* infected sheep fed a basal (BD-I) or a urea-supplemented (UD-I) diet and their respective pair-fed controls BD-PC and UD-PC



Plasma albumin

Plasma albumin concentration decreased in all the groups between day 0 and day 20 after infection (Figure 7.14). However, the plasma albumin concentration was significantly lower in the infected lambs compared with their pair-fed controls in both dietary groups ($p<0.01$) (Table 7.9). After day 20, the plasma albumin concentration remained stable in all the groups.

Figure 7.14 Mean plasma albumin (g l^{-1}) of *T.congolense* infected sheep fed a normal (BD-I) or a urea-supplemented (UD-I) diet and their respective pair-fed controls BD-PC and UD-PC

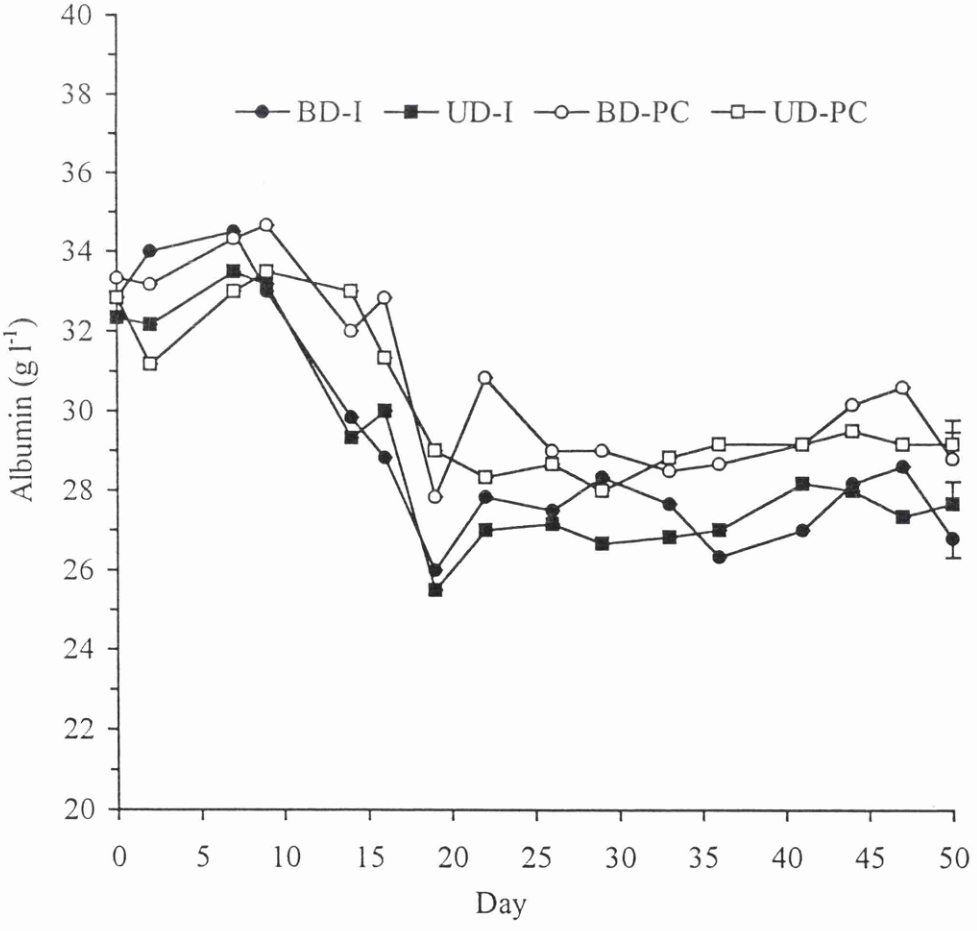


Table 7.9 Mean plasma urea (mmol l^{-1}) and albumin (g l^{-1}) concentration post-infection (day 14 - 49) of *T.congolense* infected (I) sheep and their respective pair-fed controls (PC) fed a basal (BD) or a urea-supplemented (UD) diet

Group	Urea (mmol l^{-1})	Albumin (g l^{-1})
BD-I	3.9	27.7
BD-PC	4.8	29.7
Pooled SE	.2	.5
UD-I	6.1	27.6
UD-PC	5.9	29.4
Pooled SE	.2	.4
Diet effect	**	ns
Infection effect	*	**
Interaction	**	ns

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Plasma nitrate

A slight difference in plasma nitrate levels (Table7.10) was found before infection between the two diet groups with the animals on the basal diet showing the higher levels ($p<0.05$). The plasma nitrate concentration was approximately 3 μM lower in the lambs on the basal diet. The *T.congolense* infection caused a rise in plasma nitrate concentrations (Figure 7.15) in both dietary groups. The rise was similar in both groups. In contrast, the plasma nitrate concentration decreased slightly in the pair-fed control groups during the post-infection period.

No statistically significant relationship was found between parasitaemia level and plasma nitrate concentration (Table 7.11). However, on days when the highest number of parasites were found plasma nitrate concentrations were on average 10 μM higher.

Figure 7.15 Mean plasma nitrate (μM) of *T.congolense* infected sheep fed a diet basal (BD-I) or a urea-supplemented (UD-I) diet and their respective pair-fed controls BD-PC and UD-PC

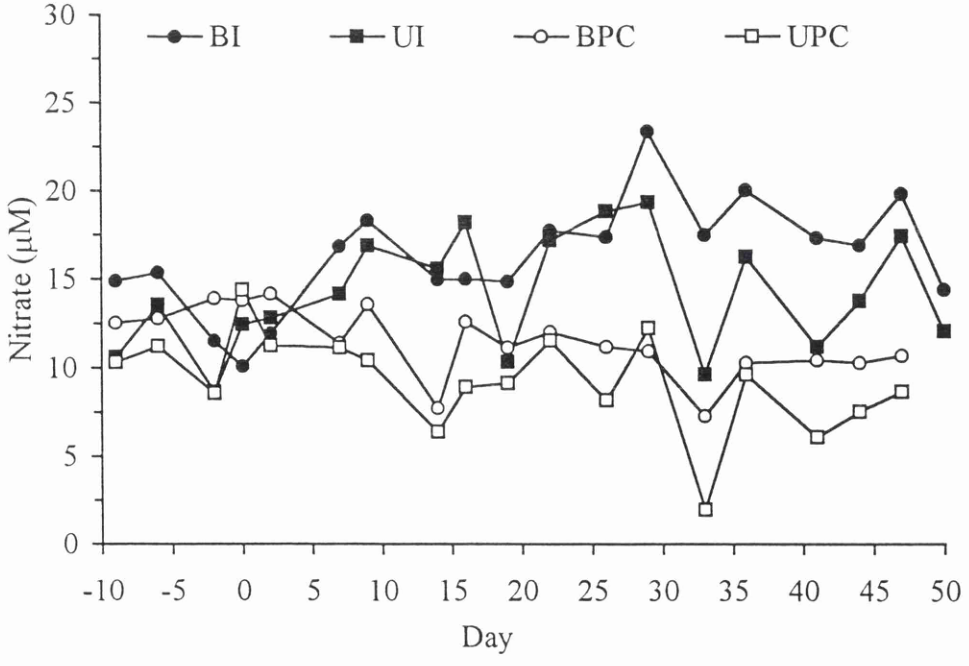


Table 7.10 Mean plasma nitrate concentration (μM) of *T.congolense* infected (I) sheep and their respective pair-fed controls (PC) fed a basal (BD) or a urea-supplemented (UD) diet during pre- (day -9 - 0) and post-infection (day 14 - 49)

	Nitrate concentration (μM)	
	Period	
Group	pre	post
BD-I	14.0	17.3
BD-PC	13.0	10.3
Pooled SE	0.8	1.4
UD-I	11.5	15.0
UD-PC	10.2	8.6
Pooled SE	0.8	1.2
Diet effect	*	ns
Infection effect	ns	**
Interaction	ns	ns

* : There is a significant difference between means ($p<0.05$)
** : There is a significant difference between means ($p<0.01$)
ns : No significant difference between means

Table 7.11 Median of plasma nitrate concentration for each intensity of parasitaemia score for *T.congolense* infected sheep

Buffy Coat (Score)	Nitrate concentration (μM)
0	17.5
1	15.0
2	16.0
3	15.1
4	16.4
5	26.5

Kruskal Wallis: $H = 5.63$, d.f. = 5, $p = 0.3$

Discussion

In this experiment the influence of urea supplementation was studied in Scottish Blackface sheep infected with *Trypanosoma congolense*. The infection was relatively mild as indicated by the small changes in packed cell volume. The urea supplementation did not appear to have a beneficial effect on the pathogenesis of the trypanosome infection.

The body weight gains of the infected lambs were significantly lower than the body weight gains of the pair-fed control lambs. The addition of urea did not result in higher body weight gains. On the contrary, the body weight gains of the infected lambs on the urea-supplemented diet appeared more affected by the infection than those on the basal diet. The amount of effective rumen degradable dietary protein (ERDP) in the normal diet was found to be limiting the microbial crude protein (MCP) supply. The urea increased the effective rumen degradable dietary protein level but did not result in an expected increase in body weight gain. One would expect this to be due to a limited availability of energy to the rumen microbes for utilising the ammonia produced. However, in this case the fermentable metabolisable energy (FME) levels were not limiting the microbial crude protein (MCP) supply. There appears to be no clear answer why the 16 g/day increase in metabolisable protein (MP) was not transformed into higher body weight gains. Whilst the fermentable metabolisable energy (FME) was calculated to be sufficient there may have been an asynchrony between the availability of nitrogen and FME in the rumen despite readily available carbohydrates in the barley grain. One of the underlying reasons may have been that the adaptation period of 4 weeks to the urea feeding was too short to get an efficient use of the urea by the lambs. It has been reported that nitrogen retention increases with the duration of urea

feeding and that this could be due to metabolic modifications attributable to rumen bacteria and host tissues (Kobayashi *et al.*, 1992).

Knox and Steel (1996) also found that in goats infected with *Trichostrongylus colubriformis* urea supplementation alone did not prove beneficial. When the goats were also supplemented with cotton seed meal which provides digestible undegraded protein, the combination of urea and cotton seed meal was found to be beneficial to body weight gains, faecal egg counts and total worm burdens at slaughter. However, for reasons unknown, no goats were supplemented with cotton seed meal only.

These results are in contrast to other findings by Knox and Steel (1996) in lambs infected with mixed infections of *Haemonchus* and *Trichostrongylus* spp. They reported a reduced level of infection and of debilitating effects of the parasites by urea supplementation. This response was thought to be partly due to the stimulatory effect of urea on feed intake counteracting the depressive effect of parasites on appetite. It was thought that the increase in feed intake alone and the consequent effect on faecal output would not be sufficient to account for the lower egg count and the probable cause was thought to be enhanced immunological regulation. However, the authors acknowledge that the effects of urea supplementation on the regulation and expulsion of the worm population was not as pronounced as those observed with by-pass protein supplements (Van Houtert *et al.*, 1995a, b). Protein supplemented diets were also found to be beneficial to *T.congolense* infected (Katunguka-Rwakishaya *et al.*, 1993), *Fasciola hepatica* infected (Berry and Dargie, 1976) and *Haemonchus contortus* infected sheep (Abbott *et al.*, 1986; Wallace *et al.*, 1995; 1996). The protein supplements used in those experiments were also at least partly protected from rumen digestion and may have been more useful to the animals than the urea supplementation.

The greater supply of essential amino acids may be a reason for the beneficial effect of digestible undegraded protein, although more research is needed to determine the effects of different amino acids on the pathogenicity of parasitic infections.

The blood haematology parameters were all significantly affected by the *T.congolense* infection, except for the white blood cell count, and followed similar patterns as in previous experiments infections (Katunguka-Rwakishaya *et al.*, 1993; Chapter 4). However, no obvious beneficial effects of the additional urea were found on the affected haematology parameters, except for mean corpuscular haemoglobin concentration which decreased less in the urea-supplemented infected sheep.

Unlike the experiment described in Chapter 4, the variance in plasma cholesterol levels between animals within groups was very low. As a consequence, the relationship between plasma cholesterol levels and parasitaemia found in the previous experiment could not be verified in this experiment.

The plasma urea concentration was higher in the urea-supplemented lambs compared with the lambs on the basal diet due to the higher metabolisable protein intake. The lower plasma urea concentration in the infected animals fed the basal diets compared with their pair-fed controls is difficult to explain. Since the animals were growing it is unlikely that body protein reserves were catabolised during infection as found in *T.congolense* infected straw fed sheep (Chapter 4). It is more likely that the infected animals on the basal diet were preserving their body protein stores, hence the lower urea concentration. However, without nitrogen balance data to show differences in nitrogen losses between groups this remains speculation.

Nitric oxide produced by macrophages during trypanosome infections has on the one hand been related to immunosuppression in the host and on the other hand

found to be cytostatic to trypanosomes *in vitro*. More recent reports, however, suggest that the cytostatic effects of nitric oxide to trypanosomes does not occur *in vivo*, possibly due to the capture of nitric oxide by haemoglobin. In fact, inhibition of nitric oxide synthesis has led to reduced parasitaemias in murine *Trypanosoma brucei* infections which may be a consequence of the inhibition of the immunosuppressive effects of nitric oxide (Vincendeau *et al.*, 1991, 1992; Mabbott *et al.*, 1994; Sternberg *et al.*, 1994).

The plasma nitrate levels were unexpectedly found to be significantly higher pre-infection in the lambs on the basal diet. One would expect more L-arginine to be available for the biosynthesis of nitric oxide (Moncada *et al.*, 1985) in urea-supplemented lambs. One plausible explanation may be that much of the L-arginine available was used for the urea cycle to detoxify ammonia in the urea-supplemented lambs.

The nitric oxide scavenging activity of haemoglobin, however, did not appear to prevent a significant increase in plasma nitrate concentration during the trypanosome infection in this experiment. It is, however, difficult to determine whether the nitrate levels were high enough to have a significant cytostatic effect on the trypanosomes. Plasma nitrate levels were highest when the number of parasites found was very high. It is likely that the high number of parasites induced the macrophages to produce nitric oxide.

Excessive production of nitric oxide has been found to lead to pathological effects such as acute and chronic inflammation (Ianaro *et al.*, 1994) and arthritis (McCartney-Francis *et al.*, 1993).

One of the properties of nitric oxide is that it reacts with superoxide, also produced by activated phagocytes (Bellavite, 1988), to produce peroxynitrite. Peroxynitrite has been found to cause aggregation of human platelets (Moro *et al.*, 1994). The sheep used in this experiment showed a decrease in numbers of platelets which may have been caused by platelet aggregation.

Conclusions

The supplementation of the diet with urea did not appear to provide beneficial effects on the haematological and biochemical parameters of sheep infected with *T.congolense*. Nitric oxide production by macrophages was found to be significantly increased only in lambs with a very high parasitaemia.

CHAPTER 8

The Effect of Dietary Arginine Restriction on the Parasitaemia Levels of Mice Infected with *Trypanosoma congolense*

Introduction

The highly inducible enzyme ornithine decarboxilase catalyses the conversion of ornithine to the polyamine putrescine which is the initial and normally rate-limiting step in polyamine biosynthesis. Polyamine synthesis is required for normal cell growth and division. Drugs which are known to inhibit the enzyme ornithine decarboxylase such as efluornitine (α-difluoroomethhylornitine, DFMO, Ornidyl$^{(R)}$) have been shown to inhibit the growth of African trypanosomes (Bacchi *et al.*, 1980).

Only small amounts of ornithine are provided by the diet and most is synthesised from arginine via the enzyme arginase and from glutamate via ornithine amino transferase through a glutamyl-γ-semialdehyde intermediate in the intestinal mucosa (Jones, 1985).

A significant amount of arginine is provided by the diet and adult mice placed upon an arginine-free diet have been shown to have markedly reduced levels of arginine and ornithine in a variety of tissues (Monso and Rubio, 1989). In recent research it has been shown that mice restricted in dietary arginine showed diminished tumorigenesis as a result of lowered pools of ornithine and the subsequent inhibition of putrescine biosynthesis (Gonzalez and Byus, 1991).

Restricted amounts of dietary arginine may also have an effect on the nitric oxide synthesis of macrophages since this is based on the conversion of arginine to nitric oxide and citrulline (Moncada *et al.*, 1985). Vincendeau and Daulouede (1991) reported that the macrophage cytostatic effect on *Trypanosoma musculi* involved a *L*-arginine dependent mechanism. The same authors (Vincendeau *et al.*, 1992) also reported that the *in vitro* cytostatic effect of macrophages on *Trypanosoma brucei gambiense* and *Trypanosoma brucei brucei* depended on nitric oxide. On the other

hand, inhibition of nitric oxide synthesis has been found to lead to reduced parasitaemia in mice infected with *T.brucei* (Sternberg *et al.*, 1994).

In this experiment the hypothesis was investigated that *T.congolense* infected mice fed an arginine-deficient diet would show lower numbers of parasites than infected mice fed a normal diet because arginine is considered to be a requirement for parasite growth. To see whether this was due to an inhibition of putrescine synthesis or other factors an extra group of mice on a restricted arginine diet received putrescine in the water.

Materials and methods

Experimental diets

The specific amino acids in the diets by percentage of weight are shown in Table 8.1. Three weeks before the trypanosome infection the animals were introduced to the diet. The diets were in the powdered form and were offered *ad libitum* to the mice. The mice were weighed on Mondays and Thursdays.

Experimental animals

Twenty five male, adult mice of approximately 40 grams were divided into five groups of 5 mice. The mice were housed individually.

Group 1: synthetic arginine-free diet + *T.congolense* infection

Group 2: synthetic arginine-free diet + 1% putrescine H_2O + *T.congolense* infection

Group 3: synthetic complete amino acid control diet + *T.congolense* infection

Group 4: synthetic arginine-free diet

Group 5: normal diet

Table 8.1 The percentage weight of the diet components in the control and arginine deficient diets (Special Diet Services, Witham, Essex)

Diet components	Control Diet	Arginine Deficient Diet
cornflour snowflake	57.6	57.6
corn oil	8.0	8.0
dextrin	5.0	5.0
sulkafloc	5.0	5.0
sugar gran	5.0	5.0
AIN 76 vit mix	1.0	1.0
AIN 76 min mix	4.0	4.0
L-arginine	1.2	0.0
L-glutamic acid	1.2	2.4
glycine	1.2	1.2
L-histidine	1.2	1.2
L-isoleucine	1.2	1.2
L-leucine	1.2	1.2
L-lysine	1.2	1.2
methionine	1.2	1.2
L-phenylalanine	1.2	1.2
L-threonine	1.2	1.2
L-tryptophan	1.2	1.2
L-valine	1.2	1.2

Experimental infection

Mice in the groups 1, 2 and 3 were infected with *T.congolense* 1180 (GRVPS 57/17) isolated in Serengeti, Tanzania (Nantulya *et al.*, 1984). Each mouse was inoculated intraperitoneal with 1 x 10^4 trypanosomes in 0.2 ml phosphate buffered saline (PBS) (containing 1.5% glucose). The infection lasted 17 days after which the animals were humanely killed.

Parasitaemia measurements

Parasitaemias were determined three times a week using the improved Neubauer haemacytometer. One microliter (µl) of mouse blood, taken from the tail, was diluted 100 times in PBS containing 1.5% glucose.

Results

One of the animals in group 3 was not used in the final analysis because of a problem with its tail.

Body weight

The mice on the synthetic diets initially lost some body weight (figure 8.1) which resulted in their weights being slightly lower during the pre-infection period compared to the animals on the normal control diet. The decrease in body weight was highest in the animals fed the arginine-free diet plus 1% putrescine (group 2) and the body weight of that group was significantly lower pre-infection than in the group receiving the normal control diet (Table 8.2; $p<0.05$). After infection the body weights

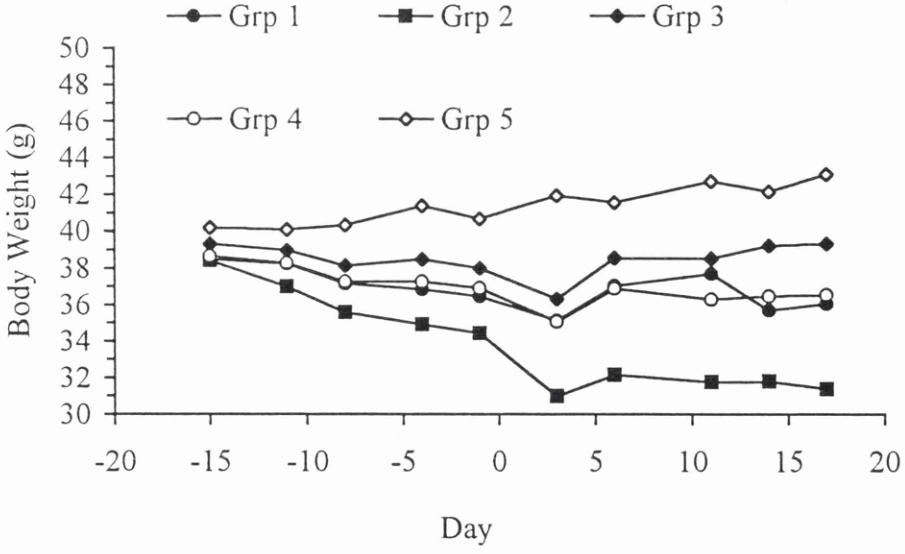
of the mice on the synthetic diets had stabilised but were significantly lower than the ones on the normal control diet (Table 8.2; $p<0.05$).

Table 8.2 Mean body weight ± standard error (g) of *T.congolense* infected mice fed special synthetic amino acid diets and their controls during the pre- (day -15 - 0) and post-infection (Day 1 - 17) periods

Group	Pre-infection	Post-infection
Synthetic arginine-free diet + *T.congolense* Infection	37.4 ± 0.86^{a}	36.3 ± 0.77^{a}
Synthetic arginine-free diet + *T.congolense* Infection + 1% Putrescine H2O	36.0 ± 0.67^{b}	31.6 ± 0.87^{ab}
Synthetic control diet + *T.congolense* Infection	38.6 ± 1.05^{c}	38.4 ± 0.98^{b}
Synthetic Arginine-free diet	37.7 ± 1.63^{d}	36.2 ± 1.94^{c}
Normal control diet	40.5 ± 1.22^{b}	42.3 ± 1.21^{abc}

Means of groups with the same letter within columns are significantly different $p<0.05$

Figure 8.1 Mean body weight (g) of *T.congolense* infected mice fed an arginine-free diet (Grp 1), an arginine-free diet plus putrescine in water (Grp 2), a synthetic control diet (Grp 3) and control mice on a arginine-free (Grp 4) and a normal diet (Grp 5)



Level of parasitaemia

Parasites were detected in 4 out of 5 animals in the group receiving the synthetic arginine-free diet on day 7 after infection, whereas the animals in the other two groups were all negative (figure 8.2). Seventeen days after infection one of the animals in the group receiving arginine-free diet plus 1% putrescine in the water and two animals in synthetic control diet were still negative. Average parasitaemia was significantly higher in the group receiving the synthetic arginine-free diet than in the group on the synthetic control diet ($p<0.05$). The parasitaemia of the group fed the arginine-free diet plus putrescine in the water appeared to be intermediate (table 8.3).

Figure 8.2 Mean parasitaemia (log(10) Tryps/ml) of *T.congolense* infected mice fed a synthetic arginine-free diet (Grp 1), a synthetic arginine-free diet plus putrescine in water (Grp 2) or a synthetic control diet (Grp 3)

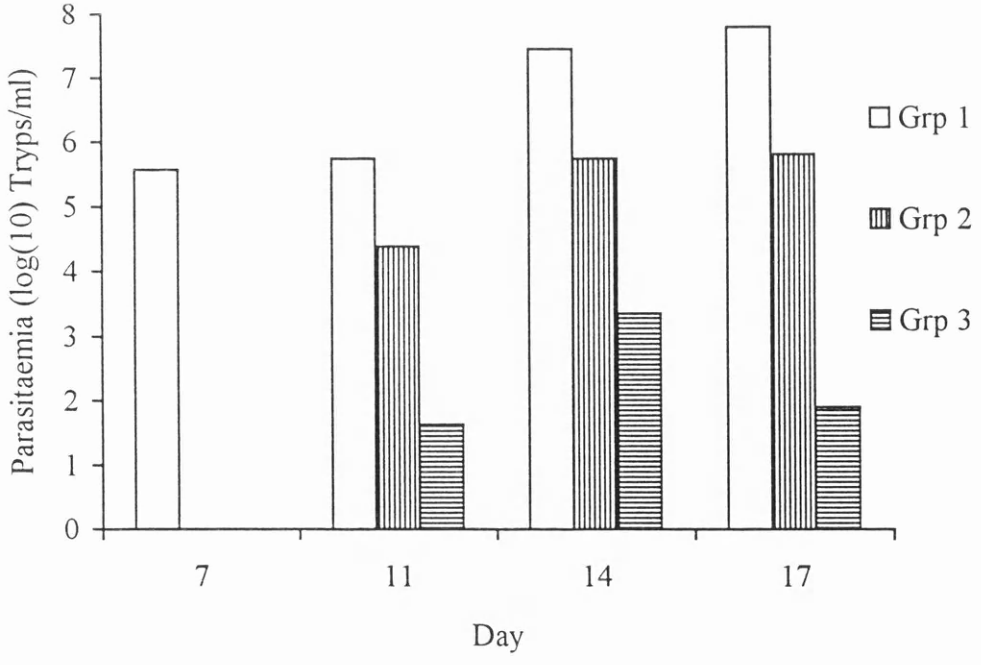


Table 8.3 ^{10}log parasitaemia (Trypanosomes/ml) ± standard deviation of *T.congolense* infected mice fed special synthetic amino acid diets and their controls (Day 0 - 17)

Group	^{10} log Parasitaemia
Synthetic arginine-free diet + *T.congolense* Infection	6.7 ± 1.5^a
Synthetic arginine-free diet + *T.congolense* Infection + 1% Putrescine H_2O	4.0 ± 2.4^b
Synthetic control diet + *T.congolense* Infection	1.8 ± 2.6^a

Means of groups with the same letter are significantly different $p<0.05$

Discussion

The results show that the hypothesis that the arginine-free diet would decrease the parasitaemia was invalid. Instead of the expected restricted cell growth of the parasites due to the limited availability of arginine to the mice, the parasites were found earlier and in greater numbers of parasites than in the mice fed the complete synthetic control diet. In two of the four control mice the parasites had not established themselves on day 17 whereas the infected mice on the arginine-free diet were all positive. The parasitaemia of the group on the arginine-free diet plus 1% putrescine in the water should be read with caution since the animals lost more weight pre-infection compared with the other groups. However, the results indicate that the added putrescine was at least in part able to make up for the arginine deficiency. Infection did not result in a further decrease in body weight in all three infected groups possibly due an increase in spleen size.

These findings are different from the findings from research into murine epidermal carcinogenesis in which a reduced number of tumours were found after feeding a diet restricted in arginine (Gonzalez and Byus, 1991). It appears that

arginine, apart from being a precursor for putrescine, is necessary for the resistance of the mice to the trypanosome infection. *L*-arginine-supplementation has been found to enhance splenocyte and thymocyte responses to polyclonal mitogens (measure of general lymphocyte reactivity). In addition, *L*-arginine has been shown to activate the macrophage cytotoxic effector mechanisms. The exact processes by which *L*-arginine mediates these immunostimulatory effects are not known but may include, e.g. increased production of hormones, enhanced polyamine synthesis, release of cytokines and via the nitric oxide pathway (Brittenden *et al.*, 1994).

Research on tumour growth has shown that the effects of *L*-arginine can be positive and negative depending on how strongly immunogenic the tumour is. When the tumour is weakly immunogenic *L*-arginine-supplementation may stimulate tumour growth with a lack of recognition by the immune system. If, however, the tumour is strongly immunogenic the effects of *L*-arginine on the host anti-tumour defence mechanisms may predominate, resulting in an inhibition of tumour growth (Brittenden *et al.*, 1994). In this experiment the effect of arginine on the immune system appears to be stronger than the effect of arginine on parasite growth.

It is not clear from the present experiment whether the arginine-free diet diminished nitric oxide production in the mice but if this were the case it did not significantly reduce parasitaemia. Sternberg *et al.* (1994) reported that inhibition of nitric oxide synthesis leads to reduced parasitaemia in *T.brucei* infected mice, possibly through the inhibition of the immuno-suppressive effect of nitric oxide. Under *in vitro* conditions several researchers (Mabbott *et al.*, 1994; Vincendeau *et al.*, 1991, 1992; Sternberg *et al.*, 1994) have found a cytostatic effect of nitric oxide on trypanosomes,

however, Mabbott *et al.* (1994) and Sternberg *et al.* (1994) found this is not the case under *in vivo* conditions.

It may be useful to conduct further experiments to investigate whether arginine-supplementation enhances the immune response to trypanosomiasis. A positive effect of arginine on the immune system during trypanosomiasis could also be exploited in ruminants. It appears that the availability of arginine to ruminants can be manipulated by changing the quality (i.e. digestibility) of the dietary protein. Low dietary protein quality reduced the levels of arginine in the plasma of dairy cows (Ruckebusch *et al.*, 1991).

Conclusions

The *T.congolense* infected mice on the arginine-free diet had a higher parasitaemia score than the mice on the diet adequate in arginine. In this experiment the effects of arginine on the immune system appeared to be greater than the effects on parasite growth.

CHAPTER 9

General Discussion

Experimental animals and infection

The Scottish Blackface lambs used in the experiments showed only moderate clinical signs to infection with *T.congolense* 1180 (Nantulya *et al.*, 1984). This might have been due to the low virulence of the *T.congolense* strain used and/or to the breed of sheep. Experiments in the past using Scottish Blackface sheep have shown similar results (Katunguka-Rwakishaya, 1992). Although Scottish Blackface sheep do not come into contact with trypanosomes under natural conditions they seem to be able to respond to trypanosome infections in a similar manner as trypanotolerant breeds of sheep (Katunguka-Rwakishaya, 1992). This resistance might be due to the hardiness of the animals since Scottish Blackface sheep have to survive under the difficult circumstances of the Scottish Highlands. However, the pathophysiological effects of the *T.vivax* Leeflang infection (Leeflang *et al.*, 1976) used in the experiment described in Chapter 6 were greater and one of the lambs had to be withdrawn from the experiment. The virulence of the *T.vivax* Leeflang infection appeared to be similar to that observed in West African Dwarf goats infected with the same strain (Wassink *et al.*, 1993).

In all the experiments pair-fed control animals were used. With pair feeding the effects of nutrient utilisation can be separated from the effects on appetite (Abbott *et al.*, 1986; Kroonen *et al.*, 1986). However, a major problem using pair-fed control animals occurs when the voluntary intake of the infected animals is higher than the intake of the control animals before infection as was the case with the lucerne hay fed lambs in Chapter 4. Crompton *et al.* (1981) reported another problem using pair-fed control animals in that the physiology of the control rats differed considerably from the *Nippostrongylus* infected ones. They attributed these changes to either the response of

the pair-fed rats to the stress of not having enough food or the consequences of rapidly eating the limited rations they were offered.

The experimental lambs were kept in individual pens in the experiments described in Chapters 4 and 7, and kept in metabolism stalls in the experiments described in Chapters 5 and 6. One has to realise that the results obtained from those experiments may have been affected by a changed physiological state due to the confinement (Marsden and Wood-Gush, 1986), especially in the lambs kept in metabolic stalls. Bowers *et al.* (1993) reported that confinement of lambs in metabolism stalls led to increased adrenal function, thyroxine and motivation for movement.

Experimental diets

Results from an experiment conducted in The Gambia indicated that *T.congolense* infected N'dama heifers reduced their intake of high fibre/low protein roughage but consumed the medium fibre/medium protein roughage and low fibre/high protein concentrate offered (Romney *et al.*, 1994). Based on these results high fibre/low protein barley straw and high fibre/high protein lucerne hay were chosen as the basis for the first experiment (Chapter 4). The aim of the experiment was to investigate whether there is interaction between the quality of the roughage offered and the pathophysiology of a trypanosome infection with particular regard to digestive function.

The lambs in the experiments described in Chapters 5 and 6 were given diets containing different amounts of roughage and concentrate. The diets offered were isocaloric but differed in the levels of digestible undegraded protein (DUP). The levels

of effective rumen degradable protein (ERDP) were similar in both dietary groups. The aim of the experiments was to investigate the effects of the level of digestible undegraded protein (DUP) on the pathophysiology of trypanosome infections. Especially the digestive function and nitrogen balance were investigated.

Chapter 7 outlines the effects of urea supplementation on the pathophysiology of trypanosomiasis. It was assumed that the supplement would increase the effective rumen degradable protein (ERDP) and thereby increase the protein available to the animal. The aim of the experiment was to investigate whether similar results could be obtained as in research based on protein supplementation which increased the digestible undegraded protein (DUP) in parasitised animals (Abbott *et al.*, 1985a; 1985b; 1986; Katunguka-Rwakishaya *et al.*, 1993).

Research on mice had shown that arginine-free diets can reduce the growth of tumours (Gonzalez and Byus, 1991). The reason behind this effect is that arginine is a precursor for ornithine which is converted to the polyamine putrescine. The polyamine putrescine is essential for normal cell growth and division. Since trypanosomes would also need putrescine for their cell growth and division an experiment was conducted to investigate whether a lack of dietary arginine would also decrease the levels of parasitaemia in trypanosome-infected mice.

Feed intake and body weight changes

The organic matter intake was significantly decreased in all the experiments following trypanosome infection (Chapter 4, 5 and 6), where the roughage was fed *ad libitum*. In the experiment on urea supplementation (Chapter 7) the animals were fed a restricted amount which the lambs consumed completely throughout the experiment.

The large decrease in lucerne hay intake between day 12 and 21 after the *T.congolense* infection in the lambs of Chapter 4 was almost certainly stress induced. The lambs were fitted with faecal bags on day 12 after infection. The decrease in barley straw intake during the same period was much smaller. The lambs on barley straw appeared less affected by the stressful event. These differences in feed intake responses are likely to have been caused by the different properties of the roughages involved. The differences in feed intake pattern between barley straw and lucerne hay found in Chapter 4 supports this conclusion. Whereas the intake of the lucerne hay was spread throughout the day, the intake of barley straw was restricted to a short period just after feeding. It is worth noting that although the organic matter intake of lucerne hay in the lambs of Chapter 4 was much greater than the intake of the lambs fed barley straw, the dietary fibre intakes were remarkably similar between the two dietary groups.

Van Dam (1996) also found a higher feed intake of West African Dwarf goats fed lucerne pellets compared with those fed grass straw but, in contrast to the Scottish Blackface lambs, he reported no difference in the percentage decrease of digestible organic matter intake due to *T.vivax* infection between the two diets.

The diets used in the experiments of Chapters 5 and 6 were very similar to each other. However, possibly due to age differences the intake of the *ad libitum* fed barley straw of the lambs was different between both experiments. Whereas the older lambs

of Chapter 5 fed a diet high in barley concentrate and low in grass hay responded by having a higher barley straw intake than the lambs on the diet low in barley concentrate and high in grass hay, the younger lambs of Chapter 6 did not compensate the lower fibre intakes.

Although the feed intake reduction (15%) after the more pathogenic *T.vivax* infection (Chapter 6) was slightly greater than for the *T.congolense* infection (11%) (Chapter 5) the intake reduction was relatively small compared with the findings of Van Dam (1996). Van Dam (1996) using the same strain of *T.vivax* concluded that the reduction in feed intake of the West African Dwarf goats was considerable and ranged between 20 and 62%. The reason may lie in differences in feed intake responses to trypanosome infections between the Scottish Blackface sheep and West African Dwarf goats. Previous studies indicate a high repeatability of the feed intake response to successive trypanosome infections which may imply an innate characteristic of genetic origin (Clausen *et al.*, 1993; Wassink *et al.*, 1993). Van Dam (1996) found some evidence of a functional relationship between polymorphism of genes in the caprine leucocyte antigens (CLA) region and the degree of anorexia.

Wassink *et al.* (1993) reported a ranking (Pearson) correlation of -0.60 between the dry matter intake after a *T.congolense* infection and rectal temperature in West African Dwarf goats. However, it is not likely that fever affects feed intake itself. Both the anorexia and fever are likely to have been triggered by cytokines (Van Miert *et al.*, 1986; 1992).

The body weight gains of the lambs on the barley straw and lucerne hay roughages (Chapter 4) were much more affected by the type of diet than by the *T.congolense* infection. This may be an indication that the *T.congolense* infection was

relatively mild, although the dry matter content of the carcase was found to be slightly lower in the infected lambs. Van Dam (1996) found that both the *T.vivax* infection and the type of diet (lucerne pellets or grass straw) significantly affected body weight changes in West African Dwarf goats but no interaction effect between diet and infection on body weight gain was observed. However, in contrast to the control sheep of the experiment in Chapter 4 the control goats were fed *ad libitum* and a large part of the decrease in weight gain found in the *T.vivax* infected goats can be attributed to the approximately 35% decrease in digestible organic matter intake.

The *T.congolense* infected lambs in Chapter 5 were growing slower than their infected pair-fed controls. However, differences were small. The higher digestible undegraded protein (DUP) in the lambs fed Diet GH was not reflected in higher body weight gains in these lambs. Katunguka-Rwakishaya *et al.* (1993) found a positive effect of protein supplementation on the body weight changes in *T.congolense* infected lambs compared to those on a low protein diet. The dietary protein source used would have contained high levels of digestible undegraded protein (DUP). However, it is difficult to compare the results on body weight gains of Katunguka-Rwakishaya *et al.* (1993) with the results of this chapter 5 since the differences in intake of metabolisable protein (MP) in their experiment were much greater between the groups.

Although the *T.vivax* infection appeared more pathogenic than the *T.congolense* infection the changes in body weight gains were relatively small (Chapter 6). Whereas the *T.vivax* infected lambs lost some weight their pair-fed controls gained some weight. There was an indication that the lambs on the diet with the higher digestible undegraded protein (DUP) lost less weight than the lambs on the other diet but differences were not statistically significant. Since the sheep were pair-fed the

results indicate that both the *T.congolense* and *T.vivax* infected lambs lost some weight gain potential due to infection compared with their pair-fed controls.

The supplementation with urea to the lambs in the experiment described in Chapter 7 did not result in higher weight gains compared with the lambs on the basal diet. It is not clear why the additional urea was not beneficial, since it increased the microbial protein supply to the lambs. There may have been an asynchrony between the available nitrogen and fermentable metabolisable energy (FME) in the rumen. The body weight gains decreased due to the *T.congolense* infection in the lambs on both diets to a similar extent. It appears that the extra urea did not ameliorate the effects of the trypanosome infection.

Supplementation with urea alone reduced the effects of gastrointestinal infection and improved the productive performance of sheep (Knox and Steel, 1996). However, a large part of these positive effects could be attributed to the stimulatory effect of urea on feed intake counteracting the depressive effect of parasites on appetite. Similar studies in goats infected with *T.colubriformis* showed that urea supplementation alone had no beneficial effect but when the goats were supplemented with both urea and cotton seed meal the differences in body weight gain between infected and control goats were eliminated. However, no information was available on the response to cotton seed meal alone.

Wallace *et al.* (1994) reported that the pathophysiology of *H.contortus* infection in a genetically susceptible breed (Hampshire Down sheep) can be reduced by the addition of urea (≡ 60 g CP/kg DM) to the basal ration containing 88 g crude protein per kg dry matter but only if the feed intake is sufficient for both growth and maintenance.

From these results on research to urea supplementation in parasitised ruminants it can be concluded that the urea supplementation is only beneficial in certain circumstances which are not clear-cut. It appears that the greatest value of urea supplementation to parasitised ruminants lies in its ability to improve the quality and intake of poor quality roughages and thereby increasing the nutritional status of the animal rather than a direct effect of the extra protein available.

Digestive function

The organic matter and the related gross energy digestibility coefficients were in general slightly lower in the infected lambs compared with their pair-fed controls in the experiments of Chapters 4, 5 and 6. However, differences were very small and appeared to be unaffected by either the pathogenicity of the disease or the type of diet. Hamminga (1989) and Van Dam (1996) found no evidence of reduced digestibility coefficients during their experiments using *T.vivax* infected West African Dwarf goats. Akinbamijo *et al.* (1992) also reported no changes to the organic matter digestibility coefficients in *T.vivax* infected West African Dwarf goats. However, the control animals in all these experiments were not pair-fed.

The fibre digestibility coefficients of the experiments in Chapters 5 and 6 were unaffected by the trypanosome infections.

The crude protein or nitrogen digestibility coefficients were also slightly lower in the infected lambs compared with their pair-fed controls. The possible reasons for these lowered nitrogen digestibility coefficients are discussed in the chapter on nitrogen balance.

The organic matter digestibility coefficients were lower in the infected lambs despite the longer mean retention time of the roughage through the digestive tract found in all three experiments (Chapters 4, 5 and 6). The reason for this longer mean retention time is not clear but appears to be due to a slower rate of passage throughout the entire digestive tract. Van Miert *et al.* (1986) found inhibition of ruminal contractions during the acute phase response in *T.vivax* infected goats and later found reduced forestomach motility in West African Dwarf goats by recombinant bovine cytokines (IL-1β, IL-2) and IFN-γ (Van Miert *et al.*, 1992). The effects of the milder *T.congolense* on the mean retention did appear to be greater than the effects of the more pathogenic *T.vivax* infection indicating that the effects of trypanosomiasis on the mean retention time is independent of the pathogenicity of the disease.

A slight interaction effect between diet and infection on the mean retention time was found in the *T.congolense* infected lambs of Chapter 5 but not in the *T.vivax* infected lambs of Chapter 6. This difference may have been due to the higher straw intake of the *T.congolense* infected lambs on the high barley concentrate/low grass hay diet in Chapter 5 compared with the *T.congolense* infected lambs on the low barley concentrate/high grass hay diet. No such differences were observed in the straw intake of the two *T.vivax* infected dietary groups in Chapter 6. These results indicate that the effects of trypanosome infections on the mean retention time depend on the level and quality of the roughage intake.

Nitrogen Balance

The nitrogen balance was only measured in the experiments described in Chapters 5 and 6. No differences were observed in the nitrogen retention of the lambs infected with the mild *T.congolense* and their pair-fed controls. The nitrogen retention

of the lambs infected with the more pathogenic *T.vivax*, however, was significantly lower than that of their pair-fed controls. This effect was much greater in the lambs fed Diet BG, the diet lower in digestible undegraded protein (DUP) than in the lambs on Diet GH, the diet higher in DUP. The lower nitrogen retention in the infected lambs on Diet BG was mainly due to a higher urinary nitrogen excretion. It appears that the extra digestible undegraded protein (DUP) is very useful to the infected lambs and reduces nitrogen losses. The reason behind these reduced nitrogen losses are not clear. Since no such effect was observed in the *T.congolense* infected animals the usefulness of the digestible undegraded protein (DUP) appears to be related to the pathogenicity of the disease and/or level of nutrition. Further research is required but these results indicate that strategic feeding of ruminants at risk from trypanosome infections with diets high in digestible undegraded protein (DUP), such as legumes, may enhance the animals ability to withstand the effects of the disease.

The faecal nitrogen excretion was slightly higher in the infected lambs leading to slightly lower nitrogen digestibility coefficients compared with the pair-fed controls. Hamminga (1989) and Van Dam (1996) reported no differences in nitrogen digestibility coefficients during their nitrogen balance experiments between *T.vivax* infected West African Dwarf goats and their controls. However, none of the control animals were pair-fed which makes comparison difficult. It is not clear whether the nitrogen digestibility coefficients were reduced during this *T.vivax* infection due to increased endogenous nitrogen excretion in the faeces or to a reduced dietary nitrogen digestibility. The finding that the faecal nitrogen excretion was increased in both infected dietary groups to a similar extent rules in favour of a reduced dietary nitrogen digestibility.

Haematology

The *T.congolense* infection caused less anaemia in the Scottish Blackface lambs than during previous experiments by Katunguka-Rwakishaya (1992) using the same strain of trypanosomes and the same breed of sheep. Generally, the packed cell volume values decreased between approximately day 7 and 20 after infection and then stabilised at levels approximately 5 percentage points lower than their controls. The anaemia found during the more pathogenic *T.vivax* infection was slightly greater than the anaemia found during the *T.congolense* infection but the pattern was found to be similar. After the initial drop in packed cell volume between approximately day 7 and 20 the packed cell volume levels stabilised at around 10 percentage points below the values of the pair-fed controls. However, the average packed cell volume levels did not get below approximately 25%. The West African Dwarf goats infected with the same strain of *T.vivax* showed a more severe anaemia with average packed cell volume levels close to 20%. These results indicate that the Scottish Blackface lambs used in the experiments were able to maintain reasonable levels of packed cell volume during trypanosome infections. A dietary effect on the levels of packed cell volume was found in the experiment in Chapter 4 with the lucerne hay fed lambs having a higher packed cell volume than the barley straw fed lambs. However, no differences in the response of the packed cell volume to infection were found. Only in Chapter 5 a significant interaction effect between diet and the *T.congolense* infection was found with the lambs having the higher intake of digestible undegraded protein (DUP) having a lower decrease in packed cell volume.

The red blood cell counts and haemoglobin levels of the trypanosome-infected sheep generally followed the same pattern as the packed cell volume.

The mean corpuscular volume increased in the *T.congolense* infected lambs in response to the anaemia. The response was highest during the latter stages of the experiments and was independent of the type of diet. Surprisingly, the mean corpuscular volume was not significantly affected by the *T.vivax* infection. This lack of erythropoietic response may have been due to the low protein intake of the *T.vivax* infected lambs. Katunguka-Rwakishaya *et al.* (1993) found that the mean corpuscular volume response to anaemia was much greater in the *T.congolense* infected lambs on a high protein intake than in those on a low protein intake. Hardly any changes were found in the mean corpuscular haemoglobin but generally the mean corpuscular haemoglobin concentration was decreased after trypanosome infections.

Blood biochemistry

Plasma cholesterol concentration decreased significantly after trypanosome infection in all experiments. This is consistent with the findings of Katunguka-Rwakishaya (1992) who also reported a decrease in plasma cholesterol levels. The plasma cholesterol concentration stabilised about 20 days after infection. The plasma cholesterol concentration of the barley straw fed animals in Chapter 4 was found to be significantly higher pre-infection than the concentration of the lucerne hay fed lambs but this did not affect the response to infection. A strong positive relationship was found in Chapter 4 between the pre-infection cholesterol levels and the parasitaemia levels during the first month after the *T.congolense* infection. Trypanosomes are known to depend on the host for cholesterol for their cell growth and the higher

plasma cholesterol levels would have provided an excellent substrate for the parasites. However, this relationship was not repeated in the other experiments, possibly due to the fact that the variability of cholesterol levels between the Scottish Blackface sheep is too small. However, differences in plasma cholesterol levels are known to exist between breeds (Traore-Leroux *et al.*, 1987) and further research is needed to assess its significance.

The plasma urea levels found during the experiments were dependent upon the level of dietary protein (Chapters 4, 5 and 6) or nitrogen (Chapter 7) intake of the lambs. The effects of the trypanosome infections on the plasma urea levels were not very consistent. Both increased (Chapter 4) and decreased (Chapter 7) plasma urea levels were found due to trypanosome infections.

The plasma albumin concentration was also affected by the level of protein intake by the lambs (Experiment 4). Plasma albumin levels were found to be lowered during the trypanosome infections which is consistent with the findings of Katunguka-Rwakishaya (1992) and Van Dam (1996). The decrease in plasma albumin levels were greater during the more pathogenic *T.vivax* infection than during the milder *T.congolense* infection. No evidence was found of an interaction effect between diet and infection on plasma albumin levels which is consistent with the findings of Van Dam (1996). However, other experiments have shown greater decreases in plasma albumin levels in *T.congolense* infected lambs fed a low protein diet compared to infected lambs on a high protein diet (Katunguka-Rwakishaya *et al.*, 1993). It appears that the level of protein has to be significantly reduced before a difference in response to high protein diets during trypanosome infections can be detected. It is not clear what causes the decrease in plasma albumin levels during trypanosome infections.

Haemodilution (Katunguka-Rwakishaya, 1992), uptake of albumin by trypanosomes (Coppens *et al.*, 1987) and catabolism of albumin in favour of γ-globulin production (Beisel, 1985) have all been implicated.

Plasma nitric oxide was found to be significantly lower in lambs fed a urea-supplemented diet compared to those on a basal diet. Plasma nitric oxide levels during the *T.congolense* infection were increased in lambs showing a high parasitaemia on sampling days. Plasma nitric oxide produced by macrophages has, among others, both host immunosuppressive and trypanosome cytostatic properties. Furthermore, haemoglobin has nitric oxide scavenging abilities (Mabbott *et al.*, 1994; Sternberg *et al.*, 1994). It is not clear which feature has the upper hand and it is therefore difficult to assess the impact of nitric oxide on both the host and the trypanosomes.

Conclusions

1. Trypanosome infections lead to a reduction in feed intake in Scottish Blackface sheep. The level of the reduction in feed intake is dependent upon the quality of the roughage involved.
2. The digestibility coefficients of organic matter and gross energy are slightly decreased during trypanosome infections but the changes are relatively small and appear to be independent of the type of diet and pathogenicity of the trypanosome infection. The fibre (NDF and ADF) digestibility coefficients are unaffected by trypanosome infections. Trypanosome infections result in a small decrease in the digestibility coefficient of nitrogen independent of the type of diet and pathogenicity of the infection.
3. The mean retention time of the roughage through the digestive tract is significantly longer in trypanosome-infected Scottish Blackface sheep. The longer mean retention time is affected by the level of lower quality roughage intake but is independent of the pathogenicity of the disease.
4. The effect of trypanosome infections on the nitrogen balance is dependent upon the level of feeding and the pathogenicity of the disease. A higher content of digestible undegraded protein in the diet reduces nitrogen losses in the urine during trypanosomiasis and thereby increases the nitrogen retention. The strategic use of diets high in digestible undegraded protein in ruminants at risk from trypanosome infection may increase their ability to withstand the effects of the disease.
5. Supplementation of the diet with urea did not prove to be beneficial to trypanosome-infected Scottish Blackface sheep.

6. The anaemia observed in the trypanosome-infected Scottish Blackface sheep was relatively mild. The anaemia was independent of the type of diet but was higher in the more pathogenic *T.vivax* than in the milder *T.congolense* infected sheep. The erythropoietic response to the anaemia may be inhibited in lambs on a low intake of dietary protein.
7. Plasma cholesterol levels are significantly reduced in trypanosome-infected lambs irrespective of the type of diet or infection. There was evidence of a relationship between plasma cholesterol levels and the number of parasites. The reduction in plasma albumin levels was greater in the *T.vivax* infected sheep than in the *T.congolense* infected sheep.
8. Plasma nitric oxide concentrations are significantly increased in trypanosome-infected lambs at times of high parasitaemia.
9. In mice dietary *L*-arginine may play an important role in the host's defence mechanism against a trypanosome infection.
10. More research is needed into the advantages of feeding digestible undegraded protein to parasitised ruminants. More knowledge on how these animals use the digestible undegraded protein would be useful.

Appendix

Metabolisable energy, fermentable metabolisable energy, effective rumen degradable protein, digestible undegraded protein and metabolisable protein estimations (e.g. Chapter 4, Table 4.2, page 70 and Table 4.4, page 78)

The rate constants a, b and c were estimated from the effective nitrogen degradability using the model: $dg = a + b\{1-e^{(-ct)}\}$

where a = water soluble N extracted by cold water rinsing,

b = potentially degradable N, other than water soluble N and

c = fractional rate of degradation of feed N per hour.

The effective rumen degradable protein (ERDP) can than be calculated from:

ERDP (g/kg DM) = 0.8[QDP] + [SDP]

where QDP (g/kg DM) = Quickly Degradable Protein = a x [CP] (g/kg DM)

SDP (g/kg DM) = Slowly Degradable Protein = {(b x c)/(c + r) x [CP] (g/kg DM) (r = rumen outflow rate)

The undegradable protein (UDP) can be calculated from:

UDP (g/kg DM) = [CP] - {[QDP] + [SDP]}

The digestible undegraded protein (DUP) is then calculated from:

DUP (g/kg DM) = 0.9{[UDP] - 6.25[ADIN]}

where ADIN = Acid detergent insoluble nitrogen.

Metabolisable protein (MP) can than be calculated as:

MP (g/kg DM) = 0.6375ERDP + DUP

Calculations on the composition and pre-infection supplies of energy and protein of Diet BS (Chapter 4)

Diet BS	Composition on a dry matter basis						Supplied by diet BS						
	DM (g/kg)	ME# (MJ/kg)	FME# (MJ/kg)	CP (g/kg)	ERDP* (g/kg)	DUP* (g/kg)	DM intake (kg/day)	ME (MJ/day)	FME (MJ/day)	CP (g/day)	ERDP (g/day)	DUP (g/day)	MP (g/day)
Barley Straw	873	6.5	5.9	46	27	8	0.510	3.3	3.0	23.5	13.8	4.1	
Barley/Soya Bean Meal	862	13.3	12.7	209	138	71	0.366	4.9	4.7	76.5	50.5	26.0	
Total							0.876	8.2	7.7	100.0	64.3	30.1	71

: AFRC (1993) values
* : Calculated using the equations explained on the previous page

M/D = 8.2/0.876 = 9.4 r = 0.05 y = 9.5 L = 1.4-1.7
MCP = 73.2 from FME supply (7.7 x 9.5 = 73.2)
MCP = 64.3 from ERDP supply
ERDP is limiting MCP supply in this ration.

Calculations on the composition and pre-infection supplies of energy and protein of Diet LH (Chapter 4)

Diet LH	Composition on a dry matter basis						Supplied by diet LH						
	DM (g/kg)	ME# (MJ/kg)	FME# (MJ/kg)	CP (g/kg)	ERDP* (g/kg)	DUP* (g/kg)	DM intake (kg/day)	ME (MJ/day)	FME (MJ/day)	CP (g/day)	ERDP (g/day)	DUP (g/day)	MP (g/day)
Lucerne Hay	894	8.8	7.8	206	116	47	0.942	8.3	7.3	194.0	109.3	44.2	
Barley	862	13.3	12.7	109	84	18	0.366	4.9	4.7	39.9	30.8	6.6	
Total							1.308	13.2	12.0	233.9	140.1	50.8	140

: AFRC (1993) values
* : Calculated using the equations explained on the previous page

M/D = 13.2/1.308 = 10.1 r = 0.08 y = 10.5 L = 2.2-2.7
MCP = 126 from FME supply (12.0 x 10.5 = 126)
MCP = 140.1 from ERDP supply
FME is limiting MCP supply in this ration.

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