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The patho-physiology of *Trypanosoma congolense* in Scottish Blackface sheep. Influence of type of roughage on digestive function

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Abstract

The influence of types of roughage, barley straw (Diet B) versus lucerne hay (Diet L), on the patho-physiology of a *T.congolense* infection was compared in 8 pairs of Scottish Blackface male twin lambs. One animal of each twin pair was infected and the other used as a pair-fed control. Voluntary feed intake, body weight, digestive function, various blood haematological and biochemical parameters were measured.

Voluntary organic matter intake decreased significantly after the *T.congolense* infection, the decrease being greater in the diet L group lambs ($p<0.01$) than in the diet B group ones ($p<0.05$). The digestibility coefficients of crude protein and organic matter were significantly lower in the infected lambs ($p<0.01$). Mean retention time of the roughage through the digestive tract in the animals fed barley straw was significantly longer ($p<0.05$) due to a lower rumen outflow rate constant ($p<0.01$). Infection resulted in longer mean retention times ($p<0.01$).

Packed cell volume (PCV) was significantly lower before infection in the animals fed diet B ($p<0.01$). After infection, diet ($p<0.01$) and infection ($p<0.01$) had an additive effect on PCV. The anaemia was both macrocytic ($p<0.05$) and hypochromic ($p<0.01$).

Diet B resulted in higher plasma cholesterol ($p<0.05$), but lower plasma urea ($p<0.01$) and albumin ($p<0.01$) concentrations before infection than diet L. The *T.congolense* infection significantly lowered plasma cholesterol ($p<0.01$) and increased plasma urea ($p<0.01$) concentrations compared with the uninfected controls. Plasma albumin concentrations decreased, but were more affected by nutrition ($p<0.01$) than by infection ($p<0.05$.

It was concluded that the patho-physiological effects of the *T.congolense* infection in the Scottish Blackface lambs were affected by the type of roughage offered, but that these effects were additive rather than interactive to the effects of infection.

Keywords: Trypanosomiasis, Roughage, 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 study conducted 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 have 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 the effects of diet on an infection with *T.congolense* were investigated in Scottish Blackface lambs 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.

Materials and methods

Animals, feed and housing

Eight pairs of twin castrate Scottish Blackface lambs (aged 5 months) were selected from a local hill farm flock in the West of Scotland. Four extra lambs were slaughtered at the start of the experiment to use as baseline control animals.

Four pairs were offered chopped barley straw plus 425 g of a pelleted barley/soya bean meal concentrate mixture (diet B) and the other 4 pairs chopped lucerne hay plus 425 g of pelleted barley concentrate (diet L). The concentrates were offered in order to provide the lambs on diet B 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 I. Diet B resulted in a crude protein (CP) and metabolisable energy (ME) intake of approximately 104 g/day and 7.9 MJ/day, respectively. The CP and ME intake for the animals on diet L was approximately 250 g/day and 14.0 MJ/day, respectively. The animals were fed twice a day at 09:00 and 15:00. The animals had free access to fresh water at all times. The lambs were housed in individual pens on a concrete floor bedded with wood shavings.

Infection

Three weeks after the experiment started the lambs in group I were infected with *T.congolense* 1180 (GRVPS 57/6) isolated in Serengeti, Tanzania (Nantulya *et al.*, 1984). The trypanosomes were obtained from irradiated mice during the first rising parasitaemia. 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

Individual food intakes were recorded daily by collecting refusals between 8.00 and 9.00 h. The animals were weighed once a week.

Organic matter (OM) and crude protein (CP) digestibilities of the feed were measured during 3 digestibility periods of 7 days each, one before and 2 after infection. During each digestibility period, a representative sample was taken from the food offered and analysed for dry matter (DM), ash, and CP content (MAFF *et al.*, 1981). For each animal a composite sample of bulked refusals was taken and analysed for DM, ash and CP. Total faecal output for each individual animal was weighed and two composite samples taken. One sample was analysed for DM and ash and the other was slurried using approximately 5 ml of toluene and 20 ml of water for CP analysis. The metabolisable energy intake (ME) was estimated using the digestible organic matter intake before infection (AFRC, 1993).

The rate of passage of the roughage through the digestive tract was measured using chromium as a marker. The chromium was mordanted to the roughage fibre using the method described by Uden *et al.* (1980, 1982). After feeding 30 to 50 g of the chromium mordanted hay to the animals faecal samples were taken at 8, 11, 17, 23, 30, 38, 48, 72, 96 and 120 h. The concentration Cr in the faeces was determined using Atomic Absorption Spectroscopy after wet digestion according to the method by Christian and Coup (1954).

Thrice weekly 10 ml of blood was collected from the jugular vein for measurement of packed cell volume (PCV) and intensity of parasitaemia (Murray *et al.*, 1977; Paris *et al.*, 1982). Once a week, mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration

(MCHC) were determined by an automated blood cell counter (ABX Minos, Roche Diagnostica).

Plasma cholesterol and urea were determined using commercial kits and plasma albumin concentration by the standard biuret method using an auto analyser (Technicon, UK).

On the day the experiment finished all animals were slaughtered and skinned. The right carcase half was macerated and a sample of 500 g taken and stored at -20 ^{o}C. Later, about 200 g of the sample was freeze dried to a constant weight in a high vacuum freeze drier. The dried samples were finely chopped in a liquidiser and sub-samples analysed for protein and ether extract (MAFF *et al.*, 1981). The dry matter, protein and crude fat gain of the animals during the experimental period was determined by subtracting the values of the baseline control animals.

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 consideration of variation between treatments, between animals within treatments and interaction between treatments. Intensities of parasitaemia were evaluated by the parametric Mann-Whitney test. Growth rates were determined using linear regression analysis. Differences in organic matter intake in the group I animals before and after infection with *T.congolense* were tested using the paired student's *t*-test.

The average digestibility of the diets was calculated over three digestibility periods. Period 1 lasted from day -10 to day -3 before infection, period 2 fom day 19 to day 26 after infection and period 3 from day 50 to 57 after infection.

The model of Dhanoa *et al.* (1985) was used to analyse the chromium excretion data, which contains an exponential term and 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 retention time between the time of 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).

Results

Feed Intake

The dry matter intake (DMI) before infection of the sheep on diet L was much higher than that of the sheep on diet B ($p<0.05$). The intakes of NDF and ADF were similar between the animals on both diets, while those of CP and ME were about 2.5 and 1.8 times higher in the animals fed diet L (Table II).

The organic matter intake (OMI) was slightly higher in the I animals compared to their pair fed controls, but this was mainly the case during the pre-infection period (Figure 1). Between day 12 and 21 the organic matter intake (OMI) of the LI group fell, resulting in the OMI being

significantly different pre- and post-infection ($p<0.01$; Table III). The BI group did not show a clear depression in OMI, but the OMI decreased after infection ($p<0.01$).

It was observed that the lambs on the barley straw (diet B) 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 L) was more spread over the 24 hour period.

Digestibility of the diets

The organic matter (OM) digestibility was approximately 10 units higher in diet L than in diet B ($p<0.01$; Table IV). The OM digestibility remained stable in the I groups on both diets throughout the trial period. However, OM digestibilities increased in the PC animals as the trial progressed ($p<0.01$). The concentrate to roughage ratios did not change much in the animals fed diet B but increased in the lambs fed diet L from 0.43 in digestibility period 1 to 0.63 in digestibility period 2. In digestibility period 3 the ratio returned to 0.51.

The crude protein (CP) digestibility was approximately 8 units higher in the lambs fed diet L compared to the ones on diet B ($p<0.01$; Table IV). Whereas the CP digestibilities remained stable in the PC lambs throughout the trial period the digestibilities of the CP in the I lambs decreased ($p<0.01$). The effects of diet and infection on the OM and CP digestibilities were additive.

Rate of passage of the roughage

The mean retention time (MRT) of the animals fed diet B was significantly longer than those of the animals fed diet L ($p<0.05$) which appeared to be caused by a lower outflow rate constant k_1 ($p<0.01$) in the animals fed diet B (Table V).

Both the MRT ($p<0.01$) and transit time (TT) ($p<0.05$) were significantly longer in the I animals compared with the PC. 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 rate of passage parameters were found (Table V).

Body weight

The body weight changes of the PC followed the changes of their I counterparts closely (Figure 2). Growth rates were 50.9 ± 3.0, 37.7 ± 3.8, 184.9 ± 14.7 and 159.4 ± 20.0 grams per day for the BI, BPC, LI and LPC groups, respectively. Growth was significantly affected by nutrition ($p<0.01$) but not by the *T.congolense* infection.

Carcase composition

The carcase of the group given the B diet had a significantly lower DM content than the diet L group ($p<0.01$; Table VI). The type of diet affected both the fat and protein content ($p<0.01$) of the carcase, with the diet L fed animals showing the higher ether extract, but the lower protein content per kg DM. Total carcase DM, EE and CP gain were significantly higher in the lambs fed diet L ($p<0.01$).

Infection resulted in significantly lower DM content and total carcase DM gain ($p<0.05$). The total carcase DM gain was about 500 g lower in the I compared to the PC. Ether extract and CP contents of the carcase were not significantly different between the I and PC. Total carcase EE and CP gains were lower in the I groups compared to their PC counterparts, however, differences were not statistically significant (Table VI).

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

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 LI than in the BI (Figure 3). The second peak parasitaemia in the LI group started earlier and was higher than the parasitaemia in the HI group which gradually increased to its second peak. After that, the intensities of parasitaemia fluctuated in both groups. None of the differences between the dietary groups were found to be statistically significant.

Packed Cell Volume

The PCV before infection was found to be significantly lower in the lambs fed diet B ($p<0.01$) and tended to be lower in the group I animals compared with the PC ($p<0.05$; Table VII). After infection the PCV of the lambs on both diets showed a gradual decrease from approximately day 10 to day 20 after which the PCV stabilised at around 30% for the LI group and 27% for the BI group (Figure 4). The PCV was affected by both infection ($p<0.01$) and diet ($p<0.01$). PCV in the LI lambs seemed to be more affected by the infection than the BI (Figure 4) but no interactive effect between infection and diet was found.

Mean Corpuscular Volume

The MCV did not appear to be significantly affected by nutrition (Table VII). *T.congolense* infection resulted in a slight rise of MCV in the I groups on both diets ($p<0.05$). The MCV in the PC groups also showed a slight increase during the trial period.

Mean Corpuscular Haemoglobin Concentration

No significant differences in MCHC were found due to nutrition before or after infection. Infection resulted in a significant decrease in MCHC in group I animals on both diets compared with their PC ($p<0.01$; Table VII).

Plasma Cholesterol Concentration

Plasma cholesterol concentration was lower in lambs on the lucerne hay than those on the barley straw diet ($p<0.05$; Table VIII). The plasma cholesterol levels in the PC and I group on diet B were still rising during the pre-infection period. Infection significantly lowered plasma cholesterol concentrations ($p<0.01$; Table VIII). Figure 5 shows that there was a sharp decrease in plasma cholesterol immediately after infection in the BI group which appeared to stabilise at the same level as the control animals fed diet L. However, no significant interaction was found between diet and infection on plasma cholesterol concentration (Table VIII).

A relationship was found between the average plasma cholesterol concentration before infection and the average intensity of parasitaemia during the first month after infection ($r=.90$; $p<0.01$). However, one has to take into account that the intensity of parasitaemia is not normally distributed.

Plasma Urea Concentration

A strong nutritional effect was found before infection on plasma urea concentration ($p<0.01$; Table VIII). A sharp decrease in plasma urea concentration in both the LI and LPC groups was detected around day 23 possibly due to the feed intake depression at that time (Figure 6). Plasma urea concentration increased around day 7 after infection, especially in the LI lambs.

Infection significantly increased plasma urea concentrations ($p<0.01$). The nutritional and interaction effects on plasma urea concentration were not significant after infection.

Plasma Albumin Concentration

A significant difference in plasma albumin concentration was detected between lambs on diet B and L ($p<0.01$; Table VIII). Infection resulted in a decrease in plasma albumin concentration in the I groups fed both diets ($p<0.05$) but the nutritional effect was still greater ($p<0.01$; Figure 7). The effects of nutrition and infection on plasma albumin concentration were additive (Table VIII).

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. While the mean retention time was longer in the infected animals this did not give the expected increase in organic matter and crude protein digestibilities which were lower than in their pair-fed counterparts. However, no differences 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 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 to the effects of infection.

Although there was a large difference in dry matter intake of the lambs between the two diets the total intakes of NDF and ADF were very similar. This is in accordance with results of experiments to the relationship between feed intake and cell wall percentage (Van Soest, 1982).

The organic matter intake (OMI) of the infected group fed diet L was more depressed than the OMI of the infected group fed diet B. The severe depression in OMI between day 12 and 21 in lambs on diet L 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 seem to affect OMI in the BI animals. 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 eg. 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 (WAD) sheep fed *Panicum maximum* and cassava peel with or without a 1:1 mixture of *Leucaena leucocephala* and *Gliricidia sepium.* Depression of voluntary intake of alfalfa pellets was 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 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).

The organic matter (OM) digestibility results indicated no direct effect of the infection on OM digestibility but an increase in OM digestibility in the PC lambs ($p<0.01$), despite the shorter mean retention time (MRT). One would expect a shorter MRT to lead to lower digestibility. Verstegen *et al.* (1991) did not find any differences in DM digestibility between

T.vivax infected WAD goats and their controls, but they found a reduced feed intake after infection.

The *T.congolense* infection resulted in a decrease in crude protein digestibility ($p<0.01$). Reduced apparent digestibility of N 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). 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 might expect more urea to be recycled when the MRT is longer. The increase in plasma urea concentration was more pronounced in the T.congolense infected lambs fed barley straw which was possibly due to the fact that the feed energy level was too low to enable all the urea nitrogen to be utilised by the rumen micro-organisms.

As expected, the animals on diet B had a longer MRT than the animals fed diet L which was due to a significant lower outflow rate constant k_1 ($p<0.01$) which is considered to be the rumen outflow rate constant (Aitchison *et al.*, 1986). The MRT of the roughage was significantly longer in the infected animals than their respective PC counterparts ($p<0.01$). The results indicate that the longer MRT in the infected lambs was due to a slower rate of passage throughout the entire digestive tract. In previous experiments, 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 changes of the infected animals in both dietary groups were not significantly different from their pair-fed control counterparts. Katunguka-Rwakishaya *et al.* (1993) found that *T.congolense* infected Scottish Blackface lambs on a high protein diet (176 g/day CP and 9.8 MJ/day ME intake as compared to 250 g/day CP and 14 MJ/day ME intake of diet L in this experiment) had similar growth rates to the control animals on the high protein diet. In a similar experiment, but using two levels of energy intake, *T.congolense* infected Scottish Blackface lambs on the low level of energy intake (99 g/day CP and 5.5 MJ/day ME intake as compared to 104 g/day CP and 7.9 MJ/day ME intake of diet B in this experiment) were growing at a similar rate as their controls (Katunguka-Rwakishaya,1992). In contrast, *T.congolense* infected Scottish Blackface lambs receiving a diet low in protein (81 g/day CP and 10.1 MJ/day ME intake) and the ones on a high energy diet (101 g/day CP and 9. MJ/day ME intake) had significantly lower growth rates than the controls (Katunguka-Rwakishaya, 1992; Katunguka-Rwakishaya *et al.*,1993). These results indicate that *T.congolense* infected sheep do less well compared to their controls when the intake of crude protein is low compared to the energy intake.

In the present experiment carcase DM content and total carcase DM were significantly lower in the infected animals compared to their pair-fed controls ($p<0.05$) on both diets. One possible explanation may be that the lower plasma albumin concentration in the infected animals caused edema (Bland, 1952) which in turn might have lowered the carcase DM content. The lower carcase DM led to lower total carcase CP and EE in the infected lambs compared with the pair-fed controls, although this was not statistically significant due to the

variation in differences between the pairs. Katunguka-Rwakisjaya (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.

The dramatic increase in maintenance requirements reported by Verstegen *et al.* (1991) in *T.vivax* infected WAD goats was not obvious in this experiment. However, the lower dry matter gain and hence the lower energy retention may suggest higher maintenance requirements in infected animals.

The first two peak parasitaemias tended to be higher in the BI than in the LI. Otesile *et al.* (1991) found that pigs 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. No difference in intensity of parasitaemia could be attributed to the plane of nutrition, maintenance and sub-maintenance, in *T.vivax* infected WAD sheep (Reynolds and Ekwuruke, 1988). The theory that the parasites are affected by the nutritional status of the host is not supported by the results of these experiments. However, a significant relationship was found between the plasma cholesterol concentration before infection and the average intensity of parasitaemia during the first month after infection ($r=.90$; $p<0.01$). 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) cholesterol levels might play an important role in trypanotolerance.

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

The PCV 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 L fed animals no interaction was found between nutrition and infection on PCV. Katunguka-Rwakishaya (1991) found that the PCV 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 PCV than animals on a higher level of protein (Berry and Dargie, 1976). In contrast, no differences were observed of PCV in *T.congolense* infected sheep on two levels of protein (Katunguka-Rwakishaya *et al.*, 1993). However, in none of these experiments were dietary effects on PCV before infection observed.

The increase in MCV ($p<0.05$) and decrease in MCHC ($p<0.01$) in the *T.congolense* infected animals shows that the anaemia was both macrocytic and hypochromic. The macrocytic and hypochromic responses were similar in both dietary groups. The low digestible CP intake in the BI animals did not result in a lower erythropoietic response compared with the HI animals. 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 MCV 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 in both the MCV and MCHC 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 L was enough to support an increase in erythropoiesis. The CP intake of the lambs on the low protein diet in the experiment of Rwakishaya *et al.* (1993) was only 81 g/day whereas in this experiment it was approximately 104 g/day.

The cholesterol concentration was higher in the animals on diet B ($p<0.05$). 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 LI group suggesting direct uptake of cholesterol by trypanosomes.

Plasma urea concentrations followed digestible CP intake closely ($p<0.01$). Infection resulted in an increase in plasma urea concentration especially in the BI group possibly indicating catabolism of body protein in the BI group post-infection. These results are comparable to the findings of Abbott *et al.* (1986) in ovine haemonchosis at two levels of protein intake.

Plasma albumin concentrations were also significantly affected by nutrition ($p<0.01$). Katunguka-Rwakishaya *et al.* (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) in sheep fed a diet low in protein compared with those on a high protein diet.

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. Despite these results no difference could be observed between body weight changes of infected lambs and their pair-fed controls, although carcases of the infected lambs had lower dry matter contents. 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 energy intake. These effects were additive rather than interactive to the effects of infection.

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Table I Dry matter (DM), organic matter (OM), neutral detergent fibre (NDF), acid detergent fibre (ADF), ether extract (EE), gross energy (GE) and crude protein (CP) of the diet components

Diet Composition	Diet B		Diet L	
	Concentrate	Barley Straw	Concentrate	Lucerne Hay
DM (g/kg)	862	873	862	894
OM (g/kg DM)	944	953	956	901
NDF (g/kg DM)	229	781	256	343
ADF (g/kg DM)	52	490	46	265
EE (g/kg DM)	14	9	12	15
GE (MJ/Kg DM)	17.7	17.8	17.2	17.7
CP (g/kg DM)	209	46	109	206

Table II Dry matter (DM), neutral detergent fibre (NDF), acid detergent fibre (ADF), metabolisable energy (ME) and crude protein (CP) intake in sheep (group I; n=4) before (day -17 - 0) infection with *T.congolense* fed either diet B (barley straw) or L (lucerne hay)

Diet	DM (g/day)	NDF (g/day)	ADF (g/day)	ME (MJ/day)	CP (g/day)
B	855 ± 35	463 ± 27	163 ± 10	7.9 ± .4	104 ± 2
L	1322 ± 110	423 ± 38	177 ± 19	14.0 ± 1.1	250 ± 25
Diet effect	*	NS	NS	**	**

* : Significant difference between means ($p<0.05$)

** : Significant difference between means ($p<0.01$)

NS : No significant difference between means

Table III Organic matter intake OMI (g/kg$^{0.75}$/day) in sheep (I group; n=4) before (day -17 - 0) and after (day 1 - 58) infection with *T.congolense* fed either diet B (barley straw) or L (lucerne hay)

	Diet B (g/kg$^{0.75}$/day)		Diet L (g/kg$^{0.75}$/day)	
	I	PC	I	PC
Pre-infection (day -17 - 0)	67 ± 2.0	61 ± 2.1	95 ± 4.2	87 ± 3.7
Post-infection (day 1- 58)	63 ± 2.3	60 ± 2.2	79 ± 3.5	76 ± 3.9
Infection effect	**		**	

** · Significant difference between means ($p<0.01$)

Table IV Organic matter (OM) and crude protein (CP) digestibility of *T.congolense* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either diet B (barley straw) or L (lucerne hay) during period 1 (day -10 - -3), period 2 (day 19 - 26) and period 3 (day 50 - 57)

	OM digestibility			CP digestibility		
Period	1	2	3	1	2	3
BI	.62	.60	.61	.66	.64	.59
BPC	.60	.64	.67	.68	.68	.67
Pooled SE	.008	.010	.014	.008	.012	.019
LI	.73	.72	.72	.74	.70	.71
LPC	.72	.76	.76	.72	.74	.75
Pooled SE	.006	.009	.010	.007	.010	.015
Diet effect	**	**	**	**	**	**
Inf. effect	NS	**	**	NS	**	**
Interaction	NS	NS	NS	NS	NS	NS

** : Significant difference between means ($p<0.01$)

NS : No significant difference between means

Table V 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 B (barley straw) or L (lucerne hay) and their respective pair-fed controls (PC)

Group	MRT	TT	k_1	k_2
BI	62.67	14.50	.024	.177
BPC	55.36	12.97	.028	.213
Pooled SE	3.33	.49	.002	.017
LI	43.86	13.14	.040	.210
LPC	40.67	10.19	.040	.211
Pooled SE	1.33	.85	.001	.027
Diet effect	*	NS	**	NS
Infection effect	**	*	NS	NS
Interaction	NS	NS	NS	NS

* : Significant difference between means ($p<0.05$)

** : Significant difference between means ($p<0.01$)

NS : No significant difference between means

Table VI Carcase dry matter (DM (g/kg fresh matter (FM))), ether extract (EE (g/kg DM)) and crude protein (CP (g/kg DM)) composition and total carcase DM (g), EE (g) and CP (g) gain# of *T.congolense* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either diet B (barley straw) or L (lucerne hay)

	DM	EE	CP	DM	EE	CP
Group	(g/kg FM)	(g/kg DM)		(Total Carcase Gain (g))		
	342.1	375.1	457.3	1415.0	738.3	456.3
	375.8	405.0	438.6	1965.5	1091.4	615.7
Pooled SE	8.8	16.4	13.8	234.4	160.0	71.9
LI	432.7	536.1	326.2	5251.9	3483.7	1146.9
	451.5	542.8	327.0	5726.9	3796.3	1307.8
Pooled SE	7.0	7.8	6.6	320.6	219.2	92.3
Diet effect	**	**	**	**	**	**
Inf. effect	*	NS	NS	*	NS	NS
Interaction	NS	NS	NS	NS	NS	NS

* : Significant difference between means ($p<0.05$)

** : Significant difference between means ($p<0.01$)

NS : No significant difference between means

: Gain compared to the baseline control animals

Table VII Packed cell volume (PCV, %), mean corpuscular volume (MCV, fl) and mean corpuscular haemoglobin concentration (MCHC, %) of *T.congolense* infected (I) sheep (n=4) and their respective pair-fed controls (PC) fed either diet B (barley straw) or L (lucerne hay) during pre- (day -20 - -3) and post-infection (day 14 - 56)

	PCV		MCV		MCHC	
Group	Pre-	Post-	Pre-	Post-	Pre-	Post-
BI	30.5	26.9	26.3	28.9	36.4	35.6
BPC	31.9	29.9	27.3	28.3	36.7	36.5
Pooled SE	.8	.8	.3	.2	.2	.2
LI	33.9	30.5	26.8	29.4	36.6	36.1
LPC	36.6	35.9	26.8	27.9	36.9	37.1
Pooled SE	.7	1.1	.3	.4	.2	.3
Diet effect	**	**	NS	NS	NS	NS
Inf. effect	*	**	NS	*	NS	**
Interaction	NS	NS	NS	NS	NS	NS

* : Significant difference between means ($p<0.05$)

** Significant difference between means ($p<0.01$)

NS : No significant difference between means

Table VIII 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 B (barley straw) or L (lucerne hay) during pre- (day -20 - -3) and post-infection (day 14 - 58)

	Cholesterol		Urea		Albumin	
Group	Pre-	Post-	Pre-	Post-	Pre-	Post-
BI	1.06	.91	7.3	9.4	28.3	27.3
BPC	1.08	1.27	7.4	7.2	28.3	28.9
Pooled SE	.07	.10	.3	.5	.3	.3
LI	.76	.79	9.8	10.3	30.3	29.4
LPC	.76	.94	10.7	9.2	30.7	30.7
Pooled SE	.04	.06	.4	.4	.3	.4
Diet effect	*	NS	**	NS	**	**
Inf. effect	NS	**	NS	**	NS	*
Interaction	NS	NS	NS	NS	NS	NS

* : Significant difference between means ($p<0.05$)

** : Significant difference between means ($p<0.01$)

NS : No significant difference between means

Figure 1 Organic matter intake (OMI (g/kg$^{0.75}$ body weight/ day)) of *T.congolense* infected (I) sheep fed diet B -●- or L -■- and their respective pair fed controls (PC) -○- and -□-

Figure 2 Body weight changes (kg) of *T.congolense* infected (I) sheep fed diet B -●- or L -■- and their respective pair fed controls (PC) -○- and -□-

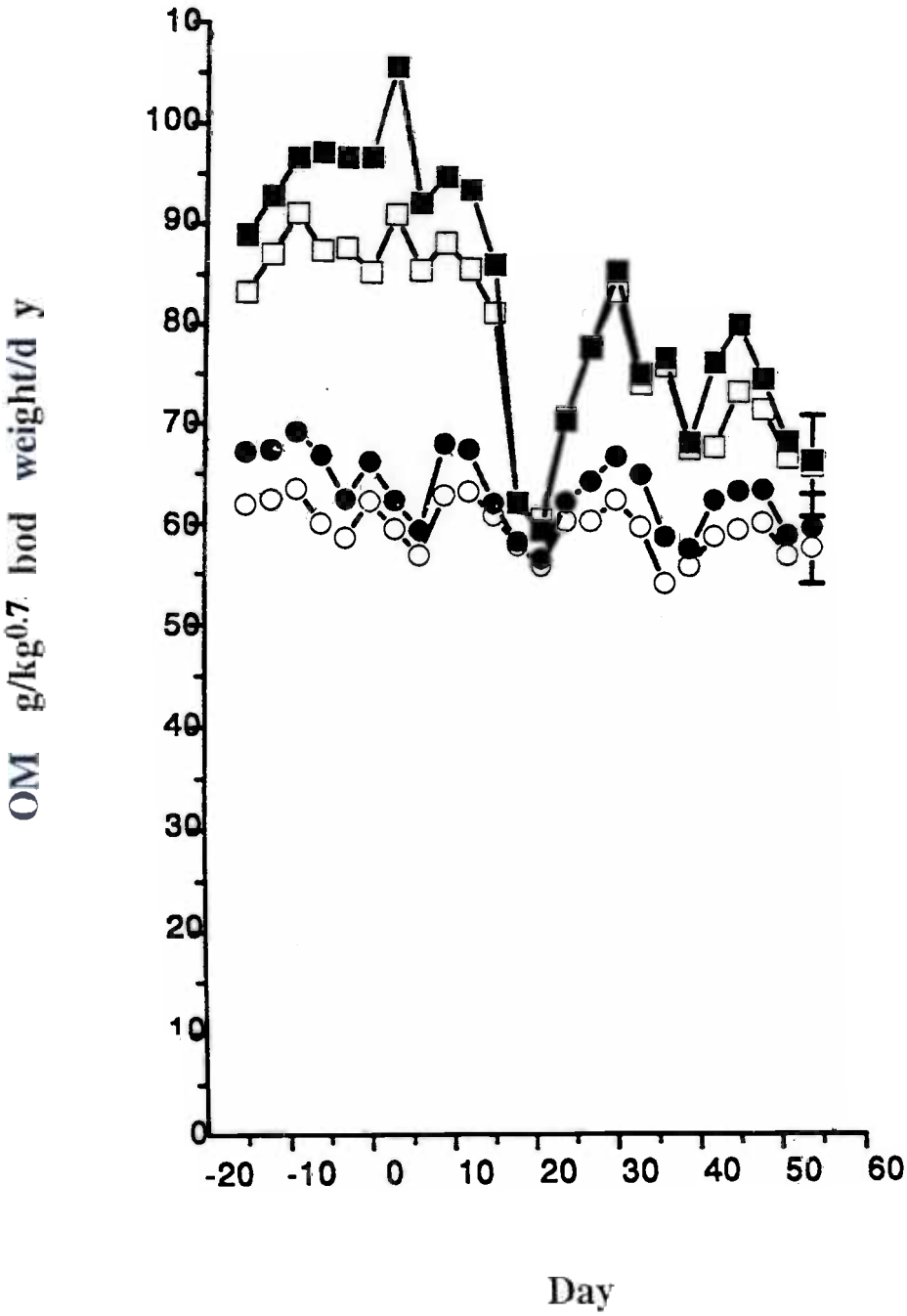
Figure 3 Intensity of parasitaemia of *T.congolense* infected (I) sheep fed diet B -●- or L -■- (n=4)

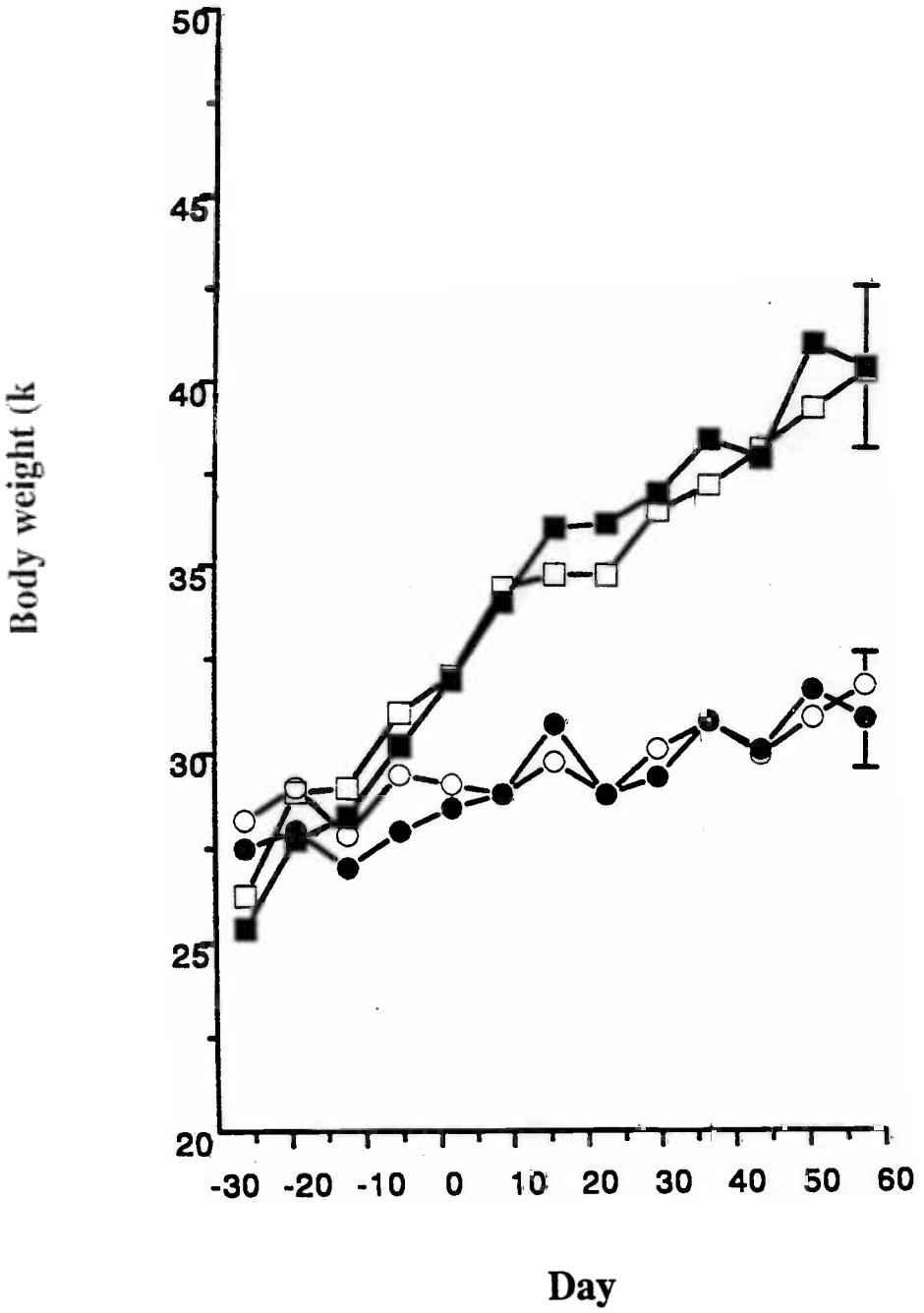
Figure 4 Packed cell volume (%) of *T.congolense* infected (I) sheep fed diet B -●- or L -■- and their respective pair fed controls (PC) -○- and -□- (n=4)

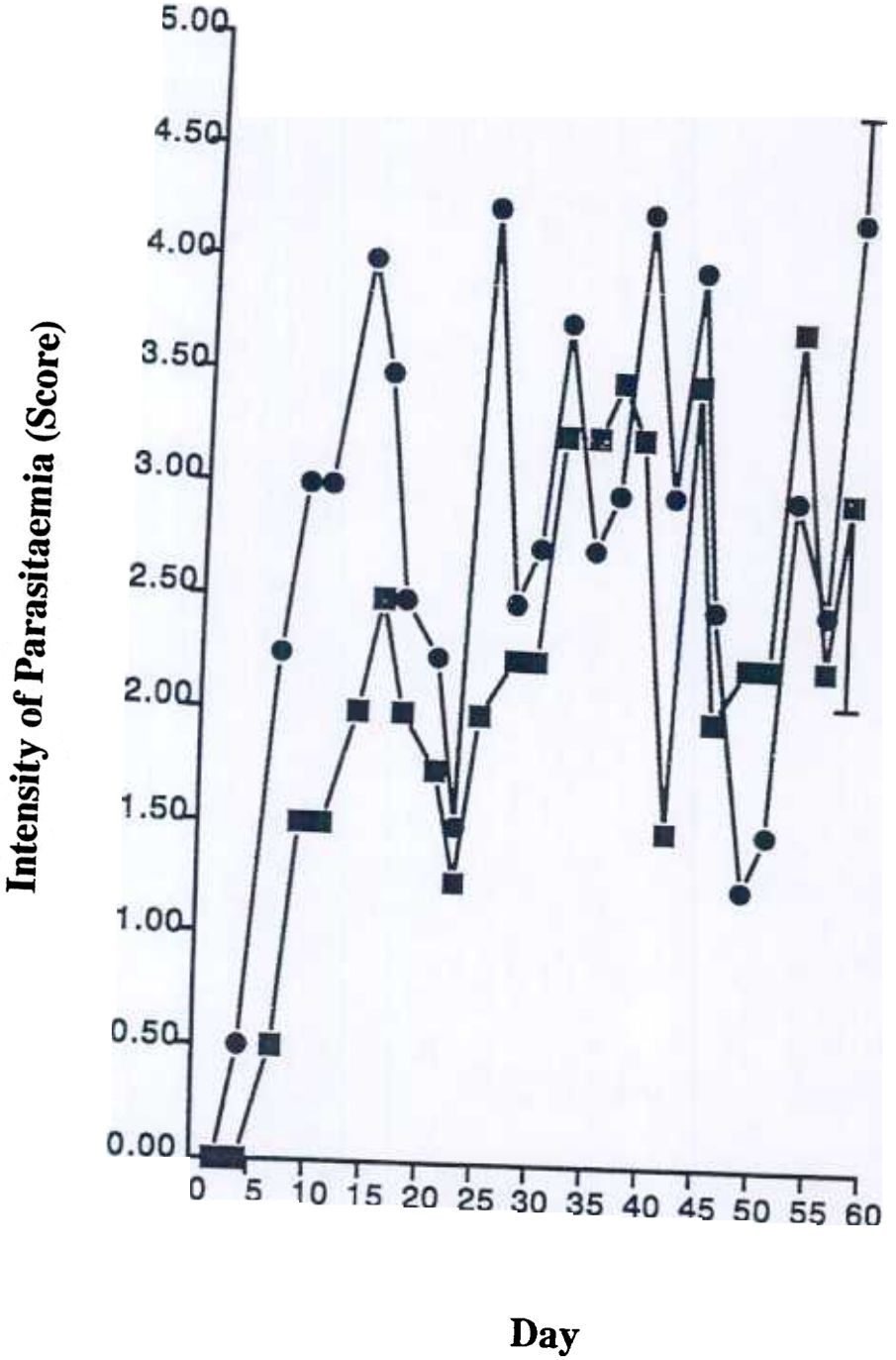
Figure 5 Plasma cholesterol concentration (mmol l^{-1}) of *T.congolense* infected (I) sheep fed diet B -●- or L -■- and their respective pair fed controls (PC) -○- and -□-

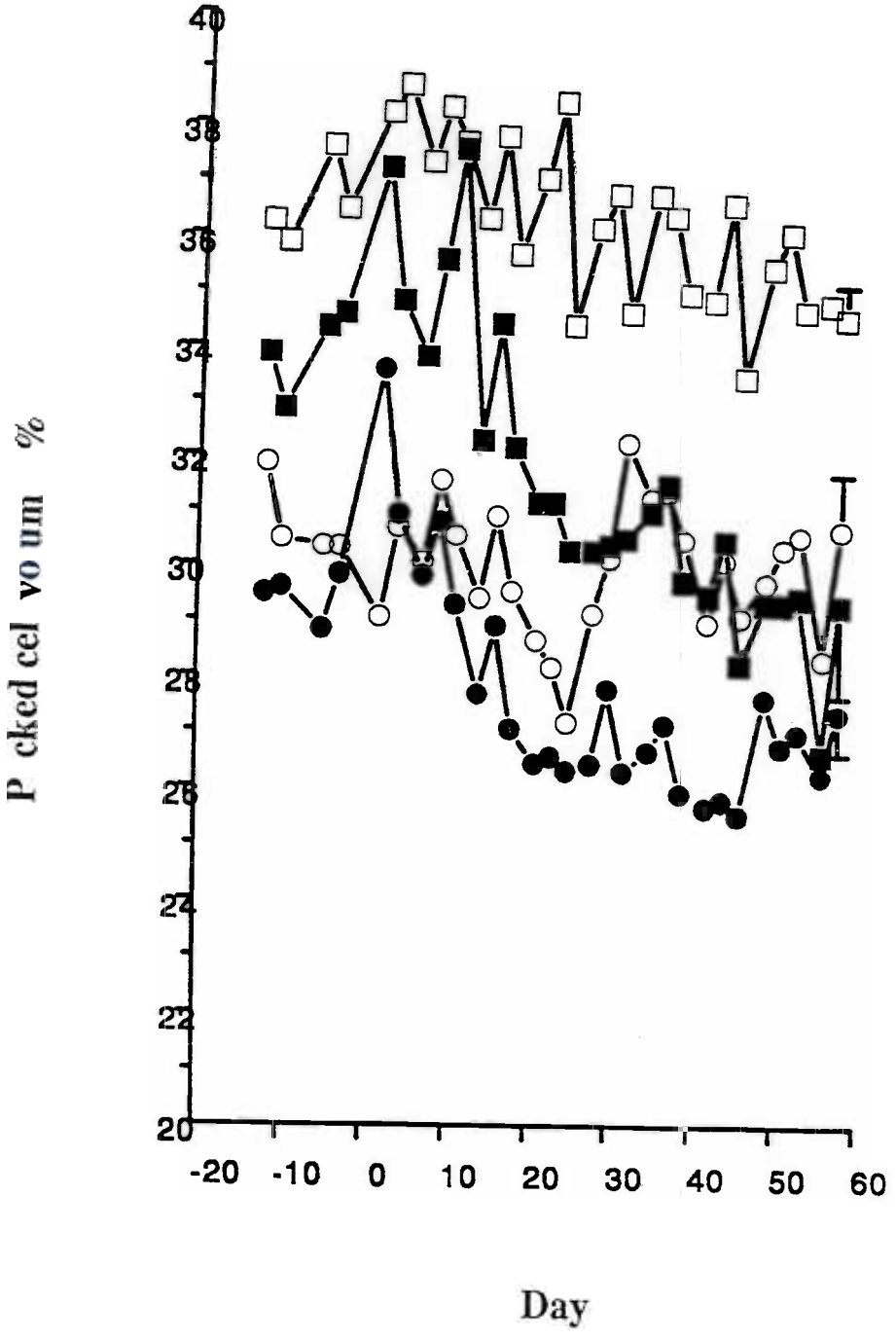
Figure 6 Plasma urea concentration (mmol l^{-1}) of *T.congolense* infected (I) sheep fed diet B -●- or L -■- and their respective pair fed controls (PC) -○- and -□-

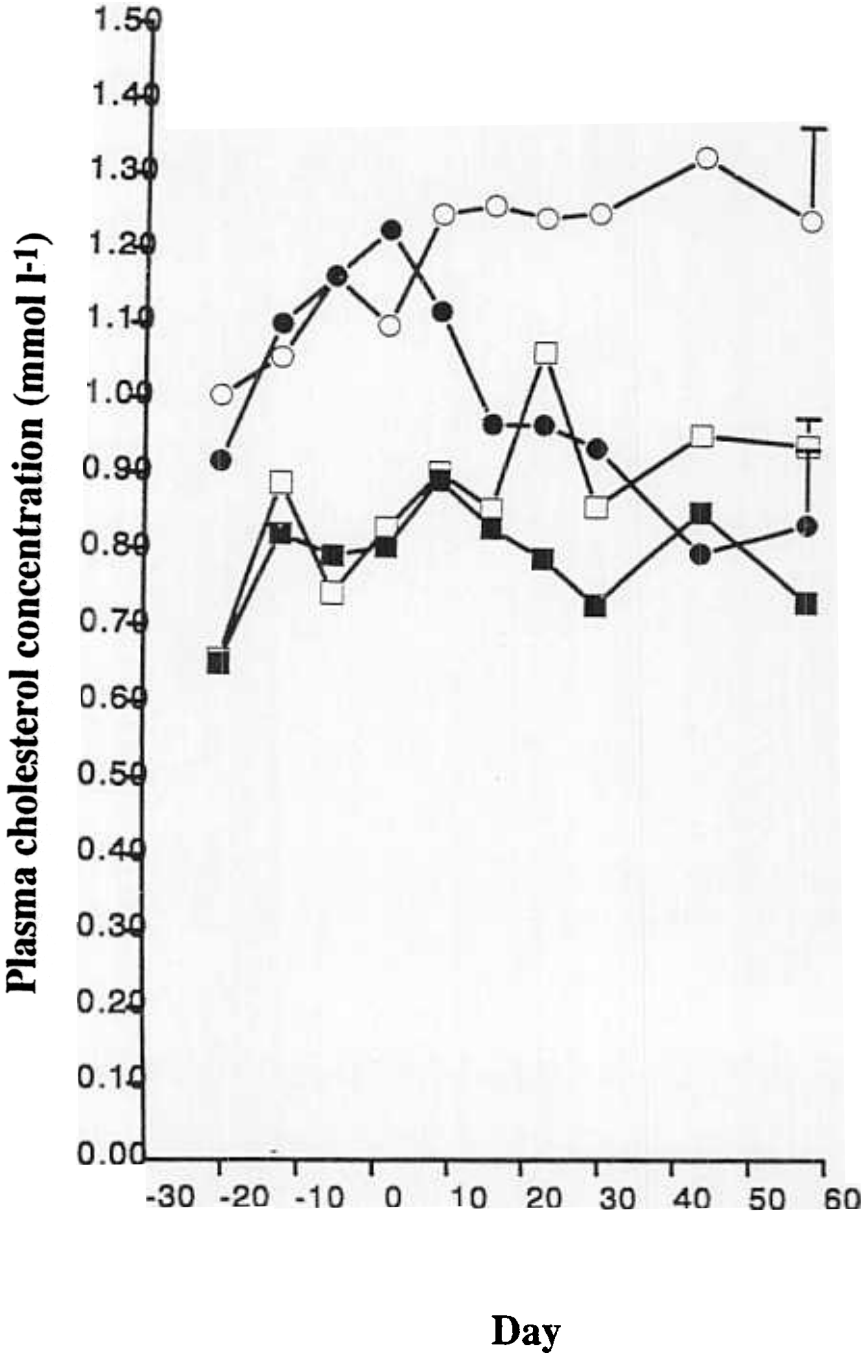
Figure 7 Plasma albumin concentration (g l^{-1}) of *T.congolense* infected (I) sheep fed diet B -●- or L -■- and their respective pair fed controls (PC) -○- and -□-

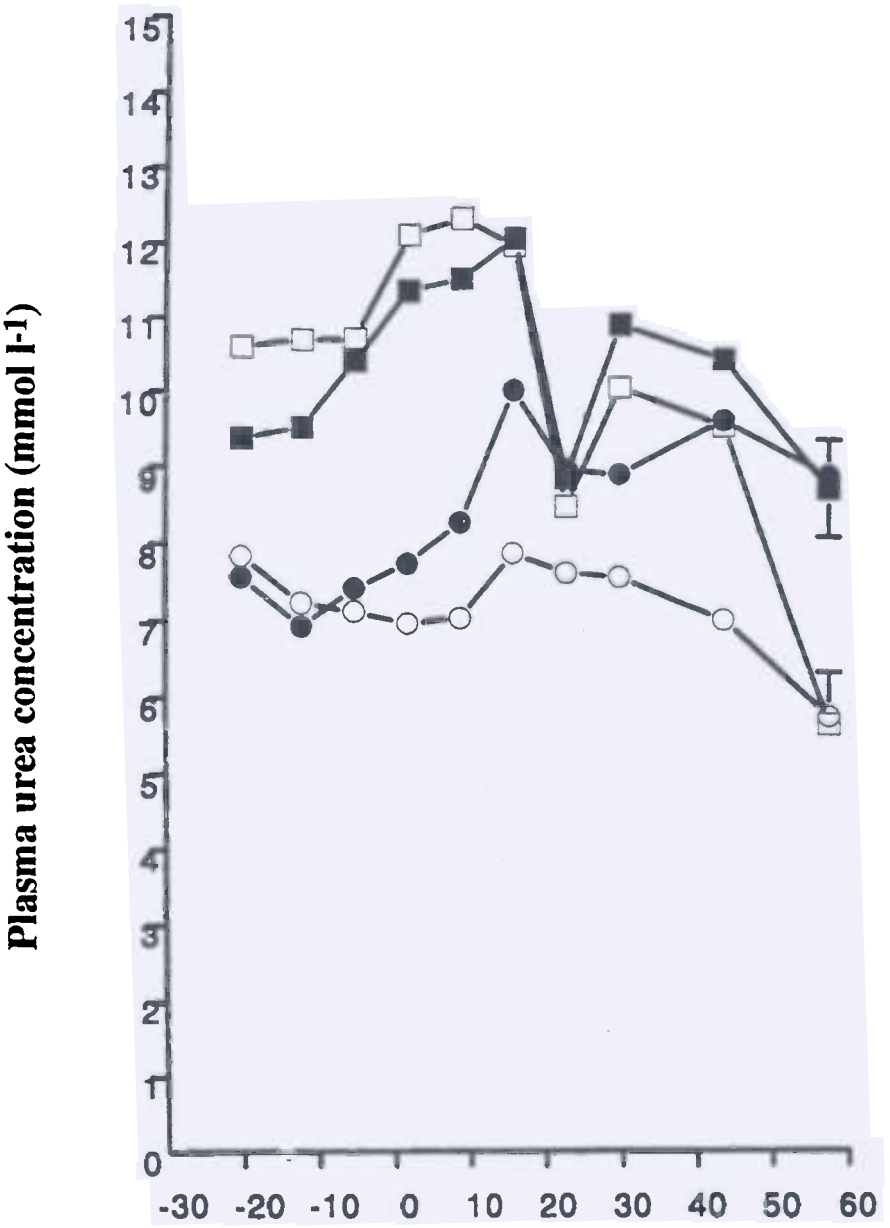

OM g/kg0.7 bod weight/d y
10
100
90
80
70
60
50
40
30
20
10
0
-20
-10
0
10
20
30
40
50
60
Day

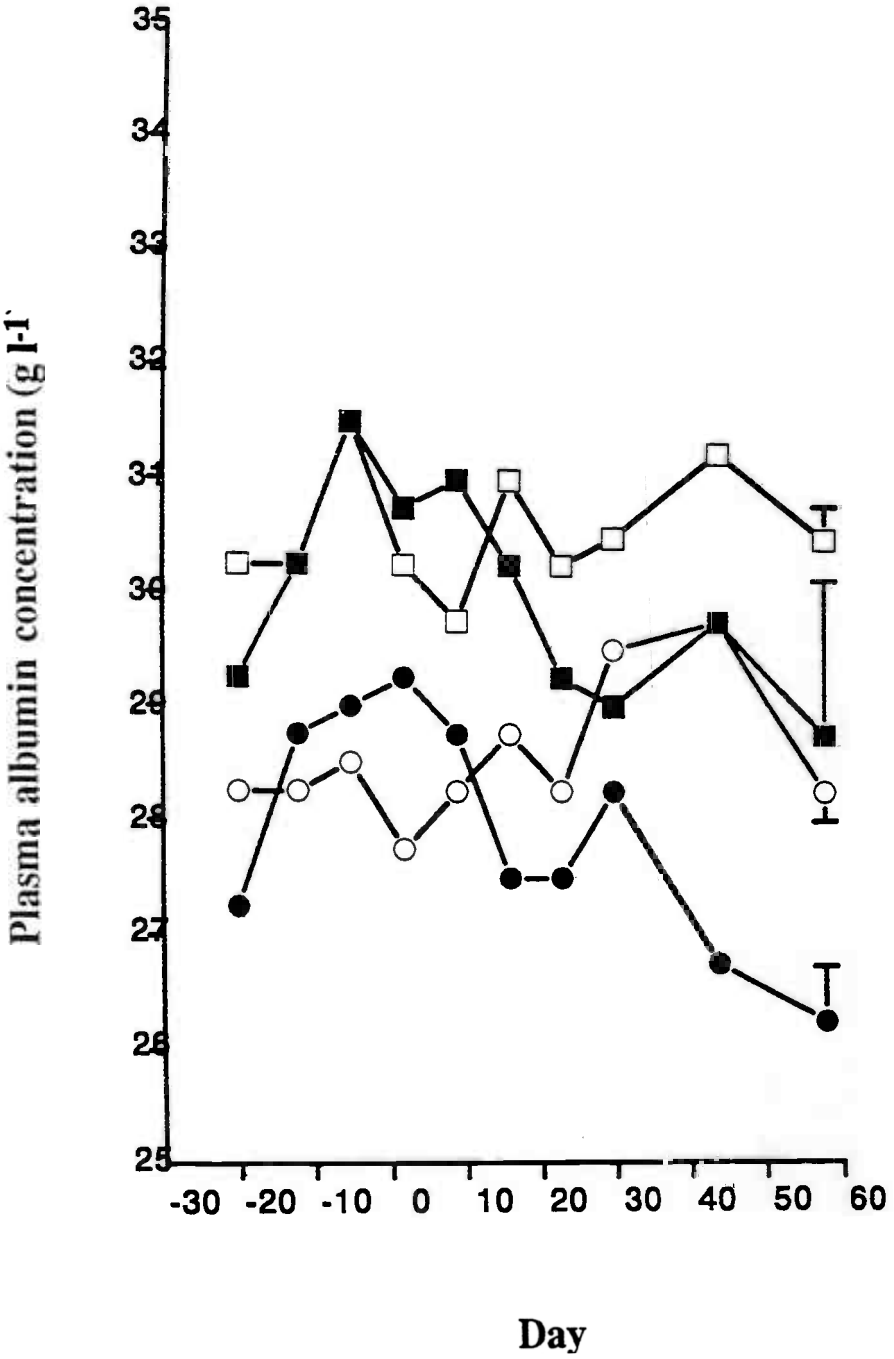

Body weight (k
50
45
40
35
30
25
20
-30
-20
-10
0
10
20
30
40
50
60
Day


Intensity of Parasitaemia (Score)
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0 5 10 15 20 25 30 35 40 45 50 55 60
Day


P cked cel vo um %
40
38
36
34
32
30
28
26
24
22
20
-20
-10
0
10
20
30
40
50
60
Day


Plasma cholesterol concentration (mmol l-1)
1.50
1.40
1.30
1.20
1.10
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
-30
-20
-10
0
10
20
30
40
50
60
Day


Plasma urea concentration (mmol l-1)
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-30
-20
-10
0
10
20
30
40
50
60
Day


Plasma albumin concentration (g l-1
35
34
33
32
31
30
29
28
27
26
25
-30
-20
-10
0
10
20
30
40
50
60
Day