DIETARY INFLUENCES ON THE PATHOPHYSIOLOGY OF OVINE HAEMONCHOSIS

by

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DECLARATION

I hereby declare that the work presented in this thesis is original and was conducted solely by the author with the exception of some of the work presented in Chapters V and VI which was carried out in collaboration with Dr. J. Rowe and Dr. S. Sivanathan respectively.

I also hereby certify that no part of this thesis has been submitted previously for the award of a degree at any university, but has been published in part as the following scientific abstracts:-

(1) Rowe, J.R., Abbott, E.M., Dargie, J.D. and Holmes, P.H. (1982)
The effect of haemonchosis and blood loss into the abomasum on N digestion in sheep.
Proceedings of the Nutrition Society, 41, 74A.

(2) Abbott, E.M., Parkins, J.J. and Holmes, P.H. (1982)
The effect of diet on the pathophysiology of ovine haemonchosis.
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CONTENTS.

SUMMARY

SUMMARY

A series of experiments were conducted to investigate, under controlled experimental conditions, the influence of host nutrition on the establishment and pathophysiological consequences of H. contortus infection in sheep. In addition to the use of conventional haematological, biochemical and parasitological techniques, extensive use was made of radioisotopic tracers. Alterations in red cell volume and quantification of gastric blood losses were measured using ^{51}Cr-labelled erythrocytes. The rate and efficiency of erythropoiesis was measured using ^{59}Fe-labelled transferrin, while changes in albumin metabolism were monitored by the use of ^{125}I-labelled albumin. Conventional digestibility and N balance studies were conducted concurrently with the radioisotopic measurements, and control animals were pair-fed to their infected counterpart during such studies.

Chapter I describes an experiment in which sheep were infected with a single dose of 50 H. contortus larvae/kg BW. They were fed either a high (hay and concentrates) or low (hay only) protein diet throughout the experiment. Infection resulted in the development of a normochromic, normocytic anaemia. Albumin metabolism was minimally affected by infection, but the infected sheep had small, but significantly greater, gastric blood losses than the controls. There were no significant differences in worm burdens between dietary groups or between breeds within the same dietary group.

In the following experiment, Chapter II, 4 month old lambs of two different breeds were infected with a single dose of 125 H. contortus larvae/kg BW. They were fed either a high (169 g CP/kg DM) or low (87.96 g CP/kg DM) protein "complete" diet from the age of 3 months/

months. The principal features of the disease were the development of a moderately severe macrocytic anaemia, hypoproteinaemia and hypoalbuminaemia. Although there appeared to be breed differences in the clinical severity of the disease, neither breed nor dietary status appeared to influence parasite establishment since all the infected groups had similar gastric blood losses, and similar worm burdens at slaughter. Regardless of dietary status, all the infected lambs mounted a vigorous erythropoietic response.

A further experiment was conducted, Chapter III, using 4 month old lambs, but on this occasion, the infective dose was increased to 350 larvae/kg BW. The diets fed were identical to those used in the previous experiment, and the dietary change was again made when the lambs were 3 months old. A further two groups of lambs were kept on the experimental diets for 5 months before infection. Despite similar gastric blood losses and similar worm burdens, the lambs fed the low protein diet suffered more severe clinical disease and appeared to be less able to withstand the pathophysiological consequences of infection relative to the better fed lambs. The development of anorexia was a feature of the disease at this level of infection, particularly in the lambs fed the low protein diet. However, regardless of dietary status, most of the infected lambs mounted a vigorous erythropoietic response. Dietary iron absorption increased in the infected lambs in response to a fall in serum iron levels and % saturation of transferrin. However, iron deficiency anaemia was only observed in two lambs, one from each dietary group, and since both lambs were severely anorectic, it was concluded that this was important in the development of such an anaemia.

The influence of dietary protein on haemonchus infection was more pronounced in the next experiment (Chapter IV), in which 4 month old lambs, on the same dietary regimen as the previous experiments, were repeatedly infected with small numbers of H. contortus larvae (200 larvae/three times weekly). The lambs fed the high protein diet were less severely affected by the disease relative to the lambs fed the low protein diet. This latter group suffered from severe clinical haemonchosis and mortality was high. However, the principal finding was that many of the lambs fed the high protein diet developed resistance to continuing infection, whereas the lambs fed the low protein diet had high worm burdens at slaughter suggesting that the immune response was impaired in the latter group. It was concluded that the failure to develop resistance to infection in this group was due to a delay in the onset of immunological competence since, in a later experiment (Chapter VI)8 month old lambs fed either a high or low protein diet were successfully vaccinated against H. contortus using radio-attenuated larvae.

There was little evidence from the digestibility studies to suggest that infection with H. contortus at any level, impaired the digestion and absorption of the experimental diets. Furthermore, whether an animal remained in positive N balance or not was largely dependent on dietary N intake as, with few exceptions, faecal and urinary N outputs were similar in both infected and pair-fed control lambs. A further experiment was conducted to investigate N digestion and absorption in adult sheep infected with H. contortus and in sheep with a simulated parasitism (Chapter V). The sheep were prepared with cannulae/

cannulae in the rumen, duodenum and ileum and a double marker technique using ^{51}Cr-EDTA and ^{103}Ru-P was used to measure N flow through the gastrointestinal tract in the two groups of sheep relative to uninfected controls. The findings of this experiment tended to substantiate the results of the more conventional techniques such as digestibility and N balance trials. They added support to the view that the depressed productivity associated with ovine haemonchosis is probably the result of a combination of reduced feed intake and increased energy requirements for blood and tissue regeneration rather than through a protein drain as a result of a failure to reabsorb the blood protein lost into the abomasum.

GENERAL INTRODUCTION

General Introduction:

Haemonchus contortus is a nematode parasite found in the abomasum of ruminants. The basic life-cycle of the parasite consists of two parts, the pre-parasitic and the parasitic phases.

The pre-parasitic phase take place outside the host and starts with the development of the fertilised egg to the first larval stage. This larva progresses by two moults to the third or infective stage (L_3). The time taken for this pre-parasitic phase of the cycle is variable depending on external factors such as temperature and humidity but, under optimal conditions, may take only 4 days.

The parasitic phase of the cycle starts with the ingestion of the infective larva (L_3) which is still enclosed in the sheath of the second larval stage. This sheath is shed in the rumen, and the exsheathed L_3, now in the abomasum, embarks on a relatively short histotrophic phase in the mucosa (Soulsby and Stewart, 1960; Silverman and Patterson, 1960; Malczewski, 1970). During development in the mucosa the L_3 moults to the fourth stage which then returns to the lumen of the abomasum. Larval development continues with a final moult to the fifth stage, the young adult, and egg production starts from day 16.

Both the adult and larval stages are haematophagic (Veglia, 1915; Andrews, 1942; Clark, Kiesel and Goby, 1964; Charleston, 1964; Malczewski, 1970; Allonby and Dargie, 1973) and thus the primary pathogenic feature of H. contortus infection is anaemia.

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A combination of less favourable climatic conditions and the extensive use of anthelmintics is probably responsible for the diminution in the importance of H. contortus infection in many temperate regions of the world. However, in tropical and sub-tropical areas, ovine haemonchosis still poses a considerable threat to the economic production of mutton and wool. In such regions, the combination of the high biotic potential of the female parasite which can lay 5,000-10,000 eggs daily, and favourable climatic conditions for the development of the pre-parasitic stages ensures a rapid build-up of infection.

As a result of their studies in East Africa, Allonby and Dargie (1973) described three forms of ovine haemonchosis (Table 1).

The least common but most dramatic form was the hyperacute syndrome which was associated with sudden death in previously healthy sheep; this was due to ingestion of massive numbers of infective larvae resulting in a severe haemorrhagic gastritis.

The most widely recognised syndrome was the acute form typified by anaemia, generalised oedema and sporadic deaths.

The third type they described was a chronic form which, although not well recognised, was reported to be widespread, and a major cause of economic loss. This form of the disease, characterised by poor growth rate and a modest anaemia, was usually associated with poor nutrition and low parasite burdens.

An association between poor nutrition and low parasite burdens in the chronic form was originally demonstrated in a field experiment with Merino sheep in Kenya (Allonby, 1974). In this study, a group of worm-free and a group of lightly infected animals were folded over the same worm-free pasture

where/

A summary of the incidence, aetiology and clinical findings of the three syndromes of ovine haemonchosis in East Africa.
(Allonby and Dargie, 1973)

	Hyperacute	Acute	Chronic
Incidence:	Uncommon	Common	Widespread
Duration:	0-7 days	1-6 weeks	2-6 months
Aetiology:	A sudden massive challenge of infective larvae.	Prevailing burden of adult parasites with continued re-infection.	A relatively low burden of adults without necessarily any re-infection.
Associated climate:	Warm, humid.	Warm, intermittent rain.	Independent of climate but effects are worse in dry weather when pasture is of poor quality.
Morbidity:	Low	Medium high	Very high
Clinical signs:	Sudden death in previously healthy sheep. Severe anaemia and dark-coloured faeces are main signs prior to death. No diarrhoea	Pale mucous membranes, generalised oedema, especially "bottle-jaw" in sheep in poor condition. Lethargy, falling wool and dark-coloured faeces are also common. No diarrhoea	Progressive insidious loss of weight resembling malnutrition. No gross anaemia or oedema making diagnosis difficult in the absence of non-infected controls. Ultimately extreme weakness and anorexia. No diarrhoea
Outcome:	Sudden death in previously healthy sheep.	Agalactia leading to death of lambs, weakness and loss in condition in ewes which is often fatal. Self-cure may give temporary alleviation at any stage of infection.	Outcome depends largely on nutritional status of host. Loss of production on good pasture, severe loss of production on poor pasture. Many deaths during extended spell of dry weather. Self-cure may alleviate condition even after the sheep have become recumbent.
Faecal egg counts:	0-400,000 e.p.g.	1,000-100,000 e.p.g.	200-2,000 e.p.g.
Numbers of parasites in abomasum:	10,000-35,000 L or immature adults.	1,000-10,000 adults ± immature adults and larval stages.	100-1,000 adults ± immature adults.
Carcase:	Few gross changes except within the abomasum.	Thin, pale with generalised oedema.	Pale carcase with generalised emaciation; little oedematous change.

where larval intake was negligible, as judged by the worm burdens of tracer sheep (0-30 adult worms). However, during spells of hot dry weather when pasture quality deterioriated, despite sufficient quantity being available, a gradual loss of condition occurred in the lightly infected sheep. This led eventually to emaciation in the adults and severe stunting of growth in the lambs. Haematological values of these sheep were always below normal but severe anaemia was only seen in terminal cases. In contrast, the worm-free sheep showed no signs of disease and they continued to thrive throughout the study. Serial kills revealed that moderate worm burdens ($\sim$ 500 adults) were maintained in the infected sheep over several months and it was suggested, therefore, that the chronic form of the disease resulted from a combination of low parasite burdens and poor nutrition.

For some considerable time it has been recognised that the interaction between parasite and host can be influenced by a variety of factors. In general terms, parasite factors include the species, the pathogenicity of particular strains and the size and frequency of the infective dose. Important host factors include genetic differences in susceptibility to infection between and within breeds, age, sex, the presence of intercurrent infections and the physiological state of the host which is, in turn, related to factors such as climatic and physical stress and nutritional status. These generalisations hold true for most gastrointestinal nematode infections.

There are numerous references to the influence of nutrition on the pathogenesis of gastrointestinal nematode parasites but only those judged to be pertinent are included in this introductory review. In addition, reference will also be made to important complementary studies in laboratory animals.

It has frequently been suggested that the nutritional status of the host animal can influence the pathogenesis of parasite infections and it is generally accepted that well nourished animals resist parasitism better than those less adequately fed (Whitlock 1949; Chandler 1953 ; Gibson, 1963). However, the mechanisms involved have rarely been elucidated. Furthermore, the diet of the host may not only play a part in host resistance to initial worm establishment or to re-infection, but may also affect the ability of the host to withstand the pathophysiological consequences of infection.

In addition, the diet of the host can influence the metabolic activity of the parasite in vivo and thus indirectly affect the host. For example, despite certain anomalies (Downey, 1966b), cobalt supplementation of the host diet has been shown to increase the fecundity of the female parasite (Richard, Shumard, Pope, Phillips and Herrick, 1954; Threlkeld, Price and Linkous, 1956; Downey, 1965; Downey, 1966a).

Both the quantity and the quality of the host diet are thought to play an important role in the ability of the host to withstand the pathogenic consequences of infection. In addition to numerous investigations into the influence of specific vitamin or mineral deficiencies on gastrointestinal tract parasitic diseases, a considerable amount of research has been directed into the study of the role of dietary protein.

Unfortunately, much of the early work on the influence of dietary protein on the resistance of sheep to helminth infections was conducted either under field conditions with mixed parasitic infections, or on housed/

housed animals with previously acquired field infections. Such studies were no doubt conducted because of both the difficulty in rearing and maintaining parasite-free animals and the problems associated with obtaining pure cultures of different parasite species.

Despite the limitations of these field studies, the results did indicate that animals on a high protein diet had smaller worm burdens than those on a low protein diet (Fraser and Robertson, 1933; Taylor, 1934; Fraser, Thomson, Robertson and George, 1938).

However, an early attempt to study the interaction of host nutrition and parasitism under carefully controlled experimental conditions was conducted by Lucker and Neumayer (1947). In their study a small number of parasite-free lambs, fed either a high or low protein diet, were artificially infected with Bunostomum trigonocephalum, a nematode parasite of the small intestine. Their results indicated that the lambs on the low protein diet were more susceptible to infection and they were also less able to compensate for the associated blood loss and thus were more severely anaemic than the better fed lambs.

Much of the early work on the influence of diet on trichostrongylid infection of sheep was reviewed by Whitlock (1949) and he concluded that animals on good quality diets had lowered mortality, smaller worm burdens and less severe clinical disease than those on basal rations.

Further evidence that a high level of dietary protein may be beneficial to the parasitised host was provided by investigations into the influence of diet on the recovery of housed sheep with heavy mixed infections which were acquired whilst at grass. Although there were differences in the design of the various experiments, the main conclusions were that animals transferred to a high protein diet generally showed an improvement in their clinical condition, a reduction in faecal egg counts/

counts and were more resistant to re-infection than animals on a basal ration (Taylor, 1934; Gordon, 1948; Laurence, Groenwald, Quin, Clark, Ortlepp and Bosman, 1951; Brunsdon, 1964). In addition, Clunies Ross and Gordon (1933) showed that acquired resistance to H. contortus infection in old sheep could be reduced by feeding a low protein diet. However, the diet had to be sufficiently low in protein content(<30gCP/kgDM) to cause weight loss before resistance was successfully overcome.

The study by Kates and Wilson (1955) is constantly referred to as one of the earlier investigations proving the beneficial effect of high protein diets on the pathogenesis of ovine haemonchosis. However, their data, as presented, is confusing, and merits some discussion. Twelve lambs were equally divided into two dietary groups. One diet was a high grade chopped alfalfa hay and the other a pelleted ration consisting of 60% alfalfa meal, 30% milo maize and 10% cane molasses. After a two-week conditioning period on the diets, three lambs in each dietary group were given a single infection of 15,000 H. contortus larvae, while the remaining three members of each group were uninfected controls. Eleven weeks later, the experiment was terminated and the average feed consumed per pound of grain (presumed to be "gain") for each group of lambs was presented (i.e. food conversion ratio (FCR):-

Pelleted infected 6.36; Pelleted control 5.98;
Alfalfa infected 7.97; Alfalfa control 6.27.

The authors then simply concluded that lambs kept on a high nutritional level (i.e. the pelleted ration) showed better feed utilisation, less severe clinical effects and had fewer worms present than lambs on a low nutritional level (i.e. the alfalfa hay diet alone).

Calculation/

Calculation of the protein and energy contents of the two diets described, using standard values obtained from the National Research Council (NRC) (1969), gave the crude protein contents (gCP/kgDM) as 185 for the alfalfa hay and 176 for the pelletted ration (if a 22% CP alfalfa meal is assumed). The calculated metabolisable energy (ME) values are 9 and 10.9 for the alfalfa and pelleted ration respectively.

Thus, contrary to popular assumption, the diets cannot be referred to as high and low protein, as they are both high protein. However, the obvious nutritional difference between them is in the energy content, and the FCR figures found for the two diets are completely predictable from standard ME calculations (Ministry of Agriculture, Fisheries and Food, Department of Agriculture and Fisheries for Scotland and Department of Agriculture for Northern Ireland (MAFF et al) 1975). No statistics are presented and it is very unlikely that the small improvements in FCR shown in the two groups of controls relative to the infected groups are significant.

More than twenty years later an experiment was conducted by Preston and Allonby (1978) to investigate the influence of high and low protein diets on the pathogenesis of ovine haemonchosis. Twelve Merino sheep, not raised parasite-free, but treated with anthelmintic and housed before the start of the experiment were divided equally into two groups, one of which was fed a diet of hay and concentrates (3.5 gCP/kg BW/d) whilst the other was fed hay only (1.7 gCP/kg BW/d). All the sheep were then infected with a single dose of 350 H. contortus larvae/kg BW. Faecal egg counts were monitored twice weekly for fourteen weeks after infection but no worm burdens were assessed. The sheep on the low protein diet had higher faecal egg counts and showed more severe/

severe clinical disease than those on the high protein diet. Thus they concluded that, since faecal outputs were similar in both groups of sheep, the higher faecal egg counts observed in the low protein group were probably due to a greater establishment of adult worms because of reduced immunocompetence. However, the possibility of increased parasite fecundity in the low protein dietary group was also considered.

Because of the limitations of the studies by Kates and Wilson (1955) and Preston and Allonby (1978) it is difficult to draw firm conclusions about the influence of dietary protein on the establishment of H. contortus.

However, evidence that initial worm establishment is probably not affected by the protein content of the host diet was presented by Bawden (1969b). Forty-five 5-month old parasite free lambs were transferred to high(190gCP/kgDM) or low(60gCP/kgDM) protein diets and 2 months later a number of lambs in each group were infected with a single dose of 1500 Oesophagostomum columbianum larvae. Some lambs in each dietary group were killed 10 days after infection, whilst the remainder were killed at day 56. The distribution and number of nodules present in the intestine, the number, sex and size of the adult parasites and the total daily faecal egg output (on a dry faecal matter basis) were recorded.

The infective larvae (L_3) of O. columbianum have a histotrophic phase deep in the mucosa of any part of the intestine, although the small intestine is more commonly affected. During this phase nodules form round the L_3 and the first parasite moult then takes place. The L_4 then emerge from the nodule to return to the gut lumen and migrate to the adult predilection site (caecum or colon) where the final moult takes place.

Since/

Since the number of nodules present in the intestines was similar in both dietary groups at 10 and 56 days post-infection, it was concluded that establishment was not affected by the protein content of the diet.

However, the number of adult worms present at slaughter in both dietary groups was different, the larger worm burdens occurring in the lambs on the low protein diet. The ratio of male : female worms was the same in both groups and, in addition, the lengths of both male and female parasites were similar. However, total daily faecal egg output was considerably greater in the low protein group and when the number of eggs per parasite was calculated, it was clearly shown that the parasites in the lambs on the low protein diet were more fecund. In addition, patency occurred earlier in the low protein group and more parasites were present in the male lambs on a low protein diet relative to the females. Another example of the influence of host sex on the parasite was the observed increase in fecundity of the parasites in female relative to male hosts.

An essentially similar investigation using the same diets and parasitic infection described above was conducted by Dobson and Bawden (1974). In addition to gross examination of gut tissue for nodules, histological sections of gut tissue were taken at intervals along the alimentary tract in infected and pair-fed control lambs on either diet. Their results confirmed the earlier findings of Bawden (1969b), namely that diet had no effect on the number or distribution of the nodules, i.e. establishment was similar in both dietary groups. However, as before, worm burdens at day 56 were greater in the animals on the low protein diet and the fecundity of the parasite was increased in this group./

group. In addition, cellular responses (macrophages, leucocytes, plasma cells, globule leucocytes, mast cells, eosinophils and mucin cells) were reduced in the lambs on the low protein diet. It was concluded that the increased susceptibility of this group to infection was associated with malfunctions in the innate immunity of the gut involving decreased peristalsis and failure of the mucin cell response with a resultant loss of immunological competence.

In an investigation into the influence of diet on the resistance of sheep to infection with the abomasal parasite, Ostertagia circumcincta, two similar experiments were conducted by using high(186gCP/kgDM) and low (120gCP/kgDM) protein diets (Downey, Connolly and O'Shea, 1972). Three-month old parasite-free lambs were artificially infected with a total of 120,000 L_3 over a period of 10 days. Their results in the first experiment indicated that the lambs on the low protein diet were more susceptible to infection, as judged by live weight gains, worm burdens and changes in certain blood constituents. However, no such differences between the two dietary groups were observed in the second experiment.

The experiments described above have been concerned with qualitative differences in the host diet. However, the influence of a quantitative reduction in the diet of sheep infected with the small intestinal nematode Trichostrongylus colubriformis was investigated by Gordon (1964). The "infected" lambs were divided into two dietary groups. One group was fed ad lib a diet containing 10% CP, whilst the other was restricted to half that consumed by the first group before infection. A third group of lambs remained as uninfected controls. After a 7-week conditioning period on the diets, the lambs in the "infected" groups were given 50,000 T. colubriformis larvae per os and a challenge dose 4 months later. Feed intakes declined rapidly in the group fed ad lib until they were consuming daily, similar quantities as the restricted group./

group. Overall mortality was similar in both groups but deaths occurred earlier in the group on the restricted diet. There was little difference in establishment, as judged by faecal egg counts and worm burdens.

Much of the evidence presented so far suggests that initial establishment of a parasite is not influenced by the level of dietary protein. However, as suggested by Dobson and Bawden (1974), protein deprived sheep may be less immunologically competent and thus less able to expel the infection. This latter suggestion would appear to substantiate the earlier reports of Taylor (1934), Gordon (1948), Laurence et al (1951) and Brunsdon (1964).

Even if the question of the influence of dietary protein on establishment and reinfection is not completely resolved, there is a considerable weight of evidence to indicate that animals on a low protein diet suffer more severe clinical disease than those on a good quality diet (Whitlock, 1949; Chandler, 1953; Gibson, 1963; Dargie, 1975). It is thus appropriate to return to the underlying pathological changes associated with gastrointestinal parasitism to find out why this may be so. If, for instance, digestion and absorption are impaired, the animal on the basal diet must then be at a disadvantage relative to the well-nourished animal.

In an excellent review of the pathology of gastrointestinal helminthiasis, Symons (1969) pertinently pointed out that although intestinal function, particularly at the site of infection, may be deranged, the experimental evidence of a failure of digestion when measured over the entire gastrointestinal tract is conflicting.

Digestibility/

Digestibility and nitrogen (N) balance trials have often been used to investigate the effects of parasites on the digestive efficiency and protein status of the host. Although it has been claimed that the digestibility of the crude protein (CP) fraction of the diet in particular has been reduced following parasitic infection, comparative studies using uninfected control animals at the same level of feed intake (pair-fed controls) were not always conducted (Stewart, 1932—1933; Spedding, 1954; Shumard, Bolin and Eveleth, 1957; Horak and Clark, 1964). Thus, the conclusions drawn from such studies have only limited value.

The use of pair-fed control animals (where the control animal is offered the same quantity of food daily as was consumed by its infected counterpart) was an important step forward in comparative studies between parasitised and uninfected control animals, particularly if differences in feed conversion efficiency, weight gain, digestibility and other parameters were to be evaluated.

The data from many experiments carried out under conditions of controlled feed intake do not support the view that digestibility is adversely affected by parasitism. For example, Andrews (1938) showed that Cooperia curticei, an intestinal nematode of sheep, had no effect on the digestibility coefficients or apparent absorption of the various components of the feed and later Andrews, Kauffman and Davis (1944) confirmed that T. colubriformis infection also had no effect on the digestibility of the diet. This finding with respect to T.colubriformis was later confirmed by Roseby (1973) and Sykes and Coop (1976). However, an increase in dry matter (DM) digestibility was observed in sheep infected with Oe.columbianum relative to uninfected but not pair-fed controls (Dobson, 1967). This increase was observed at a time when there was severe depression in

voluntary/

voluntary feed intake and thus the author concluded that the sheep used the small amounts of feed ingested during the acute stages of infection with Oe. columbianum more efficiently.

The experimental data on the influence of haemonchosis on digestibility is sparse and conflicting. Using small numbers of 9-month old lambs infected with 50,000 larvae, Owen (1973) found little difference in DM digestibility between the infected lambs and their pair-fed controls. However, in a second experiment, using 6 month old lambs and the same level of infection (Owen, 1973), dry matter digestibility was severely reduced in one of three infected lambs at weeks 2 and 3 after infection. Control lambs were not pair-fed to the infected lambs and so the significance of this finding is questionable.

Allonby and Dargie (1973) previously described weight losses in sheep infected with H. contortus. As a result of their studies, they concluded that the weight losses observed in the infected animals were not due to anorexia since these sheep were consuming more food than the controls. The weekly nitrogen intake of an unspecified number of infected and control sheep was 93.5 and 85.2 lbs. respectively. They suggested that "during the period of reduced growth, the infected sheep enter a state of reduced N balance due to increased loss of urinary and faecal nitrogen indicating that, not only are such sheep unable to reabsorb all the N lost into the gut as a result of plasma leak, but also that they actually mobilise tissue protein in order to supply the constituents necessary for synthesis of physiologically more important molecules such as albumin and haemoglobin". No data was presented to substantiate this statement other than the feed intakes and bodyweight changes mentioned earlier. Thus it is impossible to interpret these findings properly.

The experimental evidence from studies of another abomasal parasite, O. circumcincta, is also conflicting. Parkins, Holmes and Bremner (1973), using varying levels of infection, noted a reduction in crude protein (CP) digestibility when sheep were infected with one million larvae. However, when the experiment was repeated, the infected sheep had greater CP digestibility coefficients than the control sheep. A reduction in CP digestibility in lambs dosed daily with 4,000 O. circumcincta larvae for 14 weeks was observed by Sykes and Coop (1977).

Despite the known technical limitations of the N balance technique, if conducted carefully, useful information on the protein status of an animal can be obtained. However, as with digestibility trials in parasitised animals, it is essential that control animals are pair-fed to their infected counterparts if meaningful conclusions are to be drawn.

Several of the N balance studies on parasitised sheep suffered the same limitations as the early digestibility trials, namely that comparative studies with controls and/or pair-fed controls were not always conducted (Stewart, 1932-1933; Horak and Clark, 1964; Owen, 1973). Thus, their conclusions that parasitism per se resulted in a negative nitrogen balance cannot be substantiated, particularly as the parasitised animals were anorectic (Horak and Clark, 1964; Owen, 1973).

However, in their studies on ovine ostertagiasis, Parkins et al (1973) found that the infected sheep were in marked negative nitrogen balance relative to their pair-fed controls due to increased urinary N output. In contrast, Sykes and Coop (1977) found reduced N retention in lambs also infected with O. circumcincta but this was due to increased faecal nitrogen, urinary N levels being similar in both/

both the infected and pair-fed control groups. However, the lambs in this latter experiment, although younger, received a considerably smaller infective dose than those in the Parkins et al experiment. Also, although there was a reduction in voluntary feed intake after infection in Sykes and Coop's experiment, daily nitrogen intake was 32.0 g compared with 3.5g N /d in the Parkins et al experiment.

Decreased nitrogen retention, attributable to increased urinary N, was also a feature of lambs infected with T. colubriformis (Roseby, 1973), although again their daily N intake was low, $\sim$ 9 g N/day.

More direct methods of studying protein digestion in parasitised animals were reported by Symons (1969). By feeding ^{14}C-labelled Chlorella protein to rats and mice infected with the small intestinal nematode parasites Nippostrongylus brasiliensis and Nematospiroides dubius respectively, he could show no failure of protein digestion or absorption. Using the same technique, he also showed that if the parasitised rats and mice were fed either a high or low protein diet, their ability to digest and absorb protein was still not impaired. Similar studies on a limited number of cannulated sheep infected with T. colubriformis and kept on either a high or low protein diet, confirmed the findings of the studies on the rats and mice (Symons, 1969).

Although rises in gastric pH have been reported on animals with haemonchosis and ostertagiasis (Armour, Jarrett and Jennings, 1966; Malczewski, 1970), it is not known whether this could depress protein digestion by its inhibitory action on pepsin release. However, it is thought to be unlikely as protein digestion is not seriously/

seriously impaired in cases of achlorhydria in man (Symons, 1969).

The evidence presented so far would appear to indicate that impaired digestion and absorption are unlikely to be the main reason for the reduced efficiency of feed utilisation commonly associated with parasitism despite the conflicting reports on abomasal parasitism.

A relatively poor rate of growth in growing animals and even actual weight loss is one of the most widely described effects of parasitism, and anorexia has been reported almost as commonly. However, whether or not anorexia alone was solely responsible for the weight changes was not clear until pair-fed control animals were introduced into experiments with parasitised animals.

By the use of pair-feeding, Gibson (1955) was able to show that anorexia alone was not the only factor responsible for the weight changes; he found that pair-fed control lambs gained weight at a rate intermediate between uninfected lambs fed to appetite and lambs infected with the abomasal parasite Trichostrongylus axei. This finding was later confirmed by Sykes and Coop (1976, 1977).

Thus if it is unlikely that digestion and absorption are impaired by gastrointestinal parasitism and that anorexia alone is not responsible for the weight changes observed in parasitised animals, the cause of the reduced feed utilisation must be sought elsewhere.

Important/

Important work by Symons and Jones (1971, 1972) using radio-labelled amino acids helped in elucidating the metabolic changes, particularly in protein metabolism, associated with gastrointestinal parasites. In their initial experiment ^{14}C-L-leucine was injected into mice and guinea pigs infected with the intestinal nematodes N. dubius and T. colubriformis respectively. They noted that in infected animals which were anorectic and losing weight, there was reduced incorporation of the labelled amino acid into muscle but increased incorporation into liver, and uninfected mice which were also losing weight due to a quantitative reduction in their feed intake also showed reduced incorporation into muscle. However, unlike the infected animals, they did not have increased incorporation into the liver (Symons and Jones, 1971). A subsequent experiment using the same laboratory animals and infections indicated an increased rate of protein turnover (measured using ^{75}Se-selenomethionine and whole body counting) in both the infected animals which were losing weight and also in uninfected animals on a reduced ration (Symons and Jones, 1972). The authors therefore concluded that failure to grow in infected animals was due to a combination of increased rate of whole body protein catabolism and a depression of muscle protein synthesis and that reduced feed intake alone is unlikely to be the sole cause of the changes in protein metabolism.

In a further experiment using guinea pigs infected with T. colubriformis, Symons, Jones and Steel (1974) investigated the increased liver protein synthesis in greater depth. They measured gastrointestinal protein loss using ^{51}Cr-labelled albumin and albumin turnover rates and pool sizes using ^{125}I-labelled albumin. Their main findings were that there

was increased protein loss into the gut of infected animals, increased albumin turnover rates and increased incorporation of labelled amino acid into the membrane-bound ribosomes responsible for plasma protein synthesis. They concluded that the changes in liver protein synthesis were stimulated by the plasma loss into the gut and the increased plasma albumin turnover rate.

In a subsequent experiment to investigate skeletal muscle, liver and wool protein synthesis using ^{14}C-L-leucine in sheep infected with T. colubriformis, Symons and Jones (1975) found muscle protein synthesis to be equally depressed in anorectic infected sheep losing weight and in their pair-fed controls but the effect of infection on liver protein synthesis was doubtful as incorporation was increased in both groups of sheep. However, they were unable to check the significance of the relative proportions of membrane-bound and free ribosomes as in the earlier experiment with guinea pigs (Symons et al, 1974) and thus resolve the problem.

With the advent of radioisotopic tracer techniques in large animal studies, it became possible to study the turnover of red cells and plasma proteins in parasitised animals. The applications of these techniques in this field have been reviewed by Dargie (1975). From such studies it soon became clear that the anaemia and/or hypoproteinaemia commonly associated with parasitism, was due to gastrointestinal losses of red cells and/or plasma proteins. Another important fact to emerge from such studies was that, in addition to increased protein catabolism, parasitised animals were having to synthesise replacement cells and/or proteins at a greatly accelerated rate, i.e. they were hyperkinetic (Dargie, 1975). These findings in parasitised large animals are therefore complementary to the findings of Symons and Jones in infected laboratory animals.

Thus/

Thus, in parasitised animals, the distribution of synthesis between, and the extent of catabolism within, the various protein pools is altered and it would appear that emphasis is placed on synthesis of proteins most important for survival of the animal.

From the foregoing, it is apparent that the dietary requirements of a parasitised animal must lie in excess of those of an uninfected animal. The synthesis of new blood elements, repair of damaged gut tissue and the mounting of a local cellular (immune) response in the bowel wall will make additional energy and protein demands. If the parasitised animal is on a high plane of nutrition and is not anorectic, the balance between loss and synthesis may be maintained. Thus, there apparently may be a minimal effect on growth and/or production but there must be a loss in economic terms as the food conversion ratio will be increased. However, the parasitised animal on a low quality diet may have less capacity to accommodate the additional demands on energy and protein caused by parasitism. This may be exacerbated further if the infection is prolonged and anorexia develops.

To date much of the experimental work on ovine haemonchosis has centred on the pathological and immunological aspects of the disease. However, there is a dearth of information on the part played by nutrition in both the resistance to infection and in the ability of the host to withstand the pathophysiological consequences of such infection.

The pathogenesis of the anaemia of haemonchosis has been well reviewed by Campbell and Campbell-Gardiner (1960) who support the early work of Fourie (1931) and Andrews (1942) in this respect. Both Fourie and Andrews compared the development of the anaemia in infected sheep with that induced by phlebotomy and, in both cases, the blood picture was similar, namely a normochromic or hyperchromic, macrocytic (increased mean cell volume) anaemia developed. The macrocytosis was due to the release of immature red cells or reticulocytes from the bone marrow and was thus indicative of an accelerated rate of erythropoiesis. However, in fatal cases of haemonchosis, impending failure of the marrow to sustain the necessary increased production of red blood cells was heralded by the development of a microcytic, hypochromic anaemia.

Although it was fairly clear from this work that the anaemia of haemonchosis was basically haemorrhagic in character, dyshaemopoiesis may be an additional factor in parasitised sheep. When comparing the blood and bone marrow changes of both infected and phlebotomised sheep, Charleston (1964) noted the presence of megaloblastic normoblasts in the marrow of the infected sheep only. Since the bled sheep had a greater macrocytic response despite having a similar degree of anaemia but did not exhibit megaloblastic normoblasts in the marrow, he concluded that there was impaired synthesis of red cells in the parasitised sheep.

The changes in red cell and plasma protein metabolism associated with haemonchosis have been well documented (Allonby and Dargie, 1973; Dargie, 1975; Altaif, 1975; Altaif and Dargie, 1978a,b) but the role of host nutrition in the pathogenesis of the disease/

disease has not been investigated under controlled experimental conditions. However, it has been demonstrated that sheep infected with liver flukes and kept on a high plane of nutrition respond in a more sustained and positive manner to haemorrhagic stress than those on a low plane of nutrition (Berry and Dargie, 1976). Whether a similar situation is associated with haemonchosis is not known.

The application of radioisotopic tracer techniques to the study of red cell and plasma protein metabolism in parasitised sheep has been well reviewed by Dargie (1975). However, since those techniques are an integral part of this thesis, a brief description of the principles behind their application is warranted. The three radioisotopes used in the experiments described later are ^{51}Chromium-labelled erythrocytes, ^{59}Iron-labelled transferrin and ^{125}Iodine-labelled albumin.

The labelling of erythrocytes with anionic hexavalent sodium chromate ($Na_2\ ^{51}CrO_4$) was first described by Gray and Sterling (1950). After passing through the red cell membrane, the sodium chromate is reduced to the cationic trivalent chromium which then binds to the β polypeptide chains of haemoglobin (Pearson, 1963). Red cells labelled in such a way can be used to study the fate of the erythrocyte *in vivo*. For example, following normal breakdown of the red cell, the isotope is not re-utilised by other circulating erythrocytes and it is then excreted in the urine. However, if blood is lost into the gastro-intestinal tract (a common finding in several parasitic infections), the isotope is found in the faeces. Since it is not re-absorbed to any significant extent (Owen, Bollman and Grindlay, 1954), it can then be used to quantify the amount of blood loss into the gastrointestinal tract ("faecal clearance").

The rate of disappearance of the labelled red cells from the circulation can provide information on the red cell lifespan. However, although most of the radioisotope remains bound to the red cell for the duration of the life of the cell, some isotope is lost by elution, and this is particularly rapid during the first 24 hours in ruminants. Because of elution, the red cell lifespan is underestimated and thus the rate of disappearance of the labelled red cells from the blood is expressed as an "apparent" half-life value, i.e. the time taken for the blood radioactivity to fall by 50%.

In addition, by using the dilution principle, the circulating red cell volume can be determined by dividing the total ^{51}Cr injected activity by the counts/ml RBC of a blood sample collected 5-10 minutes post-injection of the labelled cells.

By analysing the pattern of ^{51}Cr loss from the body, the mechanism of an anaemia can be determined. If the anaemia is the result of intravascular haemolysis, elevated renal excretion of the radioisotope will result, whereas haemorrhage into the gut will lead to appearance of the isotope in the faeces.

Thus, ^{51}Cr-labelled red cells can give valuable information on the rates and routes of red cell breakdown. However, information on the rate and efficiency of erythropoiesis is not provided.

Radio iron was one of the first isotopes to be used for *in vivo* labelling techniques to obtain information both on normal and abnormal erythropoiesis in man (Huff, Hennessy, Austin, Garcia, Roberts and Lawrence, 1950; Wasserman, Rashkoff, Leavitt, Mayer and Port, 1962).

^{59}Fe is the tracer of choice for most studies of iron metabolism, having a half-life of 45 days, which makes it possible to monitor changes in iron metabolism over a period of weeks. If injected intravenously, it labels transferrin (i.e. the plasma iron pool) and thus allows measurement of iron clearance from the plasma and plasma iron turnover. The majority of the circulating iron is transported to the bone/

bone marrow for incorporation into the haemoglobin of the newly formed red cells. Thus, the rate of disappearance of the labelled iron from the circulation, and the speed of its reappearance in newly formed red cells permits assessment of haemoglobin synthesis and of the completeness of utilisation of the iron.

For example, where erythropoiesis is greatly accelerated, such as might occur in haemorrhagic anaemias, the disappearance of the radio-iron from the circulation is more rapid than normal, resulting in a shortened half-life of the labelled protein in the circulation. In contrast, in aplastic anaemias where red cell production is below normal, the half-life is prolonged.

Radio iron can also be used to quantify gastrointestinal blood loss in a similar manner to radiochromium. However, unlike ^{51}Cr, radio iron is reabsorbed and thus there is underestimation of blood loss. By comparing faecal clearances using both radiolabels, the amount of haemoglobin iron reabsorbed can be calculated (Roche, Pérez-Giménez and Levy, 1957).

^{125}I-labelled albumin is a valuable tool in the study of plasma protein metabolism in parasitic hypoproteinaemias. The plasma proteins are maintained in a state of dynamic equilibrium between the intravascular ($\bar{C}A$) and extravascular (EA) pools by constant synthesis and catabolism. A change in blood concentration may indicate altered distribution or a change in the rate of synthesis or catabolism. ^{125}I labelled albumin has been shown to behave metabolically like the subject's own unlabelled protein. The label remains firmly bound under physiological conditions until the albumin is degraded whereupon the label is quantitatively excreted in the urine, the thyroid having previously been blocked by the administration of inactive iodide. Once the label has been released after degradation of the albumin, it is not re-utilised for new protein synthesis.

^{125}I/

^{125}I-labelled albumin can be used to calculate the plasma volume, or more correctly the albumin space, by the dilution principle. In addition, it can provide information on the size of the intra and extravascular albumin pools and the turnover rate (F(CA)) of the albumin within the intravascular pool. It can also be used to quantify protein loss into the gut but since the label is reabsorbed in substantial amounts, faecal clearance values are a gross underestimation of the true loss.

As mentioned briefly at the beginning of this introduction, genetic factors can influence the pathogenesis of a parasitic infection. In the case of haemonchosis, the two most widely studied factors in this context are haemoglobin type and breed (Evans, Blunt and Southcott, 1963; Agar, Evans and Roberts, 1972; Allonby and Urquhart, 1976; Altaif and Dargie, 1978a,b; Preston and Allonby, 1979a,b). However, of the two, breed rather than haemoglobin type could appear to be of greater importance in the establishment of H. contortus.

Since several different breeds of sheep were used in the various experiments presented in this thesis, care was taken that the sheep within an experimental group were of the same type, i.e. mountain or lowland. Within a breed type, groups were randomised to ensure an equal distribution of haemoglobin types within the groups.

Although the sheep used in the majority of experiments were raised under conditions designed to minimise parasitic infestation, Strongyloides papillosis and coccidial infections occasionally occurred in the young lambs before the experiments started. These infections were treated promptly and successfully.

This thesis reports the results of a series of experiments designed to study the influence of dietary protein on the pathogenesis of/

of ovine haemonchosis under controlled experimental conditions. In these studies a variety of techniques were used to evaluate the pathophysiological changes in sheep under different dietary regimens and infected with different levels of Haemonchus contortus.

GENERAL MATERIALS AND METHODS

GENERAL MATERIALS AND METHODS.

A. EXPERIMENTAL ANIMALS

The sheep used were of two main types, mountain and downland. They are described in more detail in the relevant chapters.

1. Management before the experiments

The lambs used in the experiments were born indoors to ewes which had been previously treated with an anthelmintic (Panacur, 2.5% suspension, Hoechst) at housing, and also four weeks and two weeks before lambing. The lambs remained indoors from birth and for the duration of the experiments. The male lambs were castrated soon after birth. They were weaned at 6 to 8 weeks of age and given a ration consisting of a 14% crude protein commercial pellet ed feed (Lambwena, Spillers) together with hay ad libitum. Water was available at all times. They were kept either on deep littered straw beds in an open fronted Dutch barn or in concrete floored pens with a fresh straw bed daily.

The lambs were given an intramuscular injection of a selenium/vitamin E solution (Dystocel, Intervet) on two separate occasions after weaning. At this time a blood sample was collected from each lamb by jugular venepuncture for subsequent haemoglobin typing. At regular intervals after weaning and before the start of an experiment, blood samples were collected for routine haematological and biochemical analyses. Additionally, the lambs were weighed and faecal samples collected in order to confirm their parasite-free status. With the exception of Experiment b,Chapter 1,& Chapter 5, the lambs were divided into high and low protein dietary groups on a basis of haemoglobin type, sex and bodyweight. The dietary groups were further divided into "infected" and "control" groups prior to infection. The experimental diets were introduced at least/

least two weeks before infection and the lambs remained on these diets until the end of the experiment.

2. Management during digestibility and N balance studies

The lambs were normally housed in concrete floored pens, as described above. However, when complete and separate collections of urine and faeces were required, the male lambs were fitted with faecal collection bags and housed in standard metabolism cages (Duthie, 1959). Faeces was collected in 200 gauge polythene bags (Fishwick, 1973) held in place by a leather body harness. Urine passed through the mesh floor of the cage and was collected in plastic vessels which had previously been acidified with 100 ml of 5 N hydrochloric acid.

(a) Daily routine during digestibility and nitrogen balance studies

Digestibility measurements and nitrogen balance (N balance) data were conducted over 7-day collection periods which were immediately preceded by 5-day adjustment periods during which time feed intakes were stabilised.

The lambs were given their daily ration in two feeds, one at 10.00 h and the other at 17.00 h. Six litres of fresh water were offered daily to each lamb and their individual consumption recorded.

The composition and quantities of the different diets used in the various experiments are described in the relevant sections. Control lambs were pair-fed to their infected counterpart, i.e. the control lambs were offered exactly the same amount of food consumed by their infected counterpart daily throughout the adjustment and collection periods.

Faecal bags were removed every morning and the total daily faecal output recorded. A 10% sub-sample of the daily faecal output for each lamb was retained for subsequent analyses. The sub-samples were stored in plastic bags which were kept tightly closed between sampling.

Total/

Total daily urinary output for each lamb was measured volumetrically, recorded and a daily 10% sub-sample retained and stored in screw-topped plastic containers until subsequently analysed.

(b) Preparation of feed, faecal and urine samples for analysis

Samples of the diets used were taken for analyses at regular intervals throughout each experimental period.

The bulked daily 10% faecal samples obtained from each lamb during the 7-day collection period were well mixed and suitable aliquots retained for analyses (200 g for drying and subsequent analyses and 200 g for nitrogen (N) estimation).

The bulked daily 10% urine samples for each lamb were well mixed and 2 x 30 ml aliquots retained and stored at - 20°C for later analysis for N content.

B. CHEMICAL ANALYSES OF FEED, FAECES AND URINE SAMPLES

All the analytical methods used were officially established procedures i.e. MAFF et al, 1973; Fertiliser & Feedingstuffs Regulations 1968,1976 and 1982.

Dry matter (DM) The dry matter in food and faecal samples was determined by heating known quantities (~ 200g) in a hot air oven at 80°C for 36 to 48 h until a constant weight was attained.

Total nitrogen(N)Total nitrogen in food and faecal samples was measured by an automated semi-micro Kjeldahl technique (Kjell-Foss Automatic 16210) Urine nitrogen content was measured using a micro-Kjeldahl method.

Ether extract(EE), crude fibre(CF) and ash The ether extract, crude fibre and ash contents of food and faeces were determined using standard methods (The Fertisers and Feedingstuffs Regulations, 1976).

Iron (Fe) in feed and faeces The samples were ashed, dissolved in concentrated hydrochloric /

hydrochloric acid and the iron content determined by atomic absorption spectrophotometry (Fertilisers and Feeding Stuffs Regulations, 1982.

C. CALCULATION OF DIGESTIBILITY COEFFICIENTS, N BALANCE AND IRON BALANCE

"The digestibility of a food is most accurately defined as that proportion which is not excreted in the faeces and which is, therefore, assumed to be absorbed by the animal" (McDonald, Edwards and Greenhalgh, 1981). It is commonly expressed in terms of dry matter as a coefficient :-

$$\text{DM Digestibility coefficient} = \frac{\text{amount apparently absorbed}}{\text{amount in feed (DM)}}$$

The coefficients for each constituent of the DM, e.g. CP, CF, EE, etc. can be calculated in the same way.

Although the proportion of food not excreted in the faeces is commonly assumed to be equal to that which is absorbed from the digestive tract, there are objections to this assumption, particularly in the case of ruminants. For example, in ruminants methane arising from the fermentation of carbohydrate is lost by eructation and thus is not absorbed. This results in an over-estimation of the digestible carbohydrate and digestible energy of the feed. Also because of the presence of metabolic faecal nitrogen in faeces, i.e. nitrogen derived from non-dietary sources such as sloughed epithelial cells, enzymes, etc., there is under-estimation of the proportion of nitrogen absorbed by the animal. Ether extractable substances and minerals of metabolic origin are also found in the faeces, again leading to an under-estimation of their absorption. Consequently, the values obtained in digestibility studies are referred to as "apparent" digestibility coefficients.

In/

In this thesis, N Balance is expressed as g N retained or lost on a daily basis and was calculated using the following equation :-

$$\text{N balance (gN)} = \left[\text{gN feed} - (\text{gN faeces} + \text{gN urine})\right]$$

The iron content of the drinking water, feed and faeces was estimated as described earlier. The results are presented as a percentage of dietary iron apparently absorbed on a daily basis.

D. CARCASE EVALUATION

Since Kempster, Avis, Cuthbertson, and Harrington (1976) had shown that the composition of the "best neck" (i.e. 7th to 10th rib) joint was a reliable index of carcase composition, that joint was selected in this study. The complete joint (i.e.left and right sides) was removed after the carcase had been chilled for 48 hours. It was sealed in a polythene bag and stored at - 20°C for subsequent dissection.

The joints were dissected into muscle, fat and bone as rapidly as possible after thawing, care being taken to prevent excessive loss of moisture by evaporation. The joints, muscle and fat were weighed to the nearest gram and the composition expressed as the relative percentage of muscle, fat and bone.

E. RADIOISOTOPIC STUDIES

1. Daily routine

These studies were conducted concurrently with the digestibility and N balance studies, i.e. when the sheep were in metabolism cages. The sheep were dosed orally each day with 10 ml of 0.75% (w/v) potassium iodide (KI) to prevent uptake of radioactive iodine by the thyroid gland. The dosing started 4 days before injection of the radio-isotopes and continued throughout the 14 day study. Blood samples and sub-samples of the total daily faecal and urinary outputs of each animal were collected regular ly for the measurement of radioactivity (vide infra).

2./

2. Labelling techniques

a. ^{51}Cr-labelled erythrocytes Approximately 20 ml of whole blood/ lamb were collected into heparinised tubes on the day of injection of the isotope. After centrifugation at 550 g for 10 minutes, the plasma was removed and retained for subsequent labelling with radio-iron The cells were re-suspended in isotonic saline by gentle mixing. Approximately 10 ml of $Na_2{}^{51}CrO_4$ in isotonic saline (Radiochemical Centre, Amersham, U.K., specific activity 1 mc/ml, chromium content 5.4 µg/ml) was divided amongst the blood samples, each sample receiving approximately 1 mc of ^{51}Cr.

The isotope and cell suspensions were mixed gently, then incubated at 37°C for 30 minutes to allow labelling to occur. The samples were then centrifuged at 550 g for 10 minutes and the supernatant removed and discarded. The cells were then washed in isotonic saline until free of unbound ^{51}Cr (2 washes) and were finally resuspended in isotonic saline ready for injection (approximate final volume of 15 ml).

b. ^{59}Fe-labelled transferrin The retained plasma from the blood samples collected for the ^{51}Cr-labelling was pooled and mixed with ^{59}Ferric citrate (specific activity 15 µCi/ug : Radiochemical Centre, Amersham) in order to label plasma transferrin. Each sheep received approximately 170 µCi of ^{59}Fe.

c. ^{125}I-labelled albumin Commercial sheep albumin was trace-labelled with radioiodine by the iodine monochloride method of McFarlane (1958). The protein in slightly alkaline medium was treated with iodine monochloride to which the radioactive iodine had been added as carrier-free iodide.

Materials

1. Albumin Commercial sheep albumin was used (Cohn fraction V, Pentex Incorp., Illinois, U.S.A.) and a 2% solution prepared by dissolving 600 mg of the freeze-dried protein in 30 ml isotonic saline.

2. Stock/

2. Stock iodine monochloride solution This was prepared by dissolving 5.0 g of potassium iodide and 3.25 g of potassium iodate in 37.5 ml of distilled water. 37.5 ml of concentrated HCl and 5 ml of carbon tetrachloride were added to give a faint pink colour to the solution. This solution was diluted 1:350 with distilled water to give a solution containing 0.42 mg iodine/ml as iodine monochloride which was then used for the labelling procedure.

3. Glycine buffers (A and B) Buffer A (pH 8.5) was prepared by adding 9 ml molar glycine in N/4 NaCl solution to 1 ml NaOH. This was used to convert iodine monochloride to the hypoiodite.

Buffer B (pH 9.0) was prepared by adding 8 ml molar glycine in N/4 NaCl solution to 2 ml NaOH. This provided the alkaline medium necessary for the reaction to occur.

Procedure

15 ml of buffer A was added to 6 ml of the diluted stock iodine monochloride solution. The radioactive iodine ($\sim$ 10 mCi) was added to the solution and was immediately transferred to the buffered protein solution, 30 ml of 2% sheep albumin + 15 ml of buffer B. The solution was poured into a dialysis sack and carrier protein added to bring the specific activity of the protein to below 5μCi/mg protein and thus reduce the risk of denaturation of the ovine albumin. The labelled protein was dialysed for 48 hours against two changes of 20 litres of isotonic saline.

3. Preparation of standards

A known weight (approx. volume 1 ml) of each of the isotopically labelled preparations was emptied into a 100 ml volumetric flask. The contents of the flask were made up to the mark with 0.02 N NaOH. 1 ml of this solution was dispensed into counting vials and made up to 10 ml with 0.02 N NaOH. The ^{51}Cr standards were made up for each individual sheep but the ^{125}Iodine and ^{59}Iron standards were made up singly. The standards served as corrections against decay, changes in the sensitivity of the counting/

counting equipment and for calculation of the injected dose.

4. Injection of isotopes

Known weights of each isotope were injected into each sheep using a jugular catheter. The catheter was flushed with isotonic saline after the three isotopes had been injected and before withdrawing the catheter. Each sheep received its own labelled red cells.

5. Collection and preparation of blood, faecal and urine samples for counting

Blood samples (5 ml) were collected from the opposite jugular into heparinised vacutainer tubes, 10, 30, 60, 90, 120 and 180 minutes after injection of the isotopes. Thereafter samples were collected twice daily for the next three days and once daily after that for the duration of the experiment.

A packed cell volume estimation was carried out on all the blood samples, then 1 ml of whole blood and 1 ml of plasma were pipetted into counting vials and made up to 10 ml with 0.02 N NaOH.

Total daily faecal and urinary outputs were recorded. The daily faecal collection was well mixed then 2 x 10 g samples collected and packed to a volume of 10 ml in a counting vial. A 10 ml sample of the daily urine output was also retained for counting.

Radioactivity measurements

Count rates of the three isotopes in the blood, plasma, urine and faeces were determined in an automatic well-type scintillation spectrometer (Packard Tricarb Liquid Scintillation Spectrometer). The calculation of crossover factors was based on the relative count rates of the standard solutions of each isotope at each photo peak.

6. Calculation and expression of results

a. ^{51}Cr counts/ml/RBC

The radioactivity of each blood sample was corrected for background radioactivity and physical decay and expressed as counts/minute/ml (c.p.m.)/

(c.p.m.) of red cells using the PCV of each sample.

$$\text{c.p.m./ml RBC} = \frac{\text{c.p.m./ml of blood x 100}}{\text{PCV (\%)}}$$

"Apparent" half-life ($T\frac{1}{2}$)

When autologous ^{51}Cr-labelled red cells are injected into ruminants there is a rapid loss of activity over the first 24 hours due to elution. Once this rapid phase is complete, a slower exponential phase occurs and the red cell $T\frac{1}{2}$ is calculated after extrapolation of this second curve (Holmes, 1969). Since a population of red cells of all ages was labelled (random labelling) and because of elution of the isotope, the value for the half-life of the cells is an under-estimate of the true value and is thus referred to as the "apparent" half-life. The apparent half-life of the cells is the time in hours taken for the radioactivity to fall by 50%.

Calculation of circulating red cell volume (RCV)

This was calculated using the dilution principle and was expressed as ml/kg BW.

$$\underset{\text{(ml/kg BW)}}{\text{RCV}} = \frac{\text{Total injected } ^{51}\text{Cr activity cpm}}{\text{"Corrected" radioactivity 1 ml RBC at 10 mins. post-injection}} \div \text{BW(kg)}$$

Gastrointestinal blood loss (faecal clearance)

This was expressed as the quantity (ml) of RBC lost into the gastrointestinal tract daily.

$$\underset{\text{(ml/d)}}{^{51}\text{Cr Faecal Clearance}} = \frac{\text{Total faecal } ^{51}\text{Cr radioactivity over a 24 h period}}{\text{Activity(cpm)/ml RBC at start of 24 h collection period}}$$

b. ^{59}Fe-labelled transferrin

$T\frac{1}{2}$ and plasma iron turnover rate

A decrease in the plasma activity curve with time usually follows a single exponential curve when plotted on a semi-logarthmic scale. The radioactivity at the moment of injection is inferred by extrapolation/

extrapolation back to the ordinate and the time taken for the plasma radioactivity to decrease to half the initial value ($T\frac{1}{2}$) is read off the graph.

Plasma iron turnover rate, i.e. the amount of transferrin iron passing through the plasma per unit of time (PIT), was calculated using the following formulae (Finch, Deubelbeiss, Cook, Eschbach, Harker, Funk, Marsaglia, Hillman, Slighter, Adamson, Ganzoni and Giblett, 1970).

$$\underset{\text{mg/dl plasma/day}}{\text{PIT}} = \frac{\text{Serum iron (mg/dl)} \times 0.693^{*} \times 1440^{+}}{T\frac{1}{2}\ (\text{m})} \qquad (1)$$

* Natural log of 2 $\qquad$ $^{+}$ Number of minutes/day

Total PIT was calculated by multiplying the result of the above formula (1) by the plasma volume (PV)(ml) thus:-

$$\text{PIT (mg/d)} = (1) \times \frac{\text{PV } (^{125}\text{I})(\text{ml})}{100} \qquad (2)$$

PIT was then expressed as mg/d/kg BW and calculated by dividing (2) by the body weight in kg.

Red Cell Utilisation(%)(RCU)

The percentage utilisation of the injected iron by newly formed red cells was calculated every second day from day 2 until the end of the isotope study using the following formula:-

$$\text{RCU(\%)} = \frac{\text{RCV } (^{51}\text{Cr})(\text{ml}) \times 100}{\text{Total injected } ^{59}\text{Fe activity}} \times \text{cpm/ml RBC on days 2, 4, 6, etc.}$$

Estimation of iron loss and intestinal reabsorption using radiochromium and radio-iron

Measurements were made of intestinal loss and reabsorption of iron after the method of Roche, Pérez-Giménez and Levy (1957).

Radiochromium(^{51}Cr) lost from red cells which are passing into the gut as a result of haemorrhage is not significantly reabsorbed. Thus, this gives a measure of the total blood loss into the gut (faecal clearance). With a knowledge of blood haemoglobin concentration, the/

the amount of haemoglobin-iron lost into the gut can be calculated using the following formula:-

$$\text{Iron loss into gut/d (mg)} = \frac{\text{Hb (g/dl)} \times \text{whole } (^{51}\text{Cr}) \text{ blood loss (ml)} \times 347^{*}}{100 \times 100}$$

* 100 g Hb contains 347 mg Fe

Unlike radio-chromium, radio-iron lost into the gut can be reabsorbed. If gut iron loss is calculated (using ^{59}Fe data), using the same equation as for ^{51}Cr, a measure of the absorption or non-absorption of iron can be obtained:-

$$\text{Iron absorbed from gut (mg)} = \text{Iron lost into gut } (^{51}\text{Cr}) \text{ (mg)} - \text{Iron lost into gut } (^{59}\text{Fe}) \text{ (mg)}$$

c. ^{125}Iodine-labelled albumin

$T\frac{1}{2}$ (^{125}I labelled albumin) This was calculated from the plasma disappearance curve as for the other radio-isotopes.

Plasma volume (PV)

This was calculated using the dilution principle and is expressed as ml/kg BW

$$\text{PV (ml/kgBW)} = \frac{\text{Total injected } ^{125}\text{I activity (cpm)}}{\text{Corrected activity of 1 ml plasma at 10 mins. post-injection (cpm)}} \div \text{BW (kg)}$$

Intravascular pool of albumin (CA)

This was obtained by multiplying the plasma volume (l) by the albumin concentration (g/l) and dividing the result by the BW (kg) so as to express the final result as g/kg BW.

$$\text{CA (g/kg)} = \frac{\text{PV (litres)} \times \text{Serum albumin (g/l)}}{\text{BW (kg)}}$$

Total body albumin (TA)

This was calculated using the "equilibrium time" method described by Campbell, Cuthbertson, Mathews and MacFarlane (1956) which depends on the daily excreted radioactivity in the faeces and urine./

urine. The retained activity(Q_R) was obtained by subtracting the daily excreted activity from the injected activity . The extravascular activity (Q_E) was obtained as the difference each day, between Q_R and Q_P(Plasma activity). At equilibrium time the extravascular activity was maximal, thus the ratio of extravascular activity to intravascular activity was assumed to be equal to the ratio of their pool masses, i.e.

$$\frac{Q_E}{Q_P} = \frac{EA}{CA} \qquad \text{since TA = CA + EA (extravascular pool)}$$

$$TA = CA\ (g/kg) \times \frac{(Q_P + Q_E)}{Q_P}$$

The extravascular pool(EA)of albumin

This was obtained by the difference between TA and CA.

Fractional catabolic rate (F(CA))

The fraction of the intravascular albumin pool broken down each day was calculated by the method of Campbell et al (1956) and based on the excreted activity in urine and faeces. The excreted isotope in the urine and faeces was assumed to be proportional to the amount of labelled albumin in the intravascular pool where degradation occurred. The fraction of the plasma pool broken down each day was then determined from the daily 24h excreted activity(urine(U) plus faeces (F)) and the activity present in the plasma at the beginning of the 24 hour collection period.

$$F(CA) = \frac{\text{Total excreted activity (U+F) over 24 hours}}{\text{Total plasma activity at beginning of 24h collection period}}$$

The fractional catabolic rate calculated as described serves as an index of metabolism and not as an absolute value, as this would require steady-state conditions, which rarely holds true in parasitised animals (Dargie 1975).

F./

F. PARASITOLOGICAL TECHNIQUES

(1) Culture and harvesting of H. contortus larvae

H. contortus larvae were cultured from the faeces of an experimentally infected lamb which had previously been reared and maintained parasite-free. The faeces were incubated in glass jars with loosely fitting lids at room temperature for 10-14 days. At the end of this period, the jars were filled with lukewarm tap water and left for one hour to allow the larvae to migrate into the water. The fluid from the jars was then pooled and poured through a coarse sieve (60 meshes per inch) which retained the gross faecal matter. This fluid containing the larvae was then filtered through two layers of eight inch milk filters (Maxa Filters, A. McCaskie, Stirling) by suction, using a Buchner apparatus and vacuum pump. The filter paper was then removed and placed larval side uppermost on a coarse metal sieve (150 micron pores) which was placed on top of a glass funnel which was closed at the stem with a length of rubber tubing and a clip, and filled with lukewarm tap water. The motile larvae were left to migrate through the paper and sieve into the water below. They accumulated at the neck of the funnel and were collected after 12 hours.

(2) Preparation and administration of larval doses

The concentration of the larval suspension was determined by examining 40 x 0.025 ml aliquots. The suspension was mixed thoroughly during this procedure in order to keep the larvae in suspension and to avoid clumping. Once the concentration of larvae had been determined, appropriate doses for administration were pipetted during continuous mixing into narrow-necked universal bottles to which tap water was added to give a total volume of approximately 15 ml. When very small doses were required, i.e. 200 larvae (Chapter IV), the original suspension was diluted appropriately, checked by counting at least 20 x 0.025 ml aliquots and then dispensed as above. A variation of ± 10% of the desired concentration/

concentration of larvae was attained. If the larvae were not required immediately, they were stored at -4°C until use. The maximum storage time for any dose was 6 days.

After thorough mixing, the doses were administered per os Each bottle was then half-filled with water, shaken and the rinsings also given to each lamb.

(3) Faecal egg counts

Faecal samples were collected either directly from the rectum or from the faecal collection bags. The samples were examined by a modified McMaster technique (Gordon and Whitlock, 1939).

(4) Worm burdens at autopsy

The lambs were euthanased either by intravenous (i.v.) injection of pentobarbitone sodium (200 mg/ml)("Euthatal", May & Baker) or by captive bolt and immediate exsanguination. The omasum and abomasum were removed intact and the duodenum tied at the abomasal:duodenal junction. After removing the omental fat and omasum, the abomasum was opened along its greater curvature. The contents were collected in a graduated bucket and the abomasal folds were carefully washed under a slow stream of water into this bucket. The washings and contents were diluted to 2 litres with tap water and after thorough mixing, 2 x 200 ml samples were collected and stored in jars for subsequent microscopic examanation. 10 ml of 40% formalin was added to each sample as preservative. The abomasum was then laid out on a board, cut in half longitudinally and the mucosa from each half scraped off with a knife. The mucosal scrapings were digested in a pepsin-hydrochloric acid mixture (Herlich, 1956) at 42°C for 6 hours. The digests were then formalinised, diluted to 2 litres, mixed well and 2 x 200 ml samples removed for worm counting.

To/

To each 200 ml aliquot of abomasal washings and digests, 2-3 ml iodine solution (720 g potassium iodine and 450 g iodine crystals/ litre distilled water) was added and mixed thoroughly. Ten 4 ml aliquots were withdrawn by a pipette and placed in petri dishes. 2-3 ml of 50 g/l sodiumthiosulphite solution was then added to each aliquot to clear the background, leaving the parasites stained with iodine.

The aliquots were examined under a dissection microscope and the number of parasites found multiplied by the appropriate dilution factor, i.e. 50, to give the total worm burden in the original 2 litres.

G. HAEMATOLOGICAL TECHNIQUES

1. Collection of samples

Blood samples were collected by jugular venepuncture into evacuated (2.5 ml) glass tubes (Vacutainer, Becton Dickinson) containing anticoagulant. Heparin (100 i.u./ml blood) was used unless white cell counts and/or smears were to be made, in which case di-potassium sequestrene (K_2EDTA)(5 mg/2.5 ml blood) was used since this latter anti-coagulant does not discolour the smear or cause the white cells to clump. Samples were gently inverted 2-3 times immediately after collection to ensure complete mixing.

2. Analyses and calculation of red cell indices

Red cell counts (RCC) and white cell counts (WCC) were performed using an electronic particle counter (Model ZF, Coulter Electronics, Harpenden, Herts.). The machine was calibrated daily using a standard blood sample of known value (Coulter Electronics). Once calibrated, the aperture size on the machine was adjusted to allow accurate estimation of/

of sheep red cells. In addition to providing the red and white cell counts, the values for haematocrit (Hct) and mean cell volume (MCV) were also given. Haemoglobin (Hb) was measured by a colorimeter method (Coulter Haemoglobinometer) after its conversion to cyanmethhaemoglobin with Zapoglobin (Coulter Electronics). When full haematological analyses were not required, the haematocrit was measured using the microhaematocrit method.

Calculation of the red cell indices:-

Mean cell volume (MCV) (Provided by the electronic particle counter).

$$\underset{\text{(femtolitres)(fl)}}{\text{MCV}} = \frac{\text{Haematocrit (l/l) x 1000}}{\text{RCC (cells x } 10^{12}\text{/l)}}$$

Mean corpuscular haemoglobin (MCH):

$$\underset{\text{(picograms)(pg)}}{\text{MCH}} = \frac{\text{Hb(g/dl) x 10}}{\text{RCC (cells x } 10^{12}\text{/l)}}$$

Mean corpuscular haemoglobin concentration (MCHC):

$$\underset{\text{(g/dl)}}{\text{MCHC}} = \frac{\text{Hb(g/dl)}}{\text{Haematocrit(l/l)}}$$

3. Preparation of blood films

Films were made within 3 hours of collection of the sample. They were air dried immediately and then stained using the May-Grünwald Giemsa staining technique (Dacie and Lewis, 1975).

H. BIOCHEMICAL TECHNIQUES

1. Collection of samples

Blood for biochemical analyses was collected into 10 ml iron-free vacutainer tubes containing no anti-coagulant. The samples were left inverted at room temperature for 24 h before the serum was harvested. Serum was stored at -20°C for later analysis.

2./

2. Analytical Techniques

Total serum protein, albumin and urea

These were estimated by continuous flow analysis (Standard Technicon Auto-Analyser II methods). Globulin was calculated as the difference between total protein and albumin concentration.

Serum iron, total iron binding capacity (TIBC), % saturation of transferrin

Serum iron was determined spectrophotometrically using a commercial test kit (Roche Diagnostica). Within and between assay variance was <4%. Iron binding capacity was also determined using a commercial test kit (Roche Diagnostica). Control sera of known serum iron and TIBC were used in each assay.

The % saturation of transferrin with iron was calculated as follows:-

$$\% \text{ saturation} = \frac{\text{Serum iron } (\mu g/dl)}{\text{TIBC } (\mu g/dl)} \times 100$$

Haemoglobin typing

Haemoglobin typing was carried out by electrophoresis of haemolysed red cells on cellulose acetate strips, as described by Smithies (1955)

I. STATISTICAL ANALYSIS

The standard "t" test was used to analyse the results with the exception of the worm burdens for which the non-parametric Mann-Witney test was used.

Probabilities of $P < 0.05$ were considered significant.

CHAPTER I

Studies on the pathogenicity of a single infection with 50 *H. contortus* larvae/kg BW on sheep fed either a high or low protein diet

INTRODUCTION

Two experiments were conducted to investigate, under controlled experimental conditions, the chronic haemonchosis syndrome as previously described by Allonby and Dargie (1973).

In their experiment, Allonby and Dargie (1973) attempted to reproduce the field condition of chronic haemonchosis by artificially infecting a small number (5) of 3 month old, housed Merino lambs with a total of 800 H. contortus larvae/lamb (equivalent to ~ 50 larvae/kg BW) over a 7 day period. The lambs were kept on a low plane of nutrition, in the form of ad lib hay and water only, for 7 months following infection. Their subsequent progress during this time relative to uninfected controls (2 lambs) was monitored by regular weighings, haematological analyses and faecal egg counts (epg). After 7 months the infections were terminated with anthelmintic. Both groups were then restored to a high plane of nutrition, no details of which were presented, and their progress monitored for a further 4 months.

From the data presented by Allonby and Dargie, the infected lambs developed a mild anaemia (haematocrit ~ 0.23 l/l) and weight gain was retarded in the 7-month period following infection. Faecal egg counts reached maximal levels of ~ 5,000 epg at 5 weeks post-infection. However, no radioisotopic studies were conducted on these lambs, though some data is presented later in the paper on changes in albumin metabolism in sheep supposedly suffering from the field form of chronic haemonchosis.

Thus, the information to be gained from the indoor experiment of Allonby and Dargie described above is obviously limited since the/

the infection was terminated with anthelmintic (i.e. there was no assessment of worm burdens) and no pathophysiological studies were conducted.

Therefore the following experiments were conducted to confirm and extend the studies of Allonby and Dargie, attempting to reproduce the chronic haemonchosis syndrome under experimental conditions.

TABLE I.a.1: Studies on the pathogenicity of a single infection with 50 *H. contortus* larvae/kg BW on sheep kept on either a high or low protein diet - Experimental design.

Expt.	Breed	Dietary Status	Age at infection	Dose of *H. contortus*	Metabolism Cage Studies weeks post-infection	Slaughter (weeks post-infect.)
Ia	FINN DORSET Infected	High Protein	10-12m	50 l/kgBW	none	28
	FINN DORSET Infected	Low Protein	10-12m	50 l/kgBW	none	28
Ib	MERINO Infected	Low Protein	8m	50 l/kgBW	3-5w	27
	MERINO Controls	Low Protein	-	-	3-5w	27
	SCOTTISH BLACKFACE Infected	Low Protein	6m	50 l/kgBW	5→ 9w	27
	SCOTTISH BLACKFACE Controls	Low Protein	-	-	5→ 9w	27

EXPERIMENT Ia

An investigation into the pathogenesis of a single low infection with H. contortus on Finn Dorset wethers kept on either a high or low protein diet.

MATERIALS AND METHODS

Experimental design

Finn Dorset yearling wethers, fed either a high or low protein diet, were infected with 50 H. contortus larvae/kg BW. Blood and faecal samples were collected and weights were recorded weekly throughout the infection. The sheep were killed 28 weeks after infection and worm burdens were assessed (Table I.a.1)

Experimental animals

Ten Finn Dorset (FD) sheep were used. They had been reared under parasite-free conditions from birth, and at 5 months of age were divided equally, on a bodyweight basis, into two dietary treatment groups. One group received hay plus a pelletted supplement, and the other hay.

Feeding and housing

During the period of 5 to 12 months of age the sheep in the"high protein"group were offered hay (64 g CP/kg DM) to appetite and a daily allowance of 500 g of a commercial concentrate (161 g CP/kg DM). The sheep in the "low protein" group were offered hay only to appetite. The high protein ration given to 35 kg sheep (i.e. mean group bodyweight at 5 months of age) was calculated to supply sufficient daily Metabolisable Energy (ME) to provide maintenance and a daily live weight gain (DLWG) of 110 g (MAFF et al, 1975). The low protein ration was calculated to supply maintenance and allow about 50 g DLWG to sheep of similar weight.

Samples of the hay and concentrate ration were collected for the appropriate analyses at regular intervals throughout the experiment. The proximate composition of the diets used is presented in Table I.a.2).

The/

TABLE I.a.2: Proximate composition of high and low protein diets. Mean (± SE) values.

Feedstuff	D.M. Coefficient	CP	CF	EE	ASH	NFE	OM
		g/kgDM					
Hay	0.860 (0.0070)	64.66 (2.138)	384.63 (9.594)	8.39 (0.873)	69.33 (4.208)	459.75 (14.46)	927.58 (5.053)
Pelleted Ration	0.874 (0.0053)	161.2 (1.834)	104.8 (3.890)	26.3 (1.421)	137.0 (0.997)	578.03 (4.576)	862.97 (1.000)
Chopped Hay	0.853 (0.0188)	74.11 (3.172)	386.17 (12.526)	8.09 (0.941)	71.88 (5.039)	459.75 (14.46)	927.58 (5.03)

The sheep were fed twice daily and fresh water was available at all times. They were housed in concrete floored pens, as previously described in General Materials and Methods.

RESULTS

Clinical and bodyweight changes

At 5 months of age the mean group bodyweight was similar in both treatment groups, i.e. 34 kg. However, after a 7-month period on the diets, a considerable difference in mean group bodyweights existed, the mean group bodyweight of the high protein group being 57 kg whereas that of the low protein group was 42 kg (Fig. I.a.1). In the 28 weeks following infection, mean group body weights in the high protein group increased from 60 to 65 kg and in the low protein group from 43 to 46 kg.

Throughout the experiment both groups of sheep remained alert and there were no feed refusals.

Haematological and biochemical changes

Both groups of sheep developed a normocytic normochromic anaemia after infection. Haematocrit values in the high protein group fell from a pre-infection mean of 0.355 to 0.26 1/1 nine weeks after infection. Haematocrit values in the low protein group fell from 0.38 to 0.25 1/1 11 weeks after infection. Haematocrit values in the high protein group stabilised 9 weeks after infection, whereas values in the low protein group continued to fall (Fig. I.a.2). Corresponding falls in haemoglobin and red cell counts also occurred in both groups but values for the red cell indices, namely, MCH, MCV and MCHC, remained within normal limits throughout the study (Appendix A, Table 1).

Total serum protein and albumin were similar in both groups of sheep before infection. A gradual but slight fall in total protein and albumin occurred after infection but values were still within the normal range for sheep (Appendix A, Table 2).

Parasitological/

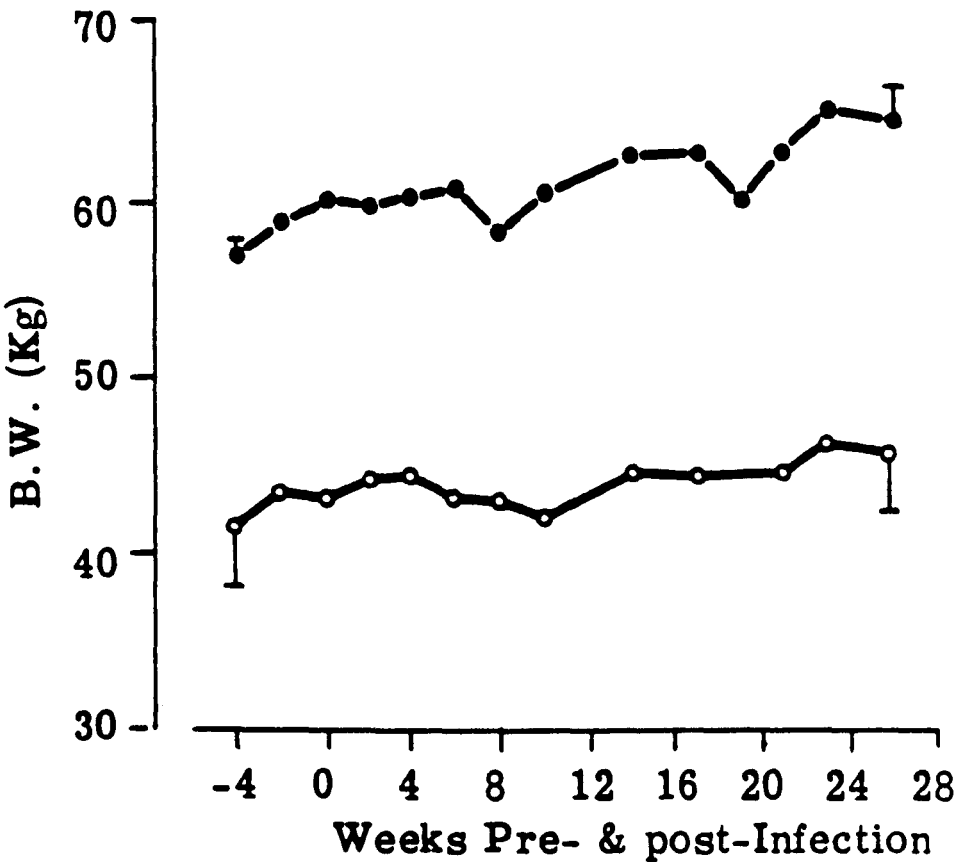

Fig. I.a.1: Mean (± SE) group body weights of yearling Finn Dorset wethers infected with 50 H. contortus larvae/kg BW and fed either a high or low protein diet.

●————● High protein o————o Low protein

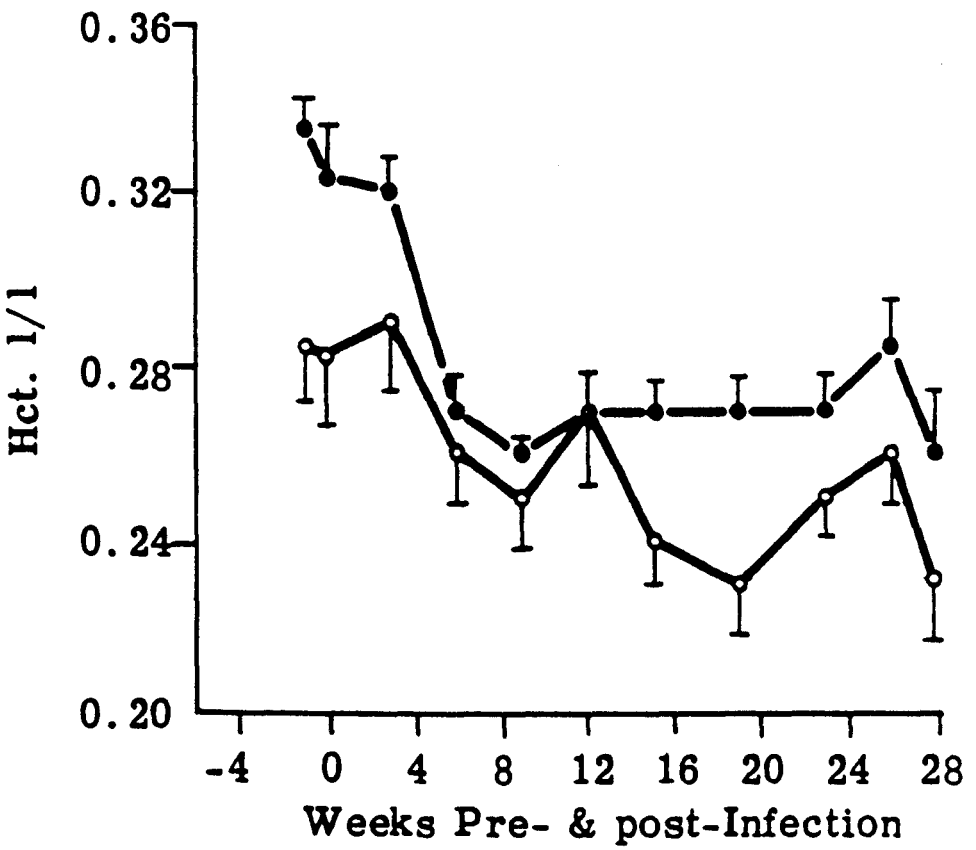

Fig.I.a.2: Mean (± SE) haematocrit values of yearling Finn Dorset wethers infected with 50 H. contortus larvae/kg BW and fed either a high or low protein diet.

●———● High Protein o———o Low Protein

Parasitological findings

Faecal egg counts rose rapidly in both groups (Fig. I.a.3). Peak values (7,790 epg) in the high protein group occurred 5 weeks after infection and maximum levels (5,410 epg) in the low protein group were reached 8 weeks after infection. From week 8 onwards counts fell gradually and in a similar way in both dietary groups to reach very low levels prior to slaughter at 28 weeks. Two animals in the high protein group had negative faecal egg counts by week 15 and they continued to be negative for the remainder of the experiment.

The two sheep in the high protein group with negative egg counts after week 15 had no worm burdens at slaughter (Table I.a.3). The remaining 3 sheep in this group had burdens ranging from 150-300 worms. Three sheep in the low protein group had negative worm burdens at slaughter, whilst the two remaining sheep had burdens of 50 and 200 respectively.

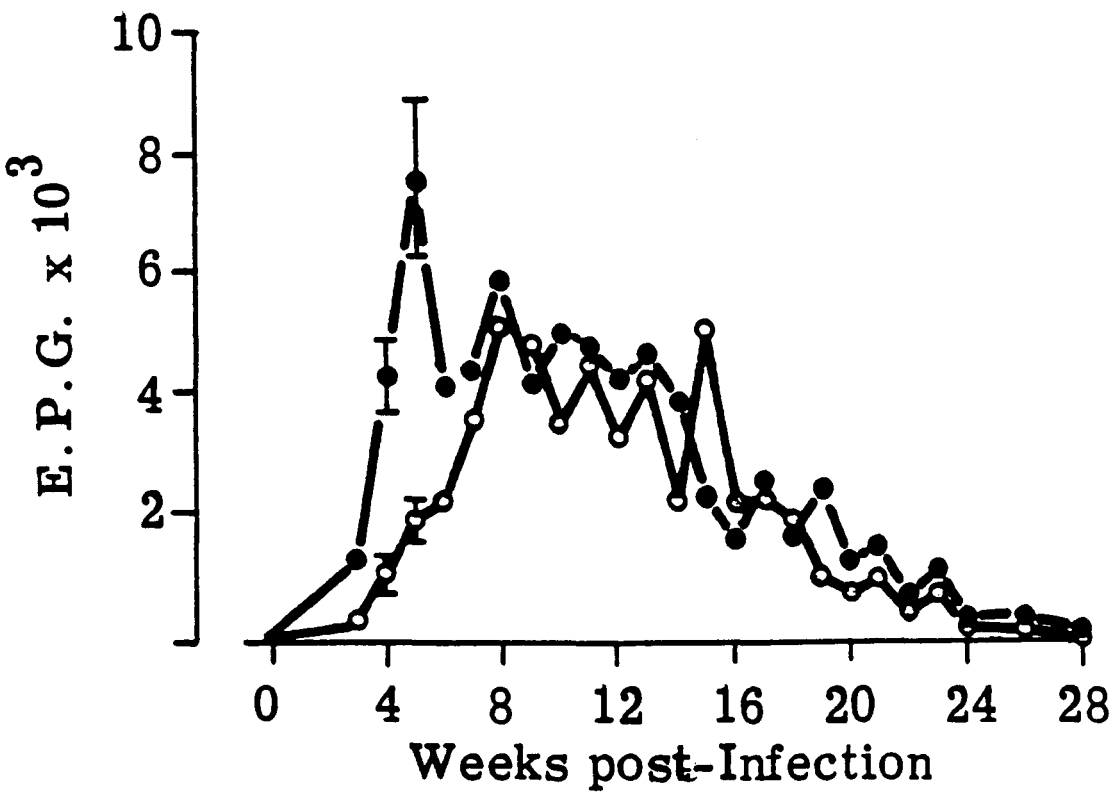

Fig. I.a.3: Mean (± SE) faecal egg counts (epg) of yearling Finn Dorset wethers infected with 50 H. contortus larvae/kg BW and fed either a high or low protein diet.

●———● High Protein o———o Low Protein

TABLE I.a.3: Individual worm burdens at slaughter: Finn Dorset wether lambs.

Group	No.	Larval Stages	Adult		Total Burden
			♂	♀	
High Protein	021	0	150	0	150
	22	0	300	0	300
	23	0	200	50	250
	24	0	0	0	0
	25	0	0	0	0
Low Protein	W68	0	0	0	0
	69	0	0	0	0
	71	0	200	0	200
	72	0	0	50	50
	73	0	0	0	0

EXPERIMENT Ib

An investigation into the pathophysiological changes associated with a single low infection with H. contortus on Merino and Scottish Blackface sheep kept on a low protein diet.

MATERIALS AND METHODS

Experimental design

Five 8-month old Merino and five 6-month old Scottish Blackface wether lambs, fed a low protein diet of hay only, were artificially infected with a single dose of 50 H. contortus larvae/kg BW. Four additional lambs of each breed, also kept on a hay only diet, were used as uninfected controls. In addition to the routine weekly collection of blood and faecal samples and weight recording, radioisotopic, digestibility and N balance studies were conducted in the early stages of the infection. The lambs were slaughtered 27 weeks after infection and the worm burdens assessed (Table I.a.1).

Experimental animals

Nine 7-month old Merino wether lambs (mean bodyweight 25 kg) which had been reared under parasite-free conditions were divided at random into infected (5 animals) and control (4 animals) groups, as described in General Materials and Methods. Nine 5-month old Scottish Blackface wether lambs (mean bodyweight 25 kg) were similarly divided into infected and control groups.

Feeding and housing

All the lambs were kept on a diet of hay only (64 g CP/kg DM) for one month before infection and throughout the experiment. The hay was on offer ad lib. whilst the lambs were in pens. During the radioisotopic and nutritional studies, the lambs were moved to standard sheep metabolism cages. In order to ensure a uniform feed intake the hay was given chopped during this study. Each lamb was offered the chopped hay to appetite (500-900 g/day) and the daily allowance was given in two feeds, one/

one in the morning and one in the afternoon, as described in General Materials and Methods. Control lambs were pair-fed to their infected counterpart during the cage study.

An average daily intake/25 kg BW lamb of approximately 1 kg of this hay may be calculated to supply enough energy and protein for a little growth (30 g/d) in excess of maintenance. Samples of the hay and chopped hay were collected for the appropriate analysis at regular intervals throughout the experiment (Table I.a.2).

Radioisotopic and nutritional studies

The radioisotopic studies using the three radioisotopes ^{51}Cr-labelled erythrocytes, ^{59}Fe-labelled transferrin and ^{125}I-labelled albumin, as described previously in General Introduction and Materials and Methods, were started on day 20 after infection in both groups of Merino lambs and continued over a 2-week period. During this time a seven-day collection period for digestibility and N balance was conducted after a preliminary adjustment period. Control lambs were pair-fed to their infected counterparts. Similar studies were carried out in the Blackface lambs starting 47 days after infection.

RESULTS

Clinical and bodyweight changes

Both groups of Merino lambs lost weight after the dietary change from the normal rearing diet, as described in General Materials and Methods. The infected lambs lost weight during the 28-week period following infection from an initial liveweight of 23 kg to about 19 kg at the end of the experiment. During this time the control Merino lambs also lost weight but their weight loss of about 3 kg,was less than that of the infected group (Fig. I.b.1).

In/

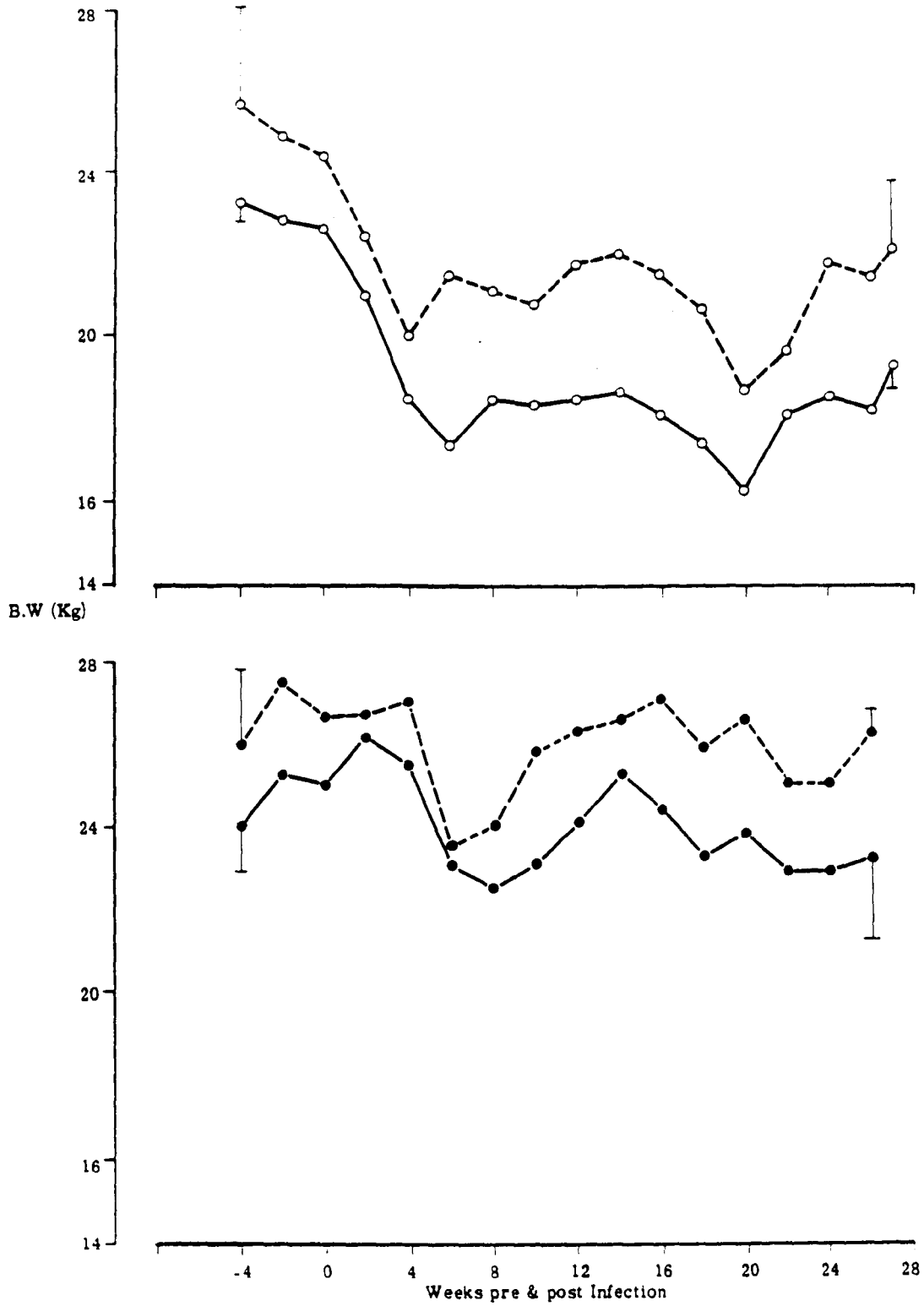

Fig. I.b.1: Mean (± SE) group body weights of infected (50 H. contortus larvae/kg BW) Merino and Blackface lambs and their respective controls. All groups were fed a low protein diet.

o——o Merino Infected ●——● Blackface Infected

o- - - - -o Merino Controls ●- - - - -● Blackface Controls

In contrast, both groups of Blackface lambs continued to gain weight after the change on to a hay only diet in the four-week period preceding infection. The infected group lost about 2 kg in weight over the 28-week infection period, whilst control sheep had the same liveweight at the end of the experiment, 25-27 kg.

During the first week of the metabolism cage study, two Merino lambs, one infected and the other its pair-fed control, became weak and unable to stand. They were removed from the experiment. Otherwise, no adverse clinical signs were observed in the infected animals.

Haematological and biochemical changes

The infected Merino lambs developed a moderate normochromic normocytic anaemia after infection, haematocrit values falling from a pre-infection mean of 0.29 to 0.24 l/l six weeks post-infection (Fig.I.b.2). Values continued to fall throughout the experiment to a pre-slaughter value of 0.21 l/l. The red cell indices (i.e. MCH, MCV and MCHC) remained within normal ranges throught the experiment (Appendix A, Table 3). Control Merino lambs also developed a moderate anaemia, their haematocrit values falling from 0.32 to 0.24 l/l during the experiment, again with no change in the red cell indices. However, the haemoglobin values, red cell counts and haematocrit values in the infected group of Merino lambs were invariably significantly lower than those in the control lambs (Appendix A, Table 3).

The infected Blackface lambs became moderately anaemic, haematocrit values falling from 0.339 to 0.25 l/l in the course of the experiment. The control lambs also became anaemic but the fall in haematocrit occurred later in the experiment relative to the Merino control lambs, i.e. at week 16 in the Blackface controls compared with week 6 in the Merino controls. The red cell indices in both groups of Blackface lambs also remained within normal limits (Appendix A, Table 4).

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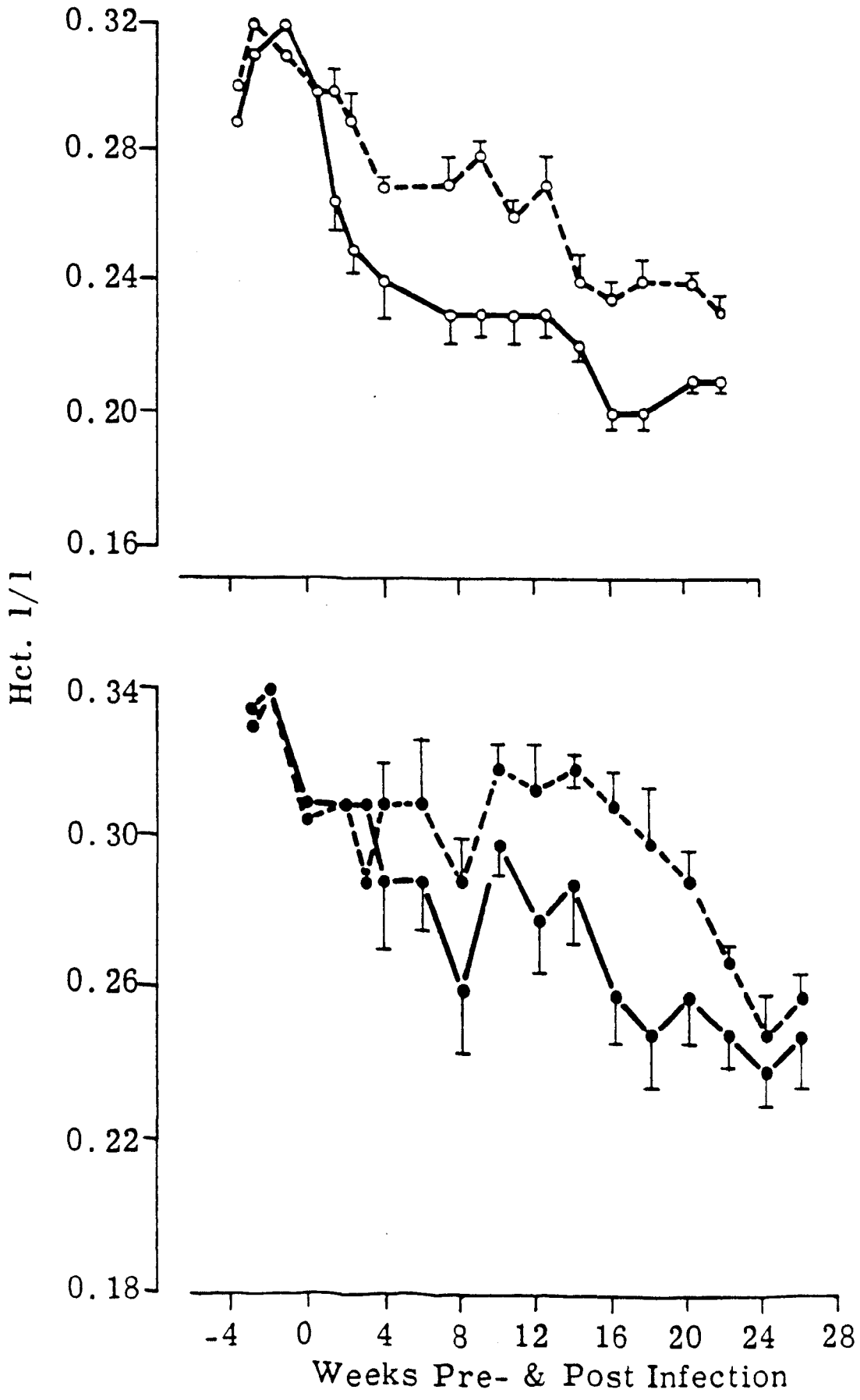

Fig. I.b.2: Mean (± SE) haematocrit values of infected (50 H. contortus larvae/kg BW) Merino and Blackface lambs and their respective controls.

o———o Merino Infected ●———● Blackface Infected

o- - - - -o Merino Controls ●- - - - -● Blackface Controls

The anaemia in the infected Merino lambs was significantly greater than that in the infected Blackface lambs between weeks 3 and 14 and 20 and 22. In addition, the Merino control lambs had significantly lower haematocrit values than the Blackface control lambs from week 6 until the end of the study.

Total serum protein and albumin levels in the infected Merino lambs fell slightly during the experiment from pre-infection values of 68 and 38 g/l respectively to 57 and 27 g/l respectively at slaughter. Globulin values fluctuated between 26 and 34 g/l. Control lambs also had slight falls in total serum protein and albumin during the experiment (Appendix A, Table 5).

Values for total serum protein and albumin were similar in both groups of Blackface lambs but a rise in globulin levels with a simultaneous rise in total serum protein concentrations was observed in both infected and control lambs in the latter half of the experiment (Appendix A, Table 6).

Serum iron levels in both groups of infected lambs ranged from 132 to 184 µg/dl with % saturation of transferrin ranging from 24 to 72%. An increase in % saturation was observed from week 15 post-infection in the infected Blackface lambs. Serum iron levels in the control lambs ranged from 134 to 200 µg/dl with % saturation ranging from 31 to 68%. An increase in % saturation was observed in the Blackface control lambs similar to that seen in the infected Blackface lambs (Appendix A, Table 7).

Parasitological findings

Faecal egg counts rose rapidly in both groups of infected lambs. Peak values (7,100 epg) occurred 6 weeks after infection in the Merino group. Counts then fluctuated between 7,000 and 4,500 epg until week 14 after/

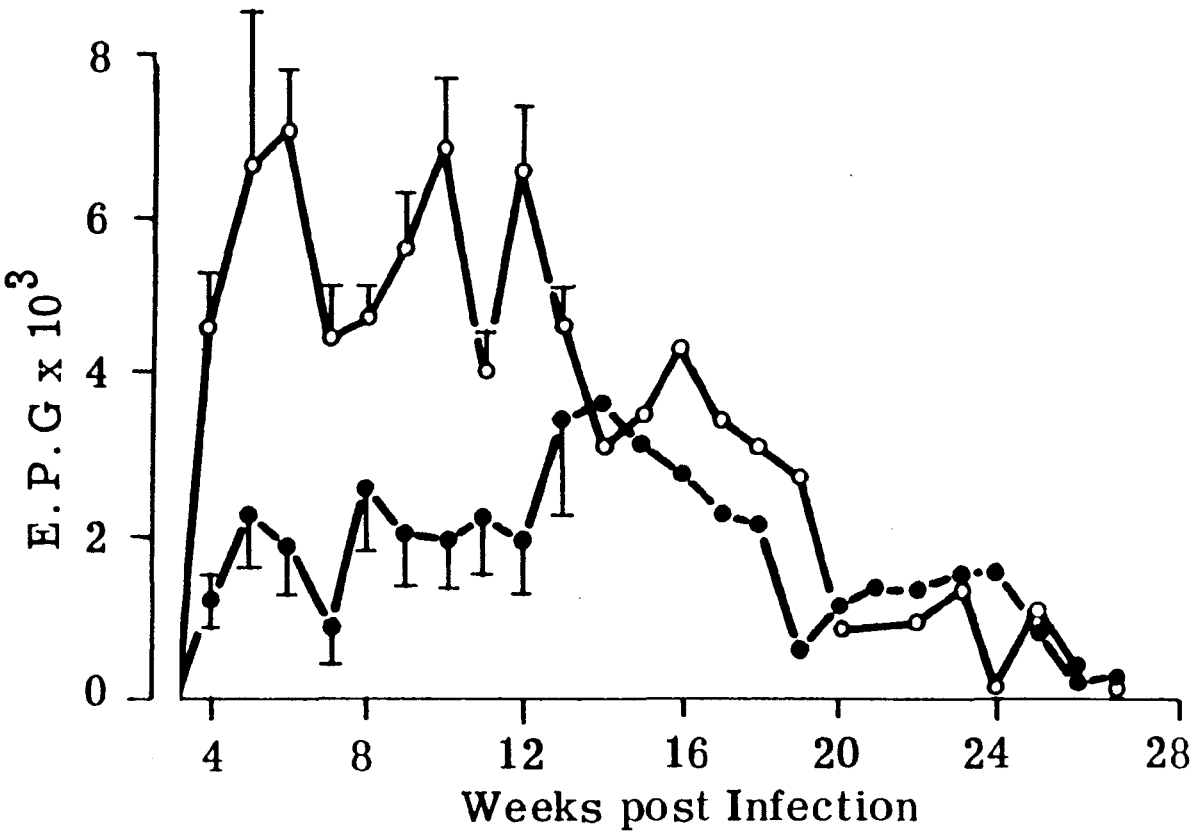

Fig. I.b.3: Mean (± SE) faecal egg counts (epg) of Merino and Blackface lambs infected with 50 H. contortus larvae/kg BW.

○——○ Merino lambs ●——● Blackface lambs

TABLE I.b.1: Individual worm burdens at slaughter: Merino and Blackface lambs.

Breed	No.	Hb type	Larval Stages	Adult		Total Burden
				♂	♀	
Merino	G40	A	0	250	50	300
	41	A	0	50	50	100
	42	A	0	50	100	150
	43	AB	0	150	0	150
	(44*)	(AB)	0	(500)	(200)	(700)
Black-face	FR31	AB	0	0	0	0
	32	AB	0	0	0	0
	33	AB	0	300	0	300
	34	A	0	100	0	100
	35	A	0	0	0	0

*Euthanased 3 weeks post-infection.

after which they fell progressively to 275 before slaughter (Fig. I.b.3).

Peak values (3,690 epg) occurred 14 weeks after infection in the Blackface lambs. Thereafter values followed a similar pattern to Merino lambs (Fig. I.b.3).

All the Merino infected lambs had worm burdens at slaughter ranging from 100 to 300, whereas only two of the five infected Blackface lambs had parasites present at slaughter (Table I.b.1). Both groups of control lambs were negative for worms at slaughter.

Radioisotopic studies

Erythrokinetics (Table I.b.2)

Circulating red cell volumes were slightly lower in the infected Merino lambs compared with their controls but this difference was not statistically significant. Similarly, although the "apparent" half-life of the ^{51}Cr-labelled red cells was lower in the infected lambs compared with controls, this,too, was not statistically significant. However, the infected Merino lambs were each losing significantly greater amounts into the gastrointestinal tract daily (approximately 5 ml) in contrast to the control lambs which were losing less than 0.5 ml red cells daily ($P < 0.001$).

Similarly, in the infected Blackface lambs red cell volumes were reduced relative to their controls but this was not statistically significant. Values for the "apparent" half-life of ^{51}Cr-labelled red cells were similar in both infected and control lambs and were greater than those recorded for both groups of Merino lambs. The infected Blackface lambs were each losing approximately 3 ml of red cells daily into the gut compared with a loss of approximately 0.3 ml in the controls ($P < 0.01$).

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TABLE I.b.2: Erythrokinetic studies: Merino and Blackface lambs.

Group	No.	Hct. l/l	RCV(^{51}Cr) ml /kg BW	PV(^{125}I) ml /kg BW	TBV ml /kg BW	$T_{\frac{1}{2}}$(^{51}Cr) (h)	^{51}Cr Faecal Clearance ml RBC/d	$\bar{x}$ ± S.D.
Merino Infected	G40	0.30	15.8	53.1	68.9	164.2	5.24	(0.493)
	41	0.27	13.7	61.7	75.4	157.3	4.28	(0.862)
	42	0.235	11.1	56.2	67.3	132.0	4.58	(1.101)
	43	0.255	14.9	56.2	71.1	186.9	4.96	(0.520)
	$\bar{x}$	0.265	13.9	56.8	70.7	160.1	4.77	
	S.E.	(0.014)	(1.02)	(1.79)	(1.76)	(11.30)	(0.211)	
Merino Control	Y17	0.285	16.8	58.8	75.6	159.3	0.29	(0.087)
	18	0.26	13.8	50.2	64.0	192.6	0.35	(0.058)
	25	0.26	14.5	49.2	63.7	171.9	0.15	(0.051)
	$\bar{x}$	0.27	15.0	52.7	67.8	174.6	0.26	
	S.E.	(0.008)	(0.91)	(7.05)	(3.92)	(9.71)	(0.059)	
B'face Infected	FR31	0.275	10.9	50.4	61.3	239.6	1.94	(0.27)
	32	0.225	9.4	52.1	61.5	254.4	3.44	(0.50)
	33	0.275	11.9	41.2	53.1	183.6	2.81	(0.48)
	34	0.28	12.6	51.6	64.2	209.2	4.29	(0.42)
	35	0.27	11.6	49.3	60.9	247.5	1.24	(0.21)
	$\bar{x}$	0.27	11.3	48.9	60.2	226.9	2.74	
	S.E.	(0.012)	(0.54)	(1.99)	(1.87)	(13.28)	(0.538)	
B'face Control	P35	0.305	18.0	50.8	68.8	236.6	0.21	(0.06)
	36	0.265	15.0	51.0	66.0	214.5	0.42	(0.12)
	37	0.25	13.3	50.3	63.6	244.5	0.20	(0.04)
	38	0.27	12.8	41.0	53.8	230.8	0.26	(0.09)
	$\bar{x}$	0.27	14.8	48.3	63.1	231.6	0.27	
	S.E.	(0.012)	(1.26)	(2.43)	(3.26)	(6.35)	(0.051)	

The gastrointestinal blood loss in the infected Merino lambs was significantly greater ($P < 0.01$) than that in the infected Blackface lambs.

Ferrokinetics (Table I.b.3)

Serum iron levels on the day of injection of ^{59}Fe-labelled transferrin were within normal limits in all groups of lambs (148 - 197 µg/dl).

Values for the half-life of ^{59}Fe-labelled transferrin were lower in the infected Merino group relative to the control group, but the difference was not statistically significant. Plasma iron turnover (PIT) was slightly greater in the infected Merinos relative to control values but again the difference was not significant (Table I.b.3). The red cell utilisation curve (Fig. I.b.4) in the infected Merinos was slightly steeper than the control group but % utilisation on day 12 after injection of the labelled protein was similar in both infected and control animals.

The half-life for ^{59}Fe-labelled transferrin was significantly reduced ($P < 0.01$) in the infected Blackface lambs, 86 minutes compared with 130 minutes in the control group. There was a corresponding increase in PIT in the infected lambs, 0.98 µg/d/kg relative to control values of 0.56 µg/d/kg BW ($P < 0.001$). The red cell utilisation curve was steeper in the infected group but differences between control and infected groups were not significant.

Albumin metabolism (Table I.b.4)

Plasma volumes were slightly increased in the infected group of Merino lambs relative to control values but this difference was not significant. However, the slight increase in the plasma volumes of the infected group was associated with an increase in the intravascular albumin pool (CA) in the infected lambs ($P < 0.02$). There was no significant difference in the extravascular albumin pools (EA) between infected/

TABLE I.b.3: Ferrokinetic studies: Merino and Blackface lambs.

Group	No.	Serum Iron µg/dl on isotope day	$T\frac{1}{2}$ (m)	Total PIT mg/d/kgBW	Day of maximal uptake of ^{59}Fe
Merino infected	G40	197	208.8	0.50	12
	41	169	159.6	0.65	10
	42	150	153.6	0.57	6
	43	149	165.0	0.51	13
	$\bar{x}$	166	171.8	0.56	10
	SE	(11.2)	(12.57)	(0.034)	(1.5)
Merino control	Y17	231	191.4	0.74	13
	18	173	263.4	0.35	10
	25	187	208.8	0.45	12
	$\bar{x}$	197	221.2	0.51	11.7
	SE	(17.5)	(21.69)	(0.117)	(0.9)
Blackface infected	FR31	159	87.5	0.92	14
	32	144	78.4	1.06	10
	33	188	87.6	0.89	14
	34	175	89.4	1.04	14
	35	180	90.7	0.97	8
	$\bar{x}$	169	85.7	0.98	12
	SE	(7.9)	(2.16)	(0.033)	(1.37)
Blackface control	P35	143	128.4	0.57	14
	36	147	139.2	0.54	14
	37	133	104.4	0.64	14
	38	170	145.8	0.48	14
	$\bar{x}$	148	129.5	0.56	14
	SE	(7.8)	(9.08)	(0.033)	(0.0)

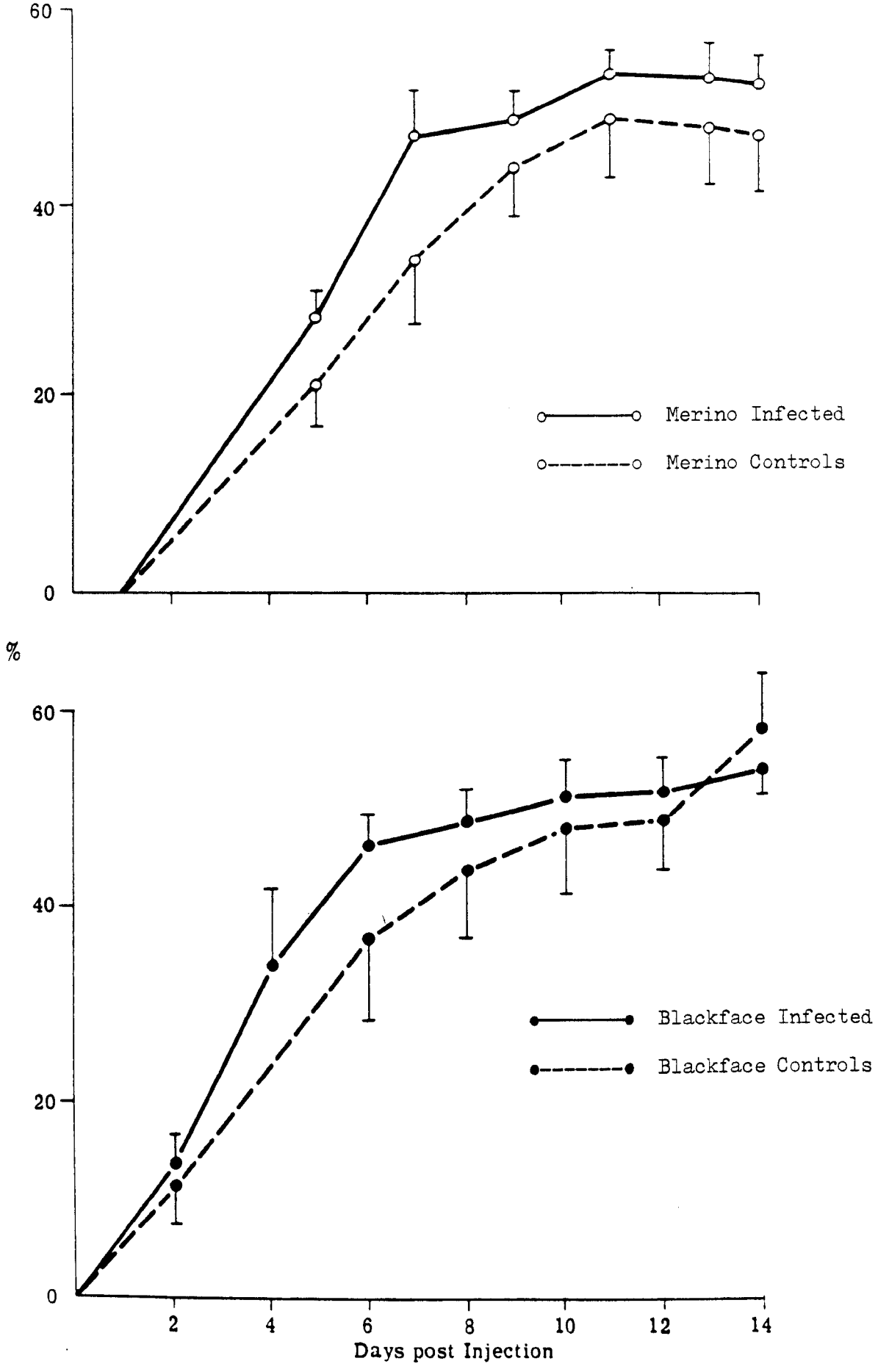


Fig. I.b.4: Mean (± SE) red cell utilisation of ^{59}Fe in Merino and Blackface lambs infected with 50 H. contortus larvae/kg BW and their respective controls.

TABLE I.b.4: Albumin metabolism studies: Merino and Blackface lambs.

Group	No.	Albumin g/l	PV (^{125}I) ml/ kg BW	CA g/kgBW	EA g/kgBW	EA/CA	$T_{\frac{1}{2}}$ (^{125}I) (h)	F(CA)
Merino infected	G40	36	53.1	1.91	2.81	1.47	363.9	0.060
	41	32	61.7	1.97	2.55	1.29	328.2	0.046
	42	33	56.2	1.86	2.15	1.16	328.9	0.061
	43	33	56.2	1.85	2.28	1.23	352.8	0.054
	$\bar{x}$	33.5	56.8	1.90	2.45	1.29	343.5	0.055
	SE	(0.86)	(1.79)	(0.028)	(0.147)	(0.066)	(8.89)	(0.003)
Merino control	Y17	30	58.8	1.76	2.33	1.32	504.1	0.046
	18	36	50.2	1.81	2.0	1.10	436.2	0.046
	25	35	49.2	1.72	2.23	1.30	404.5	0.047
	$\bar{x}$	33.7	52.7	1.76	2.19	1.24	448.3	0.046
	SE	(1.86)	(3.05)	(0.023)	(0.098)	(0.070)	(29.38)	(0.0003)
Blackface infected	FR31	29	50.4	1.46	2.05	1.40	449.9	0.058
	32	27	52.1	1.41	2.39	1.69	393.9	0.067
	33	30	41.2	1.24	2.44	1.97	364.4	0.065
	34	31	51.6	1.60	2.82	1.76	367.0	0.074
	35	34	49.3	1.68	2.76	1.64	405.1	0.059
	$\bar{x}$	30.2	48.9	1.48	2.49	1.69	396.1	0.065
	SE	(1.16)	(1.99)	(0.077)	(0.139)	(0.092)	(15.54)	(0.003)
Blackface control	P35	31	50.8	1.58	2.53	1.60	418.0	0.051
	36	31	51.0	1.58	2.26	1.43	372.5	0.054
	37	33	50.3	1.66	2.24	1.35	463.0	0.057
	38	31	41	1.27	2.23	1.75	474.0	0.062
	$\bar{x}$	31.5	48.3	1.52	2.32	1.53	431.9	0.056
	SE	(0.50)	(2.43)	(0.086)	(0.072)	(0.089)	(23.2)	(0.002)

infected and control groups and the distribution (EA/CA) between the two pools was similar. Values for the half-life of the ^{125}I-labelled albumin were significantly reduced ($P < 0.02$) in the infected group and this group also had a significant increase ($P < 0.05$) in the fractional catabolic rate (F(CA)) compared with the control groups.

There were no significant differences between the infected Blackface and their controls in the serum albumin, CA, EA, EA/CA ratio or half-life of the labelled albumin but the fractional catabolic rate (F(CA)) was significantly increased in the infected group relative to the controls ($P < 0.05$). The F(CA) in the infected Blackface lambs was significantly greater than that in the infected Merinos.

Nutritional Studies

Feed Intake

Unfortunately the chopped hay was somewhat dusty and it also became accidentally contaminated with rodent faeces. As a result, it was unpalatable, and most of the lambs, whether infected or not, were reluctant to eat it.

Average daily feed intake during the study was 591 g in the infected Merinos and 755 g in the infected Blackface lambs.

Digestibility coefficients

The results of the digestibility studies are given in Table I.b.5. The apparent digestibility of the NFE fraction of the hay was slightly reduced in the infected Merinos relative to their controls ($P < 0.05$) but there were no significant differences in the apparent digestibility coefficients of the remaining proximate fractions of the hay between the two groups.

Similarly, a reduction in the apparent digestibility of the NFE fraction was observed in the Blackface lambs relative to their controls ($P < 0.02$)/

TABLE I.b.5: Apparent digestibility coefficients of the experimental diets: Merino and Blackface lambs. Mean (±SE) values.

Group	Time post-infection	DM	CP	CF	EE	ASH	NFE	OM
Merino Infected	25-32 days	0.48 (0.008)	0.40 (0.026)	0.53 (0.014)	- 0.35 (0.321)	0.26 (0.028)	0.50 (0.014)	0.50 (0.007)
Merino Pair-fed Controls	25-32 days	0.51 (0.011)	0.44 (0.008)	0.53 (0.035)	- 0.29 (0.919)	0.30 (0.015)	0.56 (0.013)	0.53 (0.010)
Blackface Infected	54-61 days	0.56 (0.003)	0.44 (0.007)	0.56 (0.009)	0.68 (0.061)	0.34 (0.004)	0.60 (0.005)	0.57 (0.003)
Blackface Pair-fed Controls	54-61 days	0.59 (0.016)	0.46 (0.020)	0.59 (0.025)	0.30 (0.220)	0.37 (0.016)	0.64 (0.011)	0.60 (0.016)

($P < 0.02$) but no significant differences were seen in any of the remaining fractions. It is not thought that the magnitude of the differences in digestibility observed were of any biological significance.

N balance

Infected and pair-fed control Merino lambs remained in slight positive N balance during the metabolism cage study (< 1 g N retained/d) and there were no significant differences in faecal or urinary N output between the two groups (Table I.b.6).

Both infected and pair-fed control Blackface lambs remained in slightly greater N balance during the metabolism cage study relative to the Merinos ($\sim$ 2g Nretained/d). There were no significant differences in faecal or urinary N outputs between the two groups.

TABLE I.b.6: Nitrogen Balance (g N/day) : Merino and Blackface lambs. Mean (±SE) values.

	INFECTED		PAIR-FED CONTROLS	
	Merino	Blackface	Merino	Blackface
Time post-infection	25-32 days	54-61 days	25-32 days	54-61 days
Intake	5.99 (0.080)	7.53 (0.195)	5.83 (0.58)	7.43 (0.190)
Faeces	3.55 (0.38)	4.24 (0.076)	3.22 (0.27)	3.99 (0.086)
Urine	1.77 (0.20)	1.49 (0.172)	1.69 (0.18)	1.26 (0.008)
Retained	+ 0.67 (0.636)	+ 1.80 (0.198)	+ 0.92 (0.21)	+ 2.18 (0.500)

DISCUSSION

The results of these two experiments indicate that sheep infected with 50 H. contortus larvae/kg BW develop a modest but significant anaemia, the severity of which appears to be greater in animals kept on a low protein diet. Certain features of the results suggest that the chronic haemonchosis syndrome, as described by Allonby (1974), was reproduced.

A normocytic normochromic anaemia developed in all sheep after infection, the greatest degree of anaemia occurring in the sheep receiving a low protein diet. Within a dietary group, the Merino lambs appeared to be the most susceptible, this group consistently having lower haematological values than the Finn Dorset on the low protein diet or the infected Blackface lambs. The results of the erythrokinetic and ferrokinetic studies in the younger sheep confirmed the relative mildness of the anaemia in that red cell volumes were only slightly reduced in the infected lambs relative to their controls and the red cell ^{59}Fe utilisation figures were again similar to control values. Thus there was no evidence of a greatly accelerated rate of erythropoiesis in the infected lambs which would have resulted in the development of a macrocytic anaemia. This finding of a normochromic normocytic anaemia is in contrast to Allonby's (1974) findings in infected lambs under field conditions, where macrocytosis was a feature of the anaemia. However, the MCV values presented by Allonby are unusually erratic and some values presented would be regarded as being abnormally high, e.g. 66 fl. In addition, these high MCV values were associated with relatively high haematocrit values (0.26 - 0.30 l/l) and thus it is unlikely that the macrocytosis observed was a genuine physiological change.

The relative sensitivity of the radioisotopic tracer techniques to quantify gastrointestinal blood loss is illustrated by the ^{51}Cr faecal clearance values. Both groups of infected lambs were losing relatively small quantities of red cells into the gut daily (< 5 ml) and within the Merino group in particular, faecal clearances were remarkably similar.

The differences in ^{51}Cr-labelled red cell faecal clearance values between the infected Merinos and Blackface lambs would suggest a greater establishment of parasites in the former group. However, the radioisotopic measurements were made at different stages of the infection in both groups and thus it is not appropriate to place too much emphasis on these differences.

As with the ^{51}Cr faecal clearance values, faecal egg counts (epg) were consistently higher in the infected Merinos relative to the infected Blackface lambs during the first 14 weeks of infection, again suggesting greater establishment. However, total daily faecal egg output figures at the same time after infection are not available. Furthermore, worm burdens were not assessed until several weeks later when differences in faecal egg counts were not apparent. Therefore, one can only speculate as to whether establishment was different in the two breeds of lambs.

The difference in faecal egg counts between the two groups of Finn Dorset wethers is interesting in that the finding of higher faecal egg counts in the first 6 weeks of infection in the sheep on the high protein diet is contrary to most published work (see General Introduction). However, worm burdens were not assessed until 28 weeks after infection when differences in faecal egg counts were no longer obvious. As a result, one can only speculate as to the reasons for the difference in faecal egg counts. For example, a difference in fresh faecal mass as a result of/

of differences in feed consumption or physical form of the two diets may be involved. Alternatively, there may have been differences in the fecundity of the parasite. Another possibility is that, because of the great disparity in bodyweight between the two groups of wethers before infection, the animals on the high protein diet received much larger infective doses of larvae. Indeed, there was no overlap in larval doses between the two groups and thus the attempt to standardise worm burdens by infecting on a bodyweight basis may have introduced a further complicating factor.

The slight anaemia observed in both groups of control lambs in the second experiment is difficult to explain. One possibility is that it may be the "anaemia of growth" referred to by Silverman, Mansfield and Scott (1970). However, this is unlikely since a prerequisite for this type of anaemia is rapid growth which was not occurring in these animals. It is more likely that a combination of physiological adaptation to regular bleeding and the bleeding itself contributed to the development of the mild anaemia, though the low protein diet may have aggravated the situation.

Total serum protein and albumin levels were only minimally reduced by infection, and the albumin metabolism studies in the younger sheep (Merino and Blackface) confirmed this, as body albumin pools were similar in both infected and control animals. However, the fraction of the intravascular albumin pool being catabolised daily (F(CA)) was increased in both infected groups, the greater increase being observed in the Blackface lambs. The fractional catabolic rates were 5-6% which is considerably less than the rates ($\sim$14%) quoted by Allonby and Dargie (1973) in sheep supposedly suffering from chronic haemonchosis. Two features

in/

in particular,are of interest in the Allonby and Dargie work. First, the serum albumin levels in the infected sheep were very low (18 g/l) and control values were also low (26 g/l) where the normal range for sheep serum albumin is 30-40 g/l. Thus, their findings of depleted body albumin pools, reduction in $T\frac{1}{2}$ of the labelled albumin and greatly increased F(CA) values are not surprising. Secondly, the worm burdens at slaughter were $\sim$ 2,000 which is somewhat higher than would be expected in field cases of chronic haemonchosis where a maximum of 1,000 worms at slaughter is expected (Allonby, 1974). Thus, one must conclude that the findings of the present experiments as regards albumin metabolism are probably more typical of chronic haemonchosis than those of Allonby and Dargie (1973).

Another feature of the chronic haemonchosis syndrome described by Allonby (1974) was progressive insidious weight loss eventually leading to extreme weakness and anorexia. In the present experiments, weight loss was a feature of the disease in the younger animals. However, a low quality diet obviously contributed to the weight loss as the Merino control lambs also lost weight during the experiment. The influence of diet alone in effecting weight changes was well illustrated by the Finn Dorset wethers where, after 7 months on a low protein diet, the animals in this group were on average 9 kg lighter than those fed a high protein diet. However, the severe wasting and weakness observed in field cases of chronic haemonchosis was not a feature of the experimentally induced disease and one may speculate that the extra energy cost required for extensive grazing may account for these differences.

Finally, the findings of the digestibility and N balance studies show that, in these experiments, infection had little effect on the digestion and absorption of the diet of hay alone and that, despite being given this low protein diet, both groups of infected lambs remained in positive N balance.

CHAPTER II

Studies on the pathogenicity of a single infection with 125 *H. contortus*/kg BW on 4 month old Finn Dorset and Scottish Blackface lambs fed either a high or low protein diet

INTRODUCTION

The principal feature of the experiments described in Chapter I was the development of a syndrome similar to the chronic haemonchosis syndrome first described by Allonby (1974). Sheep, lightly infected with H. contortus developed a moderate anaemia, had relatively low faecal egg counts and small worm burdens (<1,000 worms) at slaughter. Younger sheep, and in particular the Merinos, kept on a low protein diet appeared to be the most susceptible. Although the pathophysiological changes were slight, both groups of younger sheep had significant blood losses into the gut relative to controls.

However, several features of the experiments disussed in the previous chapter (Chapter I) were not entirely satisfactory. Firstly, only small numbers of parasite-free sheep were available and the disparity in ages between the three breeds precluded direct comparisons between them. Furthermore, the absence of control sheep in Experiment Ia prevented an essential comparison with uninfected animals.

In the second experiment (Ib) it was not possible to have two different dietary groupings within each breed of sheep because of the small numbers of lambs available. Nevertheless, having control animals ensured that more valid conclusions could be drawn from the pathophysiological consequences associated with the infection. Unfortunately, direct comparisons between the two breeds were not possible since the lambs were of differing ages and the radioisotopic studies were conducted at different stages of the infection.

Finally, the chopped hay which was given during the metabolism cage study was not satisfactory. The dustiness and almost inevitable contamination during storage resulted in both control and infected lambs finding it somewhat unpalatable.

Because/

Because of these limitations, the following experiment was designed with a view to minimising these inadequacies. First, in Experiments IIa and b, large numbers of parasite-free lambs of two different breeds, Finn Dorset and Scottish Blackface, were used. Secondly, all the lambs were introduced to the experimental diets at 3 months of age and infection was carried out one month later. Thirdly, the pathophysiological studies were conducted at the same time after infection.

The feeding regimen was improved by using standardised "complete" diets. Two experimental diets were used, one high and one low protein. They consisted of a coarse admixture of appropriate proportions of sugar beet pulp, barley siftings ± soya bean meal, together with fully adequate minerals and vitamins. No long roughage in the form of hay or straw was required as an adequate quantity of barley siftings included in the complete diet provided the necessary crude fibre. The feeds were prepared by hand to ensure fully adequate mixing. The diets were relatively dust free and highly palatable. The energy contents of both diets were closely similar as the major componental difference was the inclusion of soya bean meal in the "high protein" diet (see Materials and Methods, Chapter II).

Since the pathophysiological changes associated with an infective dose of 50 H. contortus larvae/kg BW were relatively modest, it was decided to increase the infective dose to 125 larvae/kg BW.

Using these modifications, the following experiments were conducted to investigate in further detail, the influence of diet on the pathogenesis of H. contortus infection in 4 month old lambs of two breeds of sheep known to differ in their susceptibility to haemonchosis (Altaif and Dargie, 1978a).

Experiment IIa

An investigation into the pathophysiological changes associated with a single infection of 125 H. contortus larvae/kg BW on 4 month old Finn Dorset and Scottish Blackface lambs fed either a high or low protein diet.

MATERIALS AND METHODS

Experimental design

Finn Dorset and Scottish Blackface lambs kept on either a high or low protein diet from the age of 3 months were infected with a single dose of 125 H. contortus larvae/kg BW when 4 months old. Radioisotopic, digestibility and N balance studies were conducted 26 to 40 days after infection. Blood and faecal samples were collected, and body weights were recorded, on a weekly basis throughout the experiment. The lambs were killed 20 weeks post-infection when worm burdens were assessed (Table II.a.1).

Experimental animals

Twenty 3 month old Finn Dorset lambs of both sexes were equally divided into high and low protein dietary groups, as described in General Materials and Methods. When they were 4 months old, each dietary group was further divided into "infected" and "control" groups, the former comprising 4 castrated male and 1 female lamb and the latter 3 castrated males and 2 female lambs. All the lambs in the "infected" groups were given a single dose of 125 H. contortus larvae/kg BW per os.

Twenty Scottish Blackface lambs, all castrated males, were similarly divided into dietary groups at 3 months of age and further divided into "infected" and "control" groups one month later. The lambs in the "infected" group were given a single dose of 125 H. contortus larvae/kg BW per os.

Feeding/

TABLE II.a.1: Studies on the pathogenicity of single infection with 125 *H. contortus* larvae/kg BW on Finn Dorset and Scottish Blackface lambs kept on either a high or low protein diet - Experimental design.

Breed	Diet Change when 3m old	Infection at 4m of age (D=0)	Nutritional and Radioisotopic Studies	Slaughter Weeks post-infection
Finn Dorset & Blackface (male lambs)	High protein	125 l/kgBW	26→42d post-infection	20 weeks
	Low protein	125 l/kgBW	26→42d post-infection	20 weeks
	High protein	Controls	42→56d (pair-fed to infected counterpart)	20 weeks
	Low protein	Controls	42→56d (pair-fed to infected counterpart)	20 weeks
Blackface (female lambs)	High protein	125 l/kgBW	-	3 at 5 weeks 4 at 14 weeks
	Low protein	125 l/kgBW	-	3 at 5 weeks 4 at 14 weeks

Feeding and Housing

The basic diet consisted of a mixture of shredded sugar beet pulp (SBP), barley siftings and a mineral/vitamin/trace element mixture. Soya bean meal (SBM) was added to the high protein diet only. The proportions of the components of both diets are presented in Table II.a.2 and the composition in Table II.a.3. The degradability of the dry matter (DM) and crude protein (CP) of the SBP siftings and SBM was determined using a nylon bag technique, as described by Bass (1980). The DM degradability of the SBP and SBM were 67% and 69% respectively, and that of the siftings 12%. The crude protein in the SBP and SBM was 43% and 66% degradable respectively (Table II.a.2).

The high and low protein diets were equal in energy content, i.e.Gross Energy (GE)and determined Metabolisable Energy (ME),and so only differed essentially in protein content. Calculated requirements for a 25 kg lamb growing at 126 g/d with M/D diet of 10, is 9 MJME and 65g Digestible Crude Protein (DCP). The high protein diet offered at 1 kg fresh matter supplied this 9 MJME and double the DCP required. The low protein diet supplied a determined 25-40 DCP only.

Both diets were made up by hand in bulk each week and were fed ready-mixed during the experiment. Samples of the ready-mixed diets were taken regularly throughout the experiment for the appropriate analyses.

The lambs were housed in concrete floored pens, as described in General Materials and Methods, and the daily allowance of the appropriate diet was offered in two feeds. When in metabolism cages, the infected lambs were offered 1,000 g fresh feed daily/lamb in two feeds. However, if feed in excess of 50 g daily was rejected, the quantity of feed offered the following day would be reduced by 50 g. This was done in order to minimise feed residues. Control lambs/

TABLE II.a.2: Experimental diets: proportions of the various components. Dry matter (DM) and crude protein (CP) degradability of the sugar beet pulp, siftings and soya bean meal.

	Sugar Beet Pulp (SBP)	Barley Siftings	Soya Bean Meal (SBM)	Vitamin/Mineral/Trace element mixture
High Protein	4 parts	3 parts	2 parts	0.18 parts
Low Protein	4 parts	2 parts	0 parts	0.12 parts
Degradability %				
DM	67	12	69	-
CP	43	N.A.	66	-

TABLE II.a.3: Proximate composition of high and low protein diets

	DM Coefficient	g/kg DM					
		CP	CF	EE	ASH	NFE	OM
High Protein	0.861	169.02	195.58	8.47	101.05	525.87	898.95
Low Protein	0.857	87.96	205.64	5.77	105.96	594.67	894.04

lambs were pair fed to their infected counterpart during the metabolism cage studies.

Radioisotopic, digestibility and N balance studies

Eighteen days after infection, the infected male Finn Dorset lambs were moved to metabolism cages. Radioisotopic studies using ^{51}Cr-labelled red cells, ^{59}Fe-labelled transferrin and ^{125}I-labelled albumin started on day 26 after infection and continued for two weeks. During this time, a seven day collection period (preceded by a five-day adjustment period) for digestibility and N balance was conducted. Two uninfected female lambs were also moved to metabolism cages with the males to act as controls for the radioisotopic studies, in particular as a check that no denaturation of the albumin had occurred during the labelling with ^{125}I. The uninfected male control lambs were transferred to the metabolism cages when the studies on the infected lambs finished. Radioisotopic and nutritional studies identical to those conducted in the infected lambs were then carried out.

The Blackface lambs followed an identical protocol to that of the Finn Dorset lambs.

RESULTS

Clinical and body weight changes

Both Finn Dorset and Blackface infected lambs on the high protein diet remained alert throughout the experiment. The infected lambs on the low protein diet also were alert with the exception of one Finn Dorset lamb, B2. This lamb was very anaemic (Hct of 0.15 l/l) during the cage study, and spent a considerable amount of time in sternal recumbency. It became very inappetant (< 250 g fresh food/day) and rapidly lost weight. No sub-mandibular oedema was observed. It

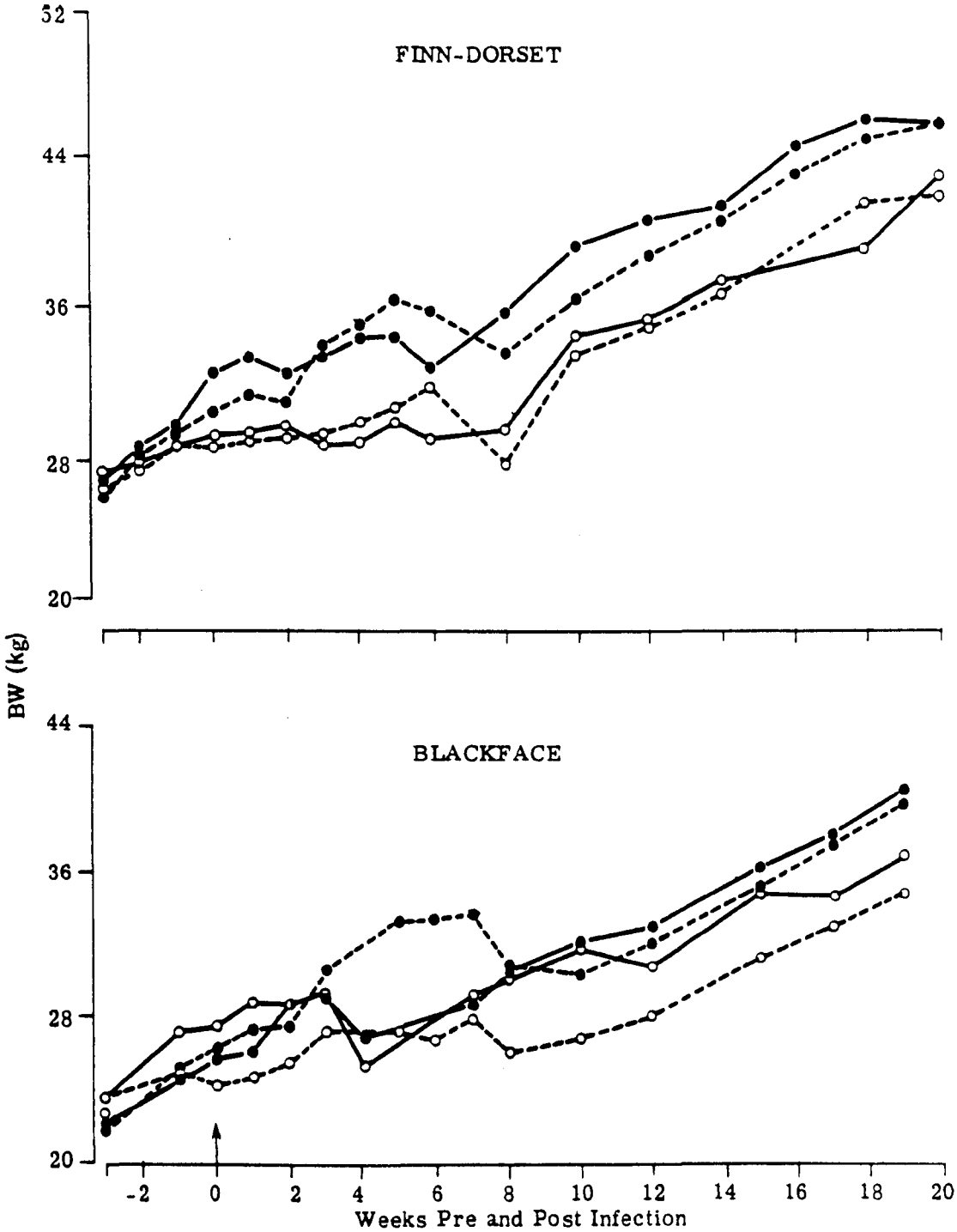


Fig.II.a.1: Mean group body weights of infected (125 larvae/kg BW) Finn Dorset and Blackface lambs fed either a high or low protein diet and their respective controls.

High Protein Infected

Low Protein Infected

High protein controls

Low protein controls

was found dead in the penned accommodation approximately four weeks later.

Both groups of infected Finn Dorset lambs failed to gain weight in the first 6 weeks of infection,but gained 14 kg from week 6 till slaughter (Fig. II.a.1). Both groups of Finn Dorset control lambs, in contrast, gained weight steadily throughout the experiment (~ 20 kg and 13 kg in the high and low protein groups respectively).

Infected Blackface lambs on the high protein diet made only minimal weight gains in the 4-week period following infection, whereas the infected lambs on the low protein diet lost ~ 2 kg in the same period. Thereafter both groups gained 13.8 kg and 11 kg in weight respectively between week 4 and slaughter. In contrast, both groups of control lambs gained weight steadily throughout the experimental period, 190 g/d and 80 g/d in the high and low protein group respectively (Fig. II.a.1).

Haematological changes

Finn Dorset lambs

A prolonged macrocytic anaemia was observed after infection in both dietary groups. Mean haematocrit values fell to minimum levels five weeks after infection (0.24 l/l and 0.21 l/l in the high and low protein groups respectively)(Fig. II.a.2). The differences in the severity of the anaemia between dietary groups was not statistically significant. The rise in MCV values started four weeks after infection and reached maximum levels of 38-40 fl at week 5 in both groups of infected lambs (Fig. II.a.3). MCV values in the infected lambs on the low protein diet remained significantly elevated relative to their controls until week 16, whereas differences in MCV values between infected and control lambs on the high protein diet were/

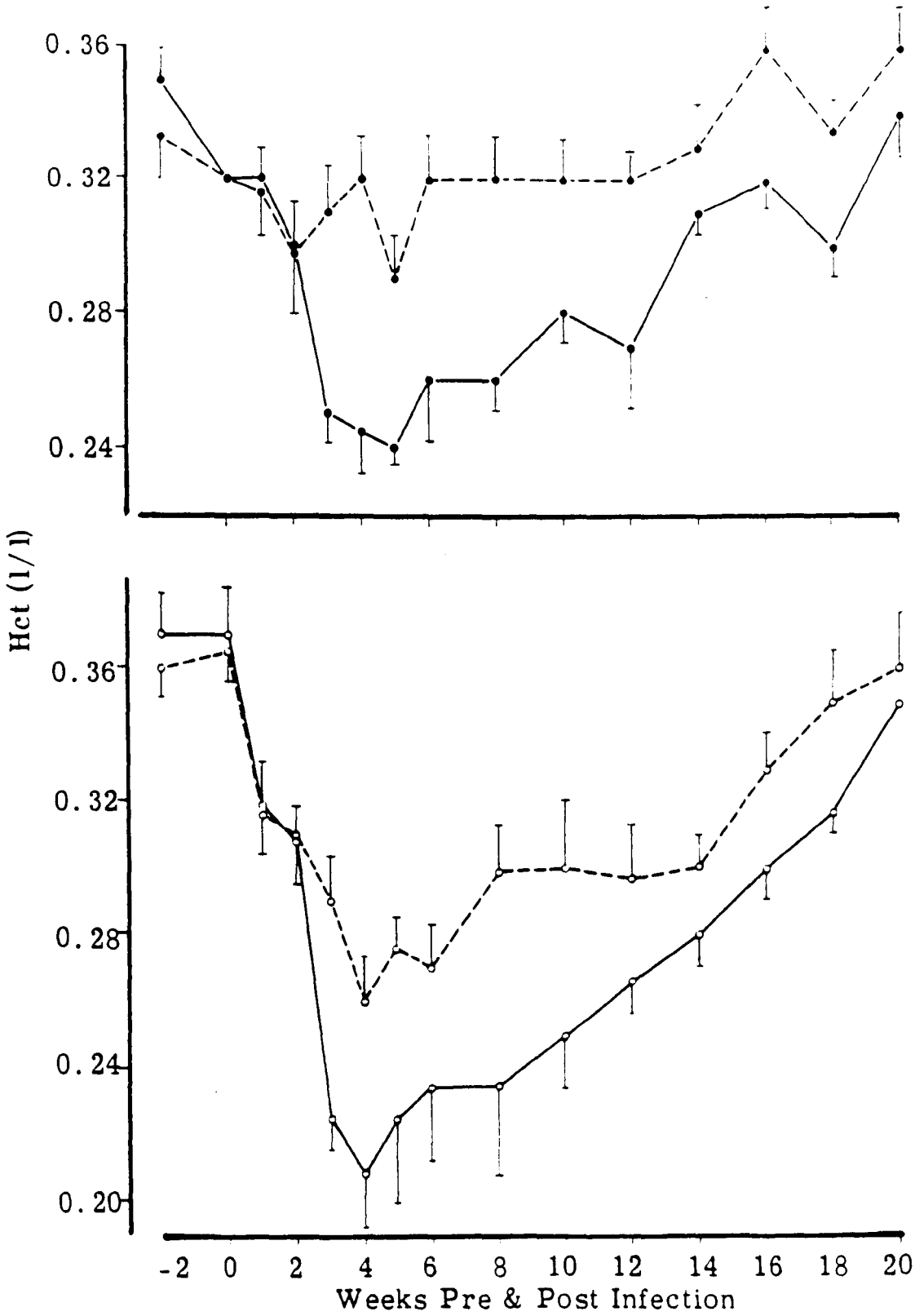


Fig. II.a.2: Mean (± SE) haematocrit values of infected (125 larvae/kg BW) Finn Dorset lambs and their respective controls.

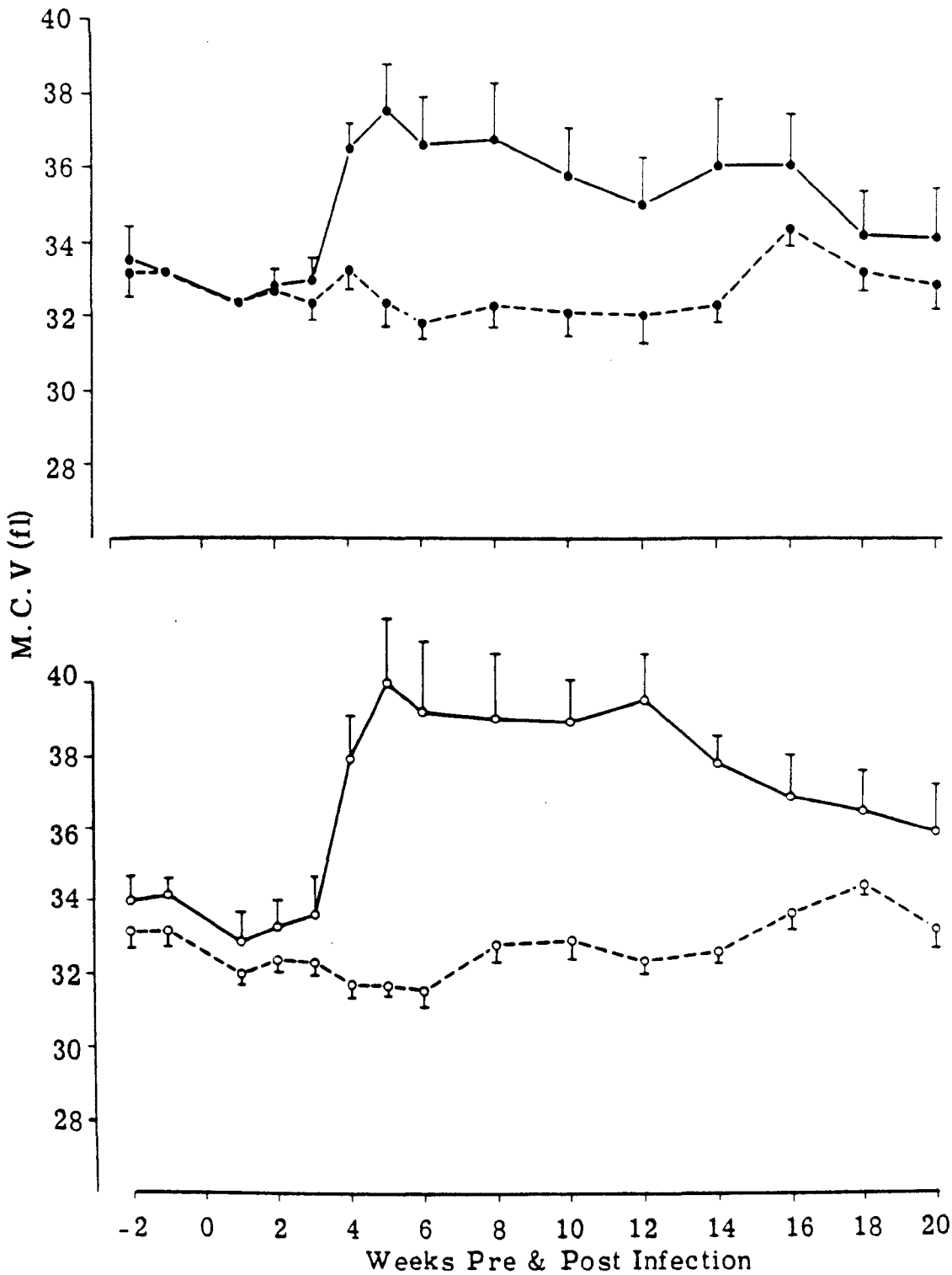


Fig. II.a.3: Mean (± SE) MCV values of infected (125 larvae/kg BW) Finn Dorset lambs and their respective controls.

●——● High Protein Infected o——o Low Protein Infected

●- - - -● High Protein Control o- - - -o Low Protein Control

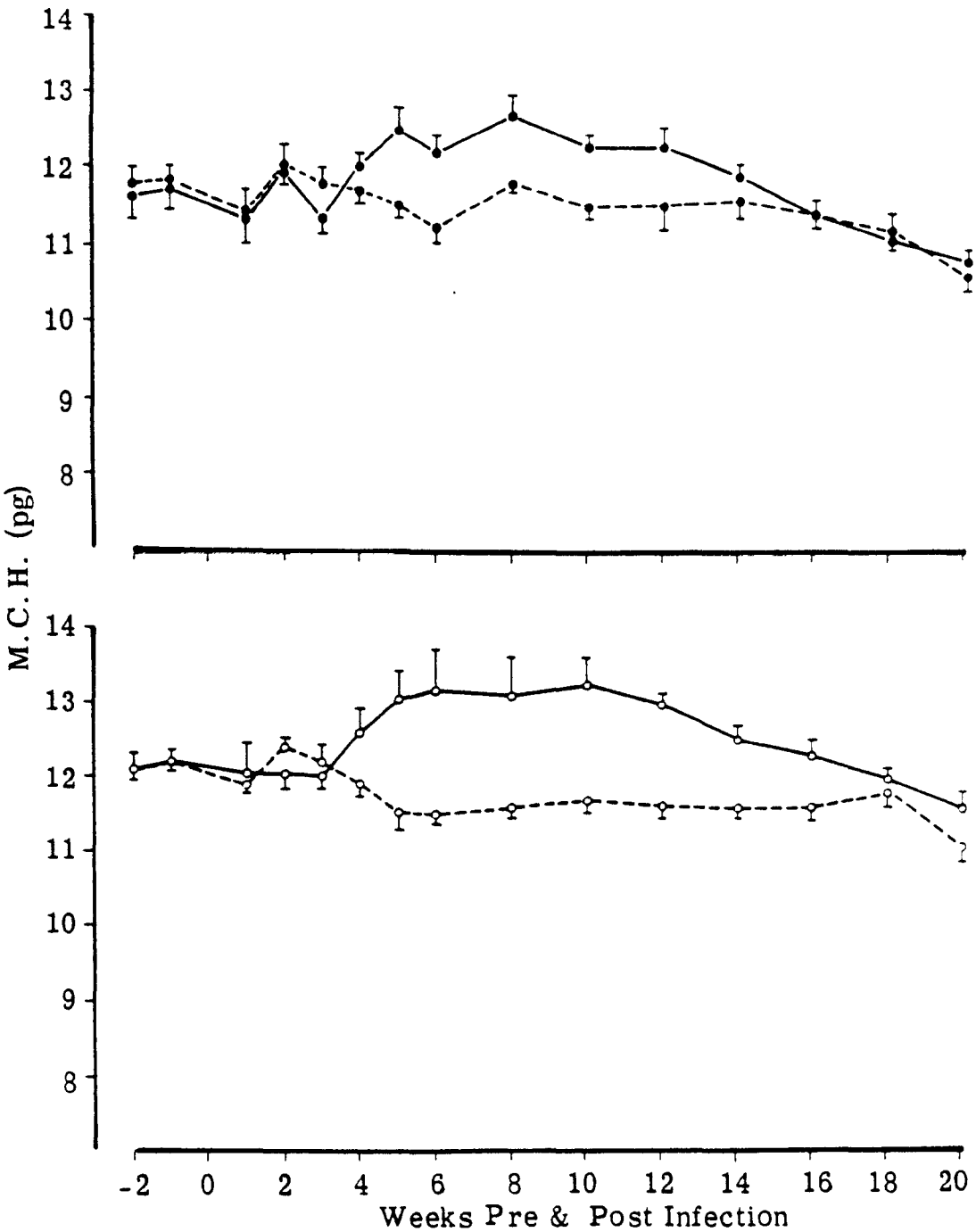


Fig. II.a.4: Mean (± SE) MCH values of infected (125 larvae/kg BW) Finn Dorset lambs and their respective controls.

●——● High Protein Infected ○——○ Low Protein Infected

●- - - -● High Protein Control ○- - - -○ Low Protein Control

were not statistically significant after week 10. MCH values rose in both groups of infected lambs parallelling the rise in MCV (Fig. II.a.4).

The haematological response of two infected lambs, one from each dietary group, differed markedly from the rest of their respective group members. Their principal haematological, parasitological and bodyweight changes are presented in Appendix B, Table 1.

The lamb from the high protein group, R 17, had a short lived macrocytic response during weeks 4 and 5, unlike the remaining members of its group, three of whom remained macrocytic virtually until the end of the experiment, whilst the remaining lamb expelled the infection and its haematological values rapidly returned to normal. Lamb R17, became inappetant during the metabolism cage studies and was consuming < 400 g fresh feed daily. However, it started to increase its food intake towards the end of the study and survived until the end of the experiment.

The lamb from the low protein group, B_2, became very inappetant also, and was consuming less than 250 g fresh feed daily. The macrocytic response of this lamb was short-lived and weak. The lambs died 9 weeks after infection.

Blackface lambs

Both groups of infected Blackface lambs developed a moderate macrocytic anaemia after infection. Haematocrit values fell to minimum levels of 0.29 l/l four weeks after infection in the lambs in the high protein group, whereas minimum values of 0.25 l/l occurred at week 8 after infection in the lambs fed the low protein diet (Fig. II.a.5).

Both/

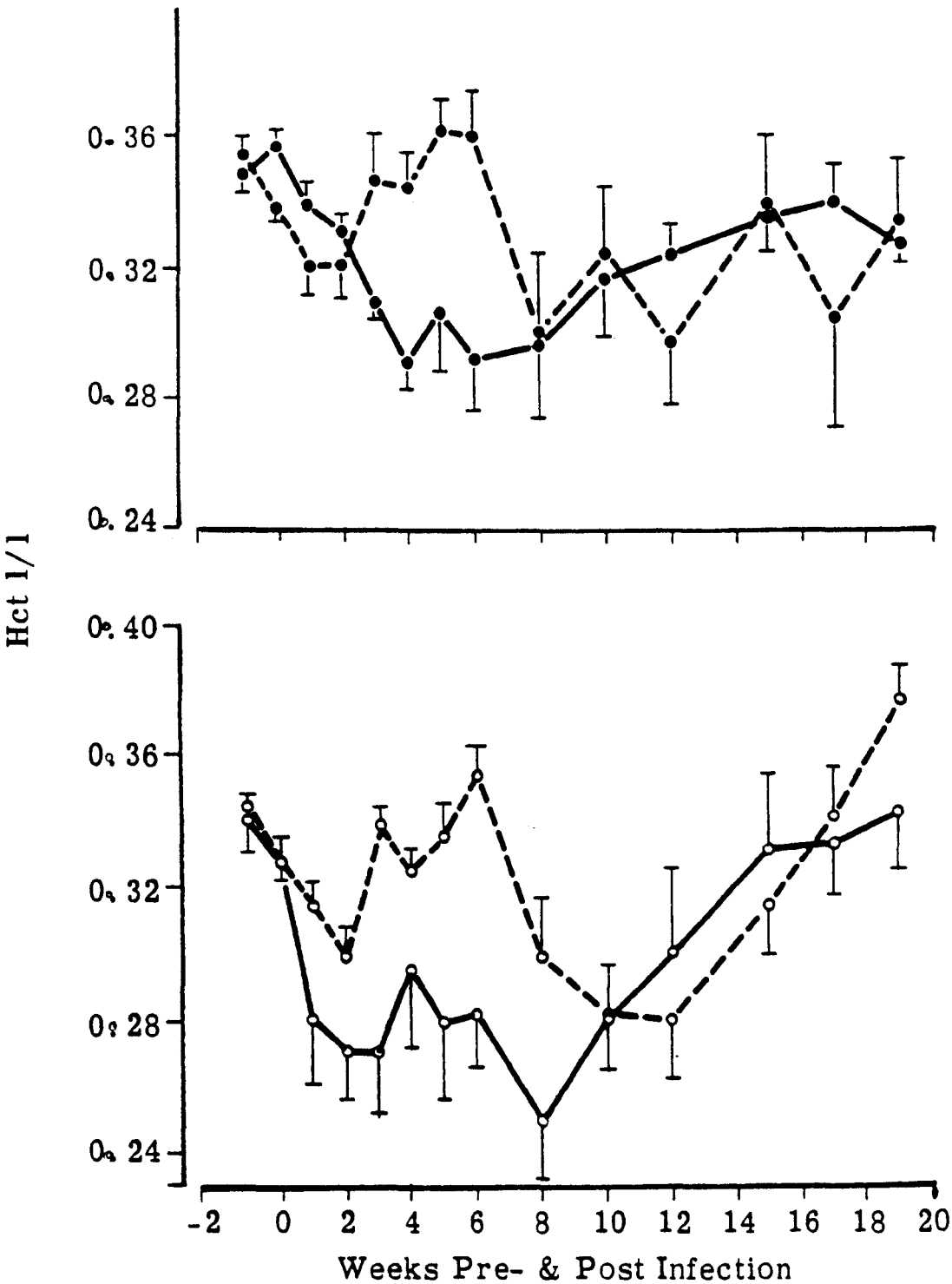


Fig. II.a.5: Mean (± SE) haematocrit values of infected (125 larvae/kg BW) Blackface lambs and their respective controls.

●——● High Protein Infected ○——○ Low Protein Infected

●- - - -● High Protein Control ○- - - -○ Low Protein Control

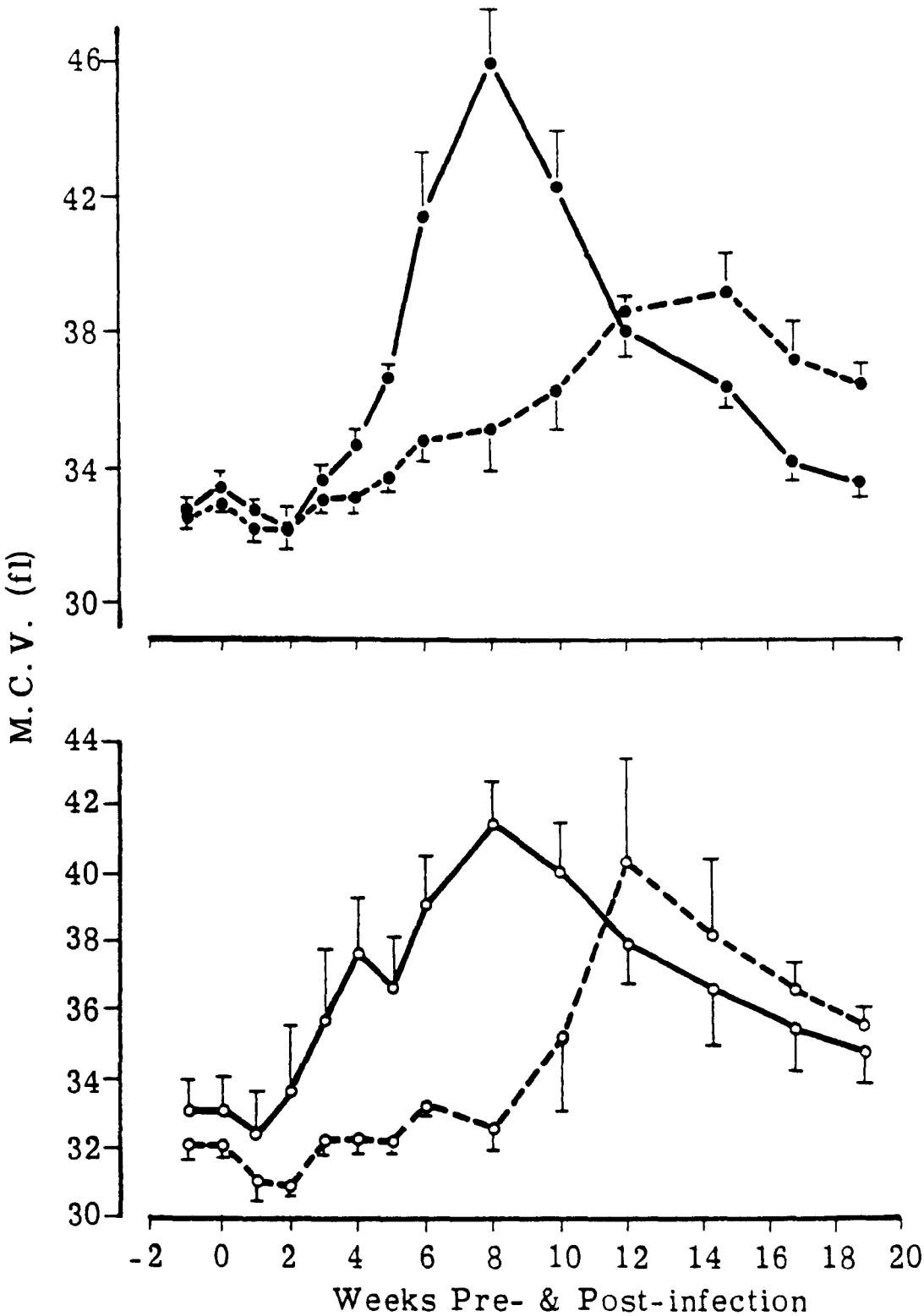


Fig. II.a.6: Mean (± SE) MCV values of infected (125 larvae/kg BW) Blackface lambs and their respective controls.

●——● High Protein Infected
o——o Low Protein Control
●-------● High Protein Control
o-------o Low Protein Control

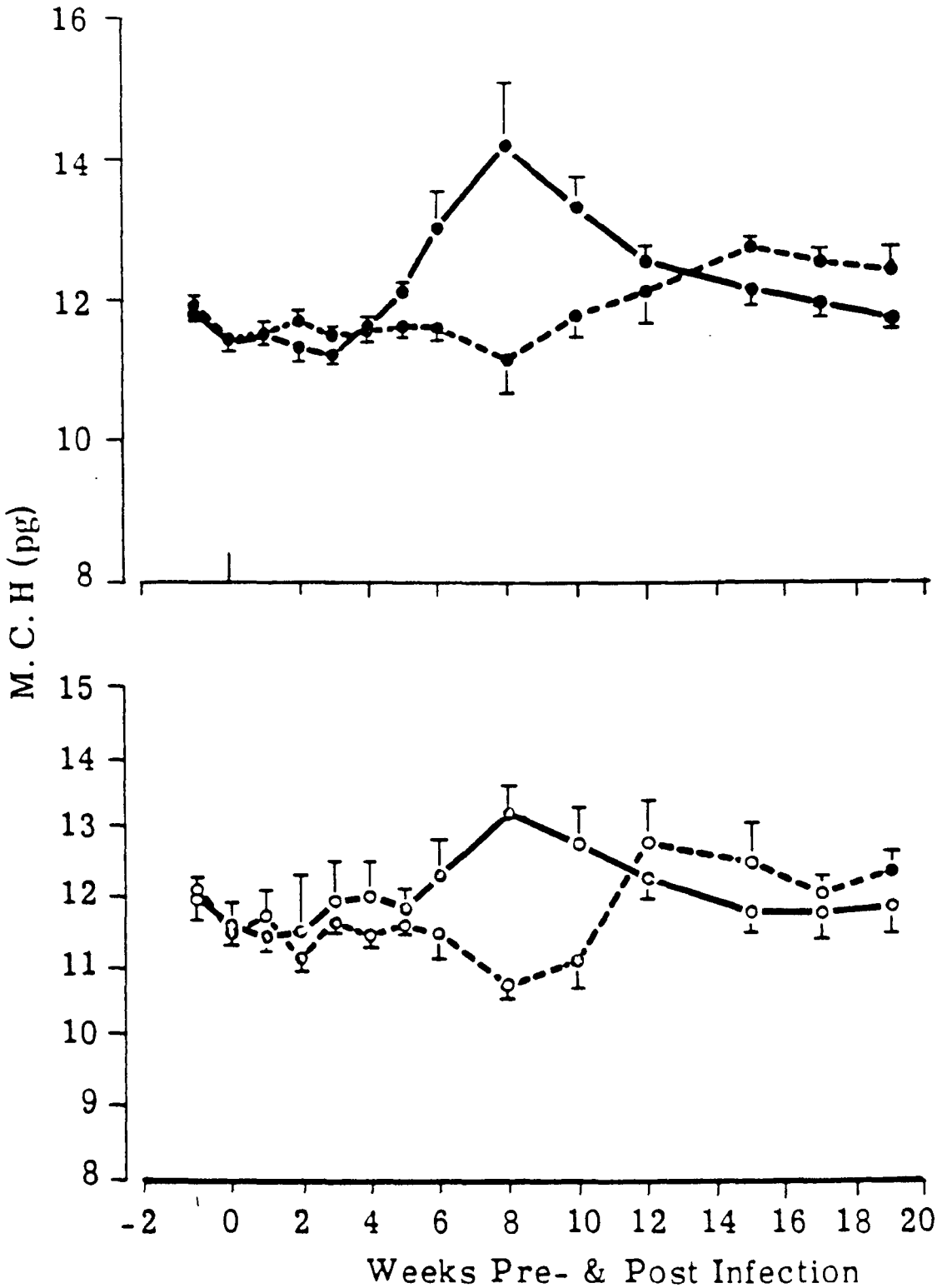


Fig. II.a.7: Mean (± SE) MCH values of infected (125 larvae/kg BW) Blackface lambs and their respective controls.

●——● High Protein Infected
o——o Low Protein Infected
●- - -● High Protein Control
o- - -o Low Protein Control

Both groups had a vigorous erythropoietic response, as illustrated by the peak MCV values of 41-46 fl at week 8 (Fig. II.a.6). Values declined gradually thereafter in both groups. MCH values rose in both groups of infected lambs parallelling the MCV changes (Fig. II.a.7).

Both groups of control lambs became slightly anaemic, haematocrit values falling rapidly between weeks 6 and 8. There was evidence of an increased erythropoietic response to the fall in haematocrit by MCV values rising. The anaemia was associated with the appearance of strongyle eggs in some of the lambs in each control group between weeks 3 and 12.

Biochemical changes

Finn Dorset lambs (Figs. II.a.8 and 9)

Both groups of infected lambs became hypoproteinaemic and hypoalbuminaemic after infection. Total serum protein, albumin and globulin values of the infected lambs on the high protein diet fell from 62 : 32 : 29 g/l to 52 : 29: 23 g/l respectively four weeks after infection, whereas values for the same in the infected lambs on the low protein diet fell from 62 : 32 : 29 g/l to 44 : 20 : 24 g/l respectively at week 6 post-infection. A rise in total serum protein, due to an increase in globulin values, was observed in the control lambs fed the low protein diet.

Blackface lambs (Fig. II.a.10)

In contrast to the Finn Dorset lambs, infection had little effect on total protein, albumin and globulin values in the Blackface lambs fed the high protein diet. However, a gradual fall in all three parameters occurred in the infected lambs on the low protein diet, pre-infection values of 65 : 32 : 33 g/l falling to 54 : 27 : 27 g/l respectively at week 8.

Although/

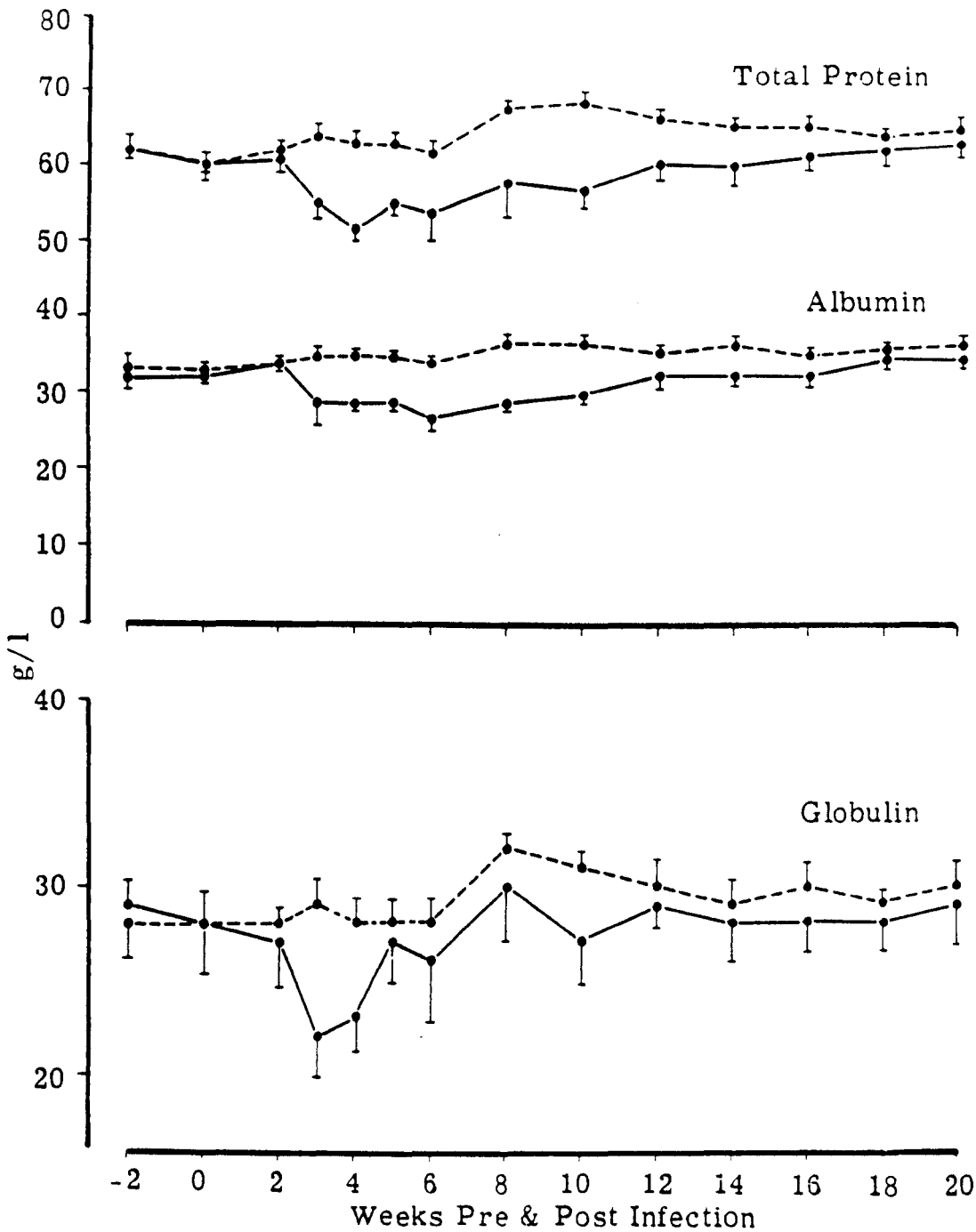


Fig. II.a.8: Mean (±SE) total serum protein, albumin and globulin values of infected (125 larvae/kg BW) Finn Dorset lambs fed a high protein diet and their respective controls.

●——● High Protein Infected

●-------● High Protein Control

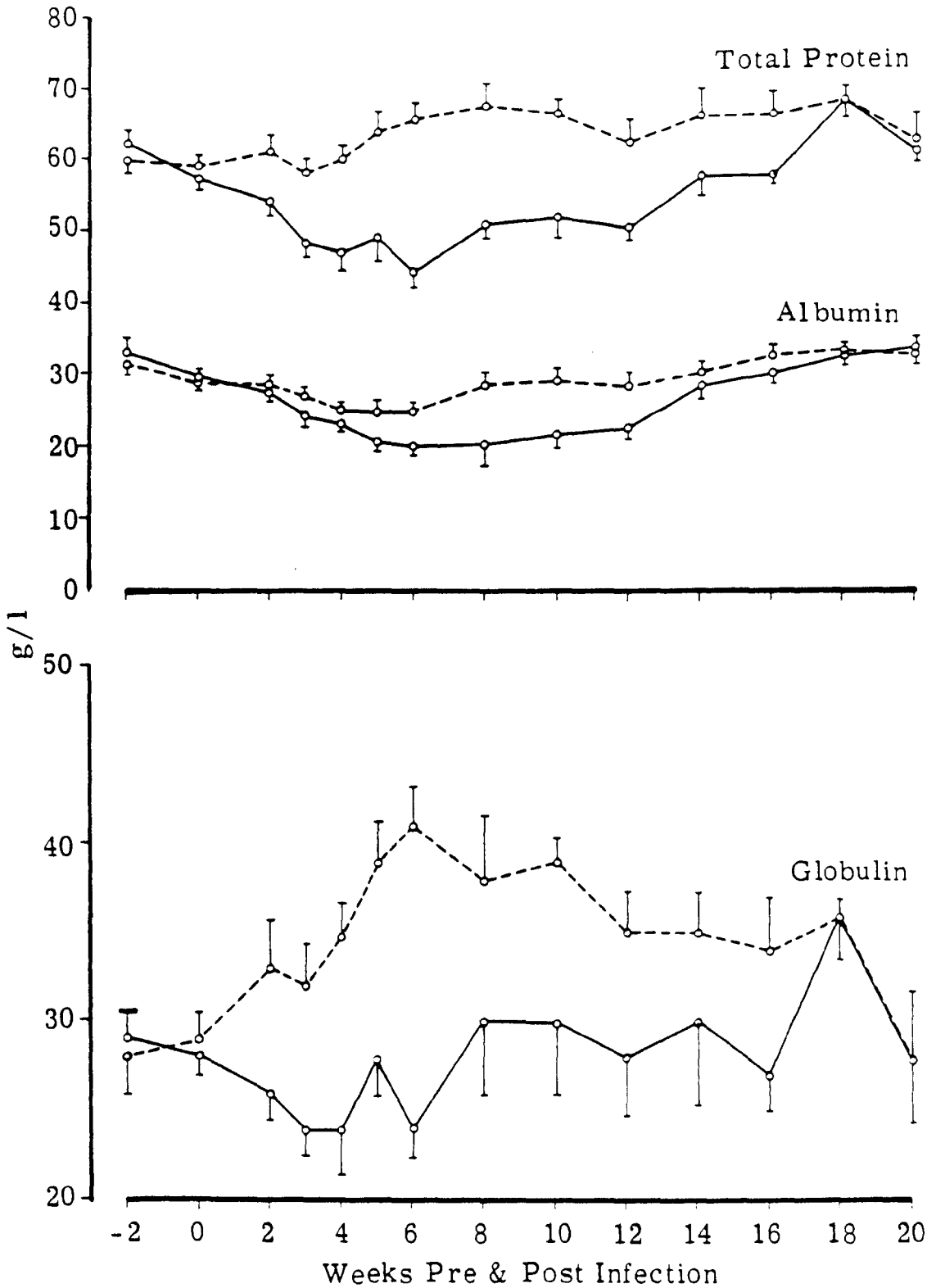


Fig. II.a.9: Mean (± SE) total serum protein, albumin and globulin values of infected (125 larvae/kg BW) Finn Dorset lambs fed a low protein diet and their respective controls

o———o Low protein infected

o-------o Low protein control

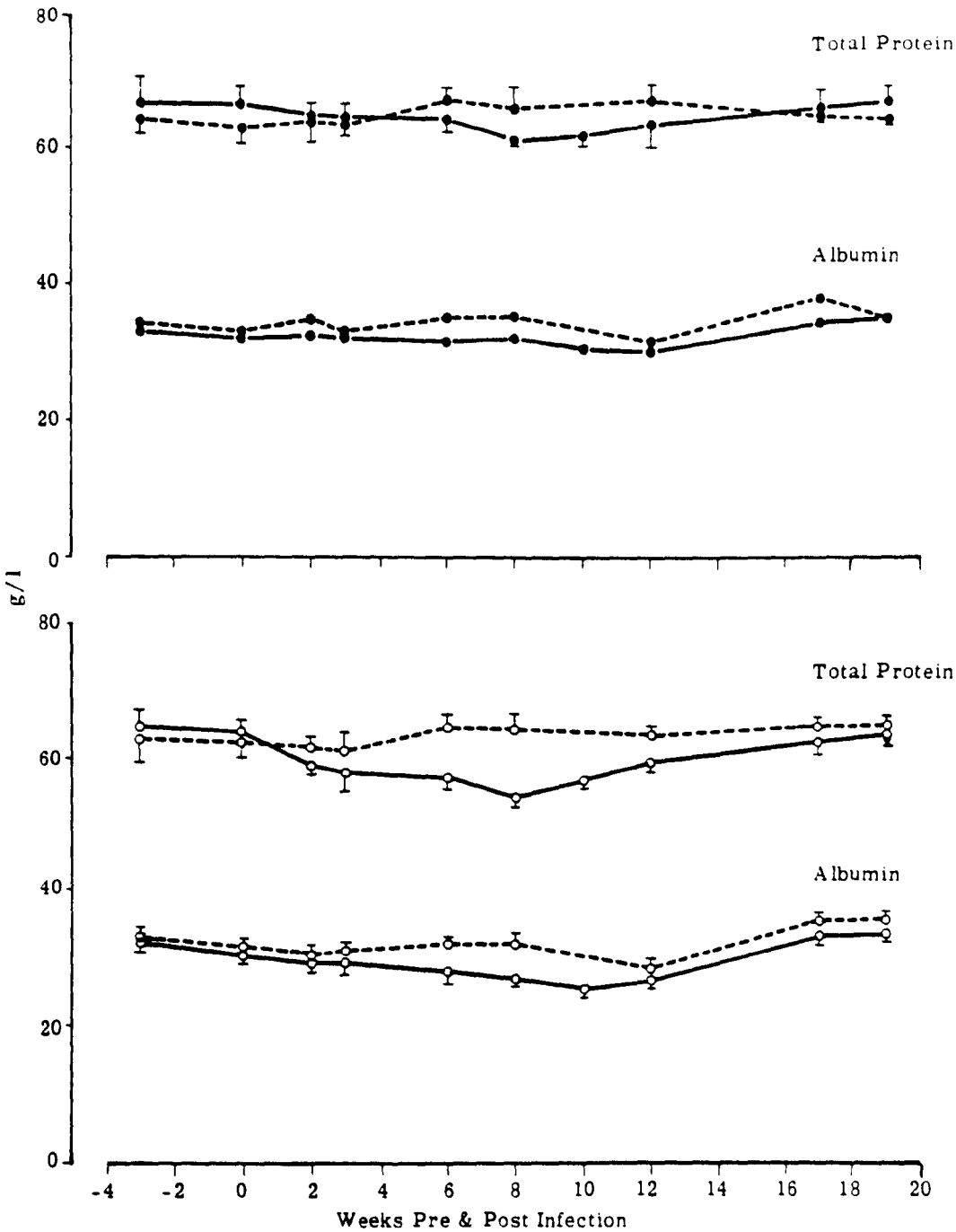


Fig. II.a.10: Mean (± SE) total serum protein and albumin values of infected (125 larvae/kg BW) Blackface lambs fed either a high or low protein diet and their respective controls.

Although plasma iron and total iron binding capacity estimations were made throughout the experiment, unaccountable contamination of the samples must have occurred during the analyses, resulting in grossly high values. Consequently, the serum iron levels presented with the ferrokinetic data are probably not acceptable as absolute values. However, they have been used purely to allow comparison between infected and control groups.

Parasitological findings

Finn Dorset lambs (Fig. II.a.11)

Faecal egg counts

Faecal egg counts rose rapidly in both groups of lambs. Values in the high protein group reached maximal levels of 21,700 epg at week 5. Values fell gradually thereafter to 4,460 at slaughter. Faecal egg counts in the low protein group reached maximal values, 34,180 epg at week 7 after infection. Since one lamb in this latter group, B2, became very inappetant during the metabolism cage study, its faecal egg counts were very high due to a greatly reduced faecal output. If the faecal egg counts for this lamb are excluded from the group mean, it can be observed that the difference in faecal egg counts between the two dietary groups is less marked and, in fact, both groups follow a very similar pattern.

However, faecal egg counts in the low protein group, even when B2 was excluded, were greater than those of the high protein group at week 4, though from week 5 onwards values were similar in both dietary groups. Total daily faecal egg output (measured during the metabolism cage study) confirmed the higher faecal egg counts observed in the low protein group but the difference between the two groups was not statistically significant(Appendix B, Table 2).

Blackface/

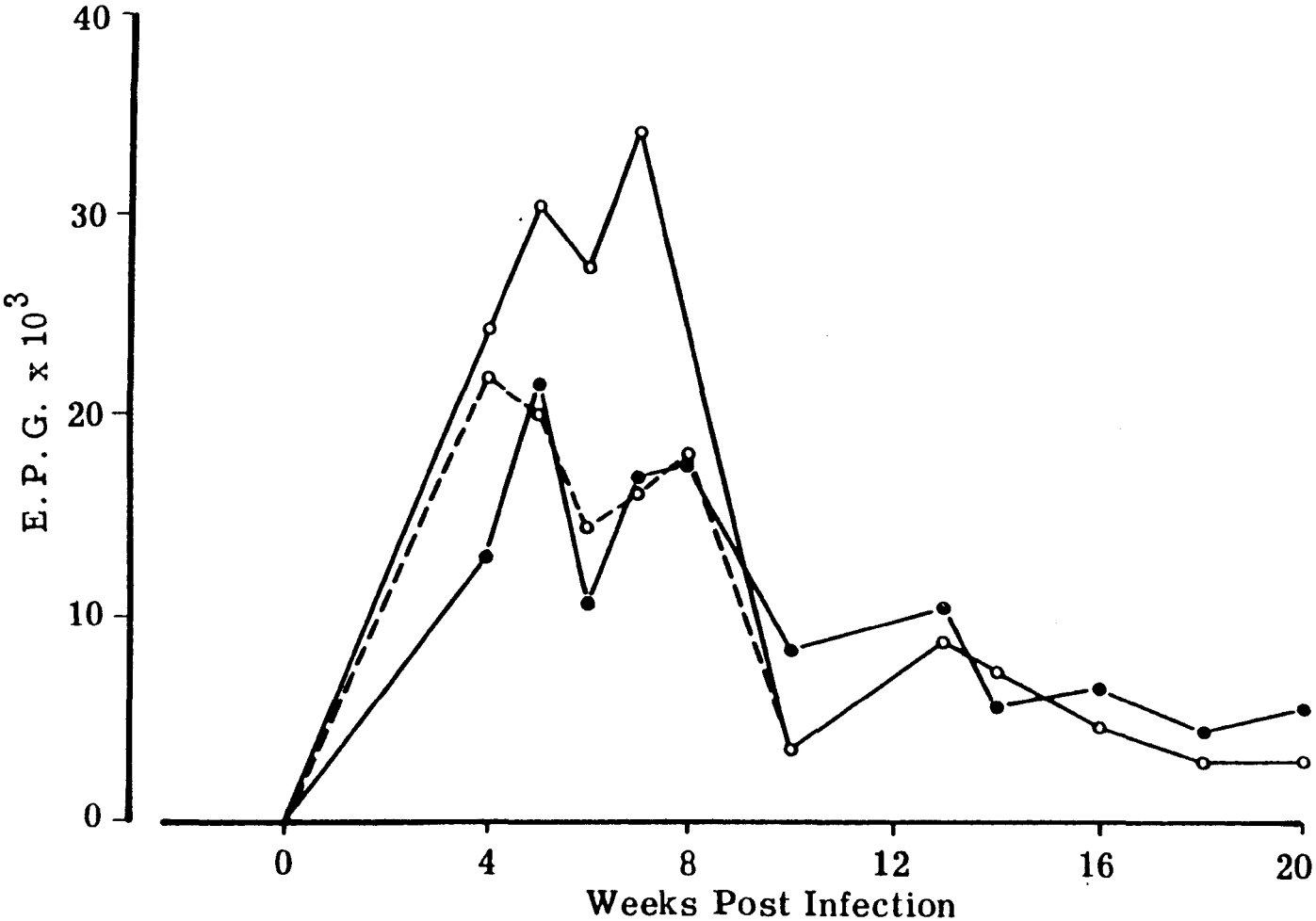


Fig. II.a.11: Group mean faecal egg counts (e p g) of Finn Dorset lambs infected with 125 *H. contortus* larvae/kg BW and fed either a high or low protein diet

●———● High protein o———o Low protein o--------o Low protein - B2 values

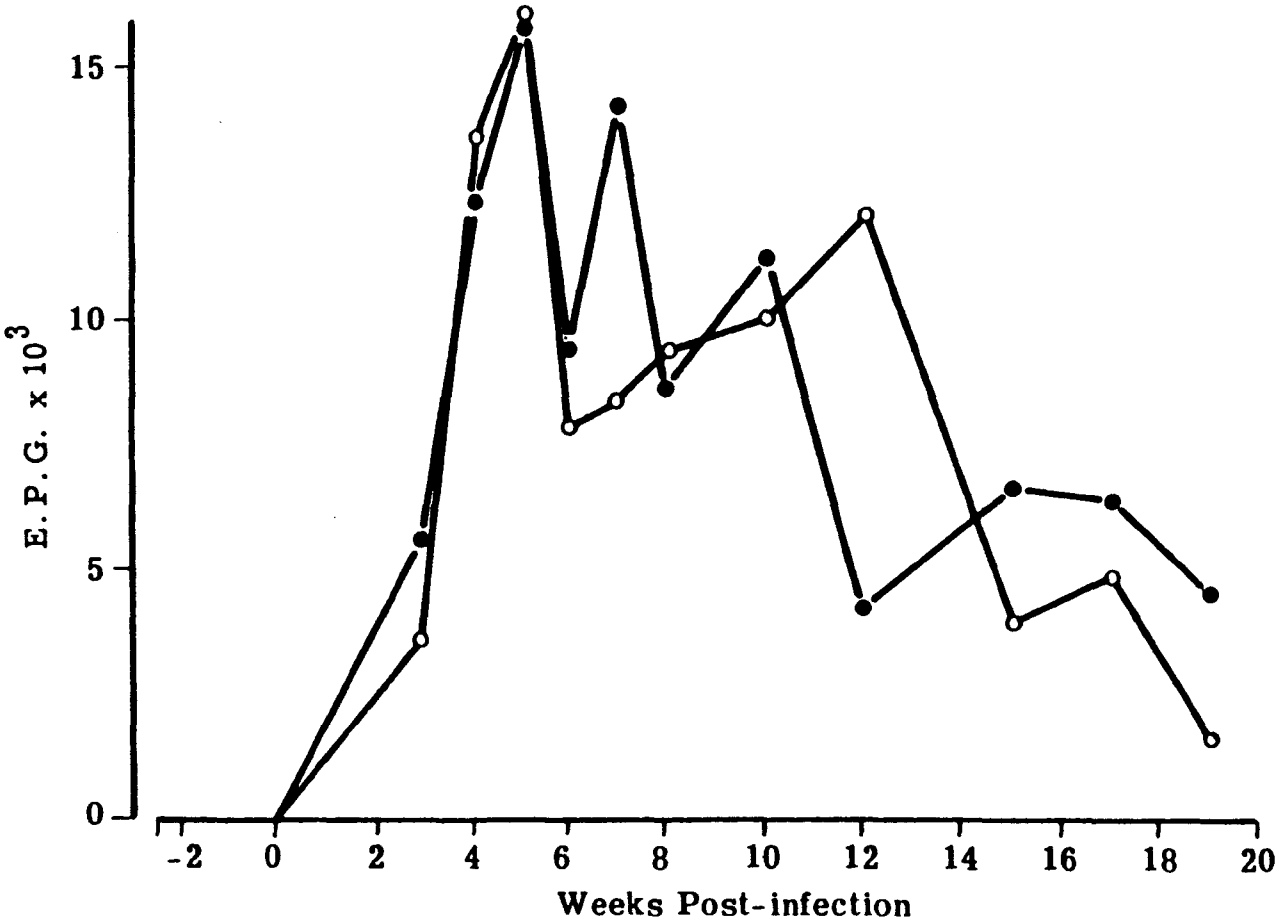


Fig. II.a.12: Group mean faecal egg counts (e p g) of Blackface lambs infected with 125 H. contortus larvae/kg BW and fed either a high or low protein diet.

●——● High protein

○——○ Low protein

Blackface lambs

Faecal egg counts (Fig. II.a.12)

Faecal egg counts rose rapidly in both dietary groups to reach similar maximum values of 16,000 epg at week 5. Thereafter, values fell gradually in both groups to 4,500 epg and 1,723 epg in the high and low protein groups respectively at slaughter. Total daily faecal egg output was similar in both dietary groups during the metabolism cage study.

Finn Dorset and Blackface lambs

Worm burdens

Worm burdens at slaughter are presented in Table II.a.4. Although there were differences between dietary groups and breeds, they were not statistically significant. All the control lambs were negative for parasites at slaughter.

Radioisotopic studies

Erythrokinetics

Finn Dorset lambs (Appendix B, Table 3)

Circulating red cell volumes were significantly reduced in both groups of infected lambs relative to their controls ($P < 0.001$), Mean group values in the infected groups were 7.5 - 8.5 ml/kg BW, compared with mean control values of 19.8 - 20.6 ml/kg BW.

Plasma volumes (measured using ^{125}I-labelled albumin) were similar in both infected and control lambs on the high protein diet, 43.8 ml/kg BW, but because of the reduction in red cell volume in the infected group, total blood volumes were significantly ($P < 0.01$) reduced in this group relative to the control group (52 ml/kg BW in the infected group compared with 64 ml/kg BW in the controls). The group mean value for plasma volume in the infected lambs on the low protein/

Table II.a.4: Individual worm burdens: Finn Dorset and Blackface lambs 20 weeks after infection with 125 H. contortus larvae/kg BW

Group	No.	Hb Type	Larval Stages	Adult		Total
				♂	♀	
Finn Dorset High Protein	R11	AB	0	50	0	50
	12	B	0	150	0	150
	16	B	0	250	250	500
	17	B	100(L_4)	0	0	100(L_4)
	20	B	0	50	0	50
Finn Dorset Low Protein	(B 2)*	(B)	(0)	(250)	(600)	(850)
	4	B	0	200	100	300
	6	AB	0	0	0	0
	9	B	0	450	50	500
	10	B	0	450	150	600
Scottish Blackface High Protein	R41	A	0	400	400	800
	42	AB	0	300	100	400
	60	A	0	0	0	0
	45	B	0	250	100	350
	46	A	0	50	0	50
Scottish Blackface Low Protein	B62	A	0	0	0	0
	63	AB	0	0	0	0
	68	A	0	400	100	500
	78	B	0	50	0	50
	79	A	0	50	0	50

* Died 9w post-infection.

protein diet was slightly elevated due to a grossly expanded plasma volume (64 ml/kg BW) in one lamb, B2. Since the red cell volume of this lamb was similar to that of other group members, the expanded plasma volume was responsible for the very low venous haematocrit (0.155 l/l) of this lamb relative to the other group members. Because of the slight increase in the infected group mean plasma volume value, total blood volume was not statistically significantly reduced in this group relative to the control group.

The "apparent" half-life of the ^{51}Cr-labelled red cells was significantly reduced in both groups of infected lambs (183 and 125 hours in the high and low protein group respectively) relative to their controls (292 and 313 hours in the high and low protein group respectively)($P < 0.01$). Values in the infected lambs on the low protein diet were significantly lower than those of the infected lambs on the high protein diet ($P < 0.01$).

Daily gastrointestinal red cell losses were greater in the infected lambs on the low protein diet relative to the infected lambs on the high protein diet (30 ml/d and 17.5 ml/d) but the difference was not quite statistically significant. Both groups of control lambs had daily gastrointestinal red cell losses of less than 0.5 ml.

Blackface lambs (Appendix B, Table 4)

Circulating red cell volumes were similar in all four groups of lambs regardless of dietary group or infection, viz:- 14.4 - 16.4 ml/kg BW. Venous haematocrit values and red cell volumes of some of the control lambs in both dietary groups were low. However, ^{51}Cr faecal clearances did not indicate that these lambs were losing blood into/

into the gastrointestinal tract. Red cell volumes of the infected Blackface lambs were considerably greater than those of the infected Finn Dorset lambs.

Plasma volumes were significantly elevated in both groups of infected lambs (52 - 55 ml/kg BW) relative to their controls. ($P < 0.01$ and $P < 0.001$ in the high and low protein groups respectively). Control values ranged from 42 - 45 ml/kg BW. As a consequence of the increase in plasma volumes of the infected lambs, total blood volumes were significantly elevated (61 - 71 ml/kg BW) in these groups relative to the control groups ($P < 0.01$ and $P < 0.001$ in the high and low protein groups respectively).

The "apparent" half-life ($T\frac{1}{2}$) of the labelled red cells was reduced in both groups of infected lambs (114 and 115 hours in the high and low protein groups respectively) relative to their controls (154 and 171 hours respectively). However, the differences were not statistically significant. $T\frac{1}{2}$ values were significantly greater in the infected Finn Dorset lambs on the high protein diet relative to the equivalent Blackface group, and Finn Dorset control values were also greater than Blackface control values.

Both groups of infected Blackface had similar gastrointestinal blood losses, namely 18 and 16 ml RBC/d in the high and low protein groups respectively. Both groups of control lambs had losses of less than 1 ml RBC/day.

Ferrokinetics

Because of the contamination of the serum iron samples, the data presented which is dependent on serum iron levels, e.g. plasma iron turnover rates, cannot be taken as absolute values.

Finn/

Finn Dorset lambs (Appendix B, Table 5)

Plasma iron levels on the day of injection of the radioisotopes were significantly elevated in the infected lambs on the high protein diet (221 μg/dl) relative to their controls (150 μg/dl)($P < 0.05$). Differences between infected and control lambs on the low protein diet (214 and 175 μg/dl respectively) were not statistically significant.

The "apparent" half-life of the labelled transferrin was significantly reduced ($P < 0.01$) in infected lambs on the high protein diet relative to their controls (47 and 106 minutes respectively). Similarly, half-life values were significantly reduced ($P < 0.01$) in the infected lambs on the low protein diet relative to their controls (52 and 89 minutes respectively). Differences between the two groups of infected lambs were not significant.

Plasma iron turnover rates were increased in the infected lambs on the high protein diet relative to their controls (2.1 and 0.66 mg/d/kg BW respectively) ($P < 0.05$). Rates were also increased in the infected lambs on the low protein diet relative to their controls (1.73 and 0.87 mg/d/kg BW) ($P < 0.01$). Rates in the infected lambs on the high protein diet were greater than those of the lambs on the low protein diet, but not significantly so.

Red cell utilisation curves rose rapidly in both groups of infected lambs to reach maximum values on day 10 in the high protein group, and on day 2 in the low protein group (Fig. II.a.13). Values in the infected lambs on the high protein diet remained fairly steady and on a plateau, in contrast to values in the low protein group where values declined over the 15 day period of observation. Control values in both dietary groups rose gradually to reach maximum values 15 days after injection /

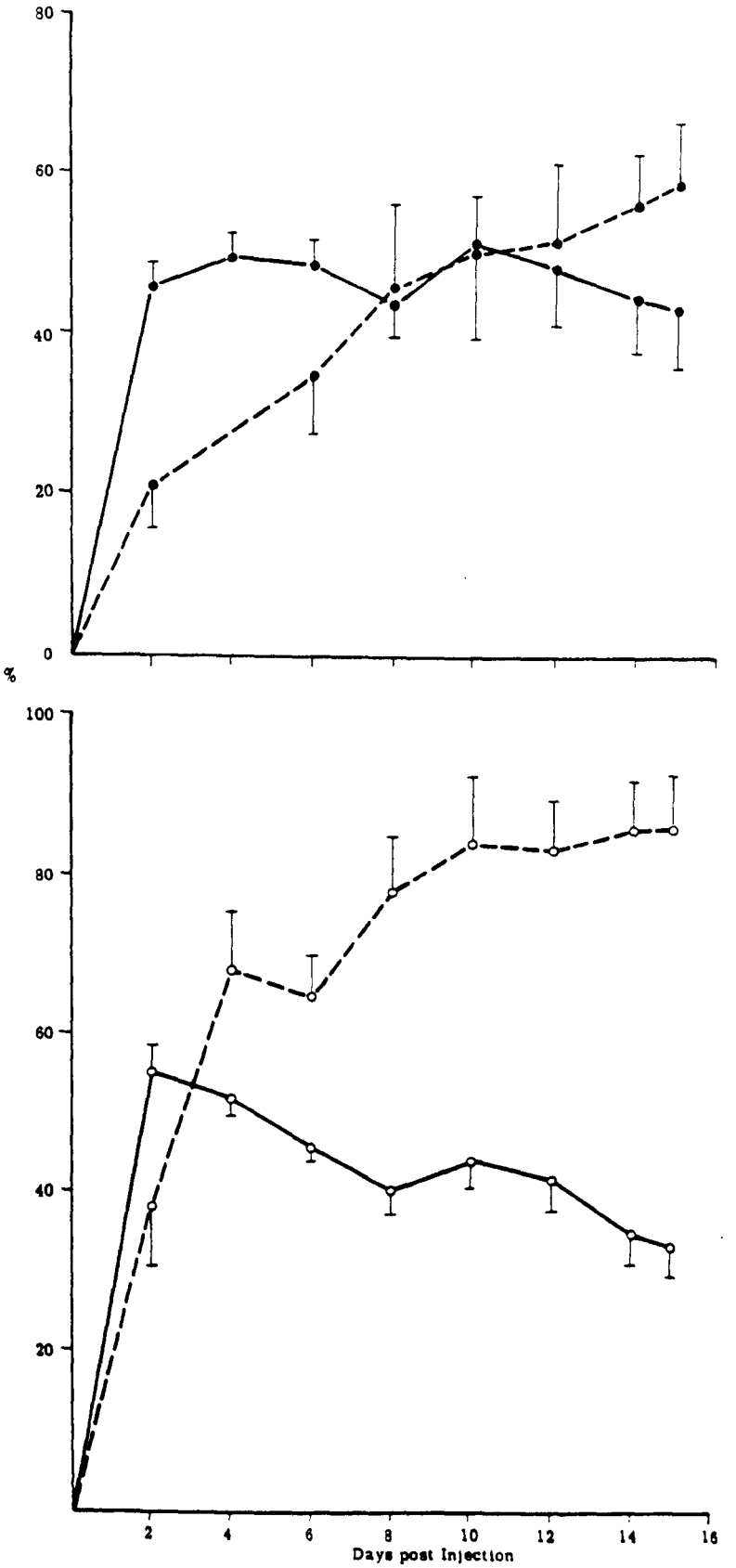


Fig. II.a.13: Mean (± SE) % red cell utilisation of ^{59}Fe in Finn Dorset lambs infected with 125 larvae/kg BW and their respective controls.

High Protein Infected
Low Protein Infected
High Protein Control
Low Protein Control

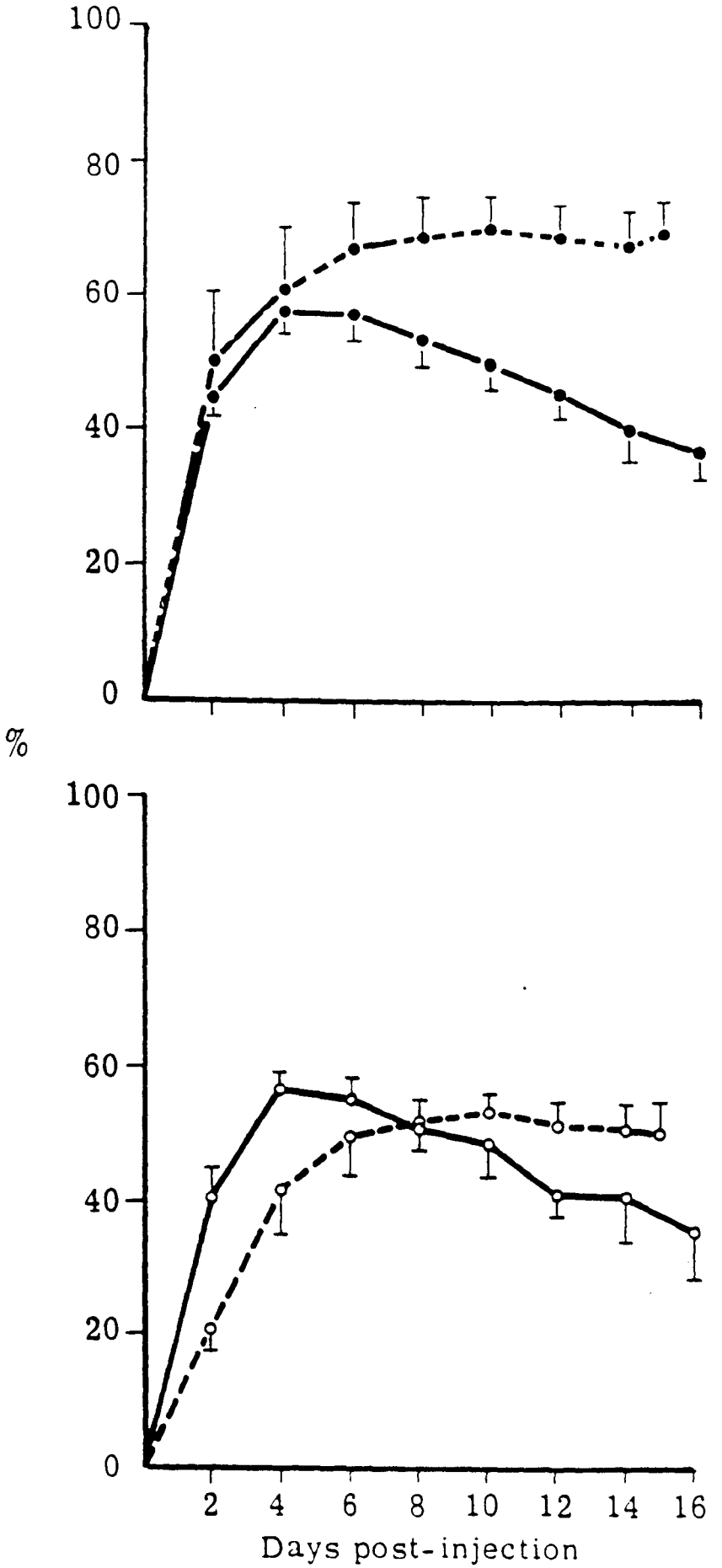


Fig. II.a.14: Mean (± SE) % red cell utilisation of ^{59}Fe in Blackface lambs infected with 125 larvae/kg BW and their respective controls.

●——● High Protein Infected o——o Low Protein Infected
●- - -● High Protein Control o- - -o Low Protein Control

after injection of the radioisotope, with a greater percentage utilisation occurring in the lambs on the low protein diet.

Blackface lambs (Appendix B, Table 6)

Plasma iron levels on the day of injection of the radioisotopes were significantly elevated in the infected lambs on the high protein diet relative to their controls, (315 and 214 μg/dl respectively) ($P<0.001$) whereas differences between both groups of lambs on the low protein diet were not significant.

Half-life values for the labelled transferrin were significantly reduced ($P<0.05$) in the infected lambs on the high protein diet relative to their controls (46 and 80 minutes respectively). Values in the infected lambs on the low protein diet were also significantly reduced ($P<0.02$) relative to their controls (47 and 85 m respectively)

Plasma iron turnover rates were increased ($P<0.001$) in the infected lambs on the high protein diet relative to their controls (3.73 and 1.56 mg/d/kgBW respectively). Rates were also increased ($P<0.01$) in the infected lambs on the low protein diet relative to their controls (2.54 and 1.07 mg/d/kg BW respectively). Rates were also significantly increased in the infected lambs on the high protein diet relative to the infected lambs on the low protein diet ($P<0.02$).

Red cell utilisation was maximal at day 4 in both groups of infected lambs (Fig. II.a.14), in contrast to the control groups where utilisation was maximal on day 10 after injection of the radioiron.

Albumin metabolism

Finn Dorset lambs (Appendix B, Table 7)

Serum albumin levels were significantly reduced in both groups of infected lambs (29.5 and 22.5 g/l in the high and low protein groups respectively) relative to their respective controls (36 and 31 g/l) ($P< 0.001$ and $P<0.01$).

The/

The differences in plasma volumes between groups have been discussed earlier (see Erythrokinetic studies).

Both the intravascular and extravascular albumin pools were reduced in the infected lambs on the high protein diet (1.29 and 1.49 g/kg BW respectively) relative to their controls(1.88 and 2.66 g/kg BW respectively)($P<0.001$). The reduction of the extravascular pool was greater than that of the intravascular pool and this resulted in a reduction in the EA / CA ratio in the infected lambs relative to their controls.

The intravascular and extravascular albumin pools were also significantly reduced in the infected lambs on the low protein diet (1.02 and 1.15 g/kg BW respectively) relative to their controls(1.38 and 2.23 g/kg BW respectively)($P<0.02$ and$P<0.01$). The reduction in the extravascular pool was again greater than that of the intravascular pool and this resulted in a decrease in the EA /CA ratio in the infected group relative to the controls ($P<0.01$).

The half-life ($T\frac{1}{2}$), of the labelled albumin was significantly reduced in the infected lambs on the high protein diet relative to their controls (285 and 449 hours respectively)($P<0.05$). Although the $T\frac{1}{2}$ was reduced in the infected lambs on the low protein diet relative to their controls, 223 and 363 hours respectively, the difference was not statistically significant.

The fractional catabolic rate (F(CA)) was considerably increased in both groups of infected lambs relative to their controls. Infected lambs on the high protein diet were catabolising 16% of the intravascular pool daily, compared with a rate of 9% in the controls ($P<0.01$). The infected lambs on the low protein diet had a catabolic rate of 20% daily compared with a 7% rate in their controls ($P<0.01$).

The/

The differences in catabolic rate between the two groups of control lambs were not significant.

Blackface lambs (Appendix B, Table 8)

Serum albumin levels were similar in all four groups of lambs during the metabolism cage study.

As mentioned in the results of the erythrokinetic study, plasma volumes were significantly elevated in both groups of infected lambs.

Within a dietary group there were no differences in the size of the intravascular albumin pools,but the infected lambs on the high protein diet had significantly larger intravascular albumin pools (1.77 g/kg BW) compared with those of the infected lambs on the low protein diet (1.41 g/kg BW)($P < 0.02$). A similar difference in pool sizes was observed in the control lambs (1.59 and 1.33 g/kg BW in the high and low protein groups respectively)($P < 0.01$).

Again, infection had little effect on extravascular albumin pool sizes but a significant dietary difference was observed, higher values occurring in both groups of lambs on the high protein diet relative to the lambs on the low protein diet (2.89 and 2.28 g/kg BW in infected and control lambs on the high protein diet compared with 1.89 and 1.96 g/kg BW in infected and control lambs on the low protein diet)($P < 0.01$ and $P < 0.05$ respectively).

Thus,there were no differences in albumin distribution as a result of infection but EA/CA ratios were higher in the infected lambs on the high protein diet relative to the infected lambs on the low protein diet (1.63 and 1.33 respectively).

Values for the apparent half-life of the labelled protein were slightly reduced in the infected lambs relative to their controls, but the difference was only significant ($P < 0.02$) in the high protein group.

Both/

Both infected and control lambs in both dietary groups had similar fractional catabolic rates (range 8-10.6%), and there were no differences between dietary groups.

Nutritional studies

Feed intake

Finn Dorset lambs

A considerable variation in the quantity of fresh feed consumed daily during the cage study existed between the infected lambs on the high protein diet, average feed intakes ranging from 550 g/d to 1236 g/d. Two lambs had steady feed intakes during the study, but the remaining lamb in the group, R17, became inappetant (Fig.II.a.15) having an average daily feed intake of 550 g/d. During this period of inappetance, the lamb lost ~4 kg in weight. Its feed intake started to increase on the last two days of the balance study.

Again, there was variation in feed intake between lambs in the low protein group. One lamb maintained a steady average daily intake of 1215 g whereas another lamb of similar body weight consumed an average of 816 g/d. The remaining lamb in the group, B2, had a severely depressed appetite (Fig. II.a.15) which started during the adjustment period and continued until the end of the study. This lamb lost approximately 2 kg in weight during the metabolism cage study and subsequently died 9 weeks after infection. Control lambs were pair-fed to their infected counterpart.

Blackface lambs

Feed intakes in both groups of infected Blackface lambs remained fairly steady throughout the study. Average daily feed intakes in the high protein group ranged from 857 to 1000 g/d, and in the low protein group from 914 to 1000 g daily. Inappetance was not observed. Control lambs were pair-fed to their infected counterpart.

Digestibility/

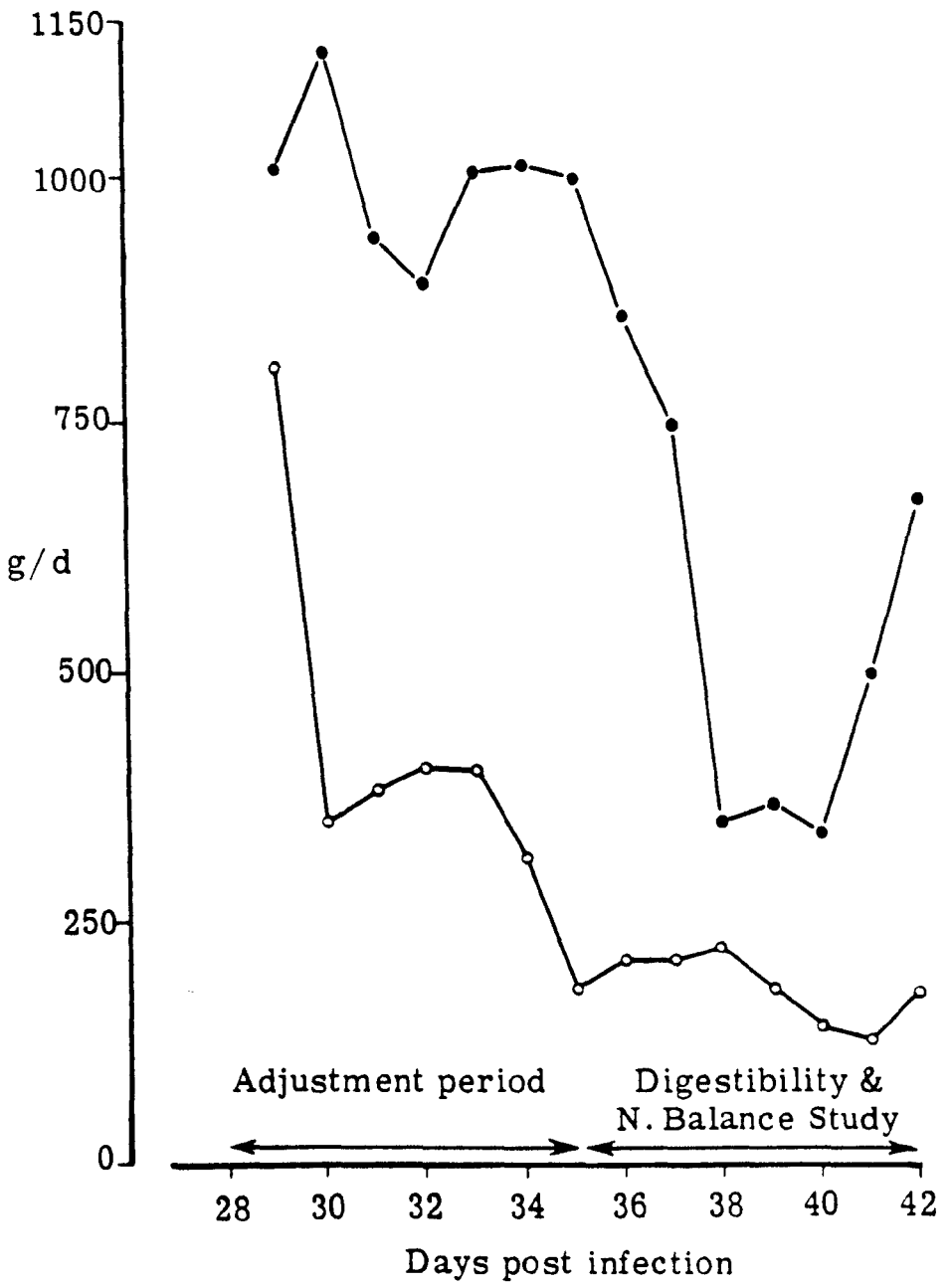


Fig. II.a.15: Daily fresh feed intakes (g/d) of two infected (125 larvae/kg BW) Finn Dorset lambs.

●——● R 17 (High protein group)

○——○ B 2 (Low protein group)

Digestibility coefficients of the experimental diets

There were no significant differences in the apparent digestibility coefficients (ADC) of the various proximate fractions of the experimental diets between infected and control Finn Dorset lambs on the high protein diet. However, crude protein and ether extract digestibility was slightly reduced in the infected lambs on the low protein diet relative to their controls ($P < 0.05$) (Appendix B, Table 9).

The apparent digestibility coefficient of the ash fraction of the diet was elevated in the infected Blackface lambs on the high protein diet relative to their controls ($P < 0.02$) but the reverse situation occurred in the two groups of lambs on the low protein diet (Appendix B,Table 10).Otherwise, there were no significant differences in the ADC of the various fractions of the two diets.

The differences in crude protein digestibility between the two dietary groups are as expected, the lowered apparent digestibility in the low protein group being attributable to the relatively higher contribution of metabolic faecal nitrogen in the faecal output of this group.

Nitrogen balance

Infected and pair-fed control lambs on the high protein diet remained in positive N balance ($\sim$ 2 g N/d), and there were no significant differences in faecal or urinary N output between the two groups. In contrast, infected Finn Dorset lambs on the low protein diet were in slight negative balance relative to their pair-fed controls (- 0.21 g N/d) but the difference was not statistically significant. Faecal N output was similar in both infected and control groups, but urinary N output was significantly greater in the infected group ($P < 0.05$)(Appendix B, Table 11)

Infected/

Infected and pair-fed control Blackface lambs on the high protein diet remained in positive N balance ($\sim$ 4 g N/d) and there were no significant differences in faecal or urinary N output between the two groups. Pair-fed control lambs on the low protein diet, however, retained significantly greater amounts of N relative to the infected lambs on the same diet ($P < 0.05$). Although faecal and urinary N output was greater in the infected group relative to the control group, the differences were not statistically significant (Appendix B, Table 12).

Experiment IIb

An investigation into the influence of high and low protein diets on parasite establishment in Blackface lambs infected with 125 H. contortus larvae/kg BW.

MATERIALS AND METHODS

Experimental design

Fourteen Scottish Blackface female lambs were kept on either a high or low protein diet and infected with a single dose of 125 H. contortus larvae/kg BW when 4 months old. This experiment was conducted in parallel with the Blackface lambs of Experiment IIa and they were infected with the same batch of larvae. Three lambs in each dietary group were killed 5 weeks post-infection and the remaining four in each group killed 14 weeks after infection and worm burdens assessed (Table II.a.1).

Blood and faecal samples were collected and weights recorded throughout the experiment. No radioisotopic, N balance or digestibility studies were conducted.

Experimental animals

Fourteen Blackface female lambs were divided into high (7 lambs) and low (7 lambs) protein dietary groups on a haemoglobin type and bodyweight basis when 3 months of age. One month later, all the lambs were infected with 125 H. contortus larvae/kg BW per os.

Feeding and housing

The diets used were those described in Experiment IIa. The lambs were housed in concrete floored pens, as described in General Materials and Methods, and were fed to appetite twice daily.

RESULTS/

Clinical and body weight changes

Both groups of lambs continued to gain weight throughout the experiment, a slightly greater increase in weight being observed in the high protein group (Fig. II.b.1). All the lambs continued to eat the food offered.

Haematological changes (Fig. II.b2)

Haematocrit values in the lambs on the high protein diet fell from a pre-infection mean of 0.34 to 0.31 l/l four weeks after infection. Values rose thereafter to 0.35 l/l and remained in excess of 0.38 l/l for the rest of the experiment. Values in the low protein group fell from a pre-infection mean of 0.31 to 0.25 l/l five weeks after infection but rose again thereafter. Although a considerable difference in haematocrit values existed between dietary groups 4-5 weeks after infection, pre-infection values were also different.

Both dietary groups had a macrocytic response of similar magnitude, peak MCV values occurring 6 weeks after infection.

Parasitological findings (Fig. II.b.3)

Faecal egg counts rose rapidly in both dietary groups to maximum values of 15,000 e.p.g. in the high protein group and 12,000 e.p.g. in the low protein group 6 weeks after infection.

Worm burdens are presented in Table II.b.1. The lambs in the high protein group which were killed at 5 weeks post-infection had a higher mean worm burden at slaughter than the low protein group, 817 (± SE 33) and 550 (± SE 176) respectively, but the difference was not statistically significant. The reverse situation occurred in the remaining lambs of both dietary groups when slaughtered 14 weeks post-infection. A higher mean worm burden occurred in the low protein group (563 ± SE 63) relative to the high protein group (363 ± SE 182) but, again, the difference was not statistically significant.

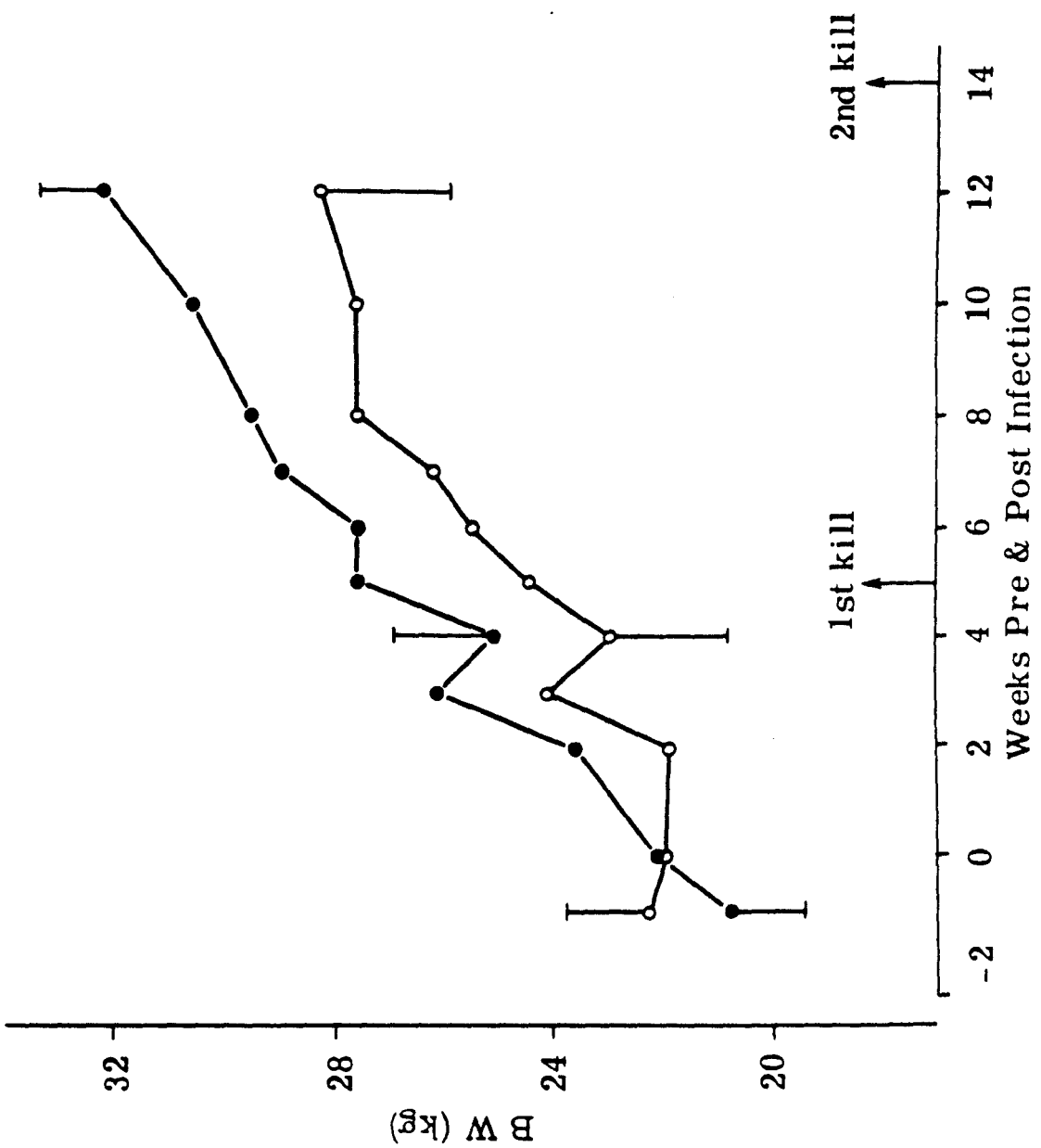


Fig. II.b.1: Mean group body weights of infected (125 larvae /kg BW Blackface female lambs fed eigher a high or low protein diet.

●——● High protein ○——○ Low protein

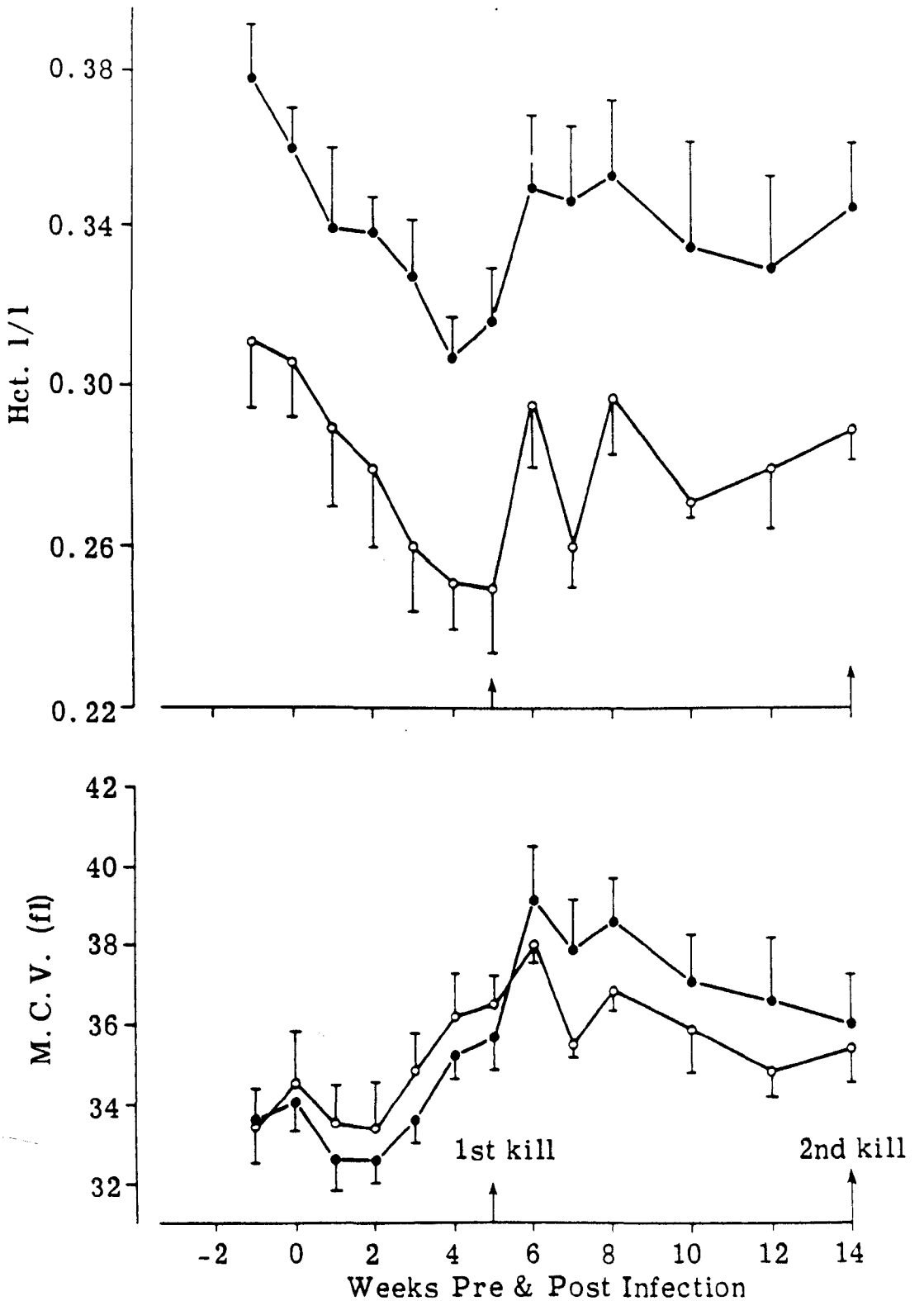


Fig. II.b.2: Mean (± SE) haematocrit and MCV values of infected (125 larvae/kg BW) Blackface female lambs fed either a high or low protein diet.

●——● High Protein ○——○ Low Protein

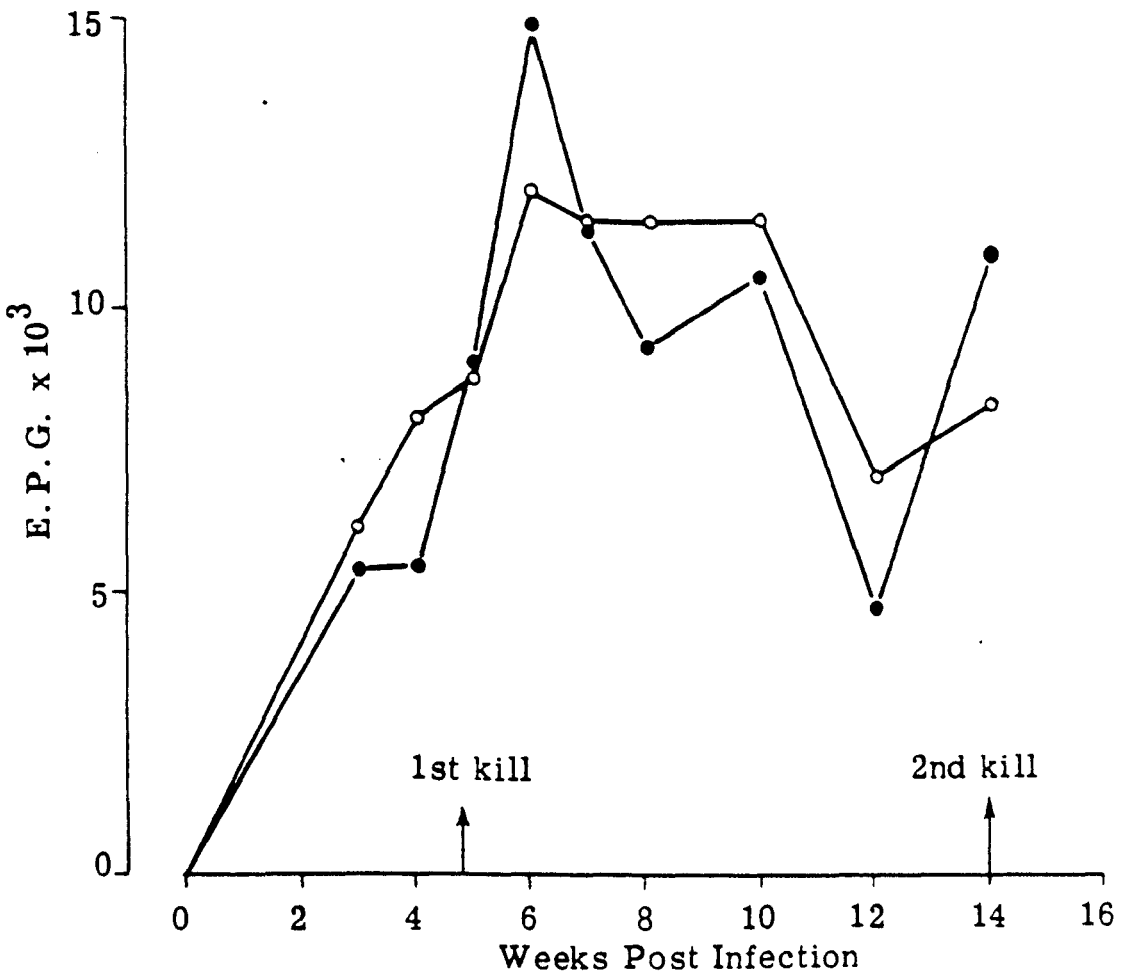


Fig. II.b.3: Mean faecal egg counts (e p g) of infected (125 larvae/kg BW) Blackface female lambs fed either a high or low protein diet.

●——● High Protein ○——○ Low Protein

TABLE II.b.1: Individual worm burdens: Blackface female lambs 5 and 14 weeks after infection with 125 *H. contortus* larvae/kg BW

Sheep No.	Dietary Status	Time of Slaughter post-infection(w)	Hb Type	Larval Stages	Adult		Total
					♂	♀	
R51	High	+5w	A	0	300	450	750
52			AB	0	400	450	850
53	Protein		A	0	250	600	850
B53	Low	+5w	A	0	250	100	350
77			AB	0	400	500	900
58	Protein		AB	0	300	100	400
R55	High	+14w	A	0	0	0	0
58			A	0	50	150	200
56			AB	0	550	300	850
59	Protein		AB	0	350	50	400
B51	Low	+14w	AB	0	400	0	400
67			A	0	500	200	700
71	Protein		A	0	450	150	600
59			A	0	400	150	550

DISCUSSION

The results of both these experiments suggest that breed, and to a lesser extent diet, influenced the pathophysiological consequences of a single low infection of 125 H. contortus larvae/kg BW but did not appear to influence parasite establishment.

The infected lambs of both breeds developed a moderately severe macrocytic anaemia and were hypoproteinaemic and hypoalbuminaemic. Gastric blood loss was similar in all the infected groups of lambs, and they all mounted a vigorous erythropoietic response regardless of dietary status. Albumin catabolism was increased after infection, particularly in the Finn Dorset lambs. Faecal egg counts and total daily faecal egg outputs were similar in all groups and there were no significant differences in worm burdens at slaughter.

Red cell volumes were reduced to a similar extent in both dietary groups of infected Finn Dorset lambs, and values were considerably lower than those of the infected Blackface lambs, yet the ^{51}Cr faecal clearances of the two breeds were not significantly different. Haemodilution was a prominent feature of infection in both dietary groups of Blackface lambs, resulting in increased total blood volumes in these two groups. In contrast, haemodilution was only observed in one infected Finn Dorset lamb, B2, from the low protein group. The grossly expanded plasma volume of this lamb was partly responsible for its very low venous haematocrit when compared with its other group members. Since plasma volumes of the remaining infected Finn Dorset lambs were within normal limits, the reduction in red cell volumes resulted in a fall in total blood volumes.

An expanded plasma volume but with a normal total blood volume has been observed by Owen (1971) in two 4-month old Merino lambs experimentally/

experimentally infected with 50,000 H. contortus. However, his findings must be accepted with caution since, with the exception of the initial occasion before infection when red cell volumes (^{51}Cr) and plasma volumes (Evans Blue) were measured simultaneously, plasma volumes only were measured subsequently, and the red cell volume calculated from the plasma volume and a "corrected" venous haematocrit. The correction factor, which is the ratio of the venous haematocrit to the whole body haematocrit, has been shown to alter in parasitised animals, and thus it is not valid to use a ratio calculated in normal unparasitised sheep for later studies when the animals are parasitised. Although no data on blood protein levels was presented, Owen commented that the plasma volumes increased in the face of a marked hypoproteinaemia, and extensive neck oedema was noted in one lamb. However, in the present experiment, oedema was not noted in the infected Blackface lambs, nor were they particularly hypoproteinaemic. Indeed, albumin metabolism appeared to be minimally disturbed in the infected lambs when compared with their controls.

However, the Blackface control lambs developed a macrocytic anaemia during the experiment which was confirmed by the radioisotopic studies. As a result, comparison between infected and control Blackface groups was made difficult. For example, circulating red cell volumes were below values usually regarded as normal in sheep, namely 19-20 ml/kg BW (Hodgetts, 1961; Holmes, 1969; Owen, 1971). The half life of the labelled red cells was reduced, especially when compared with the Finn Dorset controls. Furthermore, there was evidence of increased rates of erythropoiesis as indicated by increased plasma iron turnover rates, and fairly rapid utilisation of the radioiron. In addition to the erythrokinetic and ferrokinetic changes in the control Blackface lambs, there was also evidence of alterations in albumin metabolism. For example/

example, half life values for the labelled albumin were considerably less than values in the Finn Dorset controls, and indeed were not significantly different from the infected Blackface values. Furthermore, control fractional catabolic rates (F(CA)) were higher than expected and, again, were greater than Finn Dorset control values.

The cause of the anaemia in the control Blackface lambs is not clear. Whether it is the "anaemia of growth" referred to by Silverman et al (1970) is questionable. They attributed the anaemia of their control lambs to rapid growth rates of approximately 200 g/day, resulting in a failure to increase their blood volumes sufficiently quickly to keep pace with the increase in body size. Certainly, the LWG of ∿197 g/d of the control Blackface lambs on the high protein diet was similar to that observed by Silverman et al. However, control Blackface lambs on the low protein diet also became anaemic despite a more modest LWG of ∿90 g/day. Whether this rate of growth would result in anaemia is not known. However, since daily LWG in the Finn Dorset control groups ranged from 95-119 g/day depending on dietary regimen, and neither group became anaemic, this would suggest that, at this level of growth, the expansion of the blood volume can keep pace with the increase in body size. The appearance of strongyle eggs (maximum 250 e p g) in the faeces of some of the control Blackface lambs, may have contributed to the anaemia, yet ^{51}Cr faecal clearances were very low relative to the infected groups. However, the ^{51}Cr faecal clearances in the Blackface control lambs were double that of the Finn Dorset control lambs. A further cause of the anaemia may have been the regular withdrawal of blood for analyses throughout the experiment. However, the Finn Dorset control lambs were also bled regularly throughout the experiment. Thus, the cause of the anaemia in the Blackface control lambs remains unknown.

As mentioned earlier, albumin metabolism was less disturbed by infection in the Blackface lambs than in the Finn Dorset lambs. However, there was evidence of both breed and dietary influence on albumin metabolism. For example, both the intravascular and extravascular albumin pools were reduced in the infected Finn Dorset lambs, particularly in the group fed the low protein diet. In contrast, the expansion of the plasma volume observed in the infected Blackface group resulted in an increase in the intravascular albumin pool in both dietary groups. In both breeds of sheep, pool sizes of the control lambs on the high protein diet were greater than those of the control lambs on the low protein diet. However, there was no evidence of increased fractional catabolic rates in either breedof control lambs fed the high protein diet relative to controls on the low protein diet, as has been observed by Dargie and Berry (1979).

A further interesting observation was that, regardless of dietary status, the infected lambs of both breeds mounted a vigorous erythropoietic response. The macrocytosis observed in the peripheral blood was confirmed by the ferrokinetic studies, plasma iron turnover rates increasing in the infected lambs, together with rapid utilisation of the radioiron. The more rapid utilisation of the radioiron observed in the infected Finn Dorset lambs on the low protein diet compared with the high protein group may be associated with the slightly greater gastrointestinal blood loss in the former group.

Neither breed nor diet appeared to influence parasite establishment. The worm burdens of both groups of female Blackface lambs/

lambs which were killed 5 and 14 weeks after infection were not significantly different. Furthermore, worm burdens of the Finn Dorset and male Blackface lambs killed 21 weeks after infection were also similar, and there was no evidence of greater parasite establishment in the lambs fed the low protein diet. Any differences in faecal egg counts (e p g) between dietary groups were explained by differences in faecal mass. Furthermore, the similar gastrointestinal blood losses in the infected lambs tend to substantiate the findings of similar worm burdens in both breeds regardless of dietary status.

The findings of more severe clinical disease in the Finn Dorset lambs relative to the Blackface lambs is in general agreement with Altaif (1975) and Altaif & Dargie (1978a), who compared the effects of haemonchosis on Scottish Blackface and Finn Dorset sheep. In the earlier studies (Altaif, 1975), the pathophysiological changes observed after infection with 350 H. contortus larvae/kg BW were, in general, less severe in the Blackface lambs. Although gastric blood loss was less in the Blackface sheep relative to the Finn Dorset sheep, the difference was not statistically significant. However, since % establishment of the parasite was less in the Blackface sheep despite worm sizes being similar in both breeds, it was concluded that "the breed differences were due to differences in immunological competence". It is less easy to interpret the data of the later study (Altaif & Dargie, 1978a) as two different levels of infection were used, namely 350 or 500 larvae/kg BW. No details were given as to which breed, or which haemoglobin type within a breed, received which level of infection. Furthermore, much of the pathophysiological data is presented as a % change from pre-infection values and is, in addition, not presented on a body weight basis. Despite these shortcomings, there were indications, from the worm burdens and levels of abomasal blood loss, that parasite establishment was relatively higher in the Finn Dorset lambs compared with the Blackface lambs.

The studies of Altaif and Dargie were principally designed to investigate the influence of haemoglobin type on the susceptibility of sheep to haemonchosis. Their results indicated that in both Finn Dorset and Blackface sheep, HbAA animals were relatively more resistant to parasite establishment than AB or BB animals, though these differences were not always statistically significant. However, in the present experiment in which Hb types were carefully randomised between groups, there was no evidence that Hb type in any way influenced parasite establishment.

In conclusion, in the present experiments, although there appeared to be breed differences as regards the severity of the clinical signs observed after infection, it is unlikely that breed or diet influenced parasite establishment. However, there is evidence that breed and diet influenced some of the pathophysiological changes, in particular the changes in red cell and plasma volume, and in albumin metabolism. All the infected lambs, regardless of breed or dietary status, had similar gastric blood losses and the majority responded equally well to this haemorrhage by mounting a vigorous erythropoietic response.

CHAPTER III

Studies on the pathogenicity of a single infection with 350 *H. contortus* larvae/kg BW on 4 month old Finn Dorset/Dorset Horn lambs fed either a high or low protein diet

INTRODUCTION

The principal features of the experiments just described (Chapter II) were that a single infection with 125 H. contortus larvae/kg BW in 4 month old lambs kept on either a high or low protein diet resulted in the development of a macrocytic anaemia, hypoproteinaemia and hypoalbuminaemia. The severity of some of the pathophysiological changes was variable and appeared to be related to breed and dietary status, the Finn Dorset lambs appearing to be more severely affected by the infection compared with the Scottish Blackface lambs. However, the results also suggested that parasite establishment was similar in both breeds and thus the differences in the pathophysiological parameters could not be explained by differences in worm establishment.

The following experiments were conducted to investigate further the influence of dietary protein on the pathogenicity of infection with H. contortus. The infective dose used was increased to 350 larvae/kg BW which would allow comparison with previous work (Allonby & Dargie, 1973; Altaif, 1975; Dargie & Allonby, 1975).

The lambs used in the experiments were principally Dorset Horn or Finn Dorset and the experimental diets used were essentially similar to those used in the previous experiments (Chapter II). A similar protocol was followed, namely the dietary change was made when the lambs were 3 months old and infection was carried out one month later in the first experiment (IIIa) and 5 months later in the second experiment (IIIb). Radioisotopic and nutritional studies similar to those described in previous experiments were conducted in the first experiment and, in addition, iron intakes and faecal losses of iron were measured.

Experiment IIIa

An investigation into the pathophysiological changes associated with a single infection of 350 H. contortus larvae/kg BW on 4 month old lambs fed either a high or low protein diet.

MATERIALS AND METHODS

Experimental design

Finn-Dorset or Dorset Horn male and female lambs kept on either a high or low protein diet from the age of 3 months were given a single infection of 350 H. contortus larvae/kg BW when 4 months old. Radioisotopic, digestibility and N balance studies on the infected male lambs started 19 days after infection and continued until day 39. Blood and faecal samples were collected and weights were recorded on a weekly basis throughout the experiment. The infected male lambs were killed 41 days after infection when their worm burdens were assessed. The infected female lambs were slaughtered 11 weeks after infection when carcase quality was evaluated and worm burdens were assessed (Table III a 1).

Experimental animals

Thirty-two Finn-Dorset/Dorset Horn early weaned parasite-free lambs were used. They were divided into high and low protein dietary groups at the age of 3 months, as described in General Materials and Methods. Each dietary group comprised 8 castrated males and 8 female lambs. One month later each dietary group was further sub-divided into "infected" and control groups, each group then comprising 4 male and 4 female lambs. The lambs in the infected group were then given a single dose of 350 H. contortus larvae/kg BW per os.

Feeding and management

The experimental diets used were identical to those described in the previous experiment (Chapter II). The iron content of the high and low protein diet was 0.98 and 1.34 g/kg DM respectively. During the metabolism cage study, for each day, the separate components of/

TABLE III.a.1: Studies on the pathogenicity of a single infection of 350 H. contortus larvae/kg BW on 4 month old lambs fed either a high or low protein diet: Experimental design.

Experiment	Diet change at 3 months of age	Age at infection (m)	Dose of H. contortus /kg BW	Nutritional and radio-isotopic studies (♂lambs) (days post-infection)	Slaughter and worm burden assessment
A	High Protein (8)	4	350	19 - 39	♂ 41 days post-infection ♀ 77 days post-infection
	Low Protein (8)	4	350	19 - 39	♂ 41 days post-infection ♀ 77 days post-infection
	High Protein (8)	4	Nil	Pair-fed during metabolism cage study	♂ to Experiment B ♀ carcase evaluation
	Low Protein (8)	4	Nil	Pair-fed during metabolism cage study	♂ to Experiment B ♀ carcase evaluation
B	High Protein (4)	8	350	None	28 days post-infection
	Low Protein (4)	8	350	None	28 days post-infection

of the allocated mixture, i.e. the sugar beet pulp, siftings, soyabean meal (high protein group only), and mineral/vitamin mixture, were weighed accurately into a paper bag for each animal. The daily individual ration allowances were weighed out in advance for the whole of the experimental period, i.e. the adjustment period of 5 days and the 7 day collection period. The weighed ration allowances were then stored until use. The experimental diets were individually offered at 90% of appetite in order to minimise potential residues. The weighing of separate components of the diets individually ensured that the daily nutrient intakes were accurately known, and that uniformity of intake was achieved for the whole of the experimental period. The lambs were given approximately half of the daily ration at 10.00 h and the balance at 17.00 h. When the lambs were in penned accommodation the diets were fed ready-mixed to appetite.

Radioisotopic, digestibility and N balance studies

Twelve days after infection, the infected male lambs (4 from the high protein group and 4 from the low protein group) were moved from the penned accommodation to metabolism cages. Radioisotopic studies using ^{51}Cr-labelled red cells, ^{59}Fe-labelled transferrin and ^{125}I-labelled albumin, as described previously (Chapters I and II), were conducted over a 14 day period, starting 19 days after infection. After a 5 day adjustment period a seven day collection period for N balance and digestibility trials was conducted. In addition to the normal faecal and urinary analyses for the N balance and digestibility studies, the iron content of the feed and faeces for the 7 day period was also measured. An uninfected lamb was also transferred to the metabolism cages at this time to act as a control for the radioisotopic studies/

studies as previously described (Chapter II). After the studies on the infected lambs finished, the uninfected male control lambs (4 on the high protein diet and 4 on the low protein diet) were transferred to the metabolism cages and radioisotopic and nutritional studies essentially similar to those conducted on the infected males were carried out. Control lambs were pair-fed to their infected counterparts during the radioisotopic and nutritional studies.

RESULTS

Clincal and body weight changes

The infected male lambs on the high protein diet showed no signs of weakness or inappetance throughout the experiment. However, one infected female lamb on the high protein diet (FR5) became very inappetant and died 74 days after infection.

In contrast, sub-mandibular oedema was observed in two of the infected male lambs (P2 and P3) on the low protein diet. Both these lambs became inappetant. This was particularly marked in lamb P3 which also became increasingly reluctant to stand. Furthermore, persistent teeth grinding was frequently observed in this lamb. On days 52 and 67 post-infection two infected female lambs in the low protein group died.

Infected lambs on the high protein diet failed to gain weight during the first 6 weeks of infection, whereas the infected lambs on the low protein diet lost 1.3 kg in weight in the same period. The surviving female lambs on the high protein diet gained 3.3 kg in weight between weeks 6 and 11 post-infection, whereas the surviving female lambs on the low protein diet only gained 0.2 kg in the same period.

In/

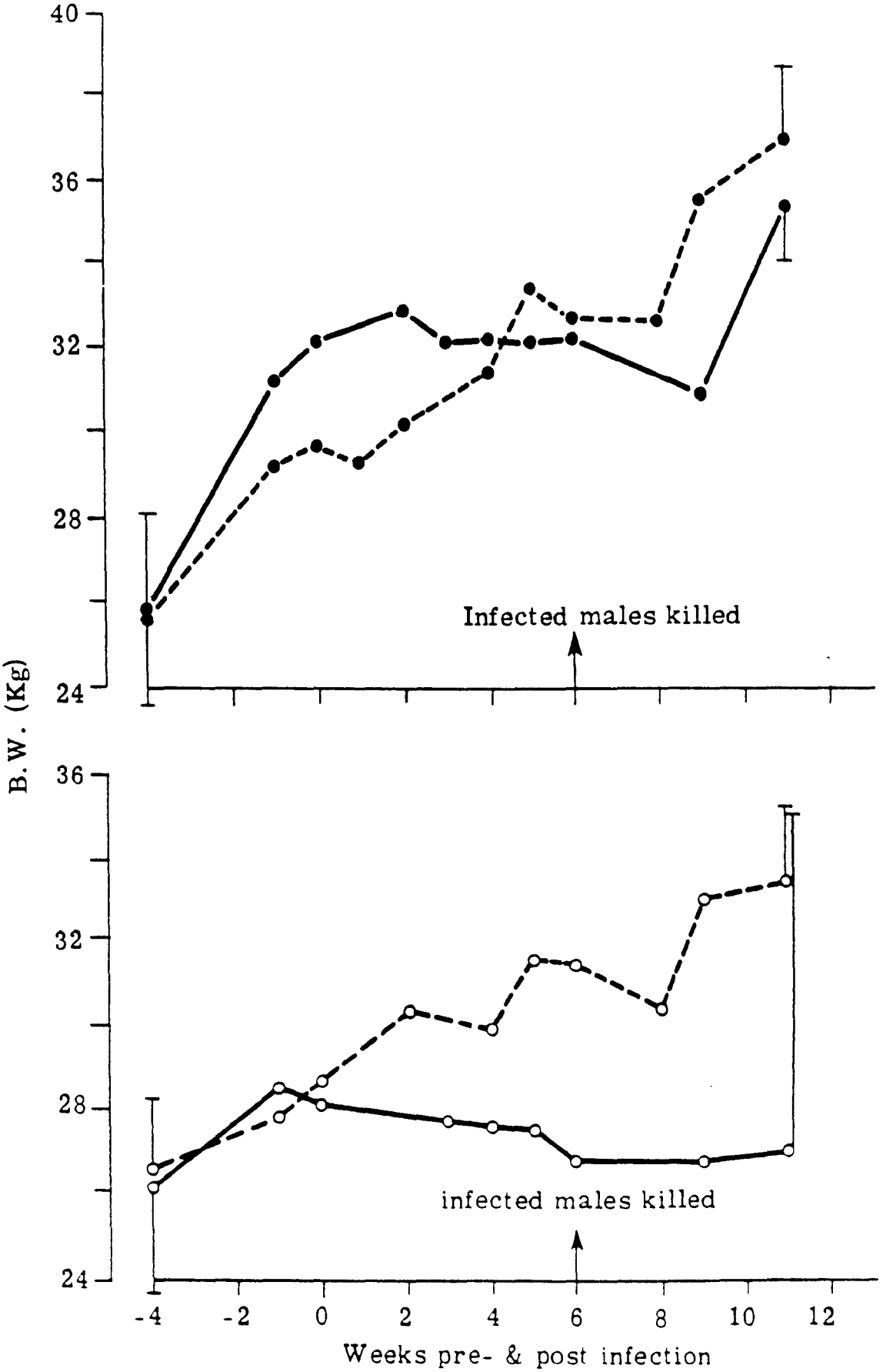


Fig. III.a.1: Mean group body weights of infected (350 H. contortus larvae/kg BW) lambs fed either a high or low protein diet and their respective controls.

●——● High protein infected
●- - - -● High protein control
o——o Low protein infected
o- - - -o Low protein control

In contrast, both groups of control lambs gained weight steadily throughout the experiment, 8 and 6.8 kg in the high and low protein groups respectively (Fig. III.a.1.).

Haematological changes

Haematological values were similar in both dietary groups before infection. After infection both groups of infected lambs developed a severe macrocytic anaemia, the greater reduction in haematocrit occurring in the lambs on the low protein diet. Haematocrit values in the infected lambs on the high and low protein diets fell to 0.20 l/l and 0.17 l/l respectively four weeks after infection (Fig. III.a.2). The macrocytic response was similar in both dietary groups of infected lambs, MCV values reaching maximum levels (39-41 fl) 4-5 weeks after infection. Values remained elevated relative to controls for the duration of the experiment (Fig.III.a.3). MCH values rose in both groups of infected lambs parallelling the MCV changes (Fig. III.a.4).

One lamb in each infected dietary group behaved differently from the rest of its respective group members, namely FR5 in the high protein group and P5 in the low protein group. Both lambs developed a severe anaemia after infection (Fig.III.a.2) but the macrocytic response was short-lived in both cases (Fig. III.a.3). The principal haematological,biochemical and parasitological changes in these two lambs are presented separately (Appendix C, Table 1). FR5 developed a microcytic hypochromic blood picture typical of iron deficiency anaemia, i.e. MCV and MCH values fell progressively to below normal, 26 fl and 7.5 pg respectively. This was associated with a fall in serum iron levels and reduction in percentage saturation of transferrin (47 µg/dl and 10% respectively, before death).

A/

Fig. III.a.2: Mean (± SE) haematocrit values of infected (350 larvae/kg BW) lambs and their respective controls.

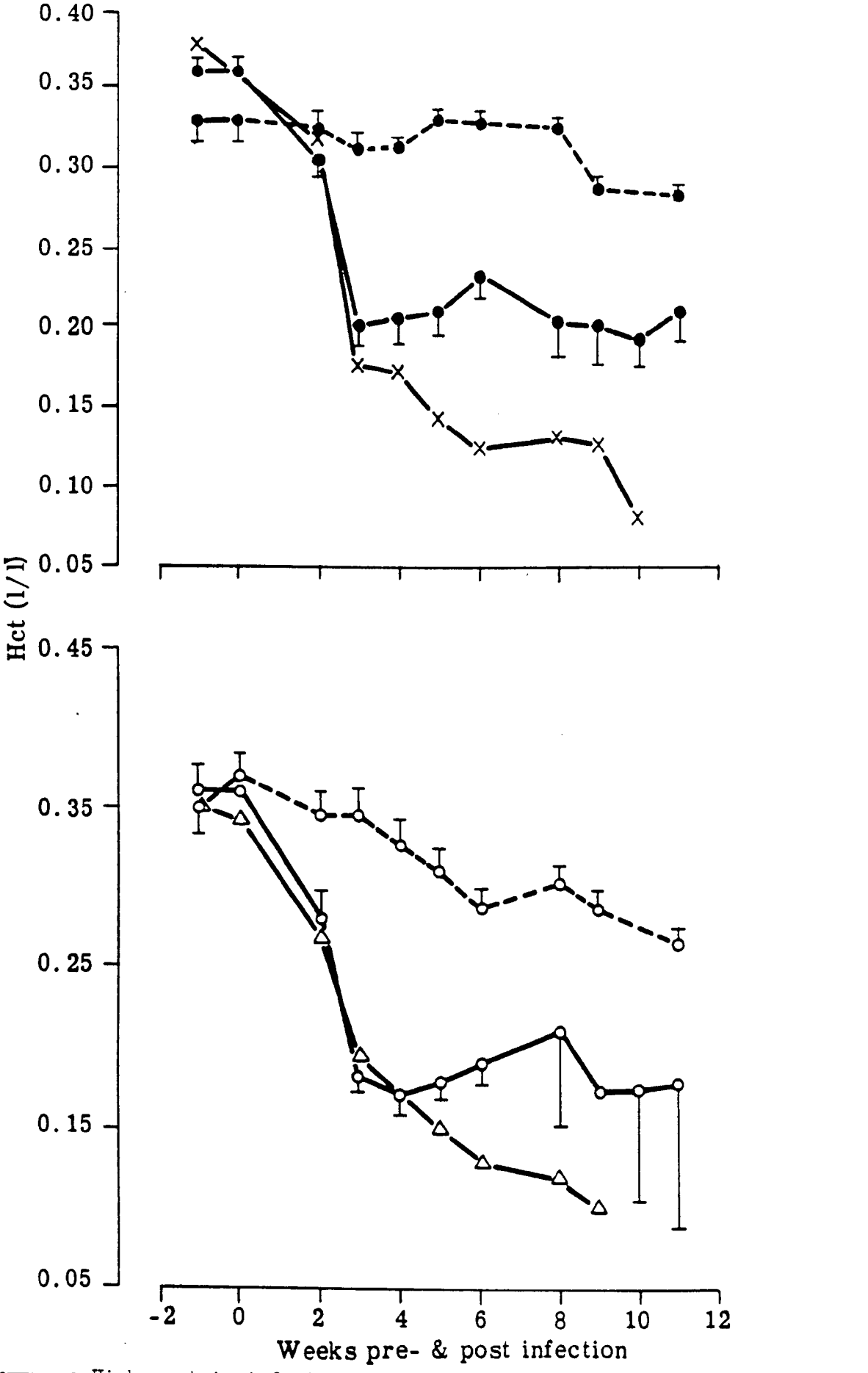


Fig. III.a.3: Mean (± SE) MCV values of infected (350 larvae/kg BW) lambs and their respective controls.

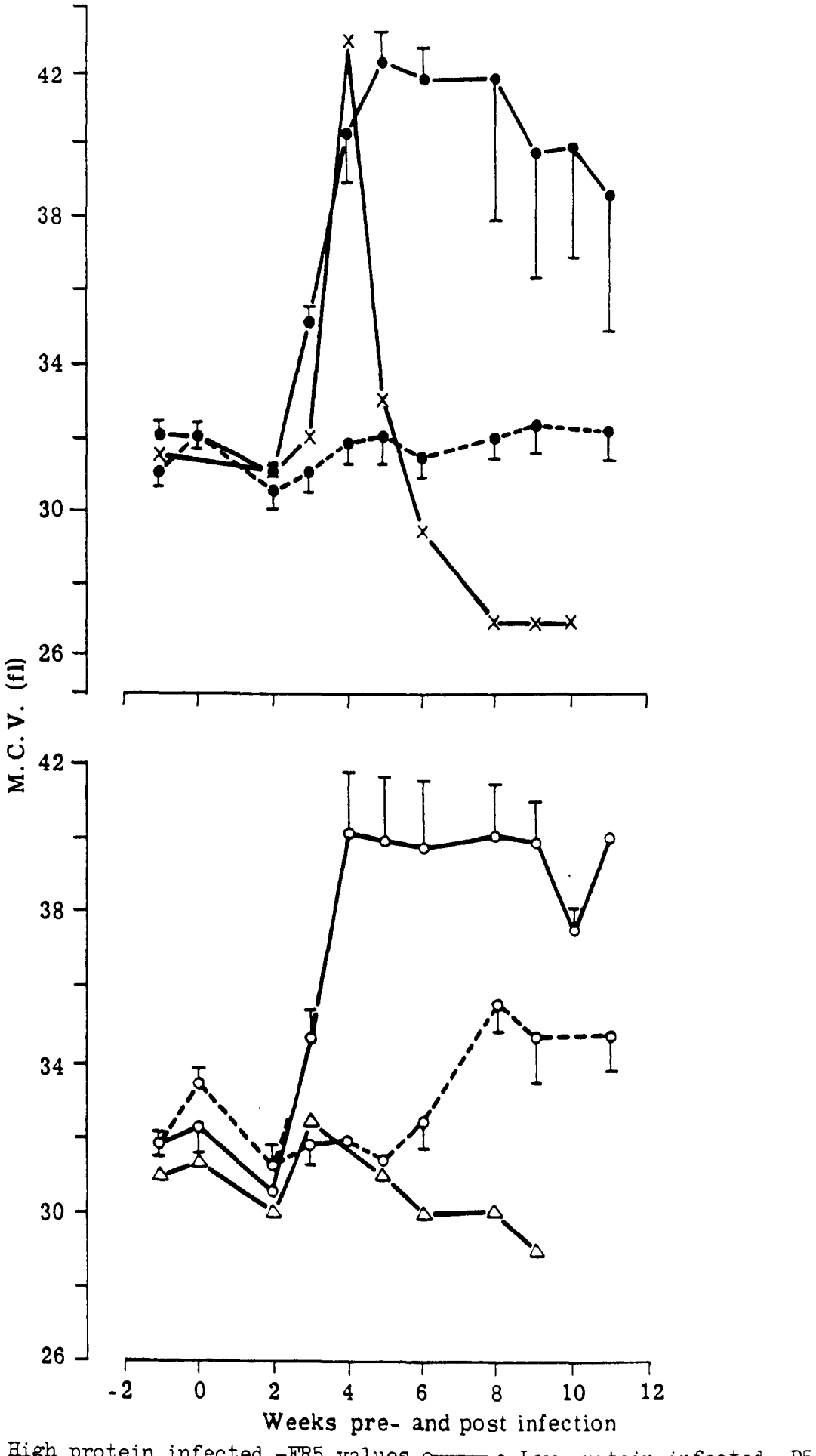
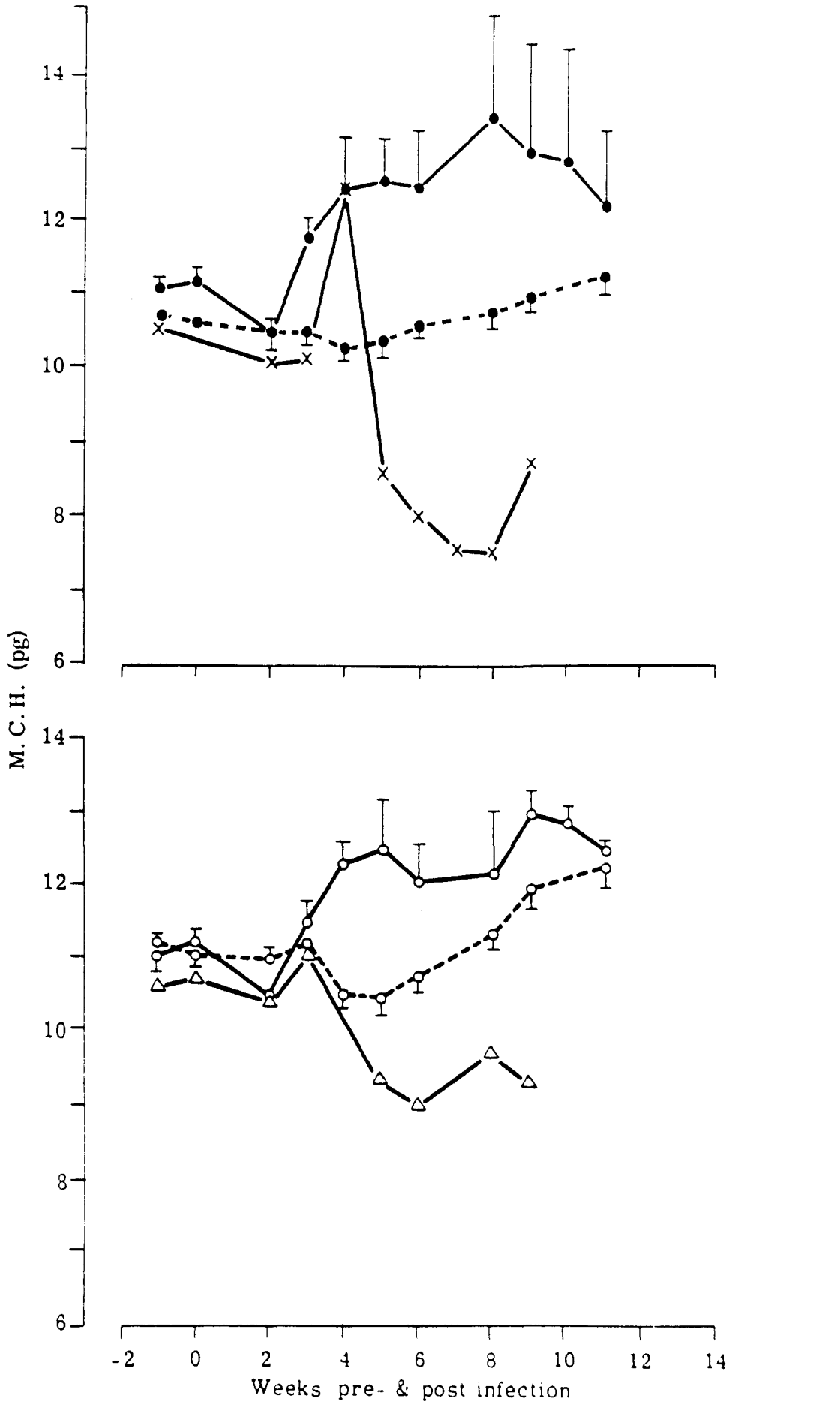


Fig. III.a.4: Mean (± SE) MCH values of infected (350 H. contortus larvae/kg BW) lambs and their respective controls.



A similar fall in serum iron levels and reduction in % saturation of transferrin occurred in the other lamb, P5. Both animals were known to have been inappetant and both lost weight rapidly before death (Appendix C, Table 1). Another infected lamb on the low protein diet group, P6, died before the end of the experiment. This lamb had a macrocytic response after infection but serum iron levels and % saturation of transferrin were reduced. This lamb also lost weight, 4.5 kg in 6 weeks, but no record of feed intake was available as the lamb died 24 hours after housing in a metabolism cage (Appendix C, Table 1).

Control lambs on the low protein diet became slightly anaemic towards the end of the experiment. MCV values rose slightly but were still considerably below the values of the infected lambs.

Biochemical changes

Both groups of infected lambs became severely hypoproteinaemic and hypoalbuminaemic after infection. Total serum protein values fell to 44 g/l ten weeks after infection in the high protein group, whilst values in the low protein group fell to 39 g/l six weeks after infection. Serum albumin levels fell to 20 g/l and 16 g/l in the high and low protein groups respectively (Fig. III.a.5).

The two infected male lambs in the low protein group in which sub-mandibular oedema was observed (P2 and P3) had total serum protein values of 34 and 36 g/l respectively, and serum albumin levels of 14 and 11 g/l respectively at slaughter.

Values for total serum protein and albumin were similar in both groups of control lambs and they remained relatively constant throughout the experiment.

Serum/

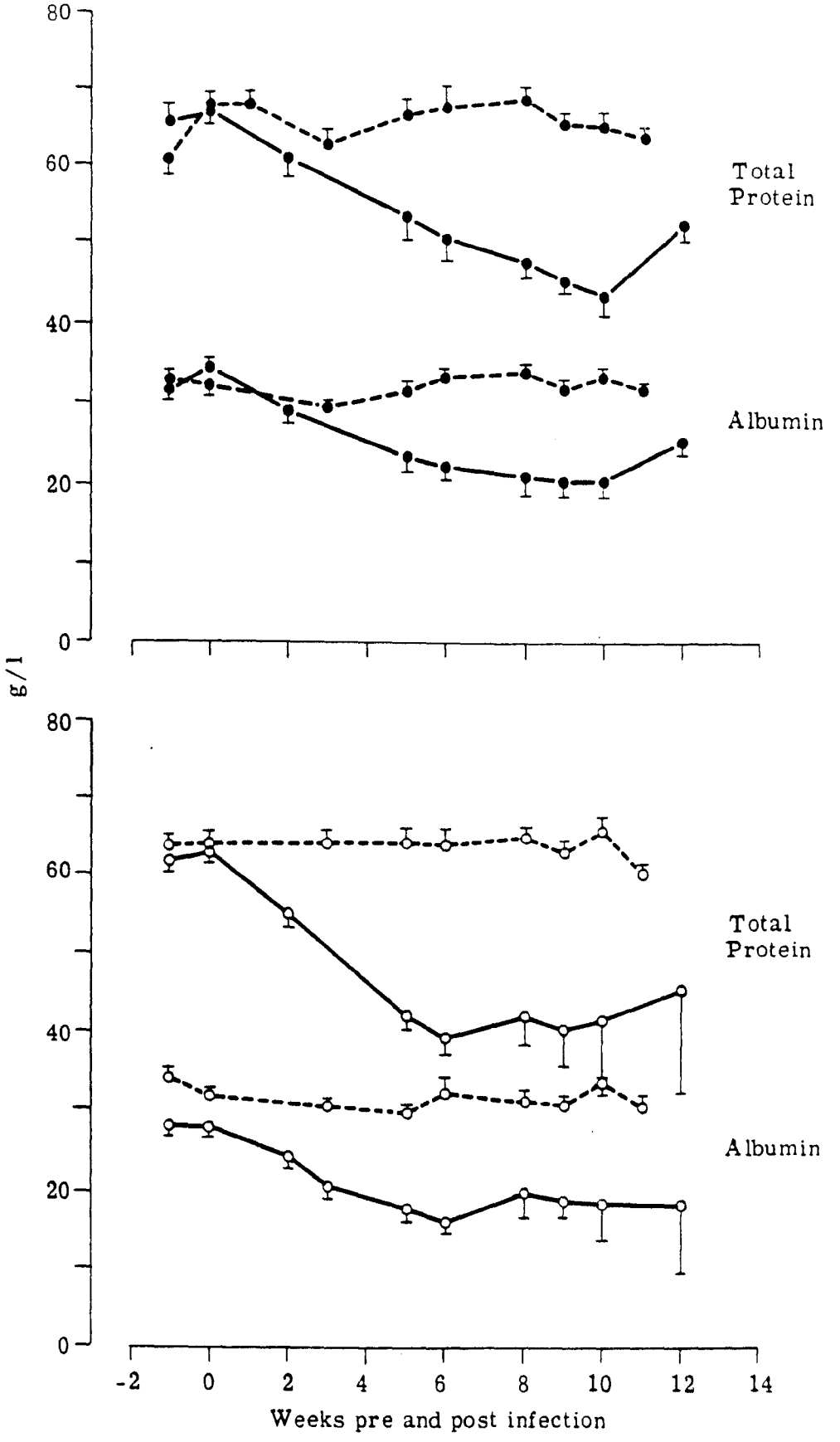


Fig. III.a.5: Mean (± SE) total serum protein and albumin values of infected (350 larvae/kg BW) lambs and their respective controls

●——● High protein infected o——o Low protein infected
●- - -● High protein control o- - -o Low protein control

Serum urea

A considerable difference in blood urea levels was observed between control groups with values in the high protein control group ($\sim$ 9 mmol/l) being almost double those of the low protein control group ($\sim$5 mmol/l)(Fig. III.a.6).

An increase in blood urea levels was also observed in both groups of infected lambs relative to their controls. The rise started 3 weeks after infection and levels remained elevated until week 6. Differences between infected and control lambs on the high protein diet were only significant at week5 ($P < 0.05$) whereas differences between infected and control lambs on the low protein diet were significant at weeks 3, 4, 5 and 6 ($P < 0.02$, < 0.001, < 0.01 and < 0.05 respectively).

Serum iron and % saturation of transferrin

Serum iron levels fell gradually in both groups of infected lambs to reach minimum values of 79 and 84 µg/dl in the high and low protein groups respectively (Figs. III.a.7 and 8). Values then rose in both groups and in the low protein group were in excess of 200 µg/dl by week 11.

The % saturation of transferrin followed a similar pattern to the iron levels, values falling during the first 6-8 weeks of infection then gradually rising, the increase being particularly marked in the low protein group.

Control serum iron levels remained between 180 and 200 µg/dl throughout, with % saturation of transferrin ranging from 30 to 48%.

Parasitological/

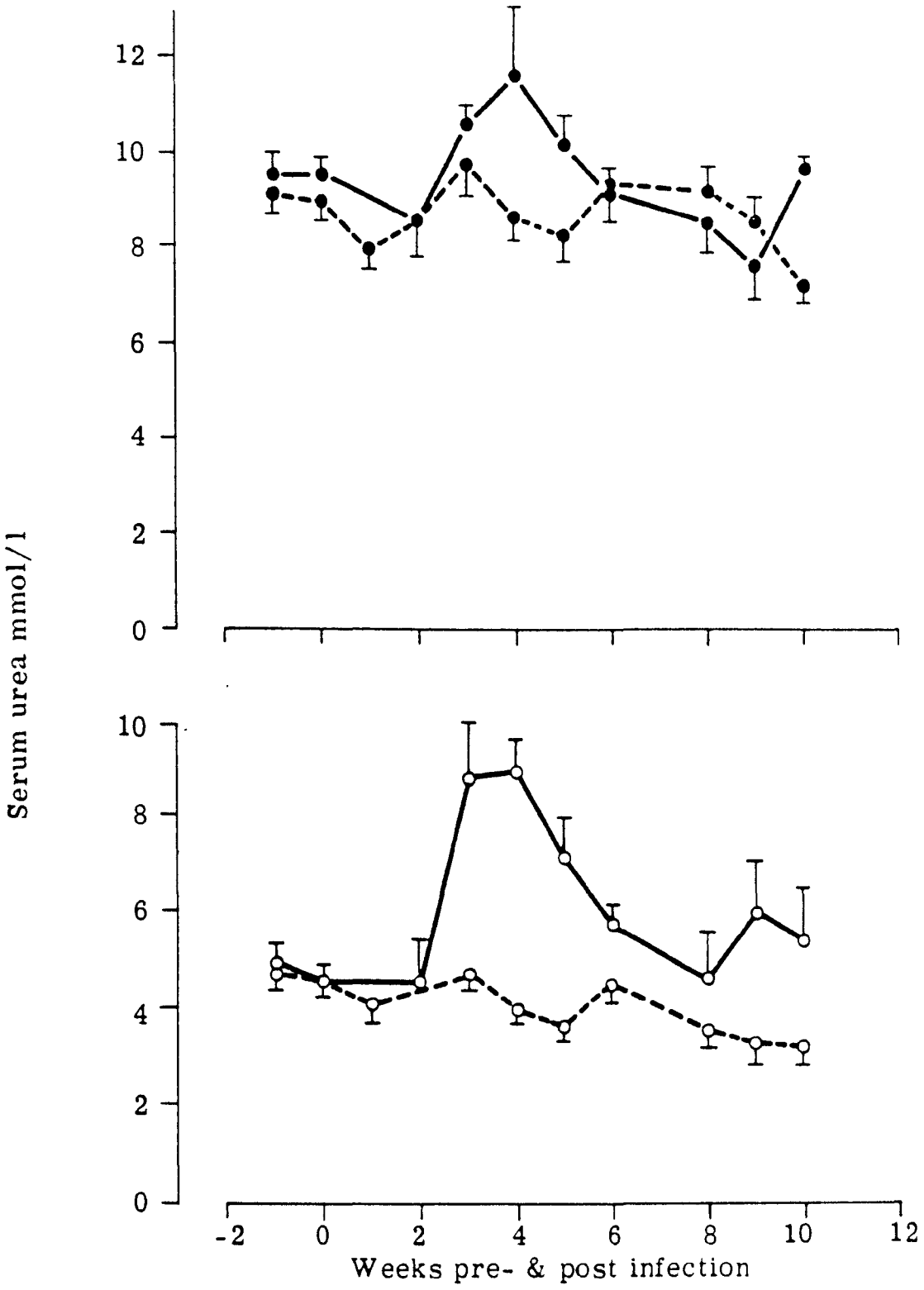


Fig. III.a.6: Mean (± SE) serum urea values of infected (350 larvae/kg BW) lambs and their respective controls.

●——● High protein infected o——o Low protein infected
●-----● High protein control o-----o Low protein control

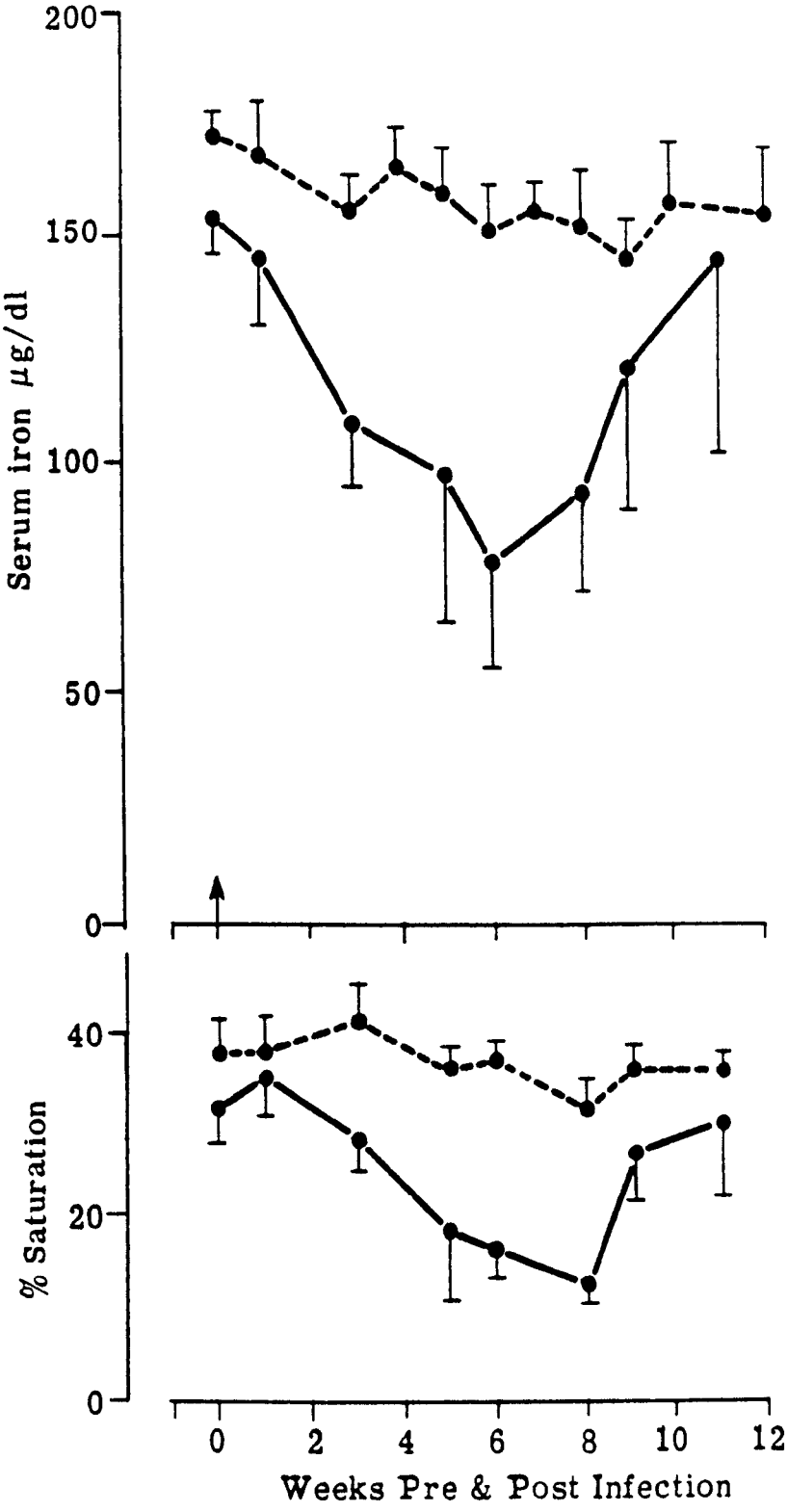


Fig. III.a.7: Mean (± SE) serum iron values and % saturation of transferrin values in infected (350 larvae/kg BW) lambs fed a high protein diet and their respective controls.

Infected Control

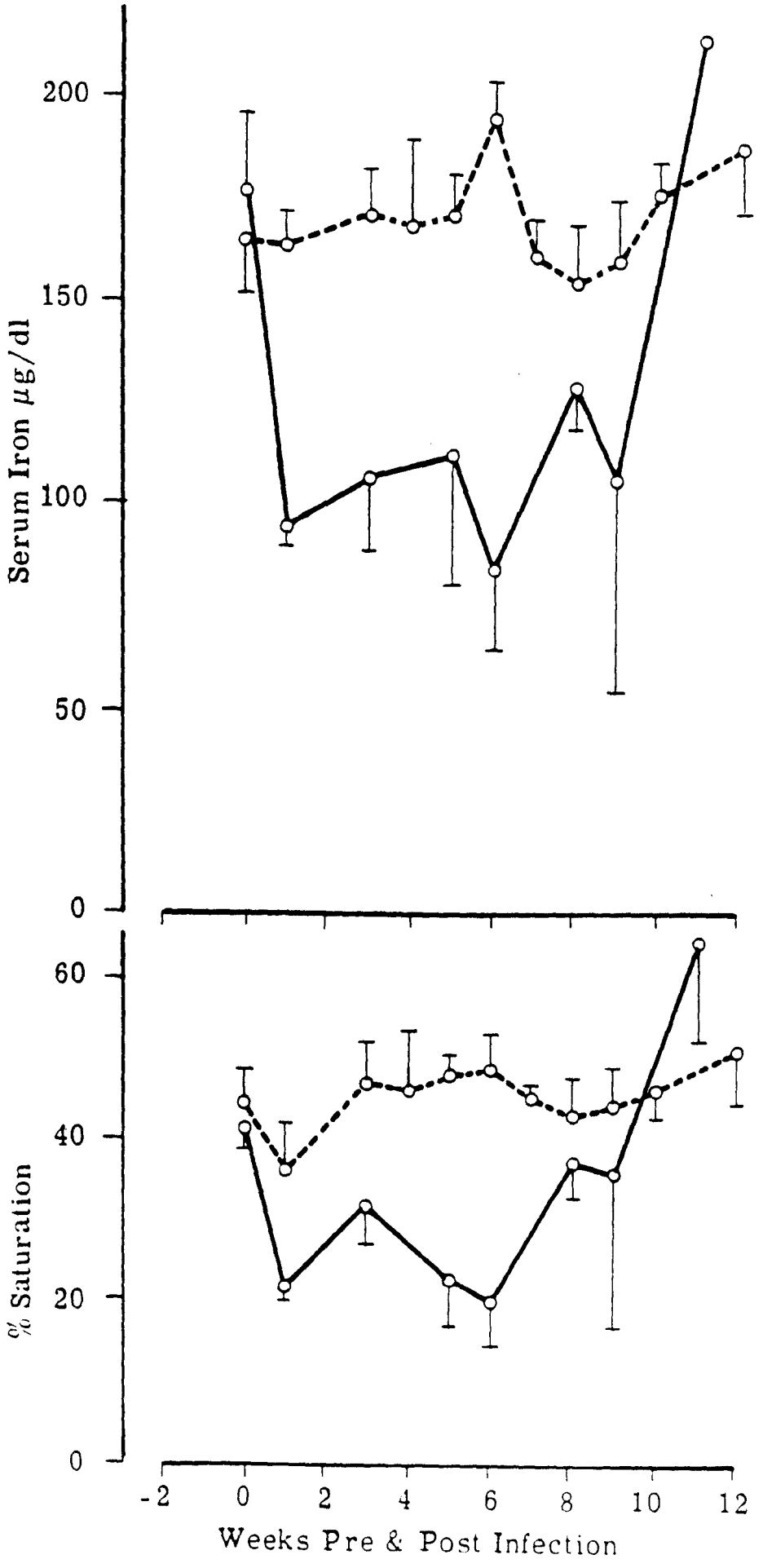


Fig. III.a.8: Mean (± SE) serum iron values and % saturation of transferrin in infected (350 larvae/kg BW) lambs fed a low protein diet and their respective controls.
o——o Infected o-----o Control

Parasitological findings

Faecal egg counts rose rapidly in both groups of infected lambs. Although there were modest differences between the two dietary groups, these were not statistically significant and were invariably due to within group fluctuations as a result of inappetance leading to reduced faecal output and resultant concentration of parasite eggs (Fig. III.a.9). Total faecal egg output of the infected male lambs was measured every second day during the metabolism cage study. Although faecal egg counts (epg) were higher in the low protein group, total faecal egg output was similar in both dietary groups (Fig. III.a.10).

Worm burdens were similar in both groups of male lambs killed 42 days after infection (3,188 ± SE 632 and 2,738 ± SE914)in the high and low protein groups respectively (Table III.a.2).

Burdens in the female lambs which died before the end of the experiment (FR5, P5 and P6) were also similar, 3,300 to 3,400. Worm burdens in the remaining female lambs in both dietary groups ranged from 1,500 to 6,200. Since only two female lambs in the low protein group survived, one of which was recovering (P7) whilst the other was not (P8), it is not appropriate to compare them as a group with the survivors in the high protein group.

The male : female worm ratios in the infected male lambs was 0.91 and 0.86 in the high and low protein groups respectively. The ratios in the surviving female lambs were 0.94 and 0.62 in the high and low protein groups respectively.

Radioisotopic studies

Erythrokinetics (Appendix C, Table 2)

Circulating red cell volumes were significantly reduced in both groups of infected lambs relative to their controls ($P < 0.01$). Mean group values in the infected groups were 10.9 and 10.4 ml/kg BW compared/

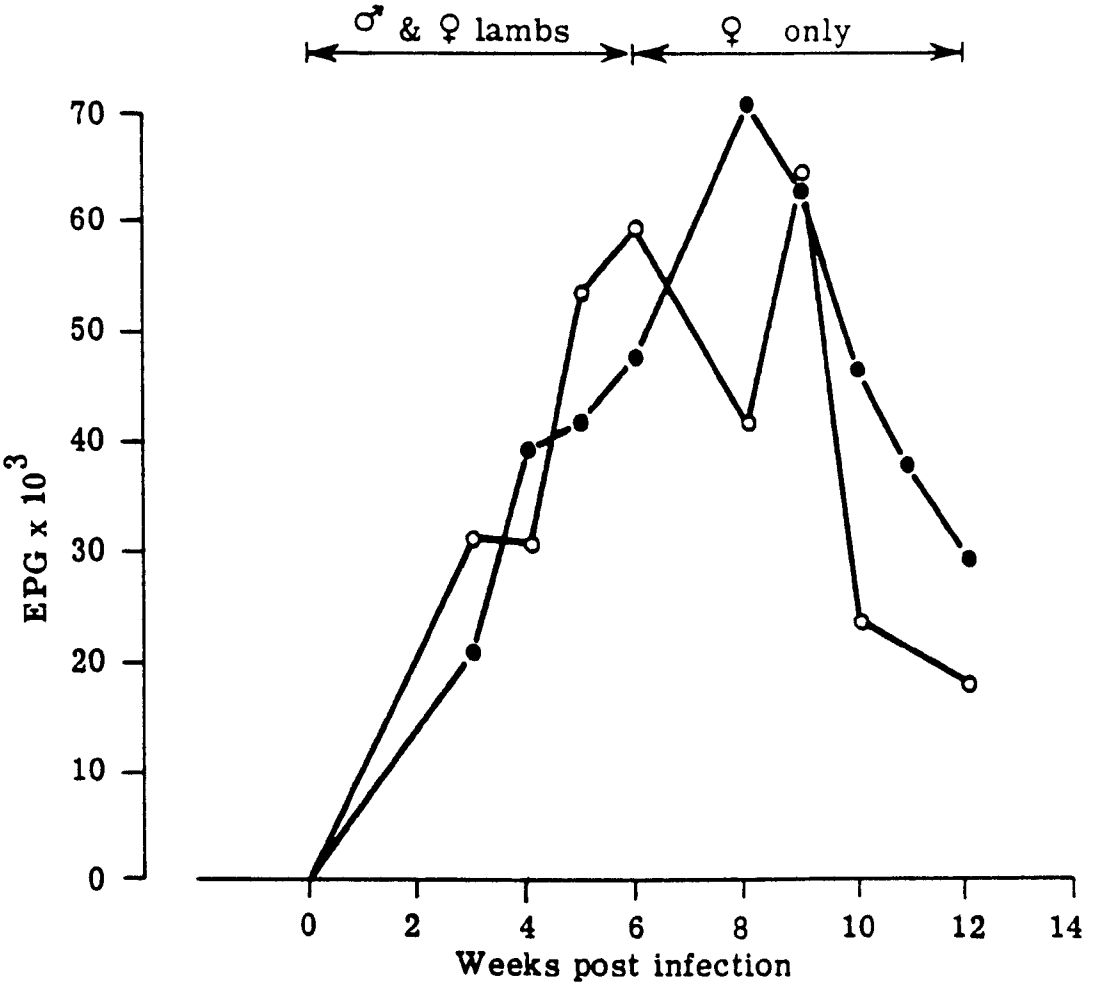


Fig. III.a.9: Mean faecal egg counts (epg) of 4 month old lambs infected with 350 H. contortus larvae/kg BW and fed either a high or low protein diet.

●——● High protein ○——○ Low protein

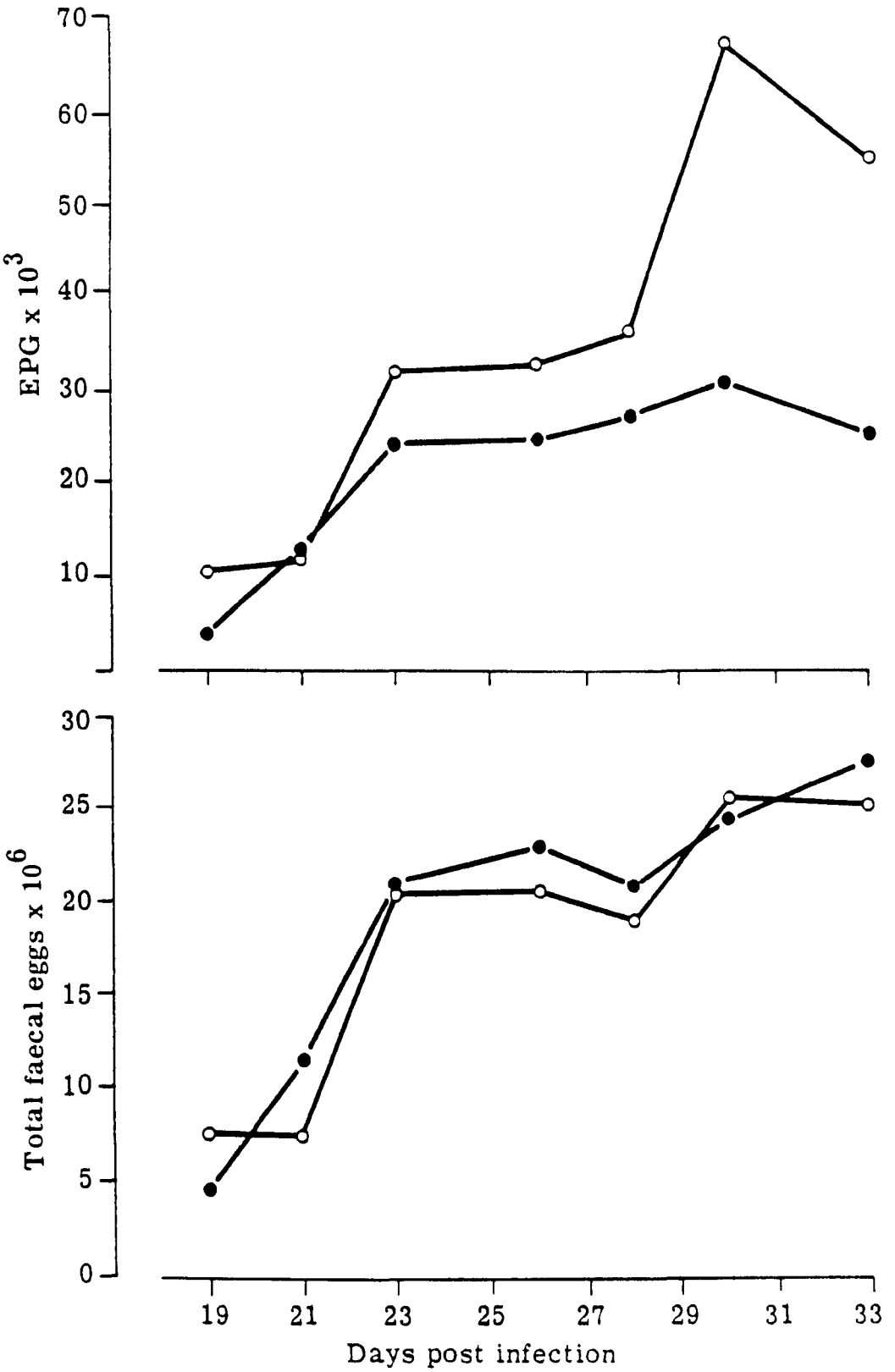


Fig. III.a.10: Faecal egg counts (epg) and total daily faecal egg output of 4 month old lambs infected with 350 H. contortus larvae/kg BW and fed either a high or low protein diet.

●——● High protein ○——○ Low protein

TABLE III.a.2 : Individual worm burdens of lambs infected with 350 H. contortus larvae/kg BW, killed either 6 or 11 weeks post-infection and fed either a high or low protein diet.

Dietary Group	No.	Hb type	Sex	Weeks after infection	Larval stages	Adult		Total
			♂					
High Protein	FR 1	B		6	0	2,000	1,900	3,900
	2	B	"	"	0	1,300	700	2,000
	3	AB	"	"	0	2,350	2,250	4,600
	4	A	"	"	50(L5)	1,000	1,200	2,250
$\bar{x}$								3,188
SE								632
Low Protein	P 1	B	♂	6	0	2,700	2,200	4,900
	2	B	"	"	0	1,600	1,750	3,350
	3	B	"	"	0	400	200	600
	4	AB	"	"	0	1,200	900	2,100
$\bar{x}$								2,738
SE								914
High Protein	FR 5	B	♀	10.5(died)	(0)	(1,500)	(1,900)	(3,400)
	6	B	"	11	0	3,100	3,100	6,200
	7	B	"	11	0	1,450	700	2,150
	8	B	"	11	0	2,050	2,400	4,450
(Survivors) $\bar{x}$								4,267
(Survivors) SE								1,173
Low Protein	P 5	B	♀	9.5(died)	(0)	(2,000)	(1,400)	(3,400)
	6	AB	"	7(died)	(0)	(1,850)	(1,450)	(3,300)
	7	B	"	11	0	800	700	1,500
	8	B	"	11	0	2,650	1,450	4,100
(Survivors) $\bar{x}$								2,800
(Survivors) SE								1,300

( ) died before end of experiment.

compared with mean control values of 17.5 and 16.8 ml/kg BW.

Plasma volumes (calculated using ^{125}I-labelled albumin) were similar in both groups of infected lambs (52-53 ml/kg BW) but were significantly elevated relative to control values (43 ml/kg BW)($P < 0.02$ and $P < 0.01$ in the high and low protein groups respectively). There were no significant differences in total blood volume between infected and control lambs on either diet (range of 59.9 to 63.6 ml/kg BW).

The "apparent" half-life ($T\frac{1}{2}$) of the ^{51}Cr-labelled red cells was significantly reduced in both groups of infected lambs (88 and 74 hours in the high and low protein groups respectively)relative to their controls (187 and 188 hours)($P < 0.01$ and $P < 0.001$ in the high and low protein groups respectively). The reduction in the $T\frac{1}{2}$ was slightly greater in the infected lambs on the low protein diet but the difference was not statistically significant.

Both dietary groups of infected lambs had similar gastrointestinal blood losses, namely 31 and 30 ml packed red cells in the high and low protein groups respectively. In contrast, both groups of control lambs were losing less than 1 ml packed RBC into the gut daily.

Ferrokinetics (Appendix C, Table 3)

Serum iron levels in infected and control lambs on either diet were similar during the metabolism cage study and ranged from 159-182 µg/dl.

The "apparent" half-life ($T\frac{1}{2}$) of the labelled transferrin was reduced in the infected lambs on the high protein diet relative to their controls (45 and 101 minutes respectively). Similarly, half-life values were reduced in the infected lambs on the low protein diet relative to their controls (37 and 69 minutes respectively). The reduction in $T\frac{1}{2}$ values was significantly ($P < 0.05$) greater in the infected/

infected lambs on the low protein diet.

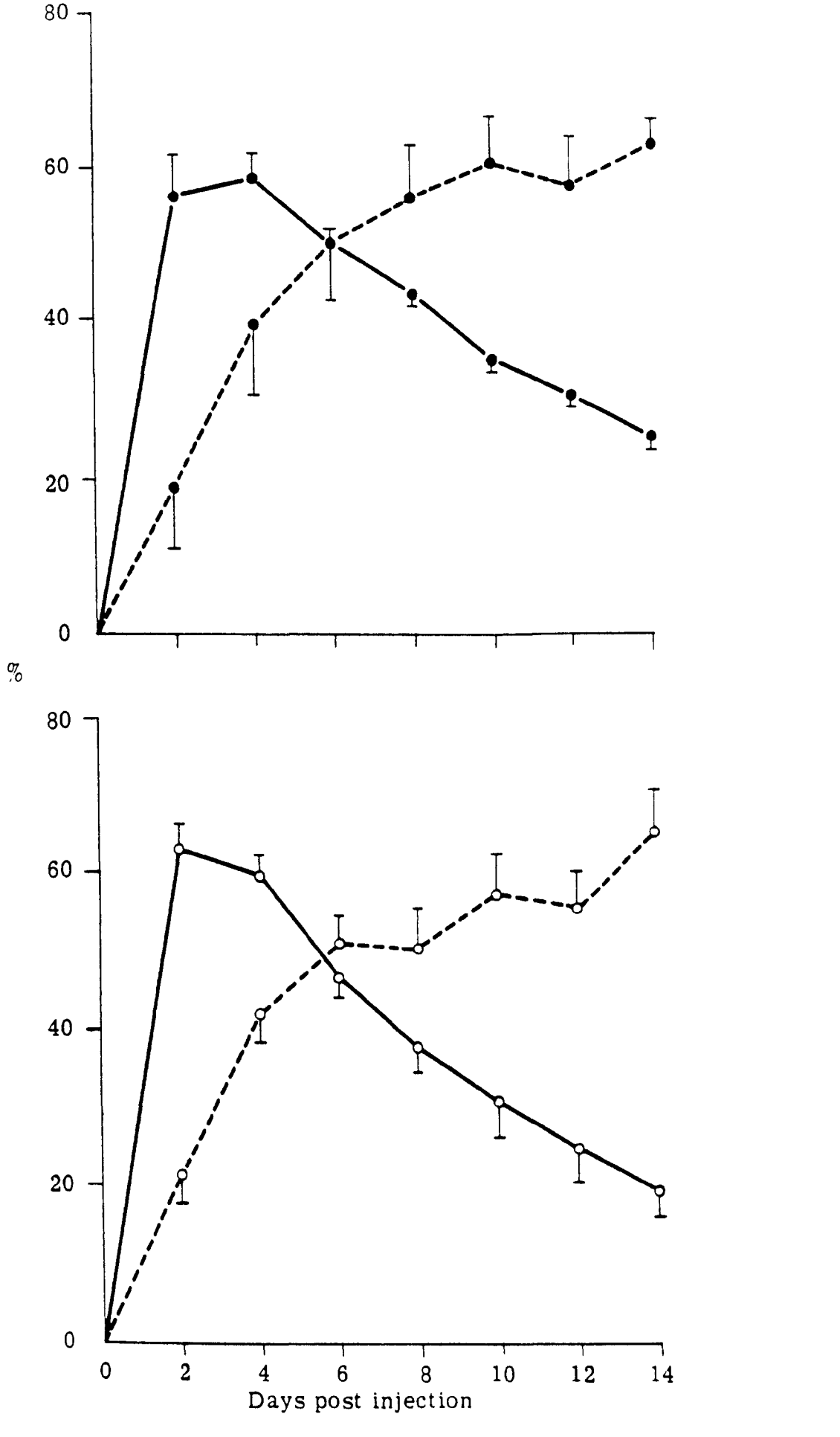
A control lamb in the high protein dietary group (G3) had a greatly reduced $T\frac{1}{2}$ (31 m). However, one week prior to the radioisotope study, this lamb developed a macrocytic anaemia of unknown origin. Its haematocrit fell from a normal value of 0.305 (± SD 0.019) to 0.24 l/l. At the same time its MCV value rose from a normal value of 29.6 (± SD 0.66) to 35 fl and remained elevated for 3 weeks before returning to normal. The fall in heamatocrit was of shorter duration, namely 1 week.

Plasma iron turnover rates were increased in the infected lambs on the high protein diet relative to their controls (1.83 and 1.18 mg/kg BW/d) but the difference was not statistically significant. In contrast, rates were significantly increased ($P < 0.001$) in the infected lambs on the low protein diet relative to their controls (2.58 and 1.12 mg/kg BW/d respectively). Turnover rates in the infected lambs on the low protein diet were significantly greater ($P < 0.02$) than those of the infected lambs on the high protein diet.

The red cell utilisation curves were maximal within 4 days of injection of radioiron in both groups of infected lambs after which time they fell rapidly (Fig. III.a.11). In contrast, the utilisation curves increased gradually in both groups of control lambs to reach maximal values on day 14.

The average daily Hb-iron losses using the faecal clearances of both the ^{51}Cr-labelled red cells and ^{59}Fe-labelled red cells are presented in Appendix C, Table 4. Both groups of infected lambs were losing similar amounts of Hb-iron into the gastrointestinal tract as indicated by the ^{51}Cr-faecal clearances, i.e. 35 and 33.5 mg/d in the high and low protein groups respectively. Faecal Hb-iron losses (calculated using the ^{59}Fe faecal clearances) were also similar in both groups of infected lambs, 38 and 39 mg/d in the high and low protein/

Fig. III.a.11: Mean (± SE) % red cell utilisation of ^{59}Fe in lambs infected with 350 H. contortus larvae/kg BW and their respective controls.



protein groups respectively. In contrast, control lambs were losing less than 4 mg of Hb-iron daily in the faeces. There was no evidence of Hb-iron absorption in either the infected or control groups on either diet.

Albumin metabolism (Appendix C, Table 5)

Serum albumin levels were significantly ($P < 0.001$) reduced in both groups of infected lambs (25 and 15 g/l in the high and low protein groups respectively) relative to their respective controls (31 g/l in both groups). Also, levels in the infected lambs on the low protein diet were significantly lower than those of the infected lambs on the high protein diet ($P < 0.001$).

The differences in plasma volumes between groups have been discussed earlier (see Erythrokinetic studies).

Intravascular (CA) and extravascular (EA) albumin pools were similar in the infected lambs on the high protein diet (1.31 g/kg BW and 2.08 g/kg BW respectively) relative to their controls (1.35 and 1.87 g/kg BW respectively). The distribution of albumin between the two pools was similar in both groups of lambs.

However, the intravascular albumin pool was significantly ($P < 0.001$) reduced in the infected lambs on the low protein diet relative to their controls (0.79 and 1.35 g/kg BW in the infected and control groups respectively). There were no significant differences in the extravascular albumin pools between the infected and control lambs but because of the reduction in the intravascular pool in the infected group, the EA/CA ratio was significantly higher ($P < 0.05$) in the infected group compared with control values.

Half-life values of the labelled albumin ($T\frac{1}{2}$) were significantly ($P < 0.001$) reduced in the infected lambs on the high protein diet relative to their controls (154 and 339 hours respectively). A similar significant/

significant ($P < 0.001$) reduction in $T_{\frac{1}{2}}$ was observed in the infected lambs on the low protein diet relative to their controls (111 and 431 hours respectively). The infected lambs on the low protein diet had significantly lower $T_{\frac{1}{2}}$ values than the infected lambs on the high protein diet ($P < 0.01$). In addition, $T_{\frac{1}{2}}$ values in the control lambs on the low protein diet were significantly higher than those of the controls on the high protein diet ($P < 0.02$).

The fractional catabolic rate (F(CA)), i.e. the fraction of the intravascular albumin pool catabolised each day, was considerably increased in both groups of infected lambs relative to their controls. Infected lambs on the high protein diet were catabolising approximately 15% of the intravascular albumin pool daily compared with 8% in the control lambs ($P < 0.01$). Infected lambs on the low protein diet had an even greater catabolic rate of the intravascular albumin pool, 21% daily, compared with a 6% rate in their controls ($P < 0.001$).

The difference in catabolic rates between the two groups of infected lambs was significant at $P < 0.01$ level. Catabolic rates in control lambs on the high protein diet were significantly ($P < 0.001$) greater than those of control lambs on the low protein diet.

Nutritional studies

Feed intakes

Feed intakes in the infected male lambs given the high protein diet were quickly stabilised after the move to the metabolism cages and all the lambs readily consumed 1000 g fresh feed daily throughout the study (Fig. III.a.12).

Individual feed intakes of the infected male lambs on the low protein diet, however, were more variable ranging from 636 to 1000 g fresh feed daily. Intakes fluctuated slightly during the first ten days/

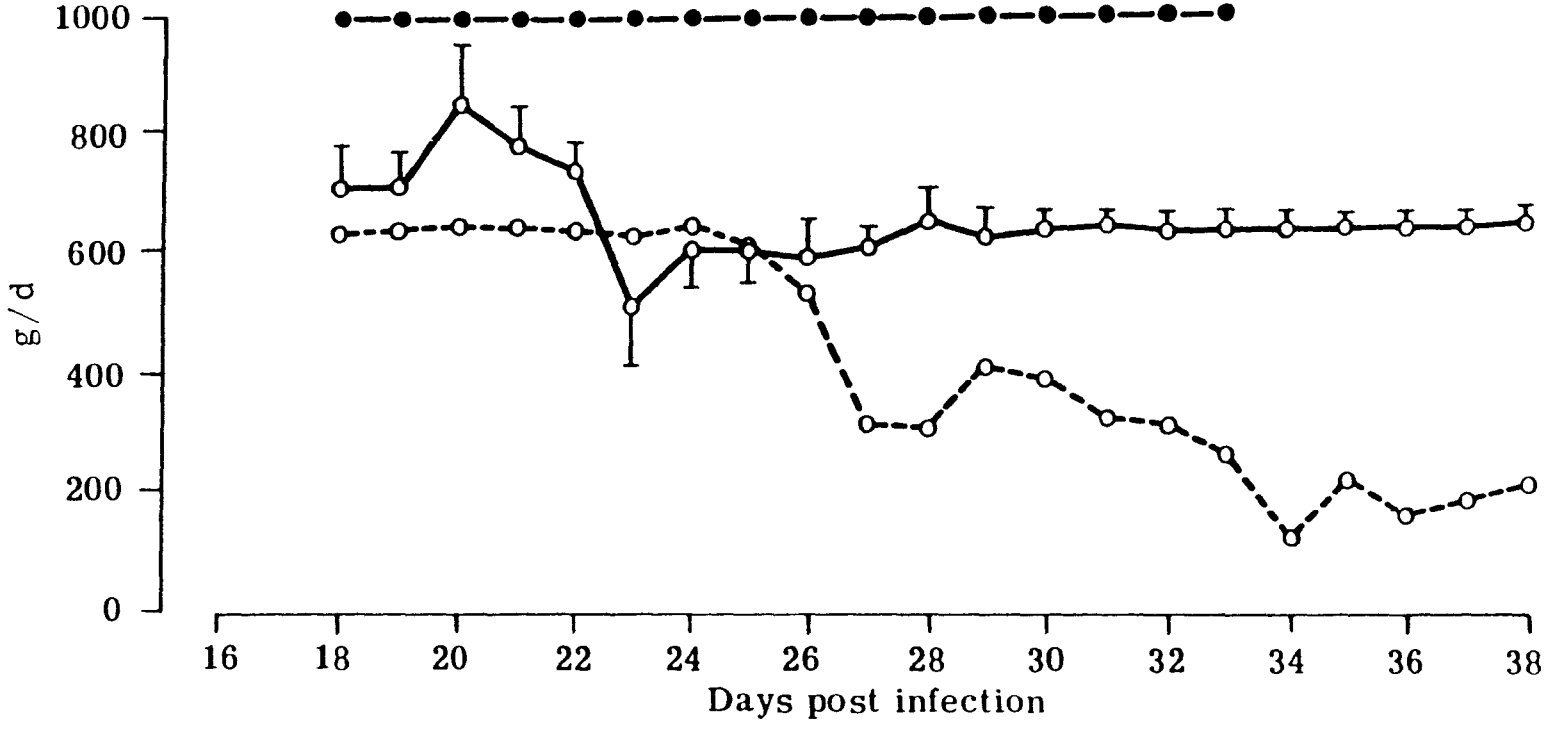


Fig. III.a.12: Mean (± SE) daily fresh feed intakes of infected (350 larvae/kg BW) lambs fed either a high or low protein diet.

●——● High protein infected

o——o Low protein infected -P3

o- - - - -o P3

days in the metabolism cages but at day 21 post-infection feed intakes started to fall in all 4 lambs. One lamb, P3, became progressively more inappetant and its daily fresh feed consumption at the end of the collection period was less than 200 g. The remaining three lambs in the group stabilised and had relatively constant daily feed intakes during the adjustment and collection periods, albeit at a lower level than that of the lambs in the high protein group.

Because of the very low feed intake of P3, no attempt was made to pair feed its control lamb.

Digestibility coefficients of the experimental diets (Appendix C, Table 6).

There were no significant differences (ether extract excepted) in the apparent digestibility coefficients of the various proximate fractions of the experimental diets between infected and control animals in either dietary group. The differences in crude protein digestibility between the high and low protein dietary groups are entirely expected where the lowered apparent digestibility in the low protein group is attributed to the relatively higher contribution of metabolic faecal nitrogen in the faecal output of this group.

N balance (Appendix C, Table 7)

Infected and pair-fed control lambs on the high protein diet remained in positive N balance during the study ($\sim$ 3 g N/d) and there were no significant differences in faecal or urinary N outputs between the two groups. In contrast, both infected and pair-fed control lambs on the low protein diet were in negative N balance during the study. However, there were no significant differences in faecal or urinary N output between the infected and control lambs in this group.

Faecal/

Faecal dry matter coefficients

Faecal dry matter was significantly ($P < 0.02$) reduced in the infected lambs on the high protein diet relative to their controls (0.33 and 0.43 respectively). An even greater reduction in faecal dry matter was observed in the infected lambs on the low protein diet relative to their controls (0.31 and 0.42 respectively)($P < 0.01$). Faecal dry matter was significantly lower in the infected lambs on the low protein diet relative to the infected lambs on the high protein diet ($P < 0.05$).

Water balance (Appendix C, Table 8)

Both groups of infected lambs had increased daily water intakes relative to their respective controls but the difference was not statistically significant. The control lambs on the high protein diet were consuming more water than the control lambs on the low protein diet ($P < 0.01$),even allowing for the slight differences between the two groups in the proportion of water from fresh food sources (139 ml/d feed water in the high protein group compared with 91 ml/d in the low protein group). Urinary fluid losses were similar in both infected and control animals within a dietary group and the lambs on the high protein diet had greater outputs than the lambs on the low protein diet.

However, faecal water content was significantly greater in both groups of infected lambs relative to their controls ($P < 0.02$ and $P < 0.01$ in the high and low protein groups respectively). Faecal water content was significantly ($P < 0.001$) greater in the control lambs on the high protein diet relative to that of the control lambs on the low protein diet.

The/

The infected lambs on the high protein diet "retained" significantly more water (1,290 ml)($P < 0.001$) than their controls (727 ml) and also significantly more ($P < 0.01$) than the infected lambs on the low protein diet (539 ml). Although the infected lambs on the low protein diet retained more water (539 ml) than their controls (264 ml), the difference was not statistically significant. The control lambs on the high protein diet retained significantly more water than the control lambs on the low protein diet ($P < 0.01$).

Iron"balance" (Appendix C, Table 9)

The iron content of both the high and low protein diets was high because of the excessively high iron content of the surgar beet, 1.905 g/kg DM.

Feed iron intakes were high in both dietary groups, the lambs on the high protein diet having a daily iron intake of 850 mg/lamb whereas intakes in the low protein group ranged from 687 - 802 mg/lamb/day.

Total faecal iron was less in the infected lambs on the high protein diet (90 mg/d) relative to their controls (107 mg/d). Total faecal iron was also significantly ($P < 0.05$) reduced in the infected lambs on the low protein diet relative to their controls (64 and 222 mg/d respectively).

Faecal non-haemoglobin iron (i.e. total faecal iron minus the calculated Hb-iron loss using ^{59}Fe faecal clearance values) was significantly ($P < 0.001$) less in the infected lambs on the high protein diet relative to their controls (52 and 104 mg iron/day respectively)./

respectively). Similarly, non-Hb faecal iron was reduced ($P < 0.02$) in the infected lambs on the low protein diet relative to their controls (21 and 220 mg/d respectively). One infected lamb, P3, in the low protein group had very low total faecal iron (28 mg/d) which was accounted for by the Hb-iron loss into the gut. Thus, non-haemoglobin faecal iron was zero in this lamb.

The "apparent" dietary iron and % absorption was significantly greater in the infected lambs of both dietary groups relative to their controls.

Carcase evaluation (Appendix C, Table 10)

The % distribution of muscle : bone : fat was almost identical in both groups of control lambs (48 : 15 : 37 and 48 : 16 : 36 in the high and low protein groups respectively). Infected lambs on the high protein diet had a similar distribution to that of the control lambs but the % of muscle present (44%) was slightly reduced.

Only two infected female lambs (P7 and P8) from the low protein groups survived to slaughter and since, clinically, they were dissimilar, the results of their carcase evaluation are presented individually. Lamb P7 was recovering from the infection and had suffered no obvious loss of appetite during the experiment. The carcase of this lamb was indistinguishable from that of a control lamb and the % distribution of muscle : bone : fat (53 : 18 : 29) was similar to control values. In contrast, the remaining lamb was still severely anaemic and had an emaciated oedematous carcase. The % muscle (35%) was considerably reduced relative to control values.

Experiment IIIb

An investigation into the influence of high and low protein diets on parasite establishment in 8 month old lambs infected with 350 H. contortus larvae/kg BW.

Experimental design

Eight 8 month old male lambs (formerly control lambs from Experiment IIIa), 4 from each dietary group, were infected with 350 H. contortus larvae/kg BW per os. Blood and faecal samples were collected regularly throughout the experiment. The lambs were killed 28 days post-infection when worm burdens were assessed (Table III.a.1).

RESULTS

These are summarised in Table III.b.1. There was little change in body weights during the experiment. Both groups of lambs developed a macrocytic anaemia after infection. Haematocrit values in the high protein group fell from 0.34 l/l to 0.23 l/l twenty-seven days after infection. Values in the low protein group fell from a pre-infection mean of 0.34 l/l to 0.27 l/l at the end of the experiment. MCV values rose in both groups (36 - 37 fl) twenty-seven days after infection. Values for total serum protein and albumin fell from 63/34 to 52/29 g/l in the high protein group and from 63/34 to 52/25 g/l in the low protein group.

Parasitological findings (Table III.b.1 and 2)

Faecal egg counts rose rapidly in both groups of lambs to reach maximum levels of 42,000 e.p.g. and 41,775 e.p.g. in the high and low protein groups at days 25 and 27 post-infection respectively. Any differences in faecal egg counts between dietary groups were not statistically/

TABLE III.b.1: Haematological, biochemical and parasitological findings in 8 month old lambs infected with 350 *H. contortus* larvae/kg BW and kept on either a high or low protein diet (Mean values)

Dietary Group		Pre-infection	Days post-infection				
			18	20	22	25	27
HP	Bodyweight	39.5					38.3
LP		33.6					34.3
HP	e p g	0	9052	13763	22483	42200	31350
LP		0	6981	30533	28088	38200	41775
HP	Haematocrit (l/l)	0.34	0.27	0.26	0.25	0.22	0.23
LP		0.34	0.28	0.265	0.27	0.24	0.27
HP	MCV (fl)	32	31.5	32.2	33.2	33.6	36.4
LP		34	33.9	34.1	34.1	34.4	37.0
HP	Total Protein (g/l)	63	56	-	-	-	52
LP		63	55	-	-	-	52
HP	Albumin (g/l)	34	30	-	-	-	29
LP		34	29	-	-	-	25
HP	Urea (mmol/l) ($\bar{x}$ ± SE)	6.2(0.8)	6.7 (0.70)	-	-	-	8.9 (0.64)
LP		2.1(0.5)	7.1 (0.7)	-	-	-	8.7 (0.8)

HP = High Protein LP = Low Protein

statistically significant. Worm burdens in the high protein group ranged from 1,050 to 6,050 (mean 3,888) and in the low protein group from 300 to 4,500 (mean 2,913) (Table III.b.2). Differences between the two dietary groups were not significant.

TABLE III.b.2: Individual worm burdens in 8 month old lambs infected with 350 H. contortus larvae/kg BW and kept on either a high or low protein diet and killed 4 weeks post-infection.

Dietary Group	No.	Hb Type	Larval	Adult		Total worms
				Male	Female	
High Protein	G 1	B	0	1900	1400	3,300
	2	B	0	3000	1950	4,950
	3	B	0	650	400	1,050
	4	B	0	3050	3000	6,050
	$\bar{x}$					3,888
	SE					(±1,088)
Low Protein	Y 1	B	100	2250	2150	4,500
	2	AB	0	1650	1750	3,400
	3	B	0	2100	1350	3,450
	4	B	0	300	0	300
	$\bar{x}$					2,913
	SE					(±907)

DISCUSSION

The results of the first experiment, IIIa, suggest that lambs on a low protein diet are less able to withstand the pathophysiological consequences of infection with 350 H. contortus larvae/kg BW than lambs fed a high protein diet. Mortality was greater in the low protein group and adverse clinical signs such as teeth grinding, dullness, weakness, anorexia, weight loss and oedema were observed more frequently in this group relative to the better fed lambs. The peripheral blood picture indicated a more severe anaemia, hypoproteinaemia and hypoalbuminaemia in the low protein group, despite similar levels of gastric blood loss (30 ml RBC/day) in both dietary groups. Furthermore, circulating red cell volumes were similar in both groups of infected lambs and plasma volumes were expanded to a similar degree in both groups. 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 relative to their controls. Loss of appetite was a feature of the disease, particularly in the lambs fed the low protein diet, but within each dietary group there were no differences in the digestibility of the various proximate fractions of the experimental diets and no differences in urinary or faecal N losses between infected lambs and their pair-fed controls. Faecal egg counts, total daily faecal egg output and worm burdens were similar in both dietary groups. The finding of similar worm burdens in both groups of infected lambs in the second experiment, IIIb, confirmed that diet appeared to have no effect on parasite establishment.

The/

The influence of diet was obvious in the albumin metabolism studies. For example, infection had little effect on pool size or distribution in the lambs on the high protein diet relative to their controls, whereas the intravascular albumin pool was reduced in the infected lambs fed the low protein diet. The fractional catabolic rate was increased in both groups of infected lambs, particularly in the lambs fed the low protein diet with a corresponding reduction in the half-life of the labelled albumin. In the control lambs on the low protein diet, the half life of the labelled albumin was extended and the fractional catabolic rate reduced relative to the controls fed the high protein diet. However, there were no differences in albumin pool sizes between both dietary groups of control lambs.

Several features of the various investigations into iron metabolism in the parasitised lambs are of interest. First, although serum iron levels fell for 8-10 weeks following infection in both dietary groups, a rise in values occurred thereafter. This rise was particularly marked in the lambs in the low protein group where group mean values were in excess of control values at the end of the experiment. In studies on calves subjected to a similar daily blood loss as the lambs in this experiment (6 ml blood/kg BW/day by phlebotomy for 14 days), Bremner and Ronalds (1965) showed a fall in serum iron levels during the period of phlebotomy but a rapid rise in values during the recovery period. This rapid rise to abnormally high levels was presumed to be due to increased absorption of dietary iron since the non-Hb iron content of the livers, which had fallen during phlebotomy, rose to normal levels in the recovery period. Certainly, dietary iron absorption was increased in the infected lambs in this experiment during the early stages of infection when compared with control values, whereas there was no evidence of reabsorption of the Hb-iron lost by haemorrhage.

This failure to show reabsorption of Hb-iron, as estimated by comparison of the ^{51}Cr and ^{59}Fe faecal clearances, is at variance with the findings of Holmes & Maclean (1969); Allonby & Dargie (1973); Altaif (1975); Dargie & Allonby (1975) and Obasaju (1981) all of whom found evidence of some reabsorption of the Hb-iron in sheep which were losing considerable quantities of blood into the gastro - intestinal tract daily. However, in the studies conducted by Holmes & Maclean (1969), Hb-iron reabsorption was only evident in one of two sheep with a heavy infection of liver fluke. In the later experiments (Allonby & Dargie, 1973; Dargie & Allonby, 1975; Obasaju, 1981), sheep infected with H. contortus (10,000 L_3 or 350 larvae/kg BW) showed evidence of some reabsorption of Hb-iron, particularly in the most anaemic animals. In these latter studies, great emphasis was placed on this inability to reabsorb the Hb-iron lost into the gut as a result of gastric haemorrhage with total disregard for absorption of iron from normal dietary sources. The use of the double isotope technique to measure gastrointestinal loss and reabsorption of iron in sheep is possibly open to criticism. For example, Hb-iron has a greater bio-availability in man, especially if iron deficient, (Bothwell, Charlton, Cook & Finch, 1979) but this has been found not to be the case in sheep. Georgi (1964) found that absorption of ^{59}Fe sulphate increased in sheep made anaemic by phlebotomy relative to controls, whereas Hb-^{59}Fe absorption was similar in both the bled and control sheep. Further evidence that dietary sources of iron could play an important role in the survival of lambs infected with H. contortus was provided by Scott, Silverman, Mansfield & Levine (1971). Lambs offered iron (as $FeSO_4$) ad libitum survived the same oral inoculations of 50,000 H. contortus larvae that previously killed 80-100% of lambs without access to supplementary iron.

A/

A correlation between serum iron levels and changes in the red cell indices was obvious in two of the fatal cases, FR5 and P5. Both lambs had very low serum iron levels and a reduced % saturation of transferrin together with the presence of a microcytic hypochromic anaemia, the changes in the red cells being particularly marked in the lamb from the high protein group. In contrast, the remaining fatality, P6, had a macrocytic blood picture with low serum iron levels. The absence of a microcytic, hypochromic blood picture in this lamb may have been due to the time lag between depletion of iron stores and the eventual effect of depletion on red cell production. Interestingly, all three lambs were severely anorectic in the weeks preceding death and, in view of the increased quantity of dietary iron apparently absorbed by the infected male lambs relative to the controls, the anorexia may well have been critical in the development of an iron-deficiency anaemia.

Anorexia is a feature common to most gastrointestinal parasitic diseases (Asghar, 1982) and several workers have observed the development of anorexia in sheep with haemonchosis (Evans, Blunt & Southcott, 1963; Pradhan & Johnstone,1972a ; Owen, 1973). However, others could find no such evidence (Allonby & Dargie, 1973; Dargie, 1980) and, in contrast, Allonby and Dargie (1973) reported that haemonchus infected sheep consumed more food than uninfected controls. However, as mentioned in the General Introduction, the evidence to support this statement is open to question.

The anorexia observed in certain of the lambs in the present experiment (IIIa) was evident both in the earlier and later stages of the study. In addition, anorexia was more frequently observed in the lambs fed the low protein diet. A number of factors may have been involved/

involved in the development of the anorexia. First, it has previously been suggested by Gibson (1955) that the pain resulting from the local damage to the bowel wall by the parasites may be responsible for the anorexia observed in sheep infected with the abomasal parasite T. axei. Thus, if worm establishment was greater in animals fed a low protein diet, it could be expected that anorexia may be more likely to develop in this group. However, in the present experiment worm burdens were similar in both dietary groups. Furthermore, since the gastric blood losses and faecal egg counts (epg and total) were also similar, this would suggest indirectly, that the parasites in both dietary groups were of a similar size and metabolic activity. Thus Gibson's (1955) suggestion of localised pain as a cause of anorexia is unlikely to be solely responsible for the increased prevalence of anorexia in the lambs in this experiment which were fed the low protein diet, although there was teeth-grinding and dullness, suggestive of abdominal pain, in the more severely affected lambs.

Secondly, the protein content of the diet per se may be a contributory factor since it is widely accepted that sheep and cattle eat less of a diet deficient in protein compared to diets with added protein or urea (Church, 1971). A deficiency of N has two serious consequences for the animal, namely a decrease in food intake and digestibility. Both factors are related to the same phenomenon, namely a depression in the potential rate of degradation of protein in the rumen with a reduction in microbial synthesis of protein and thus a consequent reduction in protein supply to the animal (Ørskov, 1982).

Thirdly, recent investigations into alterations in circulating levels of certain gut hormones, in particular gastrin, in certain abomasal/

abomasal parasitisms are possible relevant to understanding the development of anorexia following infection. Gastrin is produced by the G cells found in the antral region of the abomasum. The G cells respond to many different stimuli such as changes in composition of gastric digesta, feeding (psychic and buccal phases), vagal stimulation and local neural elements (Titchen & Anderson, 1977). Gastrin has a multiplicity of actions including stimulation of acid (HCl) secretion by the parietal cells of the abomasum and alterations in the contractility of smooth muscle in sheep resulting an in inhibition of reticulo-ruminal motility (Anderson, Hansky & Titchen, 1981). Early work by McLeay, Anderson, Bingley & Titchen(1973), who investigated acid secretion in sheep prepared with fundic pouches, indicated that an increase in acid secretion occurred in the fundic pouches following infection with O. circumcincta, whereas there was decreased acid secretion in the body of the abomasum where the parasites were present. They postulated that either mechanical or chemical stimulation of the antrum by the parasites resulted in gastrin release. This led to increased acid secretion in the fundic pouches but not in the abomasum. This latter effect was thought to be a result of substances released by the parasites which suppressed parietal cell secretion (Titchen & Anderson, 1977). This suggestion has been supported by the recent finding of Eiler, Baber, Lyke & Scholtens (1981) that extracts of O. ostertagi injected intramuscularly into rats suppressed gastric HCl secretion. By measuring blood gastrin levels during infection with O. circumcinta, both in surgically modified sheep and intact sheep, gastrin levels were found to increase during infection, decrease after anthelmintic treatment and increase again on reinfection (Titchen/

(Titchen & Anderson, 1977; Reynolds, Anderson, Stiffe, Hansky & Titchen, 1979; Anderson, Hansky & Titchen, 1981). Hypersecretion from fundic pouches has also been observed in other abomasal parasitic infections, such as H. contortus and H. placei (Titchen & Anderson, 1977). In addition to the effect gastrin has on acid secretion, reticulo-ruminal motility is also reduced (Anderson, Hansky & Titchen, 1981). This latter action of gastrin may well play a part in the development of anorexia. For example, in the normal ruminant there is much evidence that neither glucostasis nor thermostasis play an important part in controlling meal size or frequency, but most of the factors thought to be important in the regulation of feed intake involve changes in reticulo-ruminal contents and proposed receptors of various types in either the epithelium or serosa of these organs (Baile & Forbes, 1974). Furthermore, the physiological signal that limits intake due to volume may not only be distension of tension receptors but may involve gastrin release (Church, 1971). Intravenous injection of synthetic gastrin has been shown to decrease reticulo-ruminal motility (McLeay & Titchen, 1970; Ruckebusch, 1971) and to decrease digesta flow rate from the omasum of normal sheep (Onapito, Donawick & Morritt, 1978).

From the foregoing, the development of anorexia in sheep infected with H. contortus may be related to alterations in gastrin levels with a resultant decrease in reticulo-ruminal motility. However, if, as has been suggested by the parasitological findings, worm burdens were similar in the infected lambs, it is not clear why some lambs should become anorectic after infection while other lambs continue to eat all the food offered. The protein content of the diet may be a further contributory factor since anorexia was more commonly observed in the infected lambs fed the low protein diet.

The finding of increased water retention in both groups of lambs fed the high protein diet relative to the low protein group is of interest but/

but difficult to explain. However, recent work by Searle, Graham & Donnelly (1982) on the influence of the plane of nutrition on body composition of weaner sheep fed a high protein diet indicated that sheep fed the diet ad libitum (DLWG 200 g) have less fat, energy and ash and more water in their carcases than lambs restricted to 50% of the intake of the ad lib group (DLWG 100 g). Furthermore, there were no differences in the protein content of the carcases between the two groups. However, in the present experiment, although the protein content of the rib sections of the two groups of control lambs were identical, the % fat present was also similar in both groups.

The changes in blood urea levels observed in both experiments are worthy of comment. In the first experiment (IIIa) blood urea levels rose in both groups of infected lambs relative to their controls, the rise occurring 21-42 days post-infection. In the second experiment (IIIb) blood urea levels were elevated at days 18 and 27 in both dietary groups of infected lambs, the greatest change occurring in the lambs fed the low protein diet. The increase in blood urea levels in this latter group, from pre-infection levels, was four-fold. However, in the first experiment N balance studies were conducted during the period when blood urea levels were elevated but there was no evidence of elevated urinary N excretion in the infected lambs relative to their controls, in contrast to the previous findings of Parkins et al (1973) where sheep infected with O. circumcincta had high blood urea levels and high urinary N output. In the second experiment (IIIb) no N balance studies were conducted, so one can only speculate as to whether urinary N levels were elevated, particularly in the low protein group where there was a great increase in blood urea levels by 27 days post-infection.

A/

A final point of interest is the finding of similar worm burdens in both dietary groups of lambs in the second experiment (IIIb). To date, most of the experiments described in this thesis have involved the feeding of either a high or low protein diet for a relatively short period of time (i.e. one month) before infection. Thus, it could reasonably be argued that this relatively short time on a low protein diet may not be sufficiently long to place this group at a physiological disadvantage relative to the better fed lambs. However, even when the lambs have been fed the experimental diets for 5 months before infection, as in IIIb, establishment would appear to be similar in both dietary groups.

In conclusion, although dietary status did not appear to affect parasite establishment in lambs infected with 350 H. contortus larvae/kg BW, the lambs fed the low protein diet suffered more severely from the pathophysiological consequences of infection, despite being subjected to the same haemorrhagic stress as the better fed lambs. There was no evidence of a failure to respond to the haemorrhage in most of the infected lambs, as indicated by the greatly increased rates of erythropoiesis. However, inappetance was more prevalent in the low protein group and appeared to be crucial in determining the ultimate survival of the animals.

CHAPTER IV

An investigation into the pathogenesis of repeated infection with small numbers of *H. contortus* larvae on 4 month old lambs fed either a high or low protein diet.

INTRODUCTION

In the previous experiment (IIIa), there was evidence that lambs fed a low protein diet and infected with 350 H. contortus larvae/kg BW suffered more severe clinical disease, despite having similar worm burdens and similar gastrointestinal blood losses as the better fed lambs. These findings tend to suggest that the lambs on the high protein diet had a superior physiological capacity compared with the lambs fed the low protein diet. The importance of inappetance in the pathogenesis of the disease was highlighted.

The experiments described to date have been single infections with varying levels of H. contortus and do not necessarily bear a close resemblance to the type of infection found under field conditions where larval challenge can be a continuous process. Under such conditions the quality of the host diet could play a greater part in the pathophysiology of the disease. Therefore, the following experiment was conducted to investigate the influence of dietary protein on the response of 4 month old lambs subjected to regular, repeated infections with H. contortus. An essentially similar protocol was conducted, as described in the earlier experiments (IIa and IIIa), in that the dietary change was made when the lambs were 3 months old and infection with H. contortus started one month later. Radioisotopic and nutritional studies similar to those described in earlier experiments were conducted on the surviving male lambs twelve weeks after infection started.

MATERIALS AND METHODS

Experimental design

Finn Dorset or Dorset Horn castrated male and female lambs kept on either a high or low protein diet from the age of 3 months were infected with an initial dose of 100 H. contortus larvae/kg BW when 4 months old. Thereafter, each lamb received 200 larvae (H. contortus) three times weekly for the 17 weeks of the experiment. Radioisotopic, digestibility and N balance studies on the male lambs were started 12 weeks after the initial infection and finished two weeks later. Blood and faecal samples were collected, and weights were recorded on a weekly basis throughout the experiment. In addition to the normal haematological analyses, blood smears were made prior to slaughter. The smears were stained by May-Grünwald Giemsa as described in General Materials and Methods. The lambs were killed 17 weeks after the initial infection when worm burdens were assessed. Carcase quality was evaluated in the surviving female lambs (Table IV.1).

Experimental animals

Thirty-two early weaned parasite-free lambs were used. They were randomly divided into high and low protein dietary groups, as described in General Materials and Methods, when 3 months of age.

Each dietary group comprised 8 female and 8 castrated male lambs. One month later each dietary group was further divided into infected/

TABLE IV.1: Studies on the pathogenicity of repeated infection with small numbers of H. contortus larvae on 4 month old lambs fed either a high or low protein diet: Experimental design.

Diet change at 3 months of age	Age at infection (months)	Dose of H. contortus	Nutritional and Radioisotopic studies (male lambs) (weeks post-infection)	Slaughter and Worm Burden assessment
High Protein (8)	4	100/kg BW + 200/lamb/3 times weekly	12 - 14	17 weeks after initial infection Carcase evaluation female lambs
Low Protein (8)	4	100/kg BW + 200/lamb/3 times weekly	12 - 14	17 weeks after initial infection Carcase evaluation female lambs
High Protein (8)	4	Nil	Pair-fed during metabolism cage study	Males to Experiment b, Chapter III. Carcase evaluation female lambs
Low Protein (8)	4	Nil	One lamb only, pair-fed	Males to Experiment b, Chapter III. Carcase evaluation female lambs

infected and control groups, each group then comprising 4 male and 4 female lambs.

The lambs in the "infected" group were then given an initial dose of 100 H. contortus larvae/kg BW per os, followed by 200 larvae/lamb/three times weekly. Since this experiment ran concurrently with the previous acute experiment (Chapter III), the control lambs were common to both experiments.

Feeding and management

The high and low protein diets used were identical to those used in the previous experiments (Chapters II and III). They were fed to appetite with the ready-mixed diet when in penned accommodation but during the metabolism cage study, for each day, the separate components of the rations were made up accurately as described previously (Chapter III, Experiment a). Again, the diets were individually offerred at 90% of appetite in order to minimise potential residues,and they were given in two separate feeds as described previously.

Radioisotopic, digestibility and N balance studies

These studies started 12 weeks after the initial infection with 100 H. contortus larvae/kg BW. Radioisotopic studies using ^{51}Cr-labelled red cells, ^{59}Fe-labelled transferrin and ^{125}I-labelled albumin were conducted over a 14 day period on the surviving infected male lambs of both dietary groups. An uninfected control lamb was also moved to the metabolism cages to act as a control for the radioisotopic studies, as described previously (Chapters II and III).

After a 5 day adjustment period, a seven day collection period for digestibility and N balance was conducted. The iron content of the/

the feed and faeces was measured also during this collection period as described previously (Chapter IV, Experiment a).

Essentially similar studies were conducted on the male control lambs of both dietary groups once the studies on the infected lambs finished.

RESULTS

Clinical and body weight changes

Both groups of lambs on the high protein diet gained weight throughout the experiment (Fig. IV.1). The infected group gained 11.2 kg during the period from two weeks before infection to the end of the experiment, 17 weeks after the initial infection. Control lambs gained 11.7 kg in the same period. In contrast, the infected lambs on the low protein diet only gained 3.6 kg in this period, whereas their controls gained 6.2 kg.

Weakness, inappetance, dullness and the presence of sub-mandibular and/or facial oedema, were observed in five out of eight of the infected lambs on the low protein diet. Four lambs in this group died or were euthanased before the end of the experiment.

The infected lambs on the high protein diet, in contrast, remained alert throughout and all survived until slaughter at 17 weeks.

Haematological changes

Haematological values were similar in both dietary groups before infection. However, both groups of infected lambs developed a macrocytic anaemia, with the lambs on the low protein diet appearing to be more severely affected.

Haematocrit values in the infected lambs on the high protein diet fell to minimum values of 0.26 l/l 12 weeks after the initial infection, whereas minimum values of 0.175 l/l occurred at week 15 in the infected lambs on the low protein diet. Haematocrit values in

both/

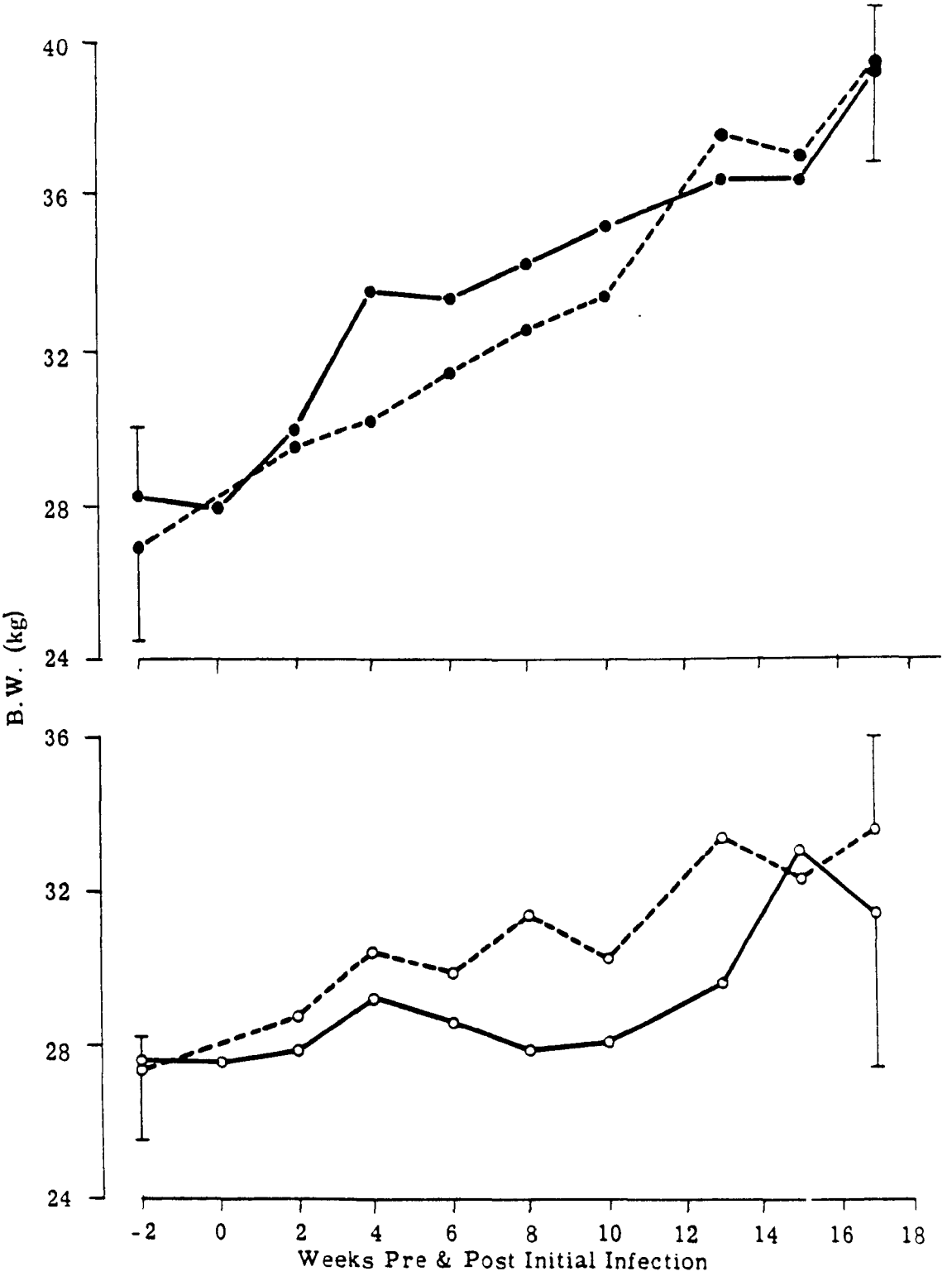


Fig. IV.1: Mean (± SE) group body weights of lambs infected with 200 H. contortus larvae/three times weekly and their respective controls.

●——● High protein infected ○——○ Low protein infected

●-----● High protein control ○-----○ Low protein control

both groups of control lambs fell slightly during the study (Fig. IV.2).

The macrocytic response was maximal in both groups of infected lambs from week 10 onwards (Fig. IV.3). The response in the infected lambs on the low protein diet was slightly greater than that observed in the high protein group. This was due to the greater uniformity in the development of the anaemia in the low protein group. Several lambs in the high protein group developed resistance to further infection and, as a consequence, their haematological values soon returned to normal, thus reducing the group mean MCV values. Control lambs on the low protein diet also developed a macrocytosis, presumably related to the fall in haematocrit observed in this group. However, the rise in MCV was less than that of the infected lambs on the same diet.

MCH values followed a similar pattern to the MCV changes, with a slightly greater response occurring in the infected lambs on the low protein diet (Fig. IV.4).

Bizarre erythrocyte shapes were seen on the blood films of both infected groups. Basophilic stippling was observed and anisocytosis was marked. In contrast, in the control lambs the red cells were more regular in size and no cells with basophilic stippling were observed.

The four infected lambs (B23, 24, 26 and 29) on the low protein diet which died or were euthanased before the end of the experiment developed a severe anaemia (Appendix D, Table 1). Haematocrit values of 0.10 l/l were recorded in three of the four lambs prior to death. Three of the four lambs had a macrocytic response. All four lambs lost weight rapidly before death and all were/

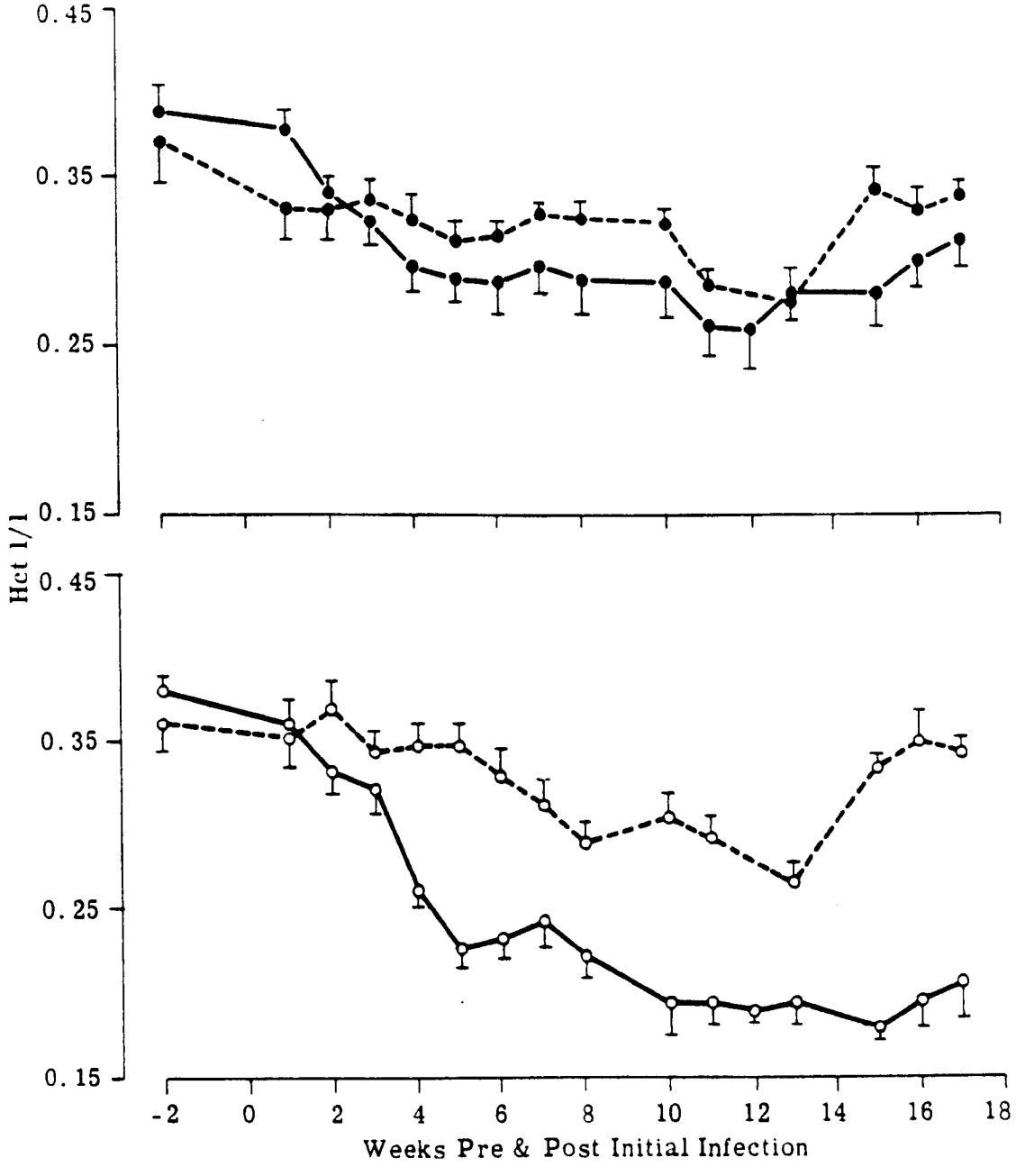


Fig. IV.2: Mean (± SE) haematocrit values of lambs infected with 200 H. contortus larvae/three times weekly and their respective controls.

●——● High protein infected o——o Low protein infected
●------● High protein control o------o Low protein control

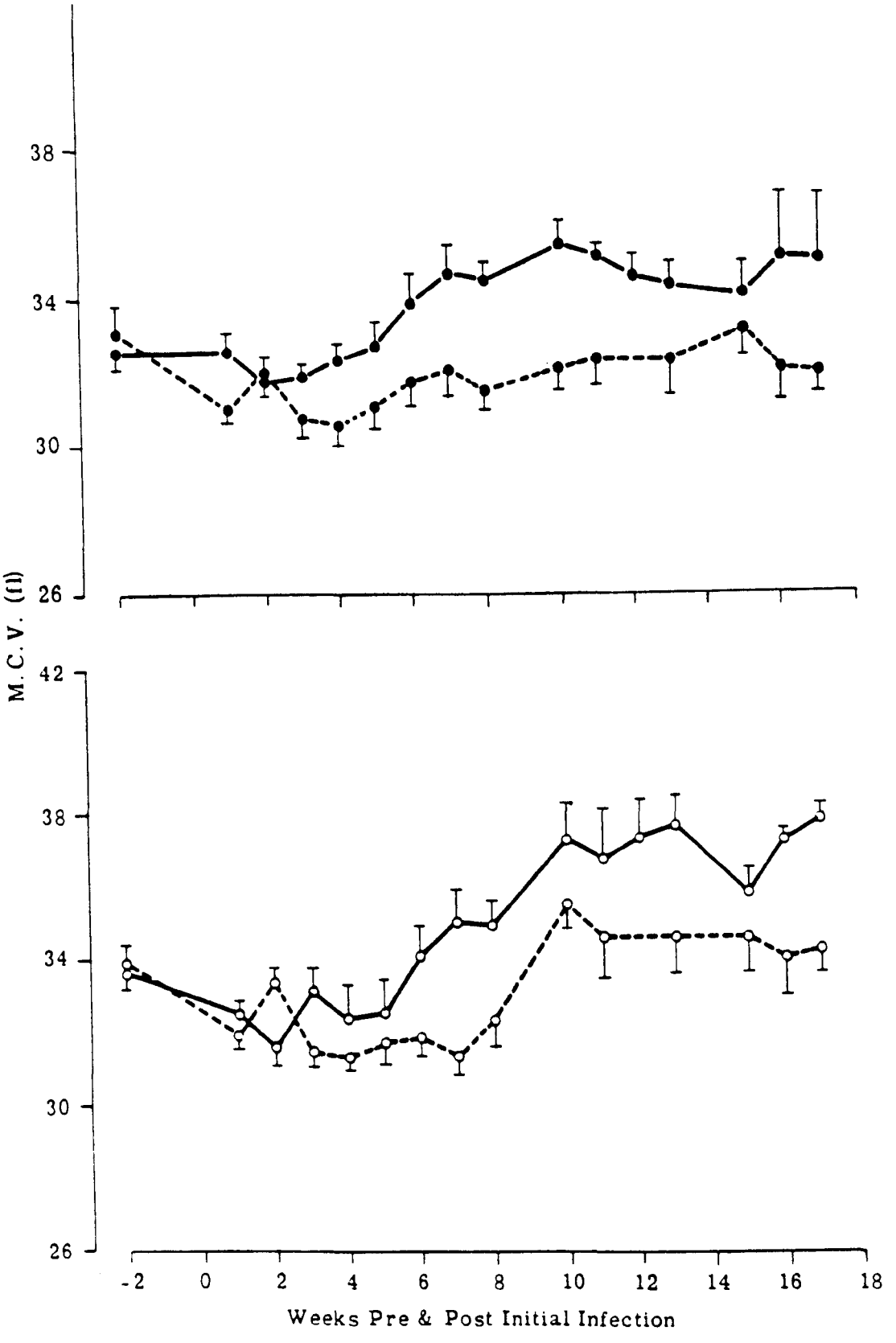


Fig. IV.3: Mean (± SE) MCV values of lambs infected with 200 *H. contortus* larvae/three times weekly and their respective controls.

●——● High protein infected ○——○ Low protein infected

●-----● High protein control ○-----○ Low protein control

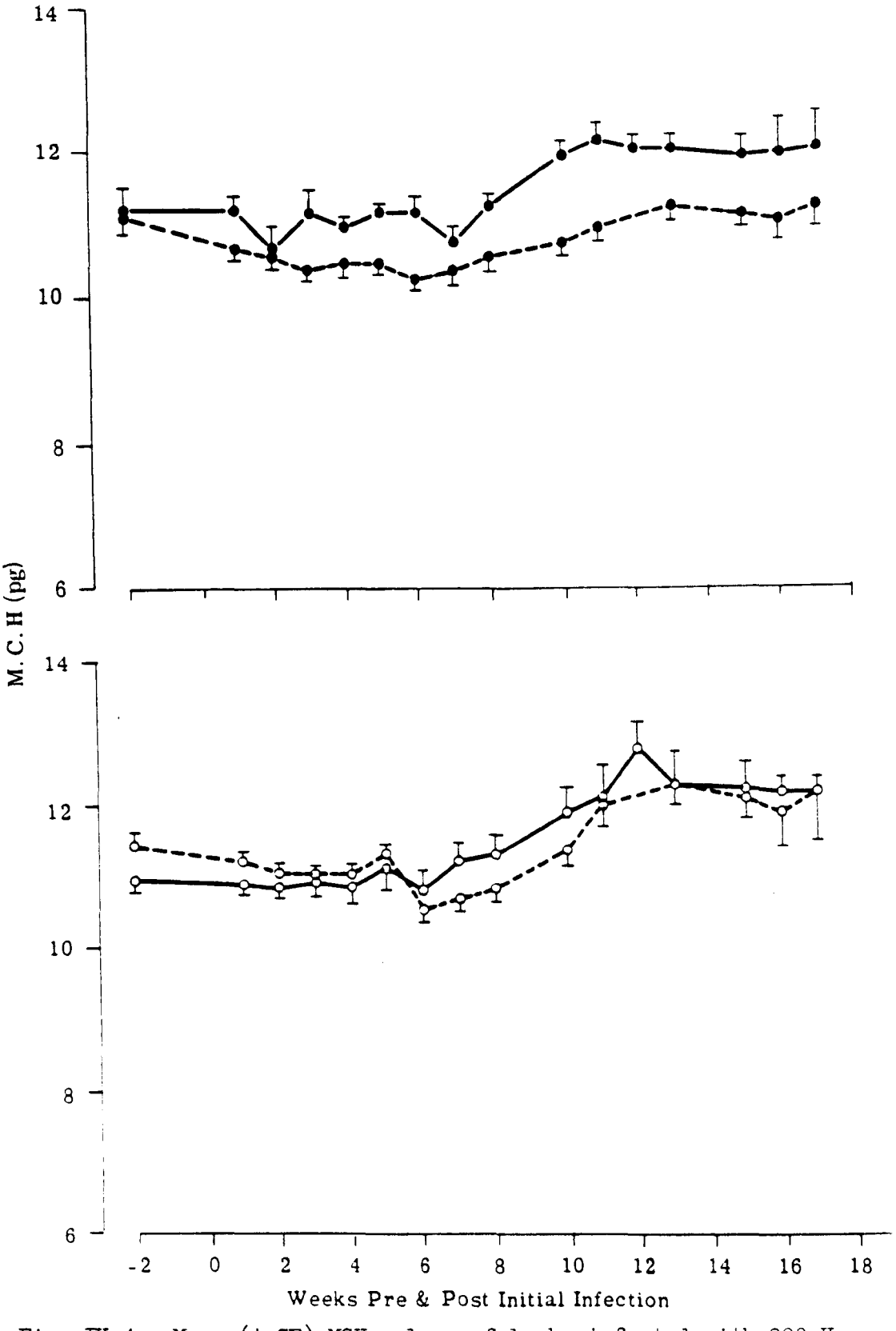


Fig. IV.4: Mean (± SE) MCH values of lambs infected with 200 H. contortus larvae/three times weekly and their respective controls.

●——● High protein infected o——o Low protein infected
●-----● High protein control o-----o Low protein control

were severely hypoproteinaemic and hypoalbuminaemic. Sub-mandibular and facial oedema was particularly marked in sheep B26. Serum iron levels and % saturation of transferrin fell in all four lambs after infection but a rise in serum iron and % saturation was observed in one lamb, B24, prior to death.

Biochemical changes

Only two of the eight infected lambs on the high protein diet became hypoproteinaemic and hypoalbuminaemic relative to controls after infection (Fig. IV.5). These two lambs, R71 and 72, had values for total serum protein and albumin of 52/29 g/l and 49/25 g/l respectively on the tenth week following the initial infection. Values for total serum protein and albumin fluctuated in the remaining six lambs in the group but were never less than 57 g/l or 29 g/l respectively.

In contrast, all eight infected lambs on the low protein diet became progressively hypoproteinaemic and hypoalbuminaemic following infection. Values for total serum protein and albumin fell from pre-infection levels of 65 g/l and 30 g/l respectively to 48 g/l and 20 g/l respectively at week 16 (Fig. IV.5). Values for total serum protein and albumin in the four infected lambs of this group which died were even lower, for example, B29 had total serum protein and albumin values of 31 and 14 g/l respectively at death (Appendix D, Table 1).

As in the "acute experiment" (Chapter III), the difference in nitrogen intake between the two dietary groups was reflected by differences in serum urea levels (Fig. IV.6). However, urea levels rose significantly ($P < 0.05$) in both groups of infected lambs relative to their controls 2 weeks after the initial infection. Thereafter/

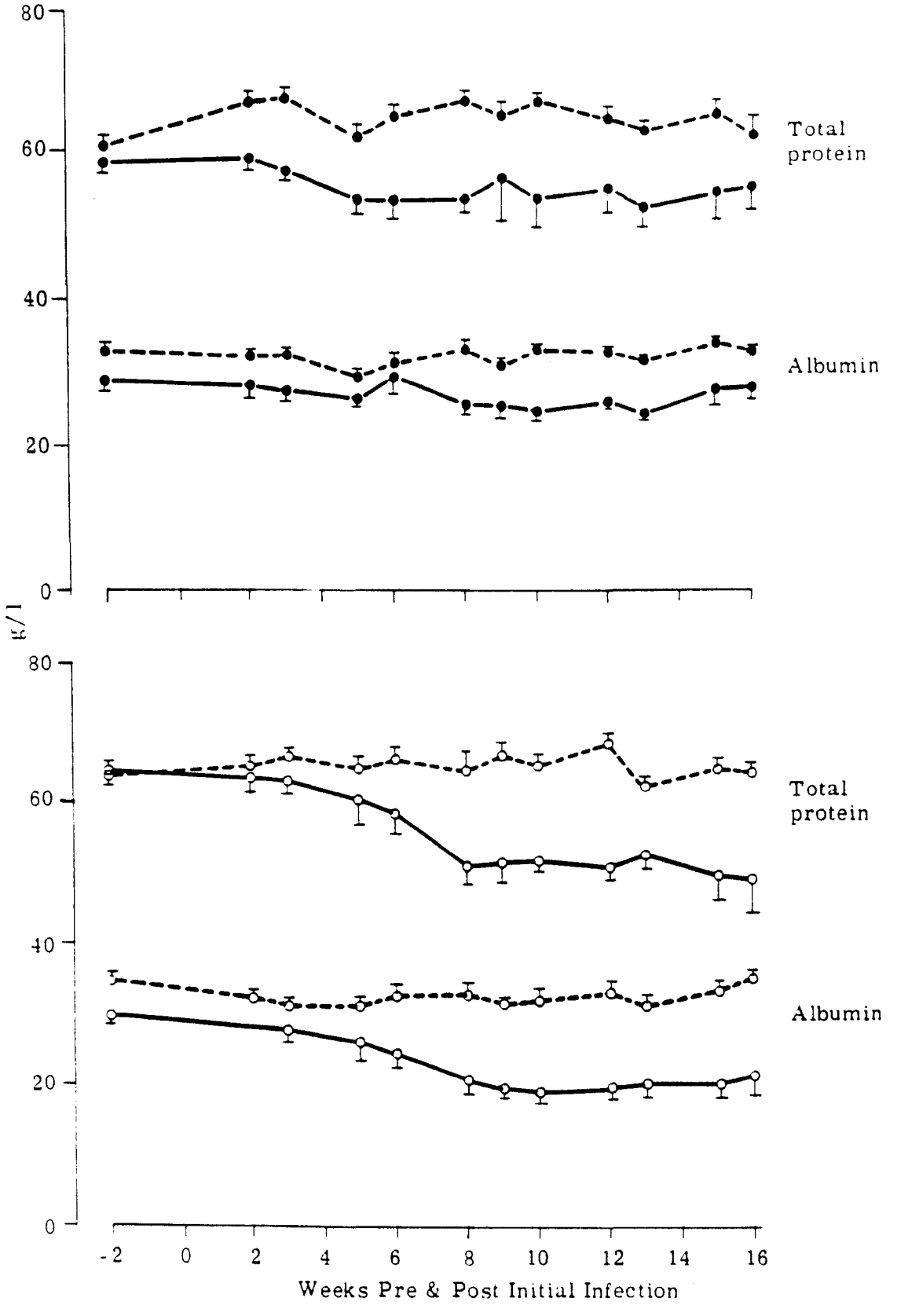


Fig. IV.5: Mean (± SE) total serum protein and albumin values of lambs infected with 200 H. contortus larvae/three times weekly and their respective controls.

●——● High protein infected o——o Low protein infected

●- - -● High protein control o- - -o Low protein control

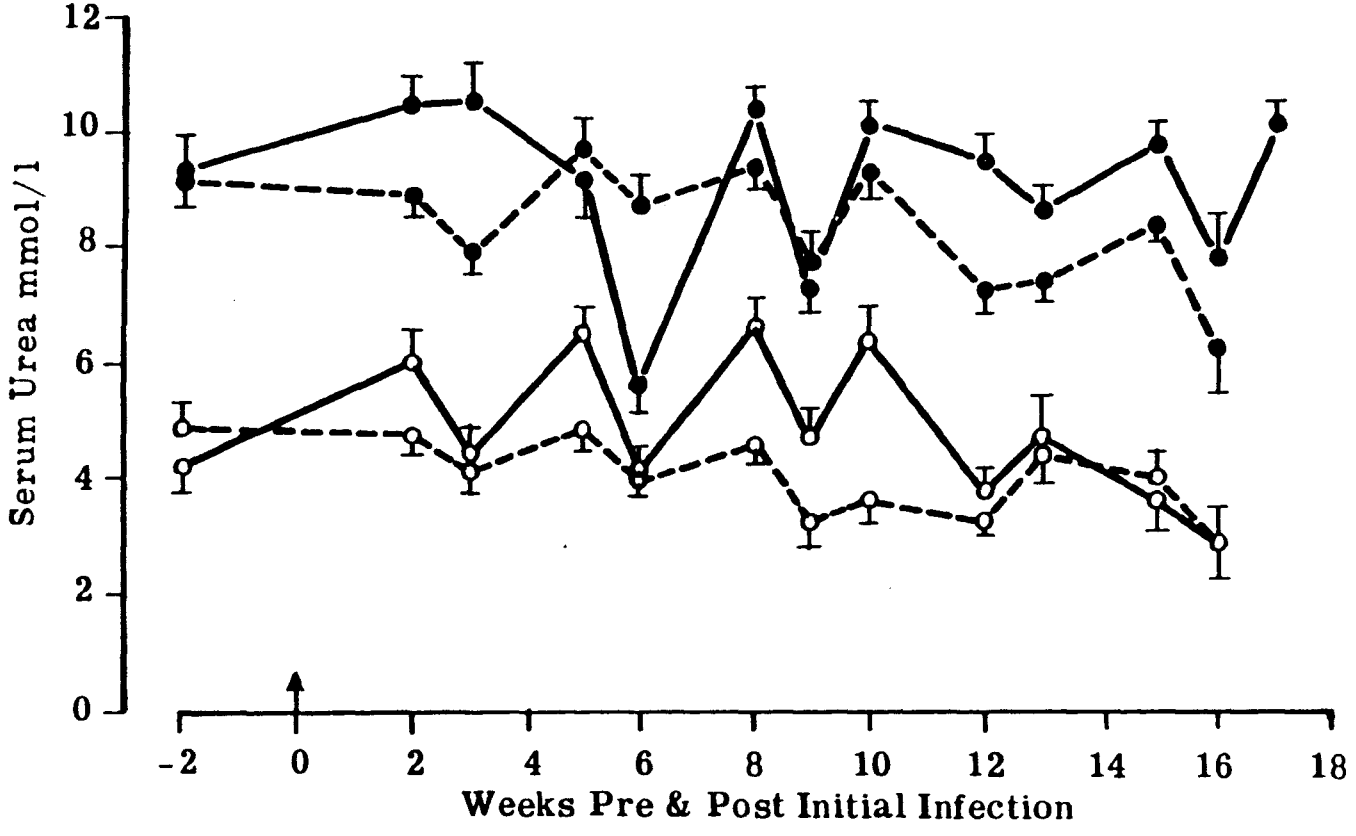


Fig. IV.6: Mean (± SE) serum urea levels of lambs infected with 200 H. contortus larvae/ three times weekly and their respective controls.

●——● High protein infected o——o Low protein infected

●-----● High protein control o-----o Low protein control

Thereafter values fluctuated in both infected groups but values in the infected lambs on the low protein diet were more often significantly elevated relative to their controls.

Serum iron levels in both infected and control lambs on the high protein diet were similar throughout the experiment and ranged from 135 to 184 μg/dl (Fig. IV.7). The % saturation of transferrin fell slightly in the infected lambs but differences between the infected and control lambs were only significant ($P < 0.05$) at week 6.

In contrast, serum iron levels in the infected lambs on the low protein diet fell after infection, minimal values occurring at week 12 (Fig. IV.8). Values were significantly lower than those of their respective controls at week 11 ($P < 0.05$) and week 12 ($P < 0.01$). The % saturation of transferrin fluctuated throughout the experiment but any differences between infected and control groups were not significant.

A fall in serum iron with a reduction in the % saturation of transferrin was observed in the four fatal cases in the low protein group. However, a rise in serum iron levels with an increase in % saturation of transferrin occurred in one lamb prior to death (Appendix D, Table 1).

Parasitological findings

Faecal egg counts rose gradually in both groups of infected lambs (Fig. IV.9). Counts in the high protein group rose to maximum levels of 13,900 e.p.g. at week 11. Thereafter counts fluctuated until week 13 and then fell to 6,000 e.p.g. at slaughter. Faecal egg/

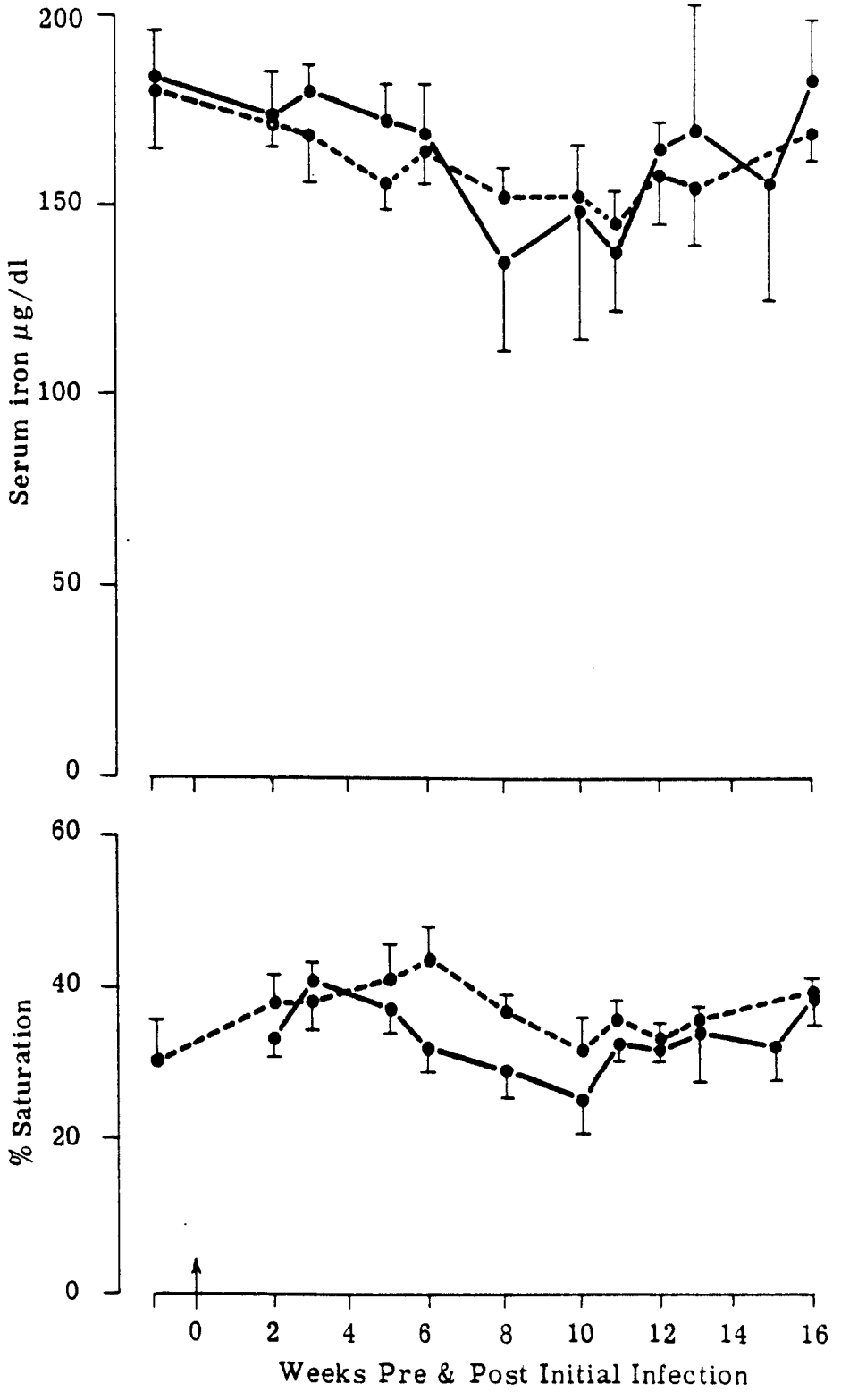


Fig. IV.7: Mean (± SE) serum iron levels and % saturation of transferrin values in lambs fed a high protein diet, infected with 200 H. contortus larvae/three times weekly and their respective controls.

●——● Infected ●------● Control

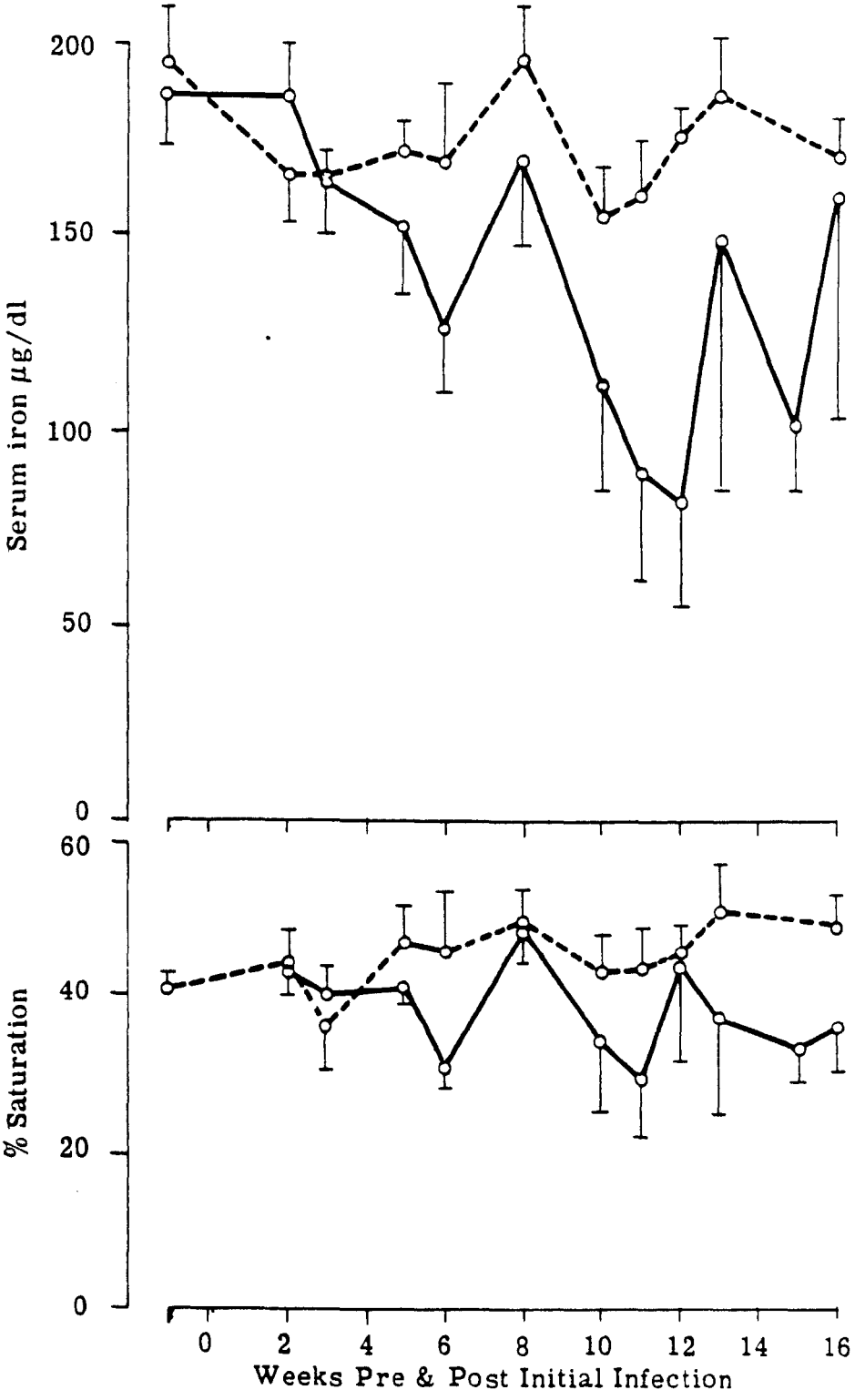


Fig. IV.8: Mean (± SE) serum iron levels and % saturation of transferrin values of lambs fed a low protein diet, infected with 200 H. contortus larvae/three times weekly and their respective controls.

o——o Infected o-----o Controls

egg counts in the infected lambs on the low protein diet rose to maximum levels of 45,000 e.p.g. at week 11 and, although their counts fluctuated after this, they were still high (40,000 e.p.g.) at the end of the experiment.

Three lambs in the high protein group (R74, 75 and 78) had low faecal egg counts from week 12 onwards. R74 and R78 had negative counts from week 14 until slaughter, and R75 was negative for the last two weeks of the experiment. A further lamb in this group (R73) had low counts on weeks 14 and 15 and negative counts from then on.

Inappetance with a resulting low faecal mass resulted in very high faecal egg counts in some of the lambs in the low protein group but, in contrast to the lambs on the high protein diet, faecal egg counts in the 4 surviving lambs were still high (8,800 - 82,200 e.p.g.) at slaughter.

Worm burdens at slaughter are presented in Table IV.2. The four lambs in the high protein group which had very low or negative egg counts, viz. R73, 74, 75 and 78, had either no worms present at slaughter or very few (e.g. 50). Worm counts in the other 2 male lambs in this group were similar (1,500 and 1,800), whereas worm burdens in the two female lambs were greater (2,600 and 3,500). Worm burdens in the surviving lambs on the low protein diet ranged from 3,250 to 3,850.

The difference in worm burdens between lambs on the high protein diet and the surviving lambs on the low protein diet was significant at $P < 0.01$ level.

Radioisotopic/

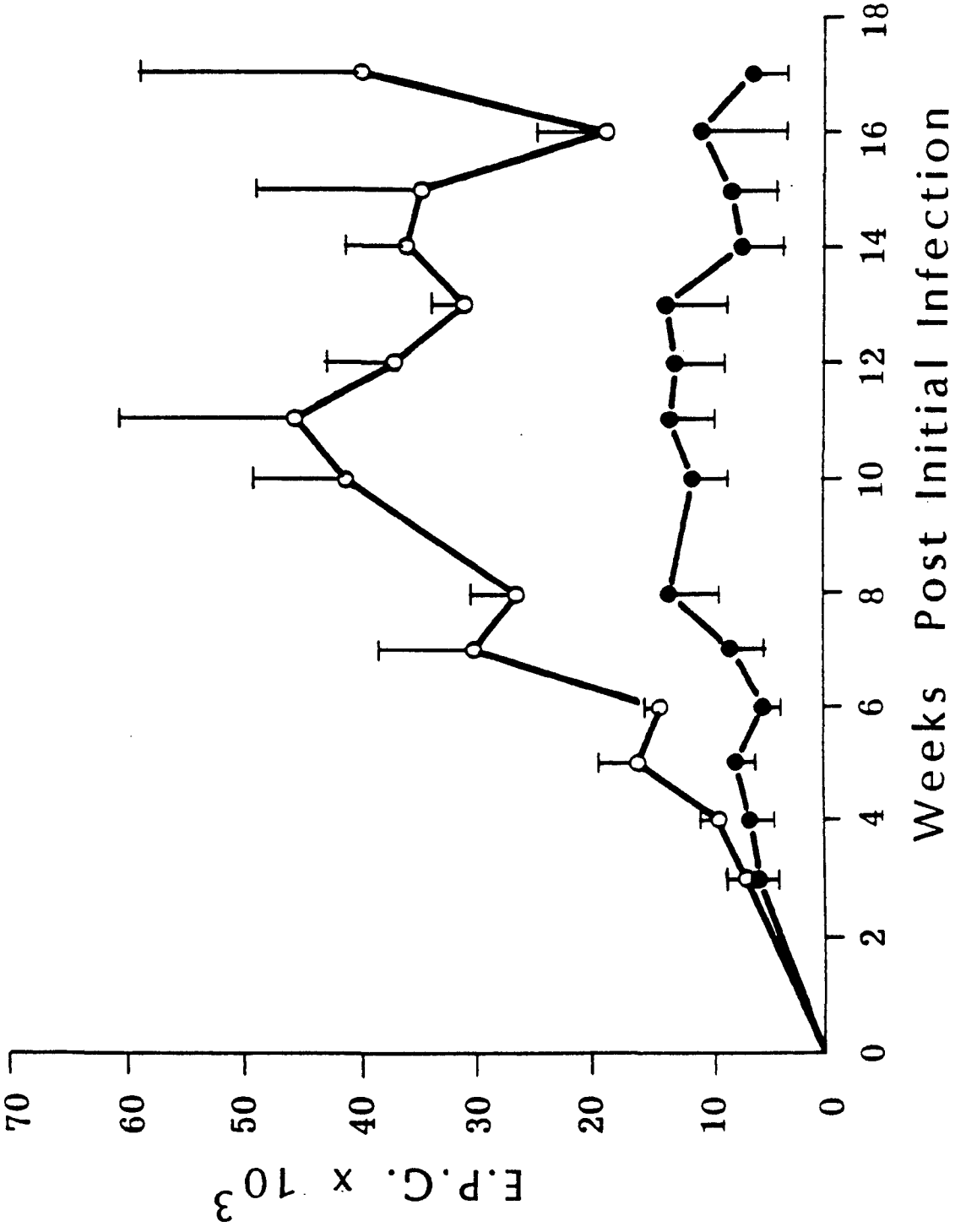


Fig. IV.9: Mean (± SE) faecal egg counts (epg) of 4 month old lambs fed either a high or low protein diet and infected with 200 H. contortus larvae/three times weekly.

●——● High protein ○——○ Low protein

TABLE IV.2: Individual worm burdens of lambs fed either a high or low protein diet and infected with 200 H. contortus larvae three times weekly.

Group	No.	Sex	Hb type	Weeks after initial infection	Larval Stages			Adult		Total
					L4	L5 ♂	L5 ♀			
HPI	R71	♂	B	17	50	150	0	800	500	1,500
	72	"	B	"	-	50	450	700	500	1,800
	73	"	B	"	0	0	0	0	0	0
	74	"	B	"	0	0	0	0	0	0
	75	♀	B	"	0	0	0	0	0	0
	76	"	B	"	0	50	300	1,300	950	2,600
	77	"	AB	"	0	50	200	1,650	1,600	3,500
	78	"	AB	"	50	0	0	0	0	50
Survivors to 17 weeks	$\bar{x}$									1,188
	S.E.									(486)
	B23	♂	AB	11	0	0	0	1,500	650	(2,150)
	24	"	B	14	0	0	0	1,800	1,450	(3,250)
	25	"	B	17	0	0	0	1,800	1,850	3,650
	26	"	AB	10	0	0	0	2,450	1,750	(4,200)
	27	♀	B	17	0	150	50	1,900	1,750	3,850
	28	"	B	17	0	0	0	1,900	1,350	3,250
	29	"	B	9	0	0	0	250	250	(500)
	30	"	B	17	0	200	200	1,250	1,600	3,250
Survivors to 17 weeks	$\bar{x}$									3,500
	S.E.									(150)

( ) Died or euthanased before 17 weeks.

Radioisotopic studies

There was considerable variation in response to continuing infection in the male lambs on the high protein diet. Therefore individual values only are presented for the infected lambs. Since only two infected male lambs on the low protein diet survived the whole metabolism cage study, their results are also presented individually. Control values are presented as group mean values (± SE) since individual values have been presented previously (Chapter III, Experiment a).

Erythrokinetics (Appendix D, Table 2)

As can be seen from the results presented in Table 2, the infected male lambs on the high protein diet fell into two distinct groups. Two lambs, R71 and 72, had a moderately severe anaemia and their red cell volumes (11 - 12 ml/kg BW) were reduced relative to control values (17.6 ml/kg BW). The remaining two infected males in this dietary group were less anaemic and their red cell volumes were only slightly reduced (15 - 16 ml/kg BW) relative to the control group. Red cell volumes in the two surviving infected lambs on the low protein diet were reduced (10 - 11.5 ml/kg BW) relative to control values (16.8 ml/kg BW) but were similar to the values observed in R71 and R72 in the high protein group.

An expanded plasma volume (measured using ^{125}I-labelled albumin) was observed in only one infected lamb, R72, in the high protein group (55.6 ml/kg BW), the remaining three lambs in this group having plasma volumes similar to control values, namely ~43 ml/kg BW. Total blood volumes were slightly reduced in these three lambs relative to control values, whereas total blood volume was slightly increased in R72.

Plasma volumes were expanded in both infected lambs on the low protein diet (~55 and 60 ml/kg BW) relative to control values of 43 ml/kg BW and their total blood volumes, although high (65 and 72 ml/kg BW), were nevertheless within the normal range.

The "apparent" half-life ($T\frac{1}{2}$) of the labelled red cells was reduced in all the infected lambs relative to the controls. In the infected lambs on the high protein diet, the reduction would appear to be related directly to the red cell volume changes, the greatest reductions in the $T\frac{1}{2}$ occurring in the lambs with the lowest red cell volume, namely R72 (93 hours). $T\frac{1}{2}$ values in the infected lambs ranged from 93 to 165 hours, compared with 187 hours in both control groups. Values in the two infected lambs on the low protein diet were similar to those of the two more anaemic lambs on the high protein diet, namely R71 and R72.

Blood loss into the gastrointestinal tract was greater in R71 and 72 (17.9 and 24.4 ml RBC/day respectively) relative to the blood loss observed in the two remaining lambs in this group, R73 and R74 (6.6 and 0.6 ml RBC/d respectively). The two infected lambs on the low protein diet had similar gastrointestinal blood losses (25.5 and 29.1 ml RBC/d) to that observed in R71 and R72. Both groups of control lambs were losing less than 1 ml packed RBC into the gastrointestinal tract daily.

Ferrokinetics (Appendix D, Table 3)

A considerable variation in serum iron levels existed between infected lambs within both dietary groups during the metabolism cage study. One infected lamb in each dietary group, R71 and B24, had reduced serum iron levels (91 and 74 µg/dl respectively). Values in the remaining infected lambs ranged from 133 - 197 µg/dl.

The/

The greatest reductions in the half-life ($T\frac{1}{2}$) of the labelled transferrin were observed in the infected lambs with the lowest serum iron levels, namely R71 and B24 (33 minutes). However, values in the remaining three infected lambs in the high protein group were reduced relative to control values. The remaining lamb in the low protein group, B25, had a half-life value of 78 minutes which was in excess of control values (69 minutes).

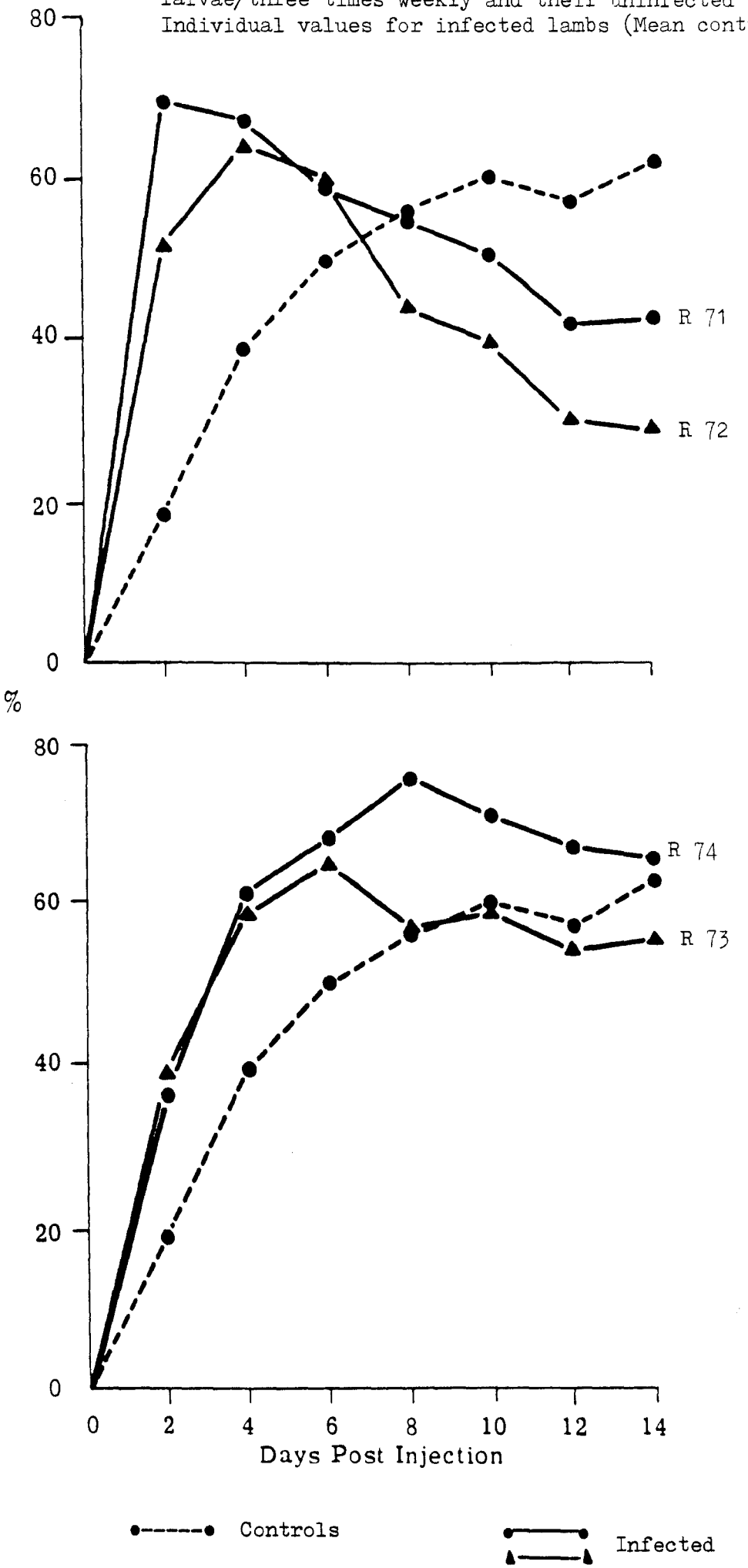
Plasma iron turnover rates were increased in only one infected lamb, R72, from the high protein group (2.24 mg/kg BW/d). Values in the remaining 3 lambs in this group were similar to control values. Both infected lambs in the low protein group had increased rates (1.23 and 1.43 mg/kg BW/d) relative to control values (1.12 mg/kg BW/d).

Red cell utilisation was maximal by day 4 after the injection of radioiron in R71 and R72. Control values, in contrast, rose gradually to maximum uptake by day 14. Red cell utilisation of ^{59}Fe was less rapid in the remaining two infected lambs (R73 and R74) in the high protein group, maximal uptake occurring on days 6 and 8 respectively. Red cell utilisation was maximal by day 4 in the two infected lambs on the low protein diet, whereas maximal uptake in the control group occurred on day 14 (Figs. IV. 10 & 11).

The average daily haemoglobin (Hb-iron) losses using the faecal clearances of both the ^{51}Cr-labelled red cells and the ^{59}Fe-labelled red cells are presented in Appendix D, Table 4. The ^{51}Cr and ^{59}Fe faecal clearances were almost identical in each lamb and there was no evidence of Hb-iron reabsorption in any of the infected lambs with the exception of R71 (0.8 mg Hb-iron/d).

Albumin/

Fig. IV.10: % red cell utilisation of ^{59}Fe: lambs fed a high protein diet and infected with 200 H. contortus larvae/three times weekly and their uninfected controls. Individual values for infected lambs (Mean control values



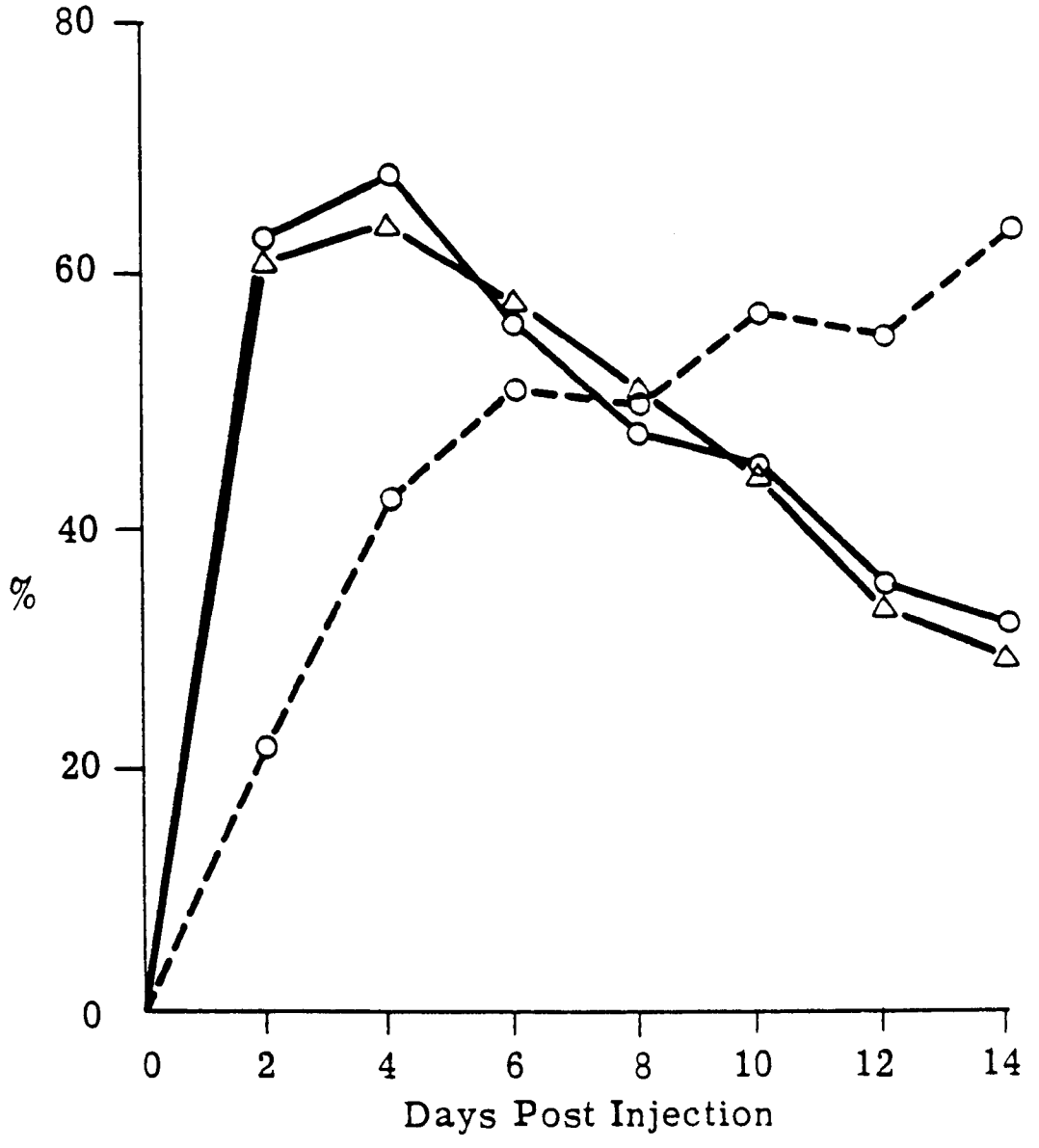


Fig. IV.11: % red cell utilisation of ^{59}Fe: lambs fed a low protein diet and infected with 200 H. contortus larvae/three times weekly, and their uninfected controls

o——o B 24 Δ——Δ B 25

o-----o Mean control values

Albumin metabolism (Appendix D, Table 5)

Lowest serum albumin levels during the metabolism cage study were observed in the two more anaemic lambs in the high protein group, R71 and R72, and in the two infected lambs on the low protein diet. Values ranged from 18 to 23 g/l, in contrast to control values of 31 g/l.

Plasma volume changes have been described earlier (see Erythrokinetics).

Intravascular (CA) and extravascular (EA) albumin pools were reduced in the infected lambs on the high protein diet relative to the control group. Although both CA and EA were reduced in the infected animals, the reduction in the EA/CA ratio in the infected group indicated that the reduction in the EA was proportionally greater than the reduction in CA.

A similar situation existed in the infected lambs on the low protein diet, both CA and EA being reduced relative to the control group. A reduction in the EA/CA ratio in the infected group again indicated a proportionally greater reduction in EA relative to CA.

The half-life ($T\frac{1}{2}$) of the labelled albumin was reduced in three of the four infected lambs (R71, 72 and 73) in the high protein group (207, 185 and 310 hours respectively), relative to the control group (339 hours). Both infected lambs on the low protein diet (B24 and 25) had greatly reduced $T\frac{1}{2}$ values (190 and 204 hours respectively) relative to control values (431 hours). $T\frac{1}{2}$ values for the control lambs on the low protein diet were significantly greater ($P < 0.02$) than those of control lambs on the high protein diet.

The/

The fractional catabolic rate (F(CA)) was increased in R71 and R72, both lambs catabolising 12% of the intravascular albumin pool daily. The remaining two infected lambs in this group had catabolic rates similar to control values, namely 7 - 8%. Fractional catabolic rates were increased in the two infected lambs on the low protein diet ~10% relative to their controls (5.9%). Fractional catabolic rates were significantly greater in the control lambs on the high protein diet relative to the control lambs on the low protein diet ($P < 0.001$).

Nutritional studies

Since only two infected male lambs on the low protein diet survived the metabolism cage study, only one of which had a pair-fed control, it was not possible to draw any firm conclusions as to the effect infection had on the digestibility of the experimental diet or on N balance. Where appropriate, brief mention will be made of any particular points of interest.

Feed intakes

Feed intakes in the infected lambs on the high protein diet rapidly stabilised after the move to the metabolism cages and all the lambs readily consumed 1000 g fresh feed daily. Of the two infected lambs on the low protein diet, one, B24, became progressively inappetant during the metabolism cage study. Its daily fresh feed intake fell from 490 to 302 g during the seven-day collection period. No attempt was made to pair-feed a control lamb to this lamb. In contrast, the remaining lamb, B25, readily consumed 1 kg fresh feed daily throughout the metabolism cage study.

Digestibility/

Digestibility coefficients of the experimental diets (Appendix D, Table 6)

There were no significant differences in the apparent digestibility coefficients of the high protein diet between infected and control lambs with the exception of a reduction in EE digestibility in the infected group.

Nitrogen balance (Appendix D, Table 7)

Infected and pair-fed control lambs on the high protein diet remained in positive N balance. The infected lambs retained significantly more N daily (6.4 g N/d) relative to the controls (2.9 g N/d)($P < 0.02$). Faecal N was similar in both groups of lambs but urinary N levels were higher (14.6 g N/d) in the control lambs relative to the infected group (11.3 g N/d).

The infected lamb (B25), and its pair-fed control on the low protein diet remained in slight positive N balance. Although faecal N was slightly elevated in the infected lamb relative to the control, urinary N was slightly greater in the control lamb.

Faecal dry matter coefficients

Faecal dry matter was significantly reduced ($P < 0.02$) in the infected lambs on the low protein diet relative to their controls (0.25 and 0.42 respectively). Although faecal dry matter was reduced in the infected lambs on the high protein diet (0.37) relative to their controls, the difference was not statistically significant.

Water balance (Appendix D, Table 8)

Daily water intake, urine output and faecal water content was similar in the two more anaemic lambs in the high protein group relative to their controls. The two remaining infected male lambs (R73 and R74) in this group were drinking more water than the controls. Faecal water content of these two lambs was similar

to/

to that of the controls but urine volume was increased in one lamb (R74). R73 and R74 were "retaining" more water relative to the other two infected lambs in the group and to the controls. Both infected lambs on the low protein diet were also drinking more water daily than their controls. Both faecal and urine water losses were elevated in these two lambs relative to the controls but "retained" water was increased in one of the infected lambs (B25).

Iron "balance" (Appendix D, Table 9)

Total faecal iron was similar in both groups of lambs on the high protein diet (107 mg/d). However, non-Hb iron in the faeces was slightly reduced in the infected group relative to the controls (89.8 and 103.6 mg/d respectively). "Apparent" iron absorption was slightly increased in the infected lambs compared with the controls. Dietary iron absorption was low in one infected lamb (B24) on the low protein diet (68%), whereas the remaining infected lamb had a similar, if slightly increased, dietary iron absorption relative to its pair-fed control (92 and 90% respectively).

Carcase evaluation (female lambs only) (Appendix D, Table 10)

The distribution of muscle : bone : fat was similar in infected and control lambs on the high protein diet, whereas a reduction in the % of muscle present occurred in the infected lambs on the low protein diet relative to their controls (41% and 47% respectively).

DISCUSSION

The principal finding of the present experiment was that if lambs repeatedly infected with small numbers of H. contortus (200 larvae/lamb, three times weekly) were fed a high protein diet, many of the lambs developed resistance to further infection. In contrast, if lambs were fed a low protein diet and were subjected to an identical infection regimen, none of the lambs appeared to develop resistance to further infection and worm burdens at slaughter were significantly greater in this group relative to the better fed lambs.

The course of the disease in the better fed lambs was less severe. Infection had little effect on growth rate; the lambs in fact gained more weight than their respective controls during the experiment. There was no evidence of anorexia and the haematological and biochemical changes were less severe than those observed in the lambs fed the low protein diet. Evidence of an increased rate of erythropoiesis in response to the gastric blood losses was provided by an increase in MCV values and was confirmed by the ferrokinetic studies in the infected male lambs. Faecal egg counts were consistently lower in this group relative to the low protein group, and four out of eight lambs had negative faecal egg counts before the end of the experiment. These four lambs had either negative or negligible worm burdens at slaughter, and overall, worm burdens were smaller in this group.

In contrast, many of the infected lambs from the low protein group suffered from severe clinical haemonchosis. Weight gain was reduced relative to their controls and four of the eight lambs died or were euthanased in extremis before the end of the experiment. The infection/

infection was characterised by the development of a severe macrocytic anaemia, hypoproteinaemia and hypoalbuminaemia, high faecal egg counts and high worm burdens at slaughter. The haematological and biochemical changes were more pronounced in the four fatal cases. Sub-mandibular and facial oedema, weakness and anorexia were other common features of the disease in this group. However, the majority of the lambs mounted an erythropoietic response and a possible iron deficiency anaemia was only observed in one of the fatal cases.

Interpretation of the findings of the radioisotopic and nutritional studies was made difficult on several counts. First, the high death rate in the infected lambs from the low protein group resulted in only two male lambs surviving the whole of the metabolism cage study. Secondly, within the group of infected male lambs fed the high protein diet, two were moderately anaemic while the faecal egg counts of the remaining two male lambs suggested that these animals had developed a considerable degree of resistance to reinfection. Finally, as mentioned earlier, anorexia was a common feature of the disease in the lambs fed the low protein diet. Of the two male lambs from this group which survived the metabolism cage study, one was severely anorectic and no control lamb was pair-fed to this lamb. Thus it was only possible to pair-feed one control lamb from the low protein group to one infected lamb from this dietary group. However, the findings of the digestibility and N balance studies showed that, in the high protein group, infection had little effect on the digestion and absorption of the experimental diet and that both infected and pair-fed control lambs remained in positive N balance. The infected lamb from the low protein diet and its pair-fed control also remained in slight positive N balance.

The/

The findings of the radioisotopic studies in the male lambs were directly related to the degree of anaemia in the lambs regardless of their dietary grouping. The two most anaemic male lambs from the high protein group and the two surviving lambs from the low protein group had the greatest reductions in red cell volume and greatest gastric blood losses. Any increase in plasma volume was observed in the more anaemic lambs, the greatest expansion occurring in the two lambs from the low protein group. This expansion of the plasma volume doubtless contributed to the severity of the peripheral blood picture in this group relative to the better fed lambs.

As mentioned above, the majority of the infected lambs, regardless of dietary status, mounted a fairly vigorous erythropoietic response, although the rise in MCV values was more gradual in this experiment relative to the earlier experiments, where a single infective dose was given (Chapters II and III). In the present study, the one lamb from the low protein group which became severely anorectic and died shortly after being transferred to the metabolism cages, failed to show any increase in MCV values, while its serum iron levels and % saturation of transferrin values fell. The remaining three fatal cases, in contrast, mounted an erythropoietic response, as indicated by a rise in MCV values. It is of interest that these fatal cases (excluding the one lamb referred to above) had moderate falls in serum iron levels, yet the % saturation of transferrin remained within the normal range or actually increased. This is in contrast to the lambs from the previous experiment (IIIa) which died or were euthanased <u>in extremis</u>, in which serum iron levels and % saturation of transferrin values were extremely low. This could well have been a reflection of the degree of depletion of the iron stores in this latter group since, in man, the adequacy of the iron supply for erythropoiesis/

erythropoiesis correlates better with the % saturation of transferrin than it does with the absolute plasma iron concentration (Bothwell et al, 1979). The % saturation value is regarded as a sensitive indicator which immediately reflects any discrepancy between supply and demand, and, in man, the lower critical level is believed to be ~15% (Bothwell et al, 1979). If one assumes that iron metabolism in the sheep is similar to that of man, these findings would suggest that iron stores were probably depleted in the fatal cases with the acute infection (IIIa), whereas in the present experiment, iron stores were maintained in the fatal cases, with the one exception mentioned above. Furthermore, dietary iron absorption was increased in the lambs with the acute haemonchus infection relative to their controls, whereas absorption was essentially similar in both infected and control lambs in the present experiment. These findings would tend to substantiate the suggestion that body iron stores were more depleted in the acute infection than in the present trickle infection.

The alterations in albumin metabolism observed after infection in the present experiment are of interest, particularly when compared with the findings of the acute experiment (IIIa). Both the intra and extravascular albumin pools were reduced in both dietary groups of infected lambs in the present experiment, the reduction of the extravascular pool being proportionally greater. This feature of haemonchus infection has also been reported by Allonby & Dargie (1973). However, in the previous "acute" experiment (IIIa) where the lambs were infected with a single dose of 350 larvae/kg BW, the intravascular pool alone was depleted and this occurred only in the low protein group. The reason for these differences in albumin distribution between the present experiment and the acute experiment could be related to the timing of the radioisotopic studies relative to the initial infection. For example, in the acute experiment (IIIa), the radioisotopic measurements were made 3-6 weeks after infection, whereas/

whereas in the present trickle infection these studies were conducted while the lambs were still being infected but 12-14 weeks after the initial infection. By this time, the lambs in the present experiment would have received 9-11,000 infective larvae i.e. a level of infection similar to the dose used in the acute experiment. Since it is probable that the parasites were still establishing, in the low protein group in particular, as judged by continuing high faecal egg counts and the magnitude of the gastric blood losses, it is not surprising that many of the pathophysiological changes observed in this group were similar to those observed in the acutely infected lambs fed the low protein diet. Furthermore, although these changes developed more slowly in the "trickle" lambs, there was evidence of increased red cell synthesis by week 8. It would not be unreasonable to assume that plasma protein synthesis was also increased by this time and thus the trickle lambs were hyperkinetic for a longer period of time, i.e. 4 weeks, before the metabolism cage studies started. This could have contributed to the greater depletion of the albumin pools, in particular the extravascular pool, in the trickle infected lambs relative to the acutely infected lambs. Furthermore, the findings of Allonby and Dargie (1973) referred to above, were from field cases of "chronic" haemonchosis in East Africa (worm burdens $\sim$ 1,600) and thus from sheep subjected to continuous infection, albeit at varying levels, throughout the year. This would tend to confirm the suggestion that prolonged exposure even to small numbers of infective larvae can have a more profound effect on body protein pools than a single high infection, particularly if the radioisotopic measurements are conducted at an early stage of infection in the latter.

A/

A further point of interest was the apparent development of resistance to continuing infection in many of the lambs fed the high protein diet. Early work by Andrews (1942) had indicated that larger total doses of infective H. contortus larvae were not fatal if given in smaller daily doses and later,Gibson (1952) working with T. axei infection in young unweaned lambs, found that daily dosing with 1,000 infective larvae for 10 days would give strong resistance to a challenge infection (40 daily doses of 4,000 larvae) at a later age (5 months). The level of the immunising dose used was critical since higher immunising doses (i.e. 1,000 larvae for 15 or 20 days instead of 10) decreased resistance to further challenge. Dineen, Donald, Wagland & Offner (1964) found that lambs given a trickle infection of H. contortus (100 L_3 for 30 days) had smaller adult worm burdens present at slaughter relative to lambs infected with a single dose of 3,000 larvae. However, there were greater numbers of inhibited larvae present in the trickle infected group and this phenomenon was regarded as evidence of an immune response. Inhibited larvae were not found in any of the lambs from either dietary group in the present experiment. This failure to show inhibition, particularly in the lambs which had lost their infection before the end of the experiment, may be a feature of the particular strain of H. contortus used in these experiments, although delayed development of patent infections after challenge has been observed in lambs hyperimmunised with irradiated larvae from the same laboratory strain (Salman, 1980). Although arrested larval development is often regarded as a manifestation of immunity (Urquhart, Jarrett & Mulligan, 1962), it has recently been recognised that,under field conditions, larvae may also be arrested in development in response to environmental stimuli (Connan, 1975; Waller & Thomas, 1975; Schillhorn van Veen, 1978; Eysker & Ogunsusi, 1979; Fabiyi, Oluyede & Negedu, 1979). This type of arrested larval development/

development is now referred to as hypobiosis, and is basically a response of the parasite to climatic conditions which are adverse for the survival and development of the pre-parasitic phase. Thus the parasite ensures the survival of its next generation by remaining inside the host during such unfavourable times.

In contrast to the lambs fed the high protein diet, the lambs on the poorer diet showed no evidence of the development of any resistance to continuing infection. Such a finding is most probably a reflection of impaired immune responses in the animals receiving the low protein diet, although there is no direct evidence to support this suggestion other than the parasitological findings.

However, the work by Bawden (1969b) and Dobson & Bawden (1974) on the influence of high and low protein diets on the establishment and survival of Oe.columbianum is of interest in this context. Although Bawden (1969) found that initial parasite establishment was similar in both dietary groups, more adult worms were present at slaughter in the low protein group and, in addition, there was evidence of delayed development but increased fecundity of the parasites in the sheep from this group. In the subsequent study (Dobson & Bawden, 1974), arrested larval development occurred in the lambs from the high protein group and patency was delayed. Furthermore, there was greater cellular proliferation in the intestines of the better fed sheep, and the authors concluded that the increased susceptibility of the sheep from the low protein group was associated with both malfunctions of the innate immunity of the gut, and with reduced lymphocyte and plasma cell reactions. However, recent investigations into the pathological changes associated with persistent low grade H. contortus infections (200 larvae for five consecutive days) in lambs fed either a high or low protein diet are less clear cut (Salman, 1980). Although faecal egg counts and worm burdens were higher in the lambs/

lambs fed the low protein diet relative to the better fed lambs, the cellular responses in the gut wall were of a similar magnitude in both dietary groups with the exception of a slight increase in mast cell numbers in the lambs fed the high protein diet (Salman, 1980).

Although mortality appeared to be greater in the low protein trickle group relative to the low protein group in the acute experiment (IIIa), there is no doubt that one, if not two, of the acutely infected male lambs from this group would have died had they remained in the experiment for the full eleven weeks and not been slaughtered at 6 weeks post-infection. Therefore, mortality in the two groups would probably have been of a similar magnitude. Furthermore, although the haematological and biochemical changes observed during the trickle infection developed more gradually relative to the acute experiment, the ultimate severity of the changes was similar in both groups of lambs fed the low protein diet. This finding is in contrast to the observations of Andrews (1942), Manton, Peacock, Poynter, Silverman & Terry (1962) and Dineen et al (1964) who reported that sheep given a trickle infection of H. contortus suffered less severe clinical disease (as judged by haematocrit values and faecal egg counts) than those receiving single infections. Later work by Pradhan & Johnstone (1972a,b), however, indicated that daily dosing with H. contortus was associated with greater pathogenicity than weekly dosing. However, the larval doses used by these authors were considerably larger than those used by Manton et al (1962) and Dineen et al (1964). Furthermore, no details of the diet of the lambs was provided by Pradhan & Johnstone, whereas the lambs in the other experiments (Andrews, 1942; Manton et al, 1962; Dineen et al, 1964) were all fed a good quality diet.

Thus, in conclusion, as far as the lambs fed the low protein diet in the present experiments (IIIa and IV) are concerned, trickle and single/

single infections are equally pathogenic. However, the better fed lambs suffered less severe clinical disease when subjected to repeated infections with small numbers of larvae than when given a single large infection and, furthermore, many developed resistance to further infection.

CHAPTER V

Studies on nitrogen digestion in sheep infected with *H. contortus* and in sheep with simulated parasitism

INTRODUCTION

It has been recognised for some considerable time that the difference between the amount of a particular nutrient eaten and the output of that nutrient in the faeces does not necessarily reflect its absorption. This is particularly the case for nitrogen. Part of the faecal matter consists of microbial protein, enzymes, bile pigments and sloughed epithelial cells and if, for example, a ruminant animal is given a N-free diet, it continues to excrete nitrogen in the faeces. This endogenous source of nitrogen is referred to as metabolic faecal nitrogen (MFN). This has been estimated by the Agricultural Research Council (ARC)(1965) as 5 g N/kg dry matter intake for sheep and cattle given normal roughage diets.

In conventional digestibility studies it is impossible to determine chemically whether a substance in the faeces is endogenous or exogenous in origin. As mentioned earlier in General Materials and Methods, the term "apparent" rather than "true" digestibility of a nutrient is used because of the losses, for example, of methane and carbon dioxide by eructation and the presence of metabolic faecal nitrogen. The "apparent" digestibility of a nutrient is a satisfactory representation of the net effect of digestive processes but provides no information on the changes occurring within the gastrointestinal tract. An important step in investigating the flow and composition of digesta within the tract was the development of surgical techniques involving the insertion of cannulae at strategic points along it, with or without the use of non-absorbable markers. Two main techniques have been developed and they will be discussed briefly .

The first technique involves the exteriorisation of the digesta flow through re-entrant cannulae. This technique allows direct measurement/

measurement of flow rate. However, it is time-consuming and, in addition, such cannulae are liable to block. There is also the more serious hazard of loss of gastrointestinal contents as a result of the connecting piece joining the two cannulae pulling apart (Ash, 1962).

In the second technique, the rate of flow of digesta is calculated by reference to indigestible markers which are present in the feed or given independently. The advantages and disadvantages of the various markers used have been reviewed by Kotb and Luckey (1972).

Certain markers, such as chromic oxide (Cr_2O_3), may be used in the intact animal in digestibility studies, where all that is required is that the marker is fully recoverable in the faeces, i.e. the marker is not absorbed during passage through the gastrointestinal tract. However, if one requires to partition digestion within the gastrointestinal tract, it is necessary either to use the system of total sampling which involves sacrifice of the experimental animal or to insert T-cannulae at various strategic points along the tract.

Although the technique of T-cannulae and indigestible markers is preferable to the use of re-entrant cannulae, there are certain problems associated with it, the most important being the difficulty in obtaining a truly representative sample of digesta flowing past the cannula. This problem can be partially overcome by the simultaneous use of two markers, one of which remains in solution, whilst the other is intimately associated with the particulate phase of digesta (Hogan and Weston, 1967; Tan, Weston and Hogan, 1971; Faichney, 1975b).

Polyethylene glycol (PEG) has been and still is used as a water-soluble marker. However, analysis may be non-specific and difficult to measure at low concentrations and thus the chromium-51 complex of ethylenediamine tetra-acetic acid (^{51}Cr-EDTA) has been found to be more suitable despite small amounts being absorbed and excreted in/

in the urine (Downes and Macdonald, 1964).

Naturally occurring markers such as lignin and plant chromogens are not totally reliable because of problems such as incomplete recovery or the lack of sufficient indicator, as in the case of chromogen in hay (Church, 1971). However, the complex of ^{103}Ru-labelled tris (1,10 phenanthroline) Ruthenium II chloride (^{103}Ru-P) has been shown to be a suitable particulate phase marker (Tan, Weston and Hogan, 1971).

The combination of ^{51}Cr-EDTA and ^{103}Ru-P has now become an established technique in digestive physiology studies in conjunction with T-cannulae. Both markers are usually infused continuously throughout the study. However, because of the difficulties in getting a representative sample of digesta using T-cannulae, it is necessary to reconstitute the true digesta either physically or mathematically (Faichney, 1975b).

The method assumes that, once equilibrium has been achieved and maintained by continuous infusion of the two markers, the concentrations of those markers in the digesta flowing past any sampling point must be equal. Thus, if sub-samples of the fluid phase are obtained by straining or centrifugation of the digesta so that fluid and digesta contain different proportions of liquid and particulate matter, the true digesta can be reconstituted by combining the fluid and digesta, either physically or mathematically, so that the concentrations of the two markers are the same (Faichney, 1975b). In the same way, the concentration of any component of digesta, e.g. organic matter or N, can be measured in true digesta by analysing the two fractions before mathematically reconstituting the digesta on the basis of the relative concentrations of the inert markers (Faichney, 1975b).

Extensive/

Extensive use has been made of markers to study various aspects of normal digestive physiology in sheep with or without cannulae fitted (Hogan, 1964; Weston and Hogan, 1967; Hogan and Weston, 1967; Grovum and Williams, 1973; Faichney, 1975a,b). However, the application of these techniques to the study of digesta flow (and composition) in parasitised sheep has been limited to two studies (Bawden, 1970; Roseby, 1977).

Bawden (1970),working with intact 7.5 month old sheep measured the rate of flow of digesta through the alimentary tract before and after infection with the intestinal nematode O. columbianum. He used stained (basic fuschin) roughage as a marker and noted its subsequent excretion in the faeces. After infection, retention times were increased to varying degrees depending on the diet and mode of infection, e.g. the greatest increase in retention time occurred in sheep fed ad lib a diet of chopped straw and infected with small doses of larvae over a 5-day period, whereas the least increase occurred in sheep given a single infective dose and fed ad lib a diet of chopped lucerne. The decreased feed intake observed after infection was thought to be directly related to the increased retention times.

Roseby (1977) used the water soluble marker ^{51}Cr-EDTA to measure the rate of passage of water soluble material through the gastrointestinal tract of 8 month old sheep infected with T. colubriformis and their pair-fed controls. Transit times through the various sections of the tract were calculated mathematically as the sheep were not fitted with cannulae. Transit time of the marker was found to be increased in the tubular intestine but not in the rumen or caecum/colon. However, he conducted a further experiment in which the mass of substrates and concentration of products of fermentation were measured in the rumen, abomasum, small intestine/

intestine, caecum-proximal colon and distal colon-rectum of anaesthetised infected sheep and their pair-fed controls (i.e. total sampling). The nitrogen content of the digesta in the abomasum, small intestine and caecum-proximal colon was higher in the infected sheep relative to controls, but similar in the distal colon-rectum. Since faecal N was not increased in the infected sheep, he concluded that the results indicated a change in the site of N absorption in parasitised sheep due to the combined effects of increased plasma protein leakage into the small intestine, along with a reduced capacity of the small intestine for digestion and/or absorption. Thus he suggested that, with a net reduction in absorption in the small intestine, a corresponding increase occurred in ammonia absorption from the large intestine as a consequence of increased fermentation at this site.

The following experiment was designed to investigate N digestion and absorption in parasitised sheep relative to uninfected controls. This was investigated in two ways. First, in sheep experimentally infected with H. contortus and, secondly, in sheep with a simulated parasitic infection. In the simulated parasitism, blood was withdrawn from the jugular vein and directly infused into the abomasum via an indwelling cannula. All the sheep were prepared with cannulae in the rumen, duodenum and ileum, and the double marker technique using ^{51}Cr-EDTA and ^{103}Ru-P was used as described by Tan, Weston and Hogan (1971).

Since digesta flow is affected by the composition, quantity and regimen of feeding (Hogan, 1964; Weston and Hogan, 1967; Faichney, 1975b), a system of continuous feeding was used in conjunction with a modified pair-feeding regimen.

MATERIALS AND METHODS

Experimental design (Table V.1)

Nine sheep were prepared with permanent cannulae in the rumen, duodenum and ileum. Three of the sheep had, in addition, a cannula into the abomasum.

The sheep were divided into three treatment groups, A, B and C, each consisting of three sheep:-

1. Group A (parasitised) Each sheep was infected with 1000 *H. contortus* larvae/kg BW *per os*, four weeks before the start of the experiment.
2. Group B (simulated parasitism): In this group, blood was collected from the jugular vein (via an indwelling cannula) into heparinised containers (200 ml/sheep/day) and was then slowly infused directly into the abomasum via an indwelling cannula during the subsequent 24 hours. This procedure started 10 days before the radioisotope study and continued throughout the experiment.
3. Group C (controls): This group consisted of uninfected control animals.

During the experiment, the sheep were fed according to a modified pair-feeding system, viz:- one sheep from each of groups B and C were pair-fed to one parasitised animal in group A. A conventional N balance was conducted over an 8 day collection period and the flow of N along the gastrointestinal tract of each animal was measured relative to the inert markers ^{103}Ru-P and ^{51}Cr-EDTA (Faichney, 1975b).

Experimental/

TABLE V.1: Studies on nitrogen digestion in sheep infected with 1000 *H. contortus* larvae/kg BW, sheep with a "simulated parasitism" and uninfected control sheep: Experimental design.

Group	Days pre-infection	Days post-infection		
	21	0	14 to 23	23 to 33
A (infected)	To metabolism cages Experimental diet introduced	Infected with 1000 *H. contortus* L_3/kg BW	-	Radioisotopic studies N balance
B (bled)	" "	Non-infected	Start pair-feeding to Group A. Simulate parasitism by removing 200 mls blood/day/sheep and infusing this blood back into the abomasum over the subsequent 24 hours	" "
C (controls)	" "	" "	Start pair-feeding to Group A	" "

Experimental animals

Nine mature Downland wethers of approximately 40 kg BW were used. They were treated orally with an anthelmintic (Levamisole HCl. 1.5% * w/v) on housing and remained housed for the duration of the experiment.

Feeding and management

Prior to surgery, the sheep were fed a diet of commercially formulated sheep pellets (120 g CP/kg DM ;ME 10.3 MJ/kg DM). Water was available at all times. The animals were moved to conventional metabolism cages four weeks after surgery and were thereafter given the experimental diet, which consisted of equal amounts of chopped hay and pelleted straw (Table V.2).

The daily ration was given continuously during each 24 hour period using an automated mechanised conveyor belt system. The parasitised sheep (Group A) were fed virtually to appetite, whilst the bled (Group B) and control (Group C) sheep were fed the amount consumed by their infected counterpart during the previous 24 hour period (pair-feeding). Pair-feeding started 10 days before making the experimental measurements. Water was freely available at all times.

Surgical techniques

The sheep were starved for 12 hours prior to surgery and the surgical techniques used were based on those described by Hecker (1974). Both flanks were clipped and prepared for surgery by scrubbing with hibitane. General anaesthesia was induced and maintained using pentobarbitone sodium (Veterinary Nembutal †). A support was placed under the shoulders during anaesthesia to allow free drainage from the buccal cavity and to minimise the risk of inhalation of rumen contents.

(a)/

* Nilverm, I.C.I., U.K.

† Ceva Ltd., U.K.

TABLE V.2: Proximate composition of experimental diet.

Component	Fresh Matter g/kg	CP g/kg DM	ME MJ/kg DM	OM g/kg DM
Chopped Hay	500	7.3	8.4	92.6
Ground Straw*	400	130.6	7.4	92.2
SoyaBean Meal	50			
Molasses	30			
Minerals & Vitamins	20			
	Pelleted			
TOTAL DIET	1,000	102	7.9	92.4

* 2% urea added

(a) Rumen fistula

The left flank was prepared for surgery using conventional aseptic techniques. A mid-flank incision was made and the various muscle layers were opened using blunt dissection. The dorsal sac of the rumen was located and exteriorised. A purse-string suture was made in the proposed site for the cannula and a hole pierced in the centre using fine scissors. The hole was stretched open using blunt scissors until it was large enough to allow insertion of the cannula. The cannula was gently eased in and the purse string suture was then pulled up and tied off. Care was taken to avoid any spillage of rumen contents. A temporary plug was fitted in the cannula to prevent spillage while the cannula was exteriorised through a stab wound made in the flank dorsal to the original incision. It was clamped in position while the main incision was closed and then fitted with a retaining flange and a removable plug.

(b) Abomasal, duodenal and ileal cannula

The right flank was next prepared for surgery and a large mid-flank incision was made, as described for the rumen cannula.

(i) Abomasal cannula The pylorus was located and then the abomasum and duodenum were gently pulled to the incision. Using fine pointed scissors a small incision was made in the abomasal wall. A silicone cannula (15 cm long, 2 mm I.D.) with a flange (1 cm diameter) was gently inserted and the incision closed with a purse-string suture. A second small flange was fitted against the external wall of the abomasum and the cannula was exteriorised together with the duodenal cannula, as described below.

(ii) Duodenal cannula This was inserted approximately 3 cm posterior to the pyloric sphincter in a similar way to the abomasal/

abomasal cannula except that the cannula was a single unit made of soft moulded plastic. To assist healing, a piece of omentum was wrapped round the cannula.

Using a bovine trocar and cannula, a stab wound was made in the abdominal wall for the abomasal cannula. It was threaded through the bovine cannula, pulled to the exterior and clamped in position. The duodenal cannula was then exteriorised through another stab incision. Both cannulae were then secured with flanges and plugs.

(iii) Ileal cannula The caecum was located and exteriorised in order to locate the position for insertion of the ileal cannula. The cannula was inserted in a similar way to the duodenal cannula and exteriorised as described above. The incision was then stitched - first peritoneum and muscle layers and then a continuous skin suture. All the sheep were given long-acting penicillin by intramuscular injection at the end of surgery. The sheep were allowed 3-4 weeks to recover from the surgery before moving into metabolism cages.

Haematological (haematocrit only) : Biochemical : Parasitological techniques

The techniques used were essentially as described in General Materials and Methods. No abomasal digests were prepared. Larval doses were prepared by the Department of Veterinary Parasitology,Glasgow.

N balance study

Total faecal and urine collections were measured over an 8-day period and a daily 10% sub-sample of each was retained and bulked for subsequent analyses. Chemical analyses of faeces and urine were made using established analytical methods (General Materials and Methods).

Blood infusion technique

The sheep in the "simulated parasitism" group (Group B) were fitted with standard indwelling catheters (14G OD, 16 G ID) into both jugular/

jugular veins. The catheters were kept patent by regular flushing with sterile heparinised saline. For ten days prior to and during the study, 200 ml of blood per sheep were withdrawn via the catheter into a heparinised bottle every morning, allowing 10 iu heparin/ml blood. The blood from each sheep was then infused back into the abomasum via the cannula at a rate of approximately 0.15 ml blood/minute over the following 24 hours.

Preparation of radioisotopes

^{103}Ru-labelled tris (1, 10 phenanthroline) Ruthenium II Chloride

This complex was prepared as described by Tan, Weston & Hogan. (1971).

^{51}Cr-EDTA This complex was prepared as described by Downes and McDonald (1964) except that the excess chromic ions were precipitated using an excess of NaOH and not NH_4OH. The filtrate was initially diluted to 200 ml with water.

Preparation of infusate

The double marker technique, as described by Tan, Weston and Hogan (1971) was used. Approximately 2 mCi ^{103}Ru-P complex and approximately 1 mCi of the ^{51}Cr EDTA complex were made up to 21 litres with water to give a final ^{103}Ru : ^{51}Cr ratio of 1.5 : 1. The solution was infused into the rumen at a rate of approximately 290 ml/sheep/day over an eight-day period. Once equilibrium was established (after 5 days of infusion), 4 samples from the duodenum and ileum were taken at intervals over a period of 2 days and bulked for each sheep. Ileal samples were collected before duodenal samples.

Preparation of samples for counting and analyses

(a) Duodenal and ileal samples

After thorough mixing, 3 sub-samples were retained as follows/

as follows:-

(1) 2 g for N determination;

(2) 20 g for DM and OM determination;

(3) 3 ml to a tared counting vial (Digesta) weighed and then counted for both ^{103}Ru and ^{51}Cr.

A further 10 ml sample was then centrifuged at 15,000 for 10 mins. and sub-samples prepared as follows:-

(1) 2 ml supernatant for N estimation;

(2) 3 ml supernatant to a tared counting vial weighed and the radioactivity of ^{103}Ru and ^{51}Cr EDTA measured (Filtrate).

(b) Faecal samples

Faecal samples were well mixed and 3 sub-samples treated as follows:-

(1) ~ 3 g to a tared counting vial, weighed and then radioactivity counted;

(2) ~ 1 g for N estimation on dry material;

(3) ~ 20 g for DM and OM estimation.

(c) Urine samples

A 3 ml sample of urine was retained for counting and a further 3 ml for N estimation.

(d) Infusate

A 1 ml sample of infusate solution was diluted to 100 ml and a 3 ml sample counted for radioactivity under the same conditions as the duodenal, ileal, urine and faecal samples. The method for preparing ^{103}Ru-P assumes 100% efficiency of the complexing. However, recovery of ^{103}Ru-P in faeces has been found to be considerably less than 100% and is probably nearer 80%. Thus, the faecal excretion of both the markers was used to calculate the infusion rate.

A/

A two-channel L K B gamma counter was used for radioactivity measurement. Counts were corrected for background and for crossover of ^{103}Ru-P into the ^{51}Cr channel. A standard counting volume of 3 ml or 3 g was used throughout to minimise any effect different sample sizes might have on counting efficiency.

Digesta flow rates were calculated according to the method of Faichney (1975b).

Autopsy Procedure

The sheep were euthanased by stunning with a captive bolt and immediate exsanguination. The abomasa of the parasitised group were removed intact, opened and washed into a graduated bucket as described in General Materials and Methods.

RESULTS

Clinical changes

One of the three parasitised sheep (4) became very inappetent and weak during the study and it was observed to grind its teeth on several occasions. The remaining two sheep in this group remained alert, although a slight drop in food intake was observed in one (7). The sheep in the "bled" group remained alert and keen to eat.

Haematological and biochemical changes

The results are presented in Table V.3. Haematocrit values in the "bled" group fell rapidly from a pre-experimental mean of 0.33 to 0.17 l/l at the end of the experiment. Sheep No. 4 in the parasitised group also became anaemic, haematocrit values falling from a pre-experimental mean of 0.34 to 0.23 l/l at slaughter. Haematocrit values in the two remaining parasitised sheep only fell marginally after/

TABLE V.3: Pre-slaughter haematological, biochemical and parasitological values in parasitised sheep (A), "bled" sheep (B) and uninfected control sheep (C).

	Low Intake			Medium Intake			High Intake		
Category	A	B	C	A	B	C	A	B	C
Sheep No.	4	1	11	7	2	8	3	5	6
Haematocrit l/l.	0.23	0.185	0.33	0.275	0.14	0.26	0.33	0.195	0.32
Total serum protein g/l	60	66	77	65	73	83	83	71	82
Albumin g/l	19	18	33	27	21	28	26	23	29
Urea mmol/l	4.9	2.8	3.0	4.4	3.0	2.7	2.4	4.1	2.9
e.p.g.	19,000	0	0	11,600	0	0	0	0	0
Total faecal eggs/d x 10^6	3.7	0	0	7.8	0	0	0	0	0
Total worm burden	700	0	0	2,500	0	0	0	0	0

after infection. Control values remained in excess of 0.30 l/l throughout.

Mean serum albumin levels in the parasitised and bled sheep were considerably lower than control values, 24, 21 and 30 g/l respectively. Although mean total serum protein values were lower in the parasitised and bled groups relative to control values, 69, 70 and 81 g/l respectively, these control values would be regarded as higher than normal. There were no significant differences in serum urea levels between the group mean values in the parasitised, bled and control groups, being 3.9, 2.9 and 3.3 mmol/l respectively.

Parasitological findings

The results are presented in Table V.3.

Infection established at a very low level in one sheep (3), its faecal egg count (epg) was only 100 on day 28 after infection and at slaughter no parasites were present in the abomasum. The remaining two sheep had increasing faecal egg counts throughout the experiment and had worm burdens of 700 (sheep 4) and 2,500 (sheep 7) at slaughter. Faecal egg counts (epg) were higher in sheep 4 due to reduced faecal output, but when total daily faecal egg output was calculated, values were lower than those of sheep 7. This latter sheep had the larger worm burden at slaughter. The ratio of male : female parasites was 0.96 and 0.76 in sheep 7 and 4 respectively.

No abomasal digests were prepared. Control and "bled" sheep had negative faecal egg counts throughout.

N balance

The results are presented in Tables V.4 and V.5.

One member of the infected group (4) only consumed 130 g DM daily during the experimental period (days 23 - 33 post-infection). Therefore, under the pair-feeding system adopted, one member of each of the bled group (B) and control group (C) was restricted to $\sim$ 220 g DM daily./

daily. This set of three animals is subsequently referred to as the low intake group (Table V.4). During the collection period, the DM intake of this group resulted in daily N intakes of only 2.5 g and 3.6 g in the parasitised and non-parasitised sheep respectively. Faecal N outputs of 1.0, 1.8 and 2.0 g/d in parasitised, bled and control animals respectively resulted in an apparent CP digestibility coefficient of 0.50 which is predictable since ~ 1 g N/d is metabolic in origin (Agricultural Research Council, 1965). The animals in this group, irrespective of whether they were infected, bled or control, had urinary N outputs of ~ 11.5 g N/d which resulted in a negative N balance ranging from -8.5 to -10.6 g N/d.

The remaining two infected sheep (7 and 3) ate considerably more during the experiment (735 and 970 g DM daily respectively) and their pair-fed partners had similar intakes. These two sets of 3 animals (one from each group A, B and C) thus constituted the medium and high intake groups (Table V.4). In these groups, the increased faecal N outputs were to be expected because of the increased N input (~ 14.5 g N/d). The resulting apparent CP digestibility coefficients of 0.60 to 0.68 were again predictable since the proportion of metabolic faecal nitrogen derived N in the faeces was less. Daily urinary N outputs in the medium and high intake groups ranged from 4.1 to 10.3 (g N/d) so that the overall N balance was positive (except control sheep 6) at about 3.5 g N/d.

The mean nitrogen balance and flow rates in the parasitised (A) and bled (B) groups relative to uninfected control sheep (C) are presented in Table V.5.

N flow rates using ^{103}Ru-P and ^{51}Cr EDTA

The results are presented in Table V.4 and V.5.

Great difficulty was experienced in obtaining suitable digesta samples/

TABLE V.4: N balance and nitrogen flow rates (gN/d) in sheep infected with 1000 H. contortus larvae/kg BW (A), "bled" sheep (B) and uninfected control sheep (C)

Parameter \ Sheep No.	Low Intake			Medium Intake			High Intake		
	A	B	C	A	B	C	A	B	C
	4	1	11	7	2	8	3	5	6
Intake	2.1	3.6	3.6	12.0	13.8	13.8	15.8	15.8	15.8
Duodenal	9.7	7.2	5.8	18.0	20.0	10.7	23.8	25.6	18.6
Ileal	1.2	3.0	-	7.1	(6.2)	6.0	(9)	10.8	8.6
Faecal	1.0	1.8	2.0	4.7	5.2	4.4	5.8	6.3	6.2
Urinary	11.4	10.3	12.2	4.1	6.2	4.6	7.1	6.2	10.3
N balance	-10.3	-8.5	-10.6	+3.2	+2.4	+4.8	+2.9	+3.3	-0.7
"Apparent"CP Digestibility Coefficient	0.52	0.50	0.44	0.61	0.62	0.68	0.63	0.60	0.61

TABLE V.5: Mean nitrogen balance and nitrogen flow rates (gN/d) in sheep infected with *H. contortus* (A) or "bled" (simulated infection)(B) relative to uninfected control sheep (C).

	A Infected	B Bled
Intake (gN/d)	- 1.7	0.0
Duodenal Flow	+ 5.5	+ 5.9
Ileal Flow	- 1.5	- 0.6
Faecal N	- 0.4	+ 0.2
Urinary N	- 1.5	- 1.4
N balance	+ 0.56	- 0.17

samples from the 3 sheep on a very low feed intake because of low digesta flow rates. Contamination of the duodenal samples with bile was another complicating factor in this group.

However, increased duodenal N flow was seen in both bled and parasitised animals in all three intake groups relative to their controls. The increase in N flow ranged from 3.6 to 9.8 g N/d in the parasitised and bled animals but overall mean values indicated an increase of 5.5 and 5.9 g N/d in the parasitised and bled group respectively relative to the controls (Table V.5).

However, both ileal N flow and faecal N flow (g N/d) were similar in infected, "bled" and control sheep within each intake group.

DISCUSSION

The results of this study indicate that both sheep with a simulated parasitic infection where 200 ml of blood was infused daily into the abomasum, and parasitised sheep, had increases of 6 g N/d in the digesta flowing from the abomasum relative to uninfected controls. However, within an intake group there were no marked differences in ileal or faecal N flow or in urinary N excretion in the parasitised and bled animals relative to their controls. This would suggest that sheep infected with H. contortus and losing considerable quantities of blood daily into the abomasum are able to reabsorb the lost blood protein in the small intestine.

A certain number of problems were encountered during the study which warrant some discussion since they made interpretation of some aspects of the results difficult, particularly when such a small number of experimental animals were available.

First, there were considerable differences in the level of establishment of the infection. Even in the sheep with the highest worm burden at slaughter, establishment was less than 10%. The reasons for this may include age resistance or the possibility of acquired resistance following previous exposure to infection. However, since it was not possible to prepare abomasal digests, it is not known if significant numbers of inhibited larvae were present.

Secondly, infection resulted in varying degrees of anorexia and this made direct comparison between groups A, B and C difficult. However, at each level of feed intake by the parasitised sheep, it was possible to compare this sheep with its two pair-fed partners.

Thirdly, the problems associated with the estimation of digesta and N flow rates were considerable. The faecal recovery of ^{103}Ru-P

was/

was considerably less than 100% and in the low intake group was less than 50%. This problem may be associated with the complexing of the compound and it is also possible that the ^{103}Ru-P was being adsorbed on to the ruminal epithelium (Rowe, personal communication). Thus the faecal excretion of both markers was used to calculated the "true" infusion rate. There were also considerable difficulties in obtaining sufficient quantity of sample for analysis in the sheep in the low intake group because of the reduction in digesta flow. Biliary contamination of the sample was a further complication in this group, resulting in tracer dilution from non-dietary sources.

The degree of anaemia observed in the "bled" group was similar to that associated with an acute haemonchus infection. If one assumes a daily blood loss of 0.05 ml/parasite (Clark, Kiesel and Goby, 1962) removal of 200 ml blood/day from each sheep would be equivalent to the blood loss associated with a burden of $\sim$ 4,000 worms.

The severity of the anaemia in the parasitised group was considerably less than that of the "bled" group, reflecting the low worm burdens present at slaughter, namely 0, 700 and 2,500 worms. As has been observed in earlier experiments (Chapters II-IV), animals exhibiting the most severe clinical signs, such as weakness, inappetance and teeth grinding, and which are severely anaemic and hypoproteinaemic, do not necessarily have high worm burdens present at slaughter. In this experiment, sheep 4, although more anaemic and exhibiting more severe clinical signs, had a worm burden of 700 at slaughter, in contrast to sheep 7 which, although less anaemic, had a parasite burden of 2,500.

The/

The mean N concentration of six different ovine whole blood samples (mean haematocrit of 0.28 1/1) was 36.7 g/litre. Thus, the calculated daily N loss into the gut from blood sources of the two more heavily parasitised sheep, namely 3 and 4, would be ~2 and 5 g respectively. Blood N in the gut of the sheep with the simulated parasitism would amount to ~7 g/day.

Sheep 3, the remaining sheep in the parasitised group, had increased duodenal N flow despite having no parasites present in the abomasum at slaughter. Furthermore, this sheep did not become anaemic or hypoproteinaemic after infection, and since its faecal egg counts were very low, it is obvious that infection established at a very low level and was of short duration. Thus the source of the increased duodenal N flow is uncertain. However, there are several possibilities as to the origin of the increased N.

First, increased mucus production by mucus producing cells in the gut mucosa and local secretion of antibody are a feature of many helminth infections including haemonchosis (Dobson & Bawden, 1974; Smith, 1977; Salman, 1980; Lloyd, 1981). Both vaccinated adult sheep and haemonchus-naïve adult sheep exposed to primary and secondary infection with H. contortus have been shown to have increased cellular responses in the gut wall. These responses included an increase in the numbers of mucus producing cells with an associated formation of a thick mucus layer on the mucosa (Salman, 1980). In addition, recent reports have suggested that larval challenge of resistant adult sheep with mainly O. circumcincta can be associated with increased plasma protein leak into the gastro-intestinal tract (Yakoob, Holmes & Armour, 1983). Furthermore, the protein leak in the challenged ewes occurred despite a very low parasite establishment (< 3%) and it was suggested that the plasma losses may be associated with a hypersensitivity reaction in the mucosa. In sheep 3 either or both of these factors may have been operating.

The/

The daily urinary N outputs of the animals in the low intake group are surprisingly high and somewhat contrary to expectations (Chapters I to IV). However, the animals used in this experiment were adult, and short-term starvation of the adult ruminant is associated initially with relatively constant blood urea levels but increased urinary N excretion (Church, 1971). However, if the source of the increased urinary N is catabolised body tissues, it is difficult to explain the low blood urea levels observed in the present experiment.

A recent study on N digestion in cannulated lambs infected with T. colubriformis was conducted by Poppi, MacRae, Corrigall & Coop (1981). They too used the dual phase markers ^{105}Ru-P and ^{51}Cr-EDTA to measure N digestion and flow rates. In addition, they measured plasma protein leak using $^{51}CrCl_3$ and digestibility and absorption of protein between the duodenum and ileum using ^{35}S-labelled bacteria. They found no difference between infected and control lambs in the digestibility of the labelled bacteria or in apparent N digestibility. Increased ileal N flow was observed in the infected lambs and they suggested that the principal source of this may be endogenous protein, in particular sloughed cells and mucin. Faecal N in the infected lambs was similar to controls but urinary N was slightly increased.

The findings of the experiment described in this chapter tend to substantiate the results of the more conventional techniques, such as digestibility and N balance trials, conducted in the earlier experiments. They add support to the view that the depressed productivity observed in ovine haemonchosis is probably the result of a combination of reduced feed intake and increased energy requirements for blood and tissue regeneration rather than through a protein drain as a result of a failure to reabsorb the blood protein lost into the abomasum.

CHAPTER VI

An investigation into the ability of 8-month old Scottish Blackface lambs kept on either a high or low protein diet to respond to vaccination with γ-irradiated *H. contortus* larvae

INTRODUCTION

In the experiments conducted so far, when lambs of varying ages and breeds were experimentally infected with a single dose of H. contortus larvae ranging from 50 to 350 larvae/kg BW, there was no real evidence to suggest that the protein content of the diet influenced parasite establishment, as judged by parasitological and pathophysiological parameters. However, as the infective dose used was increased to 350 larvae/kg BW, it became obvious that the lambs fed the low protein diet suffered more severe clinical disease despite harbouring similar worm burdens and suffering similar gastric blood losses as the better fed lambs. It was thus suggested that this difference reflected the different physiological reserves of the two dietary groups.

However, in the trickle experiment (Chapter IV), where lambs were subjected to repeated infection with small numbers of H. contortus larvae from the age of 4 months, it soon became obvious that many of the lambs on the high protein diet had developed resistance to further infection since several lambs in this dietary group had negative faecal egg counts before the end of the experiment, and had either no worms present or small worm burdens at slaughter. In contrast, mortality was high in the lambs fed the low protein diet and the surviving lambs had relatively high worm burdens at slaughter, suggesting that the immune response was impaired in these animals. In view of these findings, it was considered important to investigate the influence of dietary protein on the immune response to H. contortus in older lambs, as judged by their response to vaccination with radio-attenuated larvae.

It has been known for a considerable time that immunisation against H. contortus can be achieved under experimental conditions by the/

the use of X or γ-irradiated larvae (Jarrett, Jennings, McIntyre, Mulligan & Sharp, 1959 and 1961; Mulligan, Gordon, Stewart & Wagland, 1961). However, the success of the vaccination procedure depends on both the age and the breed of sheep used. For example, it is impossible to successfully immunise Scottish Blackface lambs using irradiated larvae if the lambs are less than 7 months of age (Urquhart et al, 1962; Urquhart, Jarrett, Jennings, McIntyre, Mulligan & Sharp, 1966a; Urquhart, Jarrett, Jennings, McIntyre & Mulligan, 1966b; Benitez-Usher, Armour, Duncan, Urquhart & Gettinby, 1977). Further evidence that the age of the lambs is an important factor in the development of resistance to H. contortus was provided by the work of Manton et al (1962) who used sensitising doses of normal larvae before challenge and found that 10-12 month old lambs completely resisted challenge, whilst 2-4 month old lambs failed to do so and were as severely affected as previously uninfected controls. The influence of breed on the development of resistance to H. contortus following vaccination was illustrated by the findings of Lopez & Urquhart (1967) who, although using the same vaccination regimen which is highly successful in Blackface sheep, were unable to protect 7 month old Kenyan Merino sheep from challenge.

The breed of lambs used in this experiment were Scottish Blackface and vaccination started when the lambs were 8 months old. Since the maximum level of protection against challenge has been achieved consistently using 40 kr of irradiation from either an X-ray or γ-ray source (Sivanathan, 1982), a regimen of two doses of 10,000 γ-irradiated larvae (40 kr) one month apart was adopted. The lambs were challenged with 10,000 normal larvae 4 weeks after the second vaccination.

MATERIALS/

MATERIALS AND METHODS

Experimental design

Scottish Blackface lambs fed either a high or low protein diet from the age of 7 months were vaccinated with 10,000 γ-irradiated larvae (40 kr) of *H. contortus* on two occasions, one month apart, starting when the lambs were 8 months of age. Four weeks after the second vaccination, the lambs were challenged with a single dose of normal 10,000 *H. contortus* larvae *per os*. An equal number of unvaccinated lambs, also kept on the different diets, were also infected with the same dose of larvae at this time (challenge controls). The lambs were killed 28 days after challenge when worm burdens were assessed. Blood samples for routine haematological analyses were collected and body weights were recorded on a weekly basis throughout the experiment. Faecal samples were collected on several occasions before challenge and on days 16, 19, 21, 23, 26 and 28 after challenge.

Experimental animals

Twenty 7 month old Blackface lambs of both sexes were divided into high and low protein dietary groups at the age of 7 months, as described in General Materials and Methods. One month later they were further divided into "vaccinated" and "challenge control" groups, each group consisting of 5 lambs.

Feeding and housing

The lambs were housed in penned accommodation throughout. The high and low protein diets used were identical to those used in previous experiments (Chapters II, III and IV). They were fed the diets ready-mixed to appetite, twice daily, and water was available *ad libitum*.

Preparation/

Preparation and administration of γ-irradiated and normal larvae

The γ-irradiated larvae were prepared and supplied by the Wellcome Laboratories for Experimental Parasitology; 10,000 infective larvae exposed to 40 kr from a ^{60}Co source was used as the immunising dose. The normal larvae were prepared as described in General Materials and Methods. Both the γ-irradiated and normal larvae were given *per os* as described in General Materials and Methods.

RESULTS

Clinical and body weight changes

All the lambs remained alert throughout the experiment. Vaccinated and control lambs on the high protein diet had a DLWG of 116 and 129 g respectively, whilst vaccinated and control lambs on the low protein diet had a DLWG of 96 and 123 g respectively.

Haematological changes (Appendix E, Table 1)

Haematocrit values fell gradually in all four groups of lambs as they became more accustomed to being handled and bled (Fig. VI.1). A rapid fall in haematocrit values occurred after challenge only in the challenge control group on the low protein diet, values falling from 0.35 l/l at challenge to 0.23 l/l three weeks later. The fall in haematocrit was associated with a sharp rise in MCV values at weeks 3 and 4 post-challenge (Fig. VI.2). Red cell indices in the remaining three groups remained within the normal range throughout, although a slight rise occurred in MCV values in the challenge control group fed the high protein diet.

Parasitological findings

During the vaccination period and before challenge, faecal samples from both the vaccinated and challenge control groups remained negative for parasite eggs.

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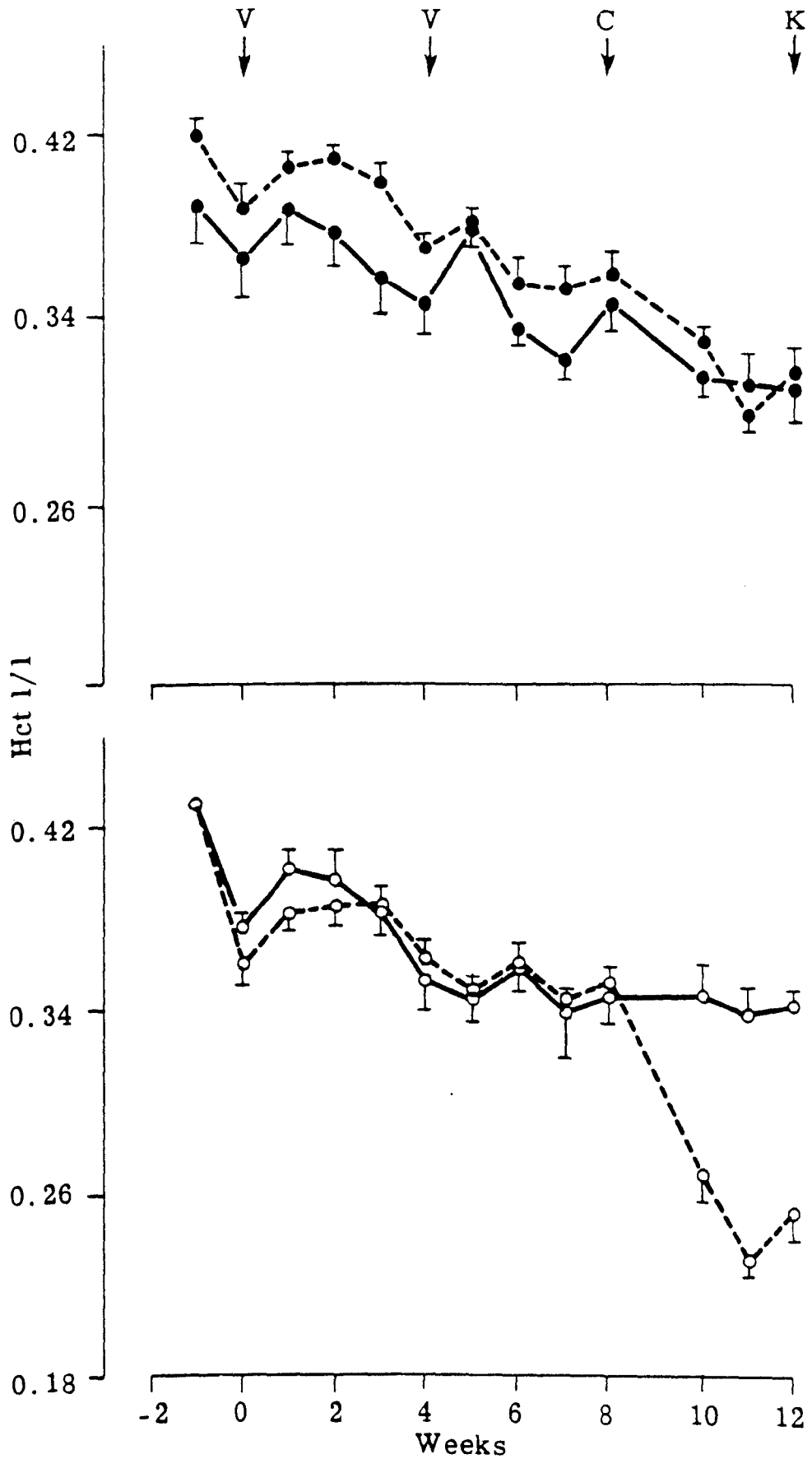

Fig. VI.1: Mean (± SE) haematocrit values of vaccinated and challenge control lambs fed either a high or low protein diet.

●——● High protein vaccinated o——o Low protein vaccinated

●-----● High protein challenge control o-----o Low protein challenge control

V = vaccination C = challenge K = kill

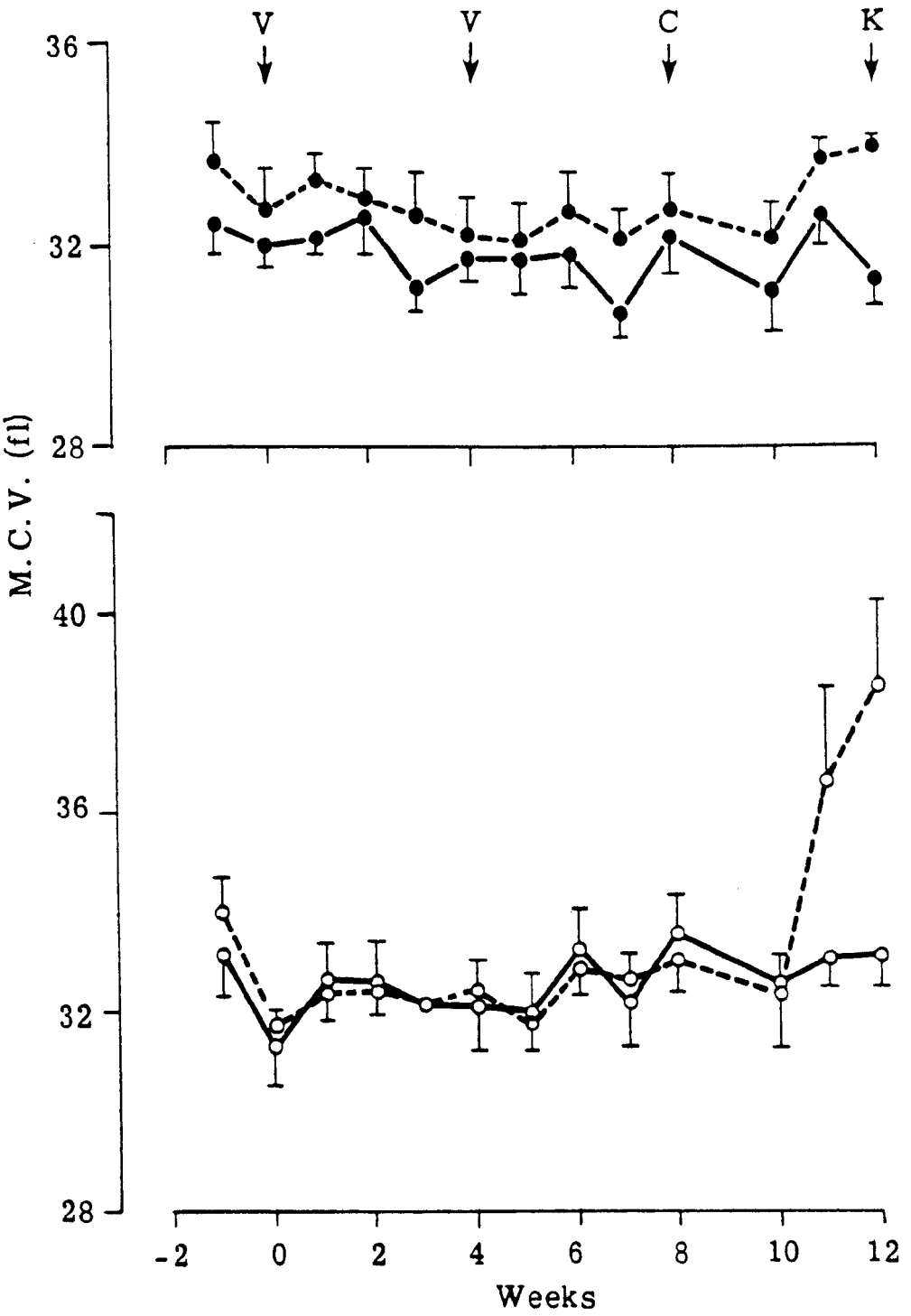

Fig. VI.2: Mean (± SE) MCV values of vaccinated and challenge control lambs fed either a high or low protein diet

●——● High protein vaccinated o——o Low protein vaccinated
●-----● High protein challenge control o-----o Low protein challenge control

V = vaccination C = challenge K = kill

By day 16 following challenge, both groups of challenge control lambs had parasite eggs present in their faeces. Counts rose rapidly to reach maximum values of 14,660 epg (± SE 5,298) on day 28 in the high protein group, and 26,440 epg (± SE 6,860) on day 26 in the low protein group (Fig. VI.3).

In contrast, only one vaccinated lamb from the high protein group (R4) had positive faecal egg counts on 3 occasions from day 23 after challenge (maximum values of 5,010 epg on day 28). Three vaccinated lambs from the low protein group (W2, 3 and 5) also had positive faecal egg counts on day 23 (50 - 200 epg) but only two of these lambs, W2 and W5, had positive counts on day 28, 1,100 and 3,650 epg respectively.

Individual worm burdens are presented in Table VI.1. Both groups of challenge control lambs had high worm burdens at slaughter, mean values of 2,550 and 2,970 in the high and low protein groups respectively. In contrast, mean group worm burdens of the vaccinated lambs were 200 and 290 in the high and low protein groups respectively. Of the vaccinated sheep which had positive faecal egg counts, R4 had 550 parasites present at slaughter, of which 200 were stunted females, and W2 and W5 had 250 and 1,200 parasites present respectively, of which 100 and 300 were stunted females.

The difference in worm burdens between vaccinated and challenge controls within a dietary group was highly significant ($P < 0.01$). However, the difference in burdens between the two groups of vaccinated lambs was not significant, nor was there any significant difference in worm burdens between both dietary groups of challenge control lambs. Stunted female parasites were only observed in the vaccinated lambs.

DISCUSSION/

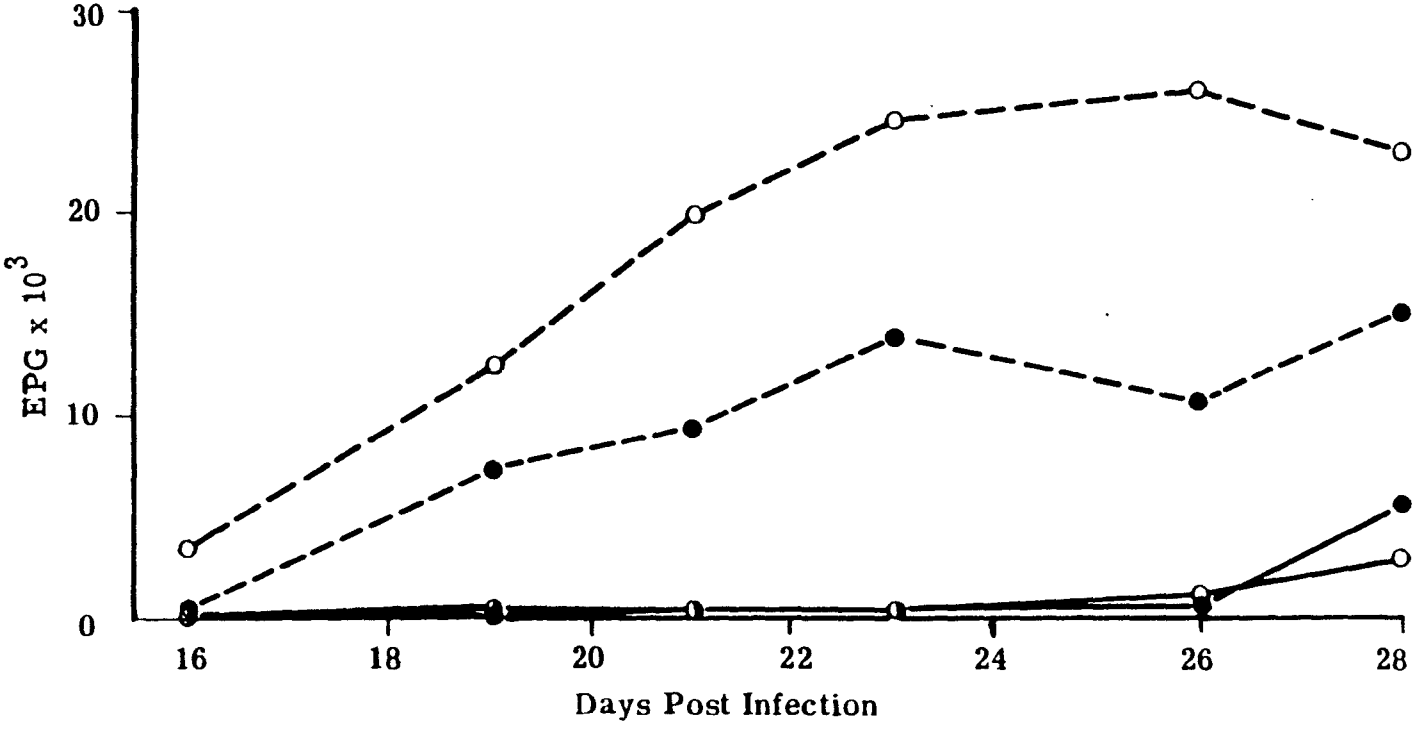

Fig. VI.3: Mean faecal egg counts (epg) of vaccinated lambs (2 x 10,000 γ-irradiated H. contortus larvae) fed either a high or low protein diet and their respective challenge controls.

●——● High protein vaccinated
●-----● High protein challenge control
o——o Low protein vaccinated
o------o Low protein challenge control

TABLE VI.1: Individual worm burdens of vaccinated lambs fed either a high or low protein diet and their respective challenge controls 28 days after challenge.

Group	No.	Larval stages	Adults				Total
			Stunted		Normal		
			♂	♀	♂	♀	
High Protein Vaccinated	R 1	0	0	50	0	0	50
	2	0	0	0	0	0	0
	3	0	0	50	0	0	50
	4	0	0	200	200	150	550
	5	0	0	250	100	0	350
	$\bar{x}$ S.E.						200 (107)
High Protein Challenge Control	FR11	0	0	0	1800	1400	3200
	12	0	0	0	750	1400	2150
	13	0	0	0	1450	1700	3150
	14	0	0	0	1500	1000	2500
	15	0	0	0	900	850	1750
	$\bar{x}$ S.E.						2550 (282)
Low Protein Vaccinated	W 1	0	0	0	0	0	0
	2	0	0	100	0	150	250
	3	0	0	0	0	0	0
	4	0	0	0	0	0	0
	5	0	0	300	300	600	1200
	$\bar{x}$ S.E.						290 (233)
Low Protein Challenge Control	LY21	0	0	0	850	1350	2200
	22	0	0	0	2050	1800	3850
	23	0	0	0	1400	650	2050
	24	0	0	0	1550	1500	3050
	25	0	0	0	2500	1200	3700
	$\bar{x}$ S.E.						2970 (371)

DISCUSSION

The principal feature of the present experiment was that vaccination with two doses of 10,000 γ-irradiated H. contortus larvae one month apart was successful in stimulating strong resistance to a challenge infection of 10,000 normal larvae in 8 month old Scottish Blackface lambs, regardless of dietary status.

Prior to challenge, the vaccinated lambs continued to gain weight, they maintained normal haematocrit values and their faeces remained negative for parasite eggs. After challenge, although some vaccinated lambs from both dietary groups had positive faecal egg counts, these were of a relatively small magnitude (maximum value of 5,010) and were considerably lower than those of the challenge controls whose faecal egg counts rose rapidly to values in excess of 14,000 epg. The worm burdens were also greatly reduced in the vaccinated groups and the % reduction in parasite establishment relative to the challenge control groups was in the order of 90-92% in both vaccinated groups.

The haematological changes observed during the vaccination and challenge period are worthy of comment. Haematocrit values fell gradually in all four groups of lambs during the vaccination period. This was doubtless due to physiological adaptation to the bleeding procedure (Hodgetts, 1961; Greenwood, 1977). However, after challenge, the challenge control lambs from the low protein group developed a moderately severe anaemia, haematocrit values falling to 0.23 l/l. A sharp rise in MCV values occurred one week after the first rapid fall in haematocrit in this group. In contrast, haematocrit values of the challenge controls fed the high protein diet remained similar to those of the vaccinated group. However, a slight rise in MCV values occurred in this group of controls before slaughter.

These differences in the haematological responses between the two groups of challenge control lambs, together with the differences in faecal egg counts, might be taken to indicate that either establishment was greater in the lambs from the low protein group or that the worms were more fecund or both. However, worm burdens were similar in both groups of challenge controls, thus the differences in faecal egg counts and haematocrits might reflect alterations in the fecundity of the parasite. Since total daily faecal egg output was not measured, nor feed intake recorded, one can only speculate as to what was responsible for the difference in faecal egg counts. However, from the work conducted to date in this thesis, it is more likely that reduced faecal output as a result of reduced feed intake was responsible for the differences in faecal egg counts, rather than increased fecundity. Furthermore, the difference in the haematological responses between the two groups may be due to haemodilution, as has previously been demonstrated in earlier experiments in this thesis (Chapters II, III and IV). These findings emphasise the care required when investigating the influence of dietary factors on parasite infestation, particularly when conclusions are based on faecal egg counts and changes in the peripheral blood only.

There is an apparent discrepancy between the findings of the present experiment and those of the trickle experiment (IV). In the present study, dietary status did not influence the ability of 8 month old lambs to be successfully vaccinated against H. contortus. However, in the earlier experiment (IV), in which younger lambs received repeated low levels of infection, diet apparently influenced the development of resistance, since lambs fed a low protein diet failed to develop resistance to continuing reinfection. A number of factors may be responsible for this discrepancy. For example, the level and duration of the antigenic priming was clearly different, viz:- 10,000 irradiated

larvae on two occasions compared with 600 normal larvae/week for 17 weeks. However, the age of the animals is probably the most crucial factor since, as was mentioned in the introduction to this experiment, it has been repeatedly demonstrated that 8 month old haemonchus-naive Blackface lambs can be successfully vaccinated against H. contortus, whereas younger lambs cannot, due to "immunological unresponsiveness" (Urquhart et al, 1962; Urquhart et al, 1966a,b; Benitez-Usher et al, 1977; Urquhart, 1980). The Finn Dorset/Dorset Horn lambs used in the trickle experiment (IV) were 4 months of age at the time of the initial infection, and could thus be regarded as being immunologically unresponsive at this age. Although it is not known at what age responsiveness occurs in these two breeds, the findings of the trickle experiment suggest that this age is probably $\sim$ 7 months, since the development of resistance in some of the better fed lambs started 10 weeks after the initial infection when the lambs were between 6 and 7 months of age. Factors which influence the timing of the onset of immunological responsiveness have not been clearly defined, but it is not unreasonable to suggest that, in common with other physiological functions, e.g. puberty, body weight and condition may be more critical than chronological age, and thus the low protein diet may have delayed its onset.

The results of the present study indicate that, under the conditions of this experiment, dietary protein status did not influence the ability of 8 month old Blackface lambs to successfully respond to vaccination against H. contortus. In contrast, the results of an earlier experiment (IV), in which younger animals received a trickle infection, indicate that dietary status could influence the development of resistance to H. contortus, possibly as a result of alterations in the onset of immunological competence.

GENERAL CONCLUSIONS AND DISCUSSION

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The experiments described in this thesis have provided a considerable amount of information on the influence of dietary protein on the pathogenesis of H. contortus infection in sheep. Furthermore, they have emphasised the necessity for a strict experimental protocol and the justification for using radioisotopic tracer techniques in such experiments, if meaningful conclusions are to be drawn from the findings.

The main conclusions to be derived from these studies were that the protein content of the diet per se did not appear to influence the establishment of a single primary infection with H. contortus but, when sheep were fed a high protein diet and subjected to repeated infection with small numbers of infective larvae, they were more likely to develop resistance to continuing infection. Furthermore, since sheep fed a low protein diet suffered more severe clinical disease, despite having similar gastric blood losses, it was suggested that such sheep have reduced physiological reserves relative to better fed sheep. Infection had little effect on the apparent digestibility and absorption of the experimental diets, and whether an infected lamb remained in positive N balance or not was principally dependent on the N intake as, with few exceptions, faecal and urinary N output were similar in both infected and pair-fed control lambs. Greatly increased urinary N levels were only observed in adult sheep on a very low daily N intake. Direct measurement of nitrogen flow through the digestive tract of parasitised sheep indicated that they were able to reabsorb the lost blood protein in the small intestine and confirmed the findings of the more conventional digestibility/

digestibility and N balance studies.

The principal pathogenic feature of haemonchus infection is the development of anaemia. Fourie (1931) was the first to describe in detail the anaemia associated with haemonchosis and, despite the technical limitations of the analytical techniques used at that time, clearly demonstrated that the anaemia was haemorrhagic in character. Thus,the increase in red cell size and mean corpuscular haemoglobin, anisocytosis and basophilic stippling observed was indicative of increased erythropoiesis. The development of a microcytic, hypochromic anaemia was associated with marrow exhaustion. In the present experiments, the type of anaemia observed after infection was dependent on the level of infection used. At low levels of infection (50 larvae/kg BW) a moderate normocytic, normochromic anaemia developed. There was no evidence of the macrocytosis or marrow exhaustion observed by Allonby (1974) in field cases of "chronic" haemonchosis. As the infective dose was increased in the present experiments, the anaemia observed was typically macrocytic in character. Accelerated erythropoiesis was confirmed by the radioisotopic studies which also emphasised the necessity for the use of such techniques, particularly when comparing different dietary groups of infected lambs. For example, although the peripheral blood picture suggested a more severe anaemia in animals which were fed a low protein diet, the radioisotopic studies indicated that, in many cases, haemodilution was responsible for such differences.

A true iron-deficiency anaemia,typified by a microcytic, hypochromic blood picture, low serum iron levels and low % saturation of transferrin, was only observed in two lambs in the acute experiment (IIIa), one from each dietary group, and one lamb from the trickle experiment/

experiment (IV) from the low protein group. Therefore, there is little evidence to support the suggestion by Allonby and Dargie (1973), Dargie and Allonby (1975) and Dargie (1975) that the anaemia of haemonchosis will invariably develop into a typical iron-deficiency anaemia. From the findings of the"iron absorption" studies in the acute and trickle experiments, it was clear that dietary iron absorption was high, both in the infected lambs and in their controls. There are several important points to emerge from this finding.

First, in the acute infection, there was a rapid loss of a considerable quantity of blood into the abomasum in the infected lambs, with a fall in serum iron levels and % saturation of transferrin relative to the controls. However, dietary iron absorption increased and was in excess of control values. Secondly, the gastric blood losses in the trickle lambs occurred more gradually and were less severe, resulting in a less dramatic fall in serum iron levels and little change in the % saturation of transferrin. Thus, not surprisingly, dietary iron absorption was similar to control values. Therefore, dietary iron absorption appeared to adjust to the needs of the animal and depended on the rate and extent to which body iron reserves were depleted by the infection. The critical factor in the development of an iron deficiency anaemia would appear to be the level of feed intake, as severe anorexia was invariably a feature common to the lambs exhibiting iron-deficiency anaemia in the present experiments. Obviously, under such circumstances, it would not be surprising if an iron-deficiency anaemia developed, since dietary iron intake would be reduced, and there is also apparently no reabsorption of the haemoglobin-iron lost into the gastrointestinal tract. Whether

anorexia/

anorexia was a feature of the iron-deficiency anaemia observed by Allonby and Dargie (1975), Dargie and Allonby (1975) and Dargie (1975) is not known, though their earlier studies (Allonby and Dargie, 1973) suggested that anorexia was not a feature of haemonchus infection under their experimental conditions.

A third feature of the iron studies which is worthy of comment is the apparently high dietary iron absorption in the control lambs. Due to accidental contamination of the sugar beet pulp, the iron content of both experimental diets was high and provided 980 to 1,340 mg Fe/kg DM. Although information on the iron requirements of sheep is scant, the minimal requirements are thought to be 30 mg Fe/kg DM (A.R.C. 1980) and it was also suggested that 500 mg Fe/kg diet should be regarded as the maximum tolerable by ruminants. Since the control lambs apparently absorbed quantities in excess of this maximum level without any obvious ill-effect, it is questionable whether the A.R.C. (1980) recommended daily allowances are, in fact, sufficient for young, rapidly growing lambs. This finding of apparently high dietary iron requirements in young, fast-growing lambs supports the findings of Scott et al (1971) who found that iron supplementation reduced mortality in lambs infected with high levels of H. contortus and, in addition, prevented the so-called "anaemia of growth" observed in young, rapidly growing, uninfected control lambs.

There was no evidence to suggest that the animals fed the low protein diet had an impaired erythropoietic response to the parasite-induced gastric haemorrhage. At the higher levels of infection, both dietary groups of infected lambs mounted an equally vigorous erythropoietic response, as indicated by the persistant rise in MCV values and the findings of the ferrokinetic studies. However, the level of protein in the diet appeared to

influence/

influence the alterations in albumin metabolism observed after infection. In general, depletion of intra and/or extravascular albumin pools was more commonly observed in the infected lambs fed the low protein diet. Furthermore, it was suggested that feeding a low protein diet may delay the onset of immunological responsiveness since the trickle infected lambs fed the low protein diet failed to develop resistance to continuing infection whereas many of the better fed lambs did. However, dietary protein did not influence the ability of "immunologically mature" lambs to respond to vaccination with X-irradiated H. contortus larvae.

Finally, the present studies confirmed the value of radioisotopic tracer techniques in evaluating factors influencing the pathogenicity of parasitic infections. Such techniques provide direct measurement of the pathophysiological changes associated with infection, in contrast to more conventional techniques such as faecal egg counts and haematological analyses which, at best, provide but an indirect measurement of pathogenicity.

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APPENDIX A

Appendix to Chapter I

TABLE 1: Mean (± SE) haematological values before and after infection with 50 *H. contortus* larvae/kg BW in yearling Finn Dorset wether lambs fed either a high or low protein diet.

Weeks pre- and post-infection	Group (n=5)	Hb g/dl	RCC cells x 10^{12}/l	Hct l/l	MCH pg	MCV fl	MCHC g/dl
-1	High Protein	-	-	0.335(0.007) •••	-	-	-
	Low Protein	-	-	0.285(0.013)	-	-	-
0	High Protein	-	-	0.32 (0.012) •	-	-	-
	Low Protein	- -	-	0.28 (0.016)	-	-	-
+3	High Protein	12.1(0.23) •	10.1(0.20)	0.32 (0.008)	12.0(0.10) ••••	32.1(0.43)	37.5(0.48) •
	Low Protein	10.6(0.53)	9.3(0.47)	0.29 (0.016)	11.4(0.05)	31.4(0.22)	36.4(0.28)
+6	High Protein	10.3(0.21)	8.4(0.09)	0.27 (0.008)	12.3(0.15) •••	32.2(0.81)	38.2(0.68)
	Low Protein	9.7(0.29)	8.3(0.28)	0.26 (0.011)	11.7(0.13)	31.5(0.49)	37 (0.48)
+9	High Protein	10.1(0.08) •	8.4(0.08)	0.26 (0.004)	12.0(0.13) •••	31.7(0.57)	38 (0.35) •
	Low Protein	9.0(0.39)	8.0(0.32)	0.25 (0.012)	11.3(0.17)	30.7(0.63)	36.7(0.29)
+12	High Protein	-	-	0.27 (0.009)	-	-	-
	Low Protein	-	-	0.27 (0.017)	-	-	-
+15	High Protein	10.3(0.27) ••	8.5(0.2)	0.27 (0.007) •	12.1(0.16) •	32.2(0.24) •	37.6(0.24)
	Low Protein	8.9(0.36)	7.7(0.32)	0.24 (0.010)	11.6(0.12)	30.8(0.47)	37.4(0.28)
+19	High Protein	10.0(0.28) ••	8.4(0.26)	0.27 (0.008) •	11.9(0.13) ••••	31.9(0.50)	36.7(0.42)
	Low Protein	8.5(0.43)	7.7(0.37)	0.23 (0.012)	11.1(0.11)	30.6(0.43)	36.5(0.42)
+23	High Protein	10.1(0.23) •	8.6(0.24)	0.27(0.008)	11.8(0.11) ••••	31.3(0.46) •	37.7(0.50)
	Low Protein	9.2(0.27)	8.3(0.23)	0.25(0.008)	11.0(0.13)	30.0(0.27)	36.8(0.39)
+26	High Protein	10.4(0.40) •	9.0(0.31)	0.285(0.010)	11.4(0.18)	31.5(0.65)	36.4(0.45)
	Low Protein	9.1(0.37)	8.3(0.33)	0.26(0.012)	11.1(0.07)	31.0(0.54)	35.8(0.54)
+28	High Protein	-	-	0.26(0.014)	-	-	-
	Low Protein	-	-	0.23(0.013)	-	-	-

• P = < 0.05 •• P = <0.02 ••• P = 0.01 •••• P = <0.001

TABLE 2: Mean (± SE) total serum protein, albumin and globulin values before and after infection with 50 *H. contortus* larvae/kg BW in yearling Finn Dorset wether lambs fed either a high or low protein diet.

Weeks pre- and post-infection	Group	Total Protein g/l	Albumin g/l	Globulin g/l
-2	HP	71 (1.29)	38 (0.86)	34 (1.78)
	LP	74 (1.41)	37 (0.90)	38 (1.71)
0	HP	70 (1.71)	37 (0.45)	32 (1.54)
	LP	74 (1.29)	37 (0.93)	38 (1.46)
6	HP	66 (1.44)	35 (0.49)	30 (1.78)
	LP	68 (0.87)	33 (0.49)	34 (1.08)
12	HP	62 (0.86)	34 (0.40)	29 (1.17)
	LP	69 (1.81)	33 (0.66)	35 (1.54)
18	HP	64 (0.84)	32 (0.20)	32 (0.80)
	LP	68 (1.84)	31 (0.48)	37 (2.12)
26	HP	64 (1.25)	34 (0.75)	31 (1.32)
	LP	68 (1.89)	31 (1.15)	37 (2.93)

TABLE 3: Mean (± SE) haematological values in infected (50 *H. contortus* larvae/kg BW) and control 8 month old Merino lambs kept on a low protein diet.

Weeks pre- and post-infection	Group	n	Hb g/dl	RCC cells x 10^{12}/l	Hct l/l	MCH pg	MCV fl	MCHC g/dl
- 2	Infected	5	-	-	0.31(0.01)	-	-	-
	Control	4	-	-	0.32(0.01)	-	-	-
0	Infected	5	-	-	0.32(0.01)	-	-	-
	Control	4	-	-	0.31(0.01)	-	-	-
+ 3	Infected	5	9.3(0.54)	8.2(0.37)	0.26(0.01) *	11.30(0.18)	32(0.6)	35(0.8)
	Control	4	9.8(0.20)	8.9(0.23)	0.30(0.01)	11.0(0.21)	33(0.6)	33(0.7)
+ 6	Infected	4	8.3(0.27) ***	7.0(0.21) ***	0.24(0.01) *	11.8(0.05) ***	33(0.5)	35(0.4)
	Control	3	9.6(0.10)	8.5(0.15)	0.27(0.00)	11.3(0.11)	32(0.5)	35(0.3)
+10	Infected	4	8.2(0.24) *	7.3(0.20) ***	0.23(0.01) *	11.1(0.11)	32(0.5)	35(0.5)
	Control	3	9.3(0.32)	8.7(0.10)	0.27(0.01)	10.7(0.26)	31(0.7)	34(0.2)
+12	Infected	4	8.4(0.31) *	7.2(0.19) ***	0.23(0.01) ***	11.6(0.18) **	32(0.2) **	36(0.6)
	Control	3	9.6(0.21)	8.5(0.15)	0.28(0.00)	11.3(0.12)	33(0.2)	35(0.4)
+14	Infected	4	8.3(0.38)	7.2(0.30) *	0.23(0.01)	11.6(0.09)	32(0.5)	36(0.6)
	Control	3	9.1(0.31)	8.1(0.13)	0.26(0.01)	11.3(0.27)	32(0.2)	35(0.7)
+16	Infected	4	8.1(0.30) *	7.1(0.25) ***	0.23(0.01) *	11.4(0.10)	32(0.2)	35(0.2)
	Control	3	9.3(0.34)	8.4(0.20)	0.27(0.01)	11.1(0.13)	32(0.4)	35(0.3)
+18	Infected	4	7.6(0.18)	6.7(0.06) ***	0.22(0.00)	11.3(0.16)	33(0.32)	35(0.5)
	Control	3	8.3(0.32)	7.5(0.14)	0.24(0.01)	11.1(0.20)	32(0.6)	35(0.10)
+20	Infected	4	7.4(0.12) *	6.4(0.09) ***	0.20(0.01) *	11.5(0.18)	32(0.8)	36(0.4)
	Control	3	8.3(0.24)	7.3(0.15)	0.235(0.006)	11.3(0.15)	32(0.6)	35(0.3)
+22	Infected	4	7.0(0.06) **	6.3(0.05) ****	0.20(0.00) **	11.2(0.05)	32(0.2)	35(0.1)
	Control	3	8.4(0.38)	7.6(0.12)	0.24(0.01)	11.1(0.34)	32(0.3)	35(0.9)
+25	Infected	4	7.6(0.13) *	6.8(0.13) ***	0.21(0.00) ****	11.2(0.13)	31(0.3)	36(0.5)
	Control	3	8.2(0.22)	7.5(0.09)	0.24(0.00)	10.9(0.17)	32(0.2)	34(0.8)

* P <0.05 *** p <0.01

** P <0.02 **** p <0.001

TABLE 4 : Mean (± SE) haematological values in infected (50 H. contortus larvae / kg BW) and control 6 month old Blackface lambs kept on a low protein diet.

Weeks pre- and post-infection	Group	n	Hb g/dl	RCC cells x 10^{12}/l	Hct l/l	MCH pg	MCV fl	MCHC g/dl
- 2	Infected	5	-	-	0.339(0.009)	-	-	-
	Control	4	-	-	0.339(0.009)	-	-	-
0	Infected	5	-	-	0.309(0.011)	-	-	-
	Control	4	-	-	0.305(0.012)	-	-	-
+ 3	Infected	5	10.7(0.461)	9.5(0.313)	0.30 (0.011)	11.3(0.253)	32.0(0.447)	35.6(0.877)
	Control	4	10.9(0.445)	9.4(0.38)	0.29(0.014)	11.6(0.205)	31.0(0.650)	37.3(0.945)
6	Infected	5	10.7(0.537)	9.5(0.349)	0.29(0.013)	11.3(0.252)	30.1(0.528)	37.5(0.188)
	Control	4	11.5(0.475)	10.1(0.500)	0.31(0.018)	11.5(0.210)	30.5(0.340)	37.6(0.855)
10	Infected	5	11.2(0.586)	10.0(0.335)	0.30(0.008)	10.9(0.263)	29.4(0.760)	37.2(1.37)
	Control	4	11.7(0.195)	10.6(0.012)	0.32(0.007)	11.1(0.225)	30.4(0.675)	36.5(0.345)
12	Infected	5	10.3(0.487) *	9.0(0.304)	0.28(0.014)	11.5(0.179)	31.6(0.631)	36.3(0.485)
	Control	4	11.7(0.19)	9.8(0.25)	0.315(0.010)	12.0(0.13)	32.0(0.255)	37.3(0.645)
14	Infected	5	10.6(0.613)	9.1(0.367)	0.29(0.016)	11.7(0.313)	31.7(0.716)	36.9(0.318)
	Control	4	11.8(0.045)	9.8(0.095)	0.32(0.004)	12.0(0.095)	32.5(0.190)	36.8(0.41)
16	Infected	5	9.7(0.470) ***	8.5(0.309) **	0.26(0.012) **	11.4(0.215) *	30.5(0.501) *	37.4(0.252)
	Control	4	11.6(0.20)	9.6(0.21)	0.31(0.009)	12.0(0.125)	32.2(0.33)	37.4(0.63)
18	Infected	5	8.8(0.496) **	7.8(0.349) **	0.25(0.013) *	11.3(0.156)	31.9(0.376)	35.3(0.331)
	Control	4	11.2(0.54)	9.4(0.355)	0.30(0.015)	12.0(0.265)	32.4(0.415)	37 (0.550)
20	Infected	5	9.4(0.452) *	8.3(0.273) **	0.26(0.012) *	11.3(0.335)	31.2(0.908)	36.5(0.[illegible])
	Control	4	10.9(0.250)	9.4(0.220)	0.29(0.008)	11.7(0.195)	31.1(0.240)	37.5(0.750)
22	Infected	5	8.9(0.415) *	7.9(0.255) *	0.25(0.009)	11.2(0.255)	31.4(0.299)	35.7(0.532)
	Control	4	10.0(0.165)	8.8(0.195)	0.27(0.004)	11.4(0.165)	31.1(0.31)	36.4(0.305)
26	Infected	5	8.9(0.541)	8.1(0.376)	0.25(0.001)	11.0(0.188)	30.6(0.492)	35.9(0.500)
	Control	4	9.5(0.250)	8.5(0.120)	0.26(0.006)	11.2(0.165)	30.3(0.470)	36.8(0.470)

* P = < 0.05
** P = < 0.02
*** P = < 0.01
**** P = < 0.001

TABLE 5: Mean (± SE) total serum protein, albumin and globulin values in infected (50 H. contortus larvae/kg BW) and control 8 month old Merino lambs kept on a low protein diet.

Time (weeks)	Group	Total Protein g/l	Albumin g/l	Globulin g/l
-4	Infected	65 (1.39)	38.2 (0.58)	26.8 (1.61)
	Control	64 (1.25)	37.5 (1.05)	26.3 (2.0)
-1	Infected	66.2 (1.30)	36.4 (1.03)	29.8 (1.39)
	Control	67 (0.65)	36.8 (1.30)	30 (1.40)
+3	Infected	67.8 (2.91)	33.0 (0.95)	34.8 (3.26)
	Control	64 (0.00)	34.7 (1.70)	29.3 (1.70)
+6	Infected	62.5 (1.00)	32.0 (0.40)	30.5 (1.20)
	Control	60 (1.21)	32.7 (0.87)	27 (0.98)
+12	Infected	62.0 (1.00)	31.0 (0.65)	31.3 (1.25)
	Control	63 (2.19)	34.7 (1.44)	28.3 (0.87)
+18	Infected	59.3 (0.65)	28.5 (0.65)	30.8 (0.65)
	Control	60 (1.50)	32 (0.98)	28 (1.15)
+24	Infected	62.8 (0.50)	31.0 (0.25)	32 (0.65)
	Control	60 (1.44)	29.3 (1.21)	30 (0.00)
+26	Infected	57.3 (0.65)	27.3 (0.65)	30 (1.10)
	Control	59 (2.19)	28 (0.98)	30.7 (2.08)

TABLE 6: Mean (± SE) total serum protein, albumin and globulin values in infected (50 *H. contortus* larvae/kg BW) and control 6 month old Blackface lambs kept on a low protein diet.

Time (weeks)	Group	Total Protein g/l	Albumin g/l	Globulin g/l
0	Infected	63.2 (1.11)	32.8 (0.72)	30.4 (0.76)
	Control	65.3 (3.05)	34.0 (0.40)	31.3 (2.85)
+3	Infected	65.4 (1.30)	33.6 (1.03)	31.8 (1.07)
	Control	62.8 (2.95)	33.3 (1.25)	29.5 (2.25)
+6	Infected	67.2 (2.15)	30. 2 (1.16)	37.0 (1.34)
	Control	67.8 (0.50)	31.5 (0.50)	36.3 (0.85)
+12	Infected	65.2 (1.61)	30.0 (0.45)	34.0 (0.72)
	Control	62.8 (0.85)	32.0 (0.70)	30.8 (0.85)
+16	Infected	71.4 (1.81)	31.6 (0.67)	39.8 (0.98)
	Control	71.5 (0.85)	34.5 (1.05)	37.0 (0.70)
+18	Infected	68.2 (0.98)	30.0 (0.99)	38.2 (0.85)
	Control	71.5 (0.65)	32.0 (0.95)	39.5 (0.50)
+20	Infected	68.6 (1.16)	28.8 (0.72)	40.2 (0.58)
	Control	68.8 (0.08)	31.3 (0.75)	37.5 (0.65)
+22	Infected	68.8 (1.21)	28.0 (0.89)	41.2 (1.02)
	Control	66.8 (0.85)	29.8 (0.85)	37 (1.20)
+24	Infected	66.0 (1.39)	28.2 (1.61)	37.4 (1.57)
	Control	62.5 (0.50)	28.8 (1.30)	37.8 (1.45)
+26	Infected	66.2 (2.06)	26.0 (1.29)	41.2 (0.98)
	Control	63.5 (2.05)	28.8 (0.45)	34.8 (1.70)

TABLE 7: Mean (± SE) serum iron levels and % saturation of transferrin of 8 month old Merino and 6 month old Blackface lambs, kept on a low protein diet and infected with 50 H. contortus larvae/kg BW and their respective controls.

	Merino				Blackface			
	Infected		Control		Infected		Control	
	Serum Fe µg/dl	% saturation	Serum Fe µg/dl	% saturation	Serum Fe µg/dl	% saturation	Serum Fe µg/dl	% saturation
Pre-infection	132(7.9)	25(0.8)	150(6.2)	33(1.2)	159(4.5)	30(2.7)	154(3.8)	28(1.3)
Weeks post-infection 3	167(11.3)	37(3.2)	200(12.7)	42(2.5)	160(7.5)	35(0.6)	134(16.6)	28(2.1)
5	142(7.4)	28(2.4)	179(5.7)	33(2.1)	165(10.4)	33(1.0)	152(10.7)	29(1.6)
7	129(8.6)	24(5.2)	139(9.7)	31(1.5)	170(7.9)	34(3.9)	149(7.7)	25(3.3)
11	165(9.8)	32(3.5)	165(18.1)	35(1.9)	175(12.7)	39(3.3)	193(9.9)	29(2.1)
15	166(12.9)	29(2.9)	159(1.6)	40(1.5)	184(10.9)	48(2.4)	185(14.3)	45(1.1)
19	164(19.1)	32(4.8)	158(7.9)	40(1.5)	157(8.9)	59(6.8)	190(11.0)	68(4.0)
27	178(11.3)	29(2.2)	167(5.3)	45(5.6)	177(11.6)	72(10.3)	193(13.0)	78(3.7)

APPENDIX B

Appendix to Chapter II

TABLE 1: Principal haematological, parasitological and body weight changes in two Finn Dorset lambs infected with 125 *H. contortus* larvae/kg BW and fed either a high or low protein diet.

Weeks post infection		2	3	4	5	6	7	8	9	10	20
	Hct l/l	0.25	0.245	0.21	0.225	0.21	0.22	0.235	0.255	0.26	0.365
R17	MCH pg	12.0	11.7	12.1	13.2	12.2	12.5	12.2	11.8	12.2	10.8
High	MCV fl	32	33	35.5	40.5	33.5	33.5	34	34	33.5	33
Protein	e.p.g.	0	–	18,000	17,100	4,700	11,600	1,600	-	6,650	550
	BW (kg)	27.5	28	29.5	29.5	NS	29	30.5	32	33	37
	Hct l/l	0.28	0.20	0.14	0.16	0.155	0.14	0.13	0.12	Died 65 days post-infection	
B2	MCH pg	12.3	11.5	11.7	11.3	11.2	11.9	11.0	12.2		
Low	MCV fl	31.5	31	34.5	34.5	32.5	32.5	32.5	34		
Protein	e.p.g.	0	15,900	33,800	70,100	77,400	104,900	NS	NS		
	BW (kg)	28	27	26	25.5	23	21	19	18		

TABLE 2: Faecal egg counts (epg) and total daily faecal egg output in infected (125 H. contortus larvae/kg BW) Finn Dorset (FD) and Blackface (BF) lambs kept on either a high (HP) or low (LP) protein diet.

Type	Group		Weeks post-infection		
			+4 w	+5 w	+6 w
FD lambs	HP	e p g	10,350(4,463)	20,275(3,268)	5,600 (2,044)
		Total daily faecal eggs (x 10^6)	10 (4.8)	18 (3.9)	4 (1.9)
	LP	e p g	25,650(3,210)	32,975(12,500)	26,825 (17,074)
		Total daily faecal eggs (x 10^6)	20 (1.9)	21 (1.4)	5 (1.6)
BF lambs	HP	e p g	12,460(2,462)	15,960(1,827)	9,420(1,789)
		Total daily faecal eggs (x 10^6)	9 (1.7)	11 (0.9)	7 (1.3)
	LP	e p g	13,780(1,622)	16,030(2,734)	7,290(1,724)
		Total daily faecal eggs (x 10^6)	11 (2.0)	11 (1.7)	7 (1.5)

TABLE 3: Erythrokinetic studies 26-40 days post-infection in Finn Dorset lambs infected at the age of 4 months with 125 H. contortus larvae/kg BW and their respective controls. The lambs were fed either a high or low protein diet from the age of 3 months.

Group	No.	Hct. l/l	RCV(^{51}Cr) ml/kgBW	PV (^{125}I) ml/kgBW	TBV ml/kgBW	$T_{\frac{1}{2}}$(^{51}Cr) h	^{51}Cr Faecal Clearance mlRBC/d	$\bar{x}$ ± S.D.
High Protein Infected	R 11	0.22	8.6	42.0	50.0	176.9	31.7	(2.56)
	12	0.19	7.2	44.3	51.5	173.1	14.7	(3.08)
	17	0.22	7.7	42.7	50.4	203.8	13.7	(3.30)
	20	0.23	10.4	46.2	56.6	187.8	11.6	(1.14)
	$\bar{x}$	0.215	8.5	43.8	52.1	185.4	17.9	
	S.E.	(0.009)	(0.70)	(0.93)	(1.52)	(6.88)	(4.64)	
High Protein Control	R 13	0.255	20.2	44.3	64.5	314.5	0.50	(0.011)
	14	0.295	20.1	44.5	64.6	264.7	0.47	(0.013)
	15	0.295	19.2	42.7	61.9	297.1	0.32	(0.07)
	$\bar{x}$	0.28	19.8	43.8	63.7	292.1	0.43	
	S.E.	(0.013)	(0.32)	(0.57)	(0.88)	(14.59)	(0.056)	
Low Protein Infected	B 2	0.155	7.6	63.8	71.4	109.9	22.7	(3.77)
	4	0.20	7.4	44.2	51.6	148.3	31.1	(4.03)
	6	0.21	8.0	36.7	44.7	118.4	35.3	(2.87)
	9	0.225	7.1	41.7	48.8	123.5	33.0	(3.62)
	$\bar{x}$	0.20	7.5	46.6	54.1	125.0	30.5	
	S.E.	(0.015)	(0.19)	(5.94)	(5.93)	(8.25)	(2.75)	
Low Protein Control	B 3	0.31	19.7	41.0	60.7	368.4	0.28	(0.09)
	5	0.255	20.3	49.1	69.4	316.1	0.21	(0.06)
	7	0.285	21.7	42.0	63.7	253.1	0.35	(0.08)
	$\bar{x}$	0.28	20.6	44.0	64.6	312.5	0.28	
	S.E.	(0.016)	(0.59)	(2.55)	(2.55)	(33.33)	(0.040)	

TABLE 4: Erythrokinetic studies 26-40 days post-infection in Blackface lambs infected at age of 4 months with 125 H. contortus larvae/kg BW and their respective controls. The lambs were fed either a high or low protein diet from the age of 3 months.

Group	No.	Hct. l/l	RCV(^{51}Cr) ml/kgBW	PV(^{125}I) ml/kgBW	TBV ml/kgBW	$T_{\frac{1}{2}}$(^{51}Cr) h	^{51}Cr Faecal Clearance mlRBC/d	$\bar{x}$ ± S.D.
High Protein Infected	R41	0.285	13.0	52.6	65.6	91.0	26.9	(1.98)
	42	0.28	15.0	53.9	68.9	66.5	19.2	(3.33)
	60	0.29	16.4	58.5*	74.9	132.0	14.6	(1.17)
	45	0.28	17.8	57.6	75.4	155.1	13.8	(1.49)
	46	0.28	17.5	50.3	67.8	126.8	15.2	(1.31)
	$\bar{x}$	0.28	15.9	54.6	70.5	114.3	17.9	
	SE	(0.002)	(0.84)	(1.54)	(1.97)	(15.75)	(2.43)	
High Protein Control	R43	0.255	13.1	43.6	56.7	128.9	0.75	(0.17)
	44	0.29	16.4	47.1	63.5	99.9	0.86	(0.31)
	47	0.34	19.1	42.8	61.9	227.8	0.65	(0.16)
	48	0.265	14.0	49.4	63.4	115.9	0.51	(0.12)
	49	0.365	19.3	41.4	60.7	198.0	0.79	(0.15)
	$\bar{x}$	0.30	16.4	44.9	61.2	154.1	0.71	
	SE	(0.021)	(1.72)	(1.47)	(1.25)	(24.89)	(0.061)	
Low Protein Infected	B62	0.32	18.7 *	48.9	67.6	165.1	13.8	(3.06)
	63	0.19	12.0	55.4	67.4	47.0	15.6	(2.24)
	68	0.25	13.3	53.9	67.2	90.7	18.7	(1.61)
	78	0.22	12.4	50.6	63	141.2	13.7	(2.09)
	79	0.29	15.6	50.4	66	133.0	18.6	(2.71)
	$\bar{x}$	0.25	14.4	51.8	66.2	115.4	16.1	
	SE	(0.023)	(1.24)	(1.21)	(0.86)	(20.29)	(1.10)	
Low Protein Control	B56	0.33	17.1	41.3	58.4	205.0	0.56	(0.09)
	73	0.28	13.2	41.6	54.8	73.4	0.71	(0.33)
	80	0.30	14.7	43.5	58.2	158.1	0.51	(0.08)
	84	0.35	18.7	38.9	57.6	255.9	0.41	(0.09)
	85	0.275	13.2*	43.2	56.4	160.4	0.61	(0.10)
	$\bar{x}$	0.31	15.4	41.7	57.1	170.6	0.56	
	SE	(0.014)	(1.0)	(0.82)	(0.67)	(30.14)	(0.050)	

*Calculated with ^{59}Fe.

TABLE 5: Ferrokinetic studies 26-40 days post-infection in Finn Dorset lambs infected with 125 *H. contortus* larvae/kg BW at 4 months of age and their respective controls. The lambs were fed either a high or low protein diet from the age of 3 months.

Group	No.	Plasma Iron µg/dl	^{59}Fe $T\frac{1}{2}$ (m)	Total P.I.T. mg/d/kg BW	Day of maximum uptake of radio-iron
High Protein Infected	R11	214	48.7	1.84	10
	12	242	37.9	2.82	4
	17	203	45.0	1.92	6
	20	225	57.3	1.81	10
	$\bar{x}$	221	47.2	2.10	6
	S.E.	(8.3)	(4.04)	(0.50)	(1.5)
High Protein Control	R13	151	110	0.61	15
	14	199	121	0.73	15
	15	130	87	0.64	15
	$\bar{x}$	150	106	0.66	15
	S.E.	(20.4)	(10.0)	(0.036)	(0.0)
Low Protein Infected	B 2	108	48.7	1.41	2
	4	265	75.0	1.56	2
	6	211	42.6	1.81	2
	9	271	41.4	2.15	2
	$\bar{x}$	214	51.9	1.73	2
	S.E.	(37.7)	(7.9)	(0.16)	(0.0)
High Protein Control	B 3	143	95.8	0.61	14
	5	178	83.0	1.05	14
	7	204	89.4	0.96	10
	$\bar{x}$	175	89.4	0.87	13
	S.E.	(17.7)	(3.7)	(0.134)	(1.3)

TABLE 6: Ferrokinetic studies 26-40 days post-infection in Blackface lambs infected with 125 H. contortus larvae/kg BW at 4 months of age and their respective controls. The lambs were fed either a high or low protein diet from the age of 3 months.

Group	No.	Plasma Iron µg/dl	^{59}Fe $T\frac{1}{2}$ (m)	Total P.I.T. mg/d/kgBW	Day of maximum uptake of radioiron
High Protein Infected	R41	331	41.6	4.18	4
	42	258	46.2	3.00	8
	60	318	49.7	3.74	4
	45	327	47.9	3.92	4
	46	339	44.9	3.79	6
	$\bar{x}$	315	46.1	3.73	5
	S.E.	(14.5)	(1.38)	(0.197)	(0.5)
High Protein Control	R43	235	47.1	2.12	10
	44	235	59.6	1.85	8
	47	185	46.4	1.70	6
	48	176	75.3	1.17	15
	49	288	122.9	0.80	6
	$\bar{x}$	214	80.3	1.56	9
	S.E.	(13.4)	(14.2)	(0.228)	(1.7)
Low Protein Infected	B62	295	72.2	1.98	10
	63	148	35.6	2.30	4
	68	239	34.2	3.76	4
	78	188	46.4	2.05	4
	79	231	44.4	2.61	6
	$\bar{x}$	220	46.6	2.54	6
	S.E.	(24.8)	(6.84)	(0.324)	(1.2)
Low Protein Control	B56	245	91.5	1.10	14
	73	224	59.3	1.57	6
	80	169	92.9	0.79	10
	84	229	116.0	0.77	14
	85	172	65.4	1.13	15
	$\bar{x}$	208	85.0	1.07	12
	S.E.	(15.6)	(10.27)	(0.145)	(1.7)

TABLE 7: Albumin metabolism studies 26-40 days post-infection in Finn Dorset lambs infected with 125 H. contortus larvae/kg BW at 4 months of age, and their respective controls. The lambs were fed either a high or low protein diet from the age of 3 months.

Group	No.	Albumin g/l	PV ml/kg BW	CA g/kg BW	EA g/kg BW	EA/CA	^{125}I $T_{\frac{1}{2}}$ (h)	F(CA)
High Protein Infected	R11	31	42.0	1.30	1.42	1.09	290.5	0.153
	12	28	44.3	1.24	1.45	1.17	228.9	0.197
	17	29	42.7	1.24	1.51	1.21	247.9	0.126
	20	30	46.2	1.39	1.57	1.13	372.5	0.167
	$\bar{x}$	29.5	43.8	1.29	1.49	1.15	285.0	0.161
	S.E.	(0.65)	(0.93)	(0.035)	(0.033)	(0.026)	(31.9)	(0.015)
High Protein Control	R13	36	44.3	1.59	2.54	1.60	504.1	0.087
	14	36	44.5	1.60	2.76	1.72	499.2	0.085
	15	36	42.7	1.54	2.67	1.74	344.8	0.092
	$\bar{x}$	36	43.8	1.58	2.66	1.69	449.4	0.088
	S.E.	(0)	(0.57)	(0.019)	(0.064)	(0.044)	(52.3)	(0.003)
Low Protein Infected	B2	18	63.8	1.15	1.56	1.36	281.0	0.170
	4	24	44.2	1.06	1.18	1.11	267.3	0.159
	6	23	36.7	0.84	0.72	0.86	162.4	0.260
	9	25	41.4	1.04	1.12	1.08	202.8	0.206
	$\bar{x}$	22.5	46.6	1.02	1.15	1.10	223.4	0.199
	S.E.	(1.55)	(5.94)	(0.065)	(0.172)	(0.102)	(27.8)	(0.023)
Low Protein Control	B3	34	41.0	1.40	2.32	1.66	498.3	0.08
	5	30	49.1	1.47	2.30	1.57	259.0	0.05
	7	30	42	1.26	2.08	1.65	332.2	0.07
	$\bar{x}$	31.3	44.0	1.38	2.23	1.63	363.2	0.067
	S.E.	(1.33)	(2.55)	(0.062)	(0.077)	(0.028)	(70.8)	(0.009)

TABLE 8: Albumin metabolism studies 26–40 days post-infection in Blackface lambs infected with 125 H. contortus larvae/kg BW at 4 months of age, and their respective controls. The lambs were fed either a high or low protein diet from the age of 3 months.

Group	No.	Albumin g/l	PV ml/kg BW	CA g/kg BW	EA g/kg BW	EA/CA	^{125}I $T_{\frac{1}{2}}$ (h)	F(CA)
High Protein Infected	R41	32	52.6	1.68	2.96	1.76	227.1	0.119
	42	31	53.9	1.67	2.33	1.39	239.8	0.115
	60	34	58.5	1.99	3.58	1.80	276.1	0.069
	45	31	57.6	1.79	2.87	1.60	250.6	0.089
	46	34	50.3	1.71	2.73	1.60	190.0	0.134
	$\bar{x}$	32.4	54.6	1.77	2.89	1.63	236.7	0.105
	S.E.	(0.68)	(1.54)	(0.059)	(0.203)	(0.073)	(14.19)	(0.012)
High Protein Control	R43	37	43.6	1.58	2.42	1.53	323.3	0.092
	44	38	47.1	1.79	2.43	1.36	306.8	0.089
	47	37	42.8	1.58	2.10	1.33	336.7	0.075
	48	31	49.4	1.55	2.28	1.47	367.9	0.067
	49	35	41.4	1.45	2.19	1.51	250.0	0.130
	$\bar{x}$	35.6	44.9	1.59	2.28	1.44	316.9	0.091
	S.E.	(1.25)	(1.47)	(0.055)	(0.064)	(0.040)	(19.51)	(0.011)
Low Protein Infected	B62	33	48.9	1.60	2.50	1.56	301.0	0.084
	63	29	55.4	1.13	1.45	1.29	212.3	0.128
	68	26	53.9	1.40	1.71	1.22	238.3	0.104
	78	32	50.6	1.62	2.06	1.27	240.6	0.111
	79	26	50.4	1.31	1.72	1.31	298.3	0.102
	$\bar{x}$	29.2	51.8	1.41	1.89	1.33	258.1	0.106
	S.E.	(1.46)	(1.21)	(0.092)	(0.181)	(0.059)	(17.68)	(0.007)
Low Protein Control	B56	34	41.3	1.40	1.79	1.28	312.9	0.080
	73	32	41.6	1.33	2.09	1.57	303.4	0.083
	80	28	43.5	1.22	2.26	1.86	276.9	0.087
	84	34	38.9	1.32	2.04	1.55	326.1	0.074
	85	32	43.2	1.38	1.60	1.16	244.1	0.088
	$\bar{x}$	32	41.7	1.33	1.96	1.48	292.7	0.082
	S.E.	(1.10)	(0.82)	(0.031)	(0.117)	(0.122)	(14.57)	(0.003)

TABLE 9: Apparent digestibility coefficients (ADC) of the various proximate fractions of the experimental diets: infected (125 H. contortus larvae/kg BW) Finn Dorset lambs and their pair-fed controls (Mean ± SE values)

Group	DM	CP	CF	EE	ASH	NFE	OM
High Protein Infected	0.64 (0.018)	0.71 (0.015)	0.50 (0.036)	0.37 (0.277)	0.46 (0.005)	0.72 (0.012)	0.67 (0.020)
High Protein Pair-fed Control	0.71 (0.056)	0.77 (0.046)	0.59 (0.063)	0.36 (0.195)	0.49 (0.107)	0.77 (0.048)	0.73 (0.051)
Low Protein Infected	0.61 (0.028)	0.32 (0.060)	0.56 (0.047)	-0.12 (0.124)	0.28 (0.049)	0.74 (0.026)	0.65 (0.031)
Low Protein Pair-fed Control	0.67 (0.030)	0.50 (0.005)	0.56 (0.047)	0.44 (0.139)	0.49 (0.082)	0.77 (0.021)	0.69 (0.024)

TABLE 10: Apparent digestibility coefficients (ADC) of the various proximate fractions of the experimental diets: infected (125 *H. contortus* larvae/kg BW) Blackface lambs and their pair-fed controls (Mean ± SE values)

Group	DM	CP	CF	EE	ASH	NFE	OM
High Protein Infected	0.68 (0.004)	0.72 (0.005)	0.55 (0.030)	0.24 (0.090)	0.51 (0.020)	0.76 (0.014)	0.70 (0.004)
High Protein Pair-fed control	0.68 (0.012)	0.73 (0.011)	0.60 (0.034)	0.35 (0.050)	0.40 (0.029)	0.74 (0.009)	0.71 (0.010)
Low Protein Infected	0.66 (0.009)	0.49 (0.016)	0.57 (0.011)	-0.12 (0.309)	0.48 (0.019)	0.75 (0.008)	0.68 (0.008)
Low Protein Pair-fed Control	0.69 (0.018)	0.55 (0.023)	0.59 (0.026)	0.30 (0.78)	0.57 (0.033)	0.77 (0.012)	0.71 (0.017)

TABLE 11: Nitrogen (N) balance (g N/d): Infected (125 H. contortus larvae/kg BW) Finn Dorset lambs and their pair-fed controls(Mean ± SE values)

	INFECTED		PAIR-FED CONTROLS	
	High Protein	Low Protein	High Protein	Low Protein
Intake	22.4 (4.90)	8.98 (3.43)	22.05 (4.65)	8.77 (2.71)
Faeces	5.28 (0.64)	4.92 (1.99)	6.09 (1.09)	4.26 (1.28)
Urine	14.7 (2.09)	4.27 (0.39)	14.8 (1.01)	3.0 (0.18)
Retained	+ 2.45 (2.42)	- 0.21 (1.84)	+ 1.19 (2.77)	+ 1.51 (1.29)

TABLE 12: Nitrogen (N) balance (g N/d): infected (125 H. contortus larvae/kg BW) Blackface lambs and their pair fed controls (Mean ± SE values)

	Infected		Pair-fed Control	
	High Protein	Low Protein	High Protein	Low Protein
Intake	22.7 (0.08)	12.4 (0.26)	22.4 (0.08)	12.4 (0.26)
Faeces	6.3 (0.24)	6.4 (0.31)	5.9 (0.23)	5.5 (0.25)
Urine	12.5 (0.57)	3.6 (0.37)	12.7 (0.58)	2.9 (0.18)
Retained	3.9 (0.69)	2.4 (0.36)	3.8 (0.97)	4.0 (0.42)

APPENDIX C

Appendix to Chapter III

TABLE 1: Principal haematological, biochemical and parasitological changes in three fatal cases of haemonchosis. The lambs were fed either a high (HP) or low (LP) protein diet from the age of 3 month and were infected with 350 larvae/kg BW one month later.

		Pre-(-1w)	+ 3w	+4w	+ 5w	+ 6w	+ 8w	+ 9w	
FR 5 HP	PCV l/l	0.38	0.17	0.17	0.14	0.125*	0.13*	0.13	*Feed consumption - 346g./d.
	MCV fl	32	32	43	33	29.5	27	26	
	MCH pg	10.6	10.2	12.5	8.6	8.1	7.6	7.5	
	e.p.g.	0	15,000		83,400	52,400	73,900	112,800	Worm burden 3,400
	BW kg.	35	35	34	32.5	32	-	26.5	
	Serum Fe µg./dl.	161	113	-	-	76	41	47	Died 74d. post-infection
	% saturation	41	33	-	-	16	10	10	
	TP/alb. g/l	60/31	42/20	-	39/17	38/16	43/15	44/15	
P 5 LP	PCV l/l	0.34	0.19	clotted	0.15	0.13*	0.12*	0.11	*Feed consumption - 350g./d.
	MCV fl	31.5	32.5	"	31	30	30	29	
	MCH pg	10.7	11.1	"	9.4	9.1	9.8	9.4	
	e.p.g.	0	50,700	23,500	59,400	84,300	49,400	-	Worm burden 3,400
	BW kg.	34	34.5	33	33	32.5	-	27	
	Serum Fe µg/dl	204	76	-	44	51		53	Perforated ulcer in abomasum at death
	% saturation	43	22	-	10	13	-	17	
	TP/alb. g/l	61/29	43/22		39/19	37/17	38/17	36/17	
P 6 LP	PCV l/l	0.43	0.18	0.21	0.185	0.20	dead		Died after move to metabolism cages
	MCV fl	33	33.5	39.5	40.5	39			
	MCH pg	11.8	12.5	12.6	13.1	12.2			
	e.p.g.	0	13,800	31,660	47,100	61,200			
	BW kg.	30.5	28	27.5	27	26			Worm burden 3,300
	Serum Fe µg/dl	212	135	-	98	49			
	% saturation	48			17	15			
	TP/alb. g/l	67/27	46/17	-	45/18	44/17			

TABLE 2: Erythrokinetic studies 19-33 days after infection: Finn Dorset/Dorset Horn lambs fed either a high or low protein diet from the age of 3 months and infected with 350 H. contortus larvae/kg BW one month later and their respective controls.

Group	No.	Hct. l/l	RCV(^{51}Cr) ml/kg BW	PV(^{125}I) ml/kg BW	TBV ml/kg BW	T ½(^{51}Cr) (h)	^{51}Cr Clearance ml RBC/d	^{51}Cr Clearance $\bar{x}$ (S.D.)
High Protein Infected	FR 1	0.215	12.1	57.2	69.3	96.3	35.5	(5.62)
	2	0.20	10.3	49.7	60.0	85.3	31.5	(2.99)
	3	0.20	11.4	52.2	63.6	83.7	29.9	(3.86)
	4	0.21	9.6	48.9	58.5	87.8	27.0	(3.20)
	$\bar{x}$	0.21	10.9	52.0	62.9	88.3	31.0	
	SE	(0.004)	(0.56)	(1.87)	(2.40)	(2.81)	(1.77)	
High Protein Control	G 1	0.34	20.1	46.9	67.0	164.0	0.85	(0.033)
	2	0.34	17.6	40.0	57.6	193.0	0.63	(0.081)
	3	0.30	15.4	45.9	61.3	140.0	1.08	(0.092)
	4	0.33	16.7	39.8	56.5	251.0	0.26	(0.039)
	$\bar{x}$	0.33	17.5	43.2	60.6	187.0	0.71	
	SE	(0.009)	(0.99)	(1.89)	(2.37)	(23.93)	(0.17)	
Low Protein Infected	P 1	0.19	11.5	51.9	63.4	85.8	35.1	(4.68)
	2	0.16	8.4	51.2	59.6	70.0	36.8	(6.56)
	3	0.17	11.9	57.6	69.5	81.9	26.7	(8.62)
	4	0.18	9.8	51.9	61.7	59.8	22.2	(4.69)
	$\bar{x}$	0.175	10.4	53.2	63.6	74.4	30.2	
	SE	(0.007)	(0.81)	(1.49)	(2.13)	(5.91)	(3.46)	
Low Protein Control	Y 1	0.33	17.1	41.1	58.2	184.0	0.47	(0.091)
	2	0.33	17.8	40.3	58.1	211.0	0.73	(0.108)
	3	0.30	15.5*	43.8	59.3	152.0	0.39	(0.060)
	4	0.305	16.7	47.4	64.1	192.0	0.31	(0.040)
	$\bar{x}$	0.32	16.8	43.2	59.9	187.8	0.48	
	SE	(0.008)	(0.48)	(1.60)	(1.42)	(12.30)	(0.091)	

* Calculated using ^{125}I PV and correction for Hct.

TABLE 3: Ferrokinetic studies 19-33 days after infection: Finn Dorset/Dorset Horn lambs fed either a high or low protein diet from the age of 3 months and infected with 350 H. contortus larvae/kg BW one month later, and their respective controls.

Group	No.	Serum Fe on day of injection of ^{59}Fe µg/dl	$T_{\frac{1}{2}}$ ^{59}Fe (m)	P.I.T. mg/kg BW/d	Day of maximal uptake of radioiron
High Protein Infected	FR 1	191	46.4	2.32	4
	2	144	38.9	1.84	2
	3	151	45.7	1.72	2
	4	148	50.5	1.43	4
	$\bar{x}$	159	45.4	1.83	3
	SE	(10.93)	(2.40)	(0.185)	(0.6)
High Protein Control	G 1	196	63.6	1.44	10
	2	150	138.0	0.43	10
	3	171	31.7	2.47	14
	4	159	170.4	0.37	14
	$\bar{x}$	169	100.9	1.18	12
	SE	(9.98)	(32.13)	(0.496)	(1.2)
Low Protein Infected	P 1	168	34.7	2.51	2
	2	192	39.6	2.47	2
	3	167	38.1	2.52	4
	4	201	36.7	2.83	2
	$\bar{x}$	182	37.3	2.58	2.5
	SE	8.57	(1.04)	(0.083)	(0.5)
Low Protein Control	Y 1	193	67.8	1.16	14
	2	142	51.0	1.12	14
	3	188	49.8	1.65	14
	4	128	108.0	0.56	14
	$\bar{x}$	163	69.2	1.12	14
	SE	16.3	(13.59)	(0.223)	(0.0)

TABLE 4: Mean (±SE) daily gastro-intestinal loss and reabsorption of Hb-iron in lambs infected with 350 H. contortus larvae/kg BW and their uninfected controls. The lambs were fed either a high or low protein diet from the age of 3 months and were infected at 4 months of age.

Group	Intestinal blood loss (^{51}Cr faecal clearance) ml RBC/d	Hb-iron loss mg/d	Intestinal blood loss (^{59}Fe faecal clearance) ml RBC/d	Hb-iron loss mg/d	Reabsorption of Hb-iron mg/d
High Protein Infected	31.0 (1.77)	35 (3.6)	35.7 (2.55)	38 (3.9)	- 3.4 (1.51)
High Protein Control	0.71 (0.17)	0.8 (0.2)	1.9 (0.49)	3.9 (1.22)	- 3.1 (1.43)
Low Protein Infected	30.2 (3.46)	33.5 (4.56)	34.5 (3.99)	39 (6.5)	- 5.8 (2.24)
Low Protein Control	0.48 (0.91)	0.65 (0.160)	1.0 (0.05)	2.0 (0.17)	- 1.4 (0.30)

TABLE 5: Albumin metabolism studies 19-33 days after infection: Finn Dorset/Dorset Horn lambs fed either a high or low protein diet from the age of 3 months and infected with 350 H. contortus larvae/kg BW one month later and their respective controls.

Group	No.	Serum albumin g/l	PV(^{125}I) ml/kg BW	CA g/kg BW	EA g/kg BW	EA/CA	$T_{\frac{1}{2}}$(^{125}I) (h)	F(CA)
High Protein Infected	FR 1	25	57.2	1.41	2.50	1.77	130	0.121
	2	27	49.7	1.34	1.85	1.38	179	0.138
	3	25	52.2	1.31	2.35	1.79	149	0.172
	4	24	48.9	1.17	1.60	1.37	158	0.177
	$\bar{x}$	25	52.0	1.31	2.08	1.58	154	0.152
	SE	(0.6)	(1.87)	(0.050)	(0.211)	(0.117)	(10.2)	(0.0135)
High Protein Control	G 1	30	46.9	1.41	1.82	1.29	355	0.075
	2	31	40.0	1.24	1.66	1.34	364	0.072
	3	33	45.9	1.51	2.00	1.32	310	0.075
	4	31	39.8	1.23	2.00	1.62	325	0.081
	$\bar{x}$	31	43.2	1.35	1.87	1.39	339	0.076
	SE	(0.6)	(1.89)	(0.068)	(0.082)	(0.077)	(12.6)	(0.0019)
Low Protein Infected	P 1	16	51.9	0.83	1.50	1.80	118	0.191
	2	17	51.2	0.86	2.21	2.57	106	0.229
	3	12	57.6	0.69	1.04	1.50	99	0.213
	4	15	51.9	0.78	1.05	1.35	122	0.221
	$\bar{x}$	15	53.2	0.79	1.45	1.81	111	0.214
	SE	(1.1)	(1.49)	(0.037)	(0.275)	(0.272)	(5.3)	(0.0082)
Low Protein Control	Y 1	30	41.1	1.23	1.59	1.29	378	0.057
	2	32	40.3	1.29	2.02	1.56	454	0.056
	3	31	43.8	1.36	2.12	1.64	410	0.061
	4	32	47.4	1.52	1.85	1.22	480	0.061
	$\bar{x}$	31	43.2	1.35	1.90	1.43	431	0.059
	SE	(0.5)	(1.60)	(0.063)	(0.116)	(0.102)	(22.7)	(0.0013)

TABLE 6: Apparent digestibility coefficients (ADC) of the various proximate fractions of the experimental diets: infected (350 H. contortus larvae/kg BW) lambs fed either a high or low protein diet and their pair-fed controls (Mean (± SE) values)

Group	DM	CP	CF	EE	Ash	NFE	OM
High Protein Infected	0.68 (0.008)	0.72 (0.009)	0.63 (0.015)	0.36 (0.100)	0.45 (0.030)	0.73 (0.008)	0.70 (0.008)
High Protein Control	0.70 (0.019)	0.75 (0.013)	0.60 (0.026)	0.65 (0.042)	0.48 (0.040)	0.76 (0.017)	0.72 (0.017)
Low Protein Infected	0.68 (0.016)	0.46 (0.014)	0.68 (0.023)	0.20 (0.021)	0.36 (0.021)	0.69 (0.066)	0.67 (0.031)
Low Protein Control	0.70 (0.022)	0.53 (0.065)	0.65 (0.036)	-0.03 (0.36)	0.48 (0.032)	0.80 (0.020)	0.73 (0.020)

TABLE 7: Nitrogen (N) balance (gN/d): infected (350 H. contortus larvae/kg BW) lambs fed either a high or low protein diet and their pair-fed controls (Mean (± SE) values)

	Infected		Pair-fed controls	
	High Protein (n = 4)	Low Protein (n = 3)	High Protein (n = 4)	Low Protein (n = 3)
Intake	23.3 (0.00)	7.6 (0.40)	23.3 (0.00)	7.6 (0.40)
Faeces	6.5 (0.21)	4.1 (0.09)	5.8 (0.31)	3.6 (0.90)
Urine	12.9 (0.24)	4.4 (0.60)	14.6 (0.83)	4.2 (0.37)
Retained	+ 3.8 (0.44)	- 0.87 (0.371)	+ 3.0 (0.88)	- 0.18 (0.635)

TABLE 8: Daily water intake and output (ml) in lambs of 4 mths of age infected with 350 *H. contortus* larvae/kg BW and kept on either a high or low protein diet and their respective controls.

		BW(kg)	Intake Feed and H_2O	Urine(U)	Faeces(F)	Retained Intake (U + F)
High Protein Infected	FR 1	36.5	2683	781	688	1214
	2	32.5	2790	931	499	1360
	3	30	4249	2494	446	1309
	4	27.5	3400	1408	716	1276
	$\bar{x}$	32	3281	1404	587	1290
	SE	(1.9)	(359.4)	(387.3)	(67.4)**	(30.6)****
High Protein Control	G 1	41	2323	1194	320	809
	2	37	2489	1341	341	807
	3	35.5	2410	1510	380	520
	4	34.5	2663	1556	336	772
	$\bar{x}$	37	2471	1400	344	727
	SE	(1.42)	(72.4)	(82.8)	(12.7)	(69.5)
Low Protein Infected	P 1	33.5	1675	621	330	725
	2	30.5	1246	544	403	298
	4	17.5	2840	1847	399	594
	$\bar{x}$	27	1920	1004	377	539
	SE	(4.9)	(476.2)	(422.1)	(23.7)***	(126.3)
Low Protein Control	Y 1	35	1289	803	221	265
	2	35.5	1259	813	183	263
	4	25	1893	1411	217	264
	$\bar{x}$	32	1480	1009	207	264
	SE	(3.4)	(206.5)	(201.0)	(12.1)	(0.58)

* $P < 0.05$ ** $P < 0.02$ *** $P < 0.01$ **** $P < 0.001$

TABLE 9: Iron input and output in 4 month old lambs infected with 350 *H. contortus* larvae/kg BW and kept on either a high or low protein diet and their respective pair-fed controls.

Group	No.	Feed Iron mg/d	Faecal Iron (mg/d)			"Apparent" dietary iron absorption	% of dietary iron absorbed
			Total	Hb-iron calculated from ^{59}Fe faecal clearance	Non-Hb-iron		
High Protein Infected	FR 1	850	97	49	48	802	94
	2	850	78	36	42	808	95
	3	850	90	37	53	797	94
	4	850	94	30	64	786	93
	$\bar{x}$	850	90	38	52	798	94
	SE	(0)	(4.2)	(4.0)	(4.7)****	(4.7)****	(0.4)****
High Protein Control	G 1	850	111	3.6	107.4	743	87
	2	850	118	4.9	103.1	737	87
	3	850	90	0.7	89.3	761	89
	4	850	111	6.5	104.5	746	88
	$\bar{x}$	850	107	2.4	103.6	746	88
	SE	(0)	(5.9)	(1.1)	(5.08)	(5.1)	(0.5)
Low Protein Infected	P 1	802	63	55	8	794	99
	2	687	67	45	22	665	97
	(3) †	(339)	(28)	(28)	(0)	(339)	(100)
	4	687	61	29	32	655	95
	$\bar{x}$	725	64	43	21	705	97
	SE	(38.3)	(1.8)*	(7.6)	(7.0)**	(44.8)	(1.2)**
Low Protein Control	Y 1	802	173	1.8	171.2	631	79
	2	687	313	1.7	311.3	376	55
	3						
	4	687	179	2.5	176.5	511	74
	$\bar{x}$	725	222	2.0	219.7	506	69
	SE	(38.3)	(45.7)	(0.25)	(45.84)	(73.7)	(7.3)

† Sheep P3 excluded from group mean. *P < 0.05 **P < 0.02 ***P < 0.01 ****P < 0.001

TABLE 10: Carcase evaluation : female lambs infected with 350 H. contortus larvae/kg BW and their uninfected controls. The lambs were fed either a high or low protein diet from the age of 3 months and they were infected when 4 months old.

Group	n	% Muscle	% Bone	% Fat
High Protein Infected (mean ± SE)	3	44 (2.7)	17 (1.8)	39 (1.6)
High Protein Control (mean ± SE)	4	48 (1.8)	15 (1.7)	37 (2.9)
Low Protein Infected (individual values)	P7	53	18	29
	P8	35	31	34
Low Protein Control (mean ± SE)	4	48 (1.9)	16 (1.1)	36 (1.7)

APPENDIX D

Appendix to Chapter IV

TABLE 1: Principal haematological, biochemical and parasitological findings in four lambs fed a low protein diet and infected with 200 H. contortus larvae three times weekly. All four lambs died or were euthanased in extremis before the end of the experiment.

Sheep No. and Hb Type		Pre-infection (- 2 w)	Post-infection (+ 3 w)	Post-infection (+ 6 w)	Post-infection (+ 10 w)	Death /Euthanased	
B 23 (AB)	Hct (l/l)	0.40	0.315	0.21	0.16	0.13	at 11-12 weeks
	MCV (fl)	32	30.5	29.5	31	32	
	epg	0	5,300	9,400	70,600	91,800	
	TP/albumin (g/l)	66/32	65/29	73/32	46/17	46/17	
	Serum Fe (µg/dl)	172	116	116	40	-	2,150 worms
	% saturation (%)	NA	26	29	12	-	
	BW (kg)	34	32.5	31	26	23.5	
B 24 (B)	Hct (l/l)	0.39	0.36	0.24	0.205	0.19	at 13-14 weeks
	MCV (fl)	34	34	33	38	36	
	epg	0	2,700	-	19,400	36,000	
	TP/albumin (g/l)	66/32	63/28	64/28	52/18	54/16	
	Serum Fe (µg/dl)	181	181	141	72	340	3,250 worms
	% saturation (%)	NA	47	42	24	73	
	BW (kg)	32	35.5	33	30	25.5	
B 26 (AB)	Hct (l/l)	0.36	0.27	0.21		0.10	at 10-11 weeks
	MCV (fl)	34.5	35	37		38	
	epg	0	5,200	11,700		52,300	
	TP/albumin (g/l)	66/28	58/24	51/20		45/14	
	Serum Fe (µg/dl)	259	229	180		97	4,200 worms
	% saturation (%)	NA	59	45		36	
	BW (kg)	20.5	23	20.5		16.5	
B 29 (B)	Hct (l/l)	0.40	0.29	0.20		0.10	at 9-10 weeks
	MCV (fl)	33	32.5	31		38	
	epg	0	5,800	-		32,100	
	TP/albumin (g/l)	60/27	53/23	46/17		31/14	
	Serum Fe (µg/dl)	180	138	124		-	500 worms
	% saturation (%)	NA	29	31		-	
	BW (kg)	23	24	21.5		17	

TABLE 2: Erythrokinetic studies 12-14 weeks after initial infection in lambs repeatedly infected with H. contortus (200 larvae/three times weekly) from the age of 4 months and their uninfected controls. The lambs were fed either a high or low protein diet from the age of 3 months.

Group	No.	Hct l/l	RCV(^{51}Cr) ml/kg BW	PV(^{125}I) ml/kg BW	TBV ml/kg BW	$T_{\frac{1}{2}}$ (^{51}Cr) (h)	^{51}Cr Clearance ml RBC/d ($\bar{x}$ ± SD)
High Protein Infected	R71	0.215	12.2	44.0	56.2	109	17.9 (3.58)
	R72	0.245	11.1	55.6	66.7	93	24.4 (3.12)
High Protein Infected	R73	0.265	15.0	41.2	56.2	140	6.6 (0.53)
	R74	0.30	15.9	42.4	58.3	165	0.6 (0.05)
High Protein Control	$\bar{x}$	0.33	17.6	43.2	61.3	187.0	0.71
	±SE	(0.009)	(1.13)	(1.89)	(3.07)	(23.9)	(0.17)
Low Protein Infected	B24	0.175	10.4	54.8	65.2	107	25.5 (4.23)
	B25	0.18	11.5	60.4	71.9	97	29.1 (2.47)
Low Protein Control	$\bar{x}$	0.32	16.8	43.2	59.9	187.8	0.48
	±SE	(0.008)	(0.49)	(1.59)	(1.41)	(12.30)	(0.091)

TABLE 3: Ferrokinetic studies 12-14 weeks after initial infection in lambs repeatedly infected with H. contortus (200 larvae/three times weekly) from the age of 4 months and their uninfected controls. The lambs were fed either a high or low protein diet from the age of 3 months.

Group	No.	Serum iron on day injection of ^{59}Fe µg/dl	$T_{\frac{1}{2}}$ ^{59}Fe (M)	P.I.T. mg/kg BW/ d	Day of maximal uptake of ^{59}Fe
High Protein Infected	R71	197	50.4	1.71	2
	72	133	33.0	2.24	4
	73	91	38.4	0.98	6
	74	183	84.0	0.92	8
High Protein Controls (x ± SE)	"G" gp	169 (9.98)	100.9 (32.13)	1.18 (0.496)	12 (1.2)
Low Protein Infected	B24	74	33	1.23	4
	B25	190	78	1.43	4
Low Protein Controls (x ± SE)	"Y" gp	163 (16.3)	69.2 (13.59)	1.12 (0.223)	14 (0.0)

TABLE 4: Daily gastro-intestinal loss and reabsorption of Hb-iron in lambs repeatedly infected with small numbers of H.contortus(200 larvae/3/week) and their uninfected controls

Group		Intestinal Blood loss (^{51}Cr faecal clearance) ml RBC/d	Hb-iron loss mg/d	Intestinal blood loss (^{59}Fe faecal clearance) ml RBC/d	Hb-iron loss mg/d	Reabsorption of Hb-iron
High Protein Infected	R 71	17.9	21	16.9	20.2	+ 0.8
	72	24.4	27	25.7	28.0	- 1.0
	73	6.6	9	6.7	9.1	- 0.1
	74	0.6	0.8	1.2	1.6	- 0.8
High Protein Controls	$\bar{x}$	0.71	0.8	1.9	3.9	- 3.1
	SE	(0.17)	(0.2)	(0.49)	(1.22)	(1.43)
Low Protein Infected	B 24	2.55	33	26.2	34	- 1.0
	B 25	29.1	40	29.6	40.5	- 0.5
Low Protein Controls	$\bar{x}$	0.48	0.65	1.0	2.0	- 1.4
	SE	(0.091)	(0.160)	(0.05)	(0.17)	(0.30)

TABLE 5: Albumin metabolism studies 12-14 weeks after initial infection in lambs repeatedly infected with H. contortus (200 larvae/three times weekly) from the age of 4 months and their uninfected controls. The lambs were fed either a high or low protein diet from the age of 3 months.

Group	No.	Serum Albumin g/l	PV(^{125}I) ml/kg BW	CA g/kg BW	EA g/kg BW	EA/CA	$T_{\frac{1}{2}}$(^{125}I) (h)	F(CA)
High Protein Infected	R71	23	44.0	1.01	1.33	1.32	207	0.120
	R72	18	55.6	1.00	1.18	1.18	185	0.128
High Protein Infected	R73	25	41.2	1.03	1.05	1.02	310	0.085
	R74	30	42.4	1.27	1.44	1.13	360	0.079
High Protein Control	$\bar{x}$	31	43.2	1.35	1.87	1.39	339	0.076
	±SE	(0.6)	(1.89)	(0.068)	(0.082)	(0.077)	(12.6)	(0.0019)
Low Protein Infected	B23	17	68.8	1.10	N.A.	N.A.	N.A.	N.A.
	B24	18	54.8	0.99	1.01	1.02	190	0.105
	B25	18	60.4	1.09	0.72	0.66	204	0.098
Low Protein Control	$\bar{x}$	31	43.2	1.35	1.90	1.43	431	0.059
	±SE	(0.5)	(1.60)	(0.063)	(0.116)	(0.102)	(22.7)	(0.0013)

TABLE 6: Apparent digestibility coefficients (ADC) of the various proximate fractions of the experimental diets: lambs fed a high protein diet and infected with 200 H. contortus larvae/three times weekly and their pair-fed controls (Mean (± SE) values)

Group	DM	CP	CF	EE	ASH	NFE	OM
High Protein Infected	0.70 (0.006)	0.76 (0.004)	0.61 (0.011)	0.24 (0.132)	0.50 (0.015)	0.75 (0.005)	0.72 (0.006)
High Protein pair-fed controls	0.70 (0.019)	0.75 (0.013)	0.60 (0.026)	0.65 (0.042)	0.48 (0.040)	0.76 (0.017)	0.72 (0.017)

TABLE 7: Nitrogen balance (mean ± SE)(gN/d) in lambs fed a high protein diet and given repeated infections of *H. contortus* (200 larvae three days weekly) and their pair-fed controls

Group	High Protein Infected (4)	High Protein Pair-fed Controls (4)
Intake	23.3 (0.00)	23.3 (0.00)
Faeces	5.6 (0.09)	5.8 (0.31)
Urine	11.3 (0.55)	14.6 (1.65)
Retained	6.4 (0.58)	2.9 (0.88)

TABLE 8 : Daily water intake and output (ml) in lambs infected with 200 H. contortus larvae/three times weekly and their uninfected controls. The lambs were fed either a high or low protein diet from the age of 3 months and infection was started one month later.

	Intake (Feed and H_2O)	Urine (U)	Faeces (F)	Intake - (U + F)
R 71	2,250	1,117	401	732
R 72	2,495	1,281	509	705
R 73	2,925	1,049	506	1,370
R 74	3,713	2,090	421	1,202
Mean control values (High Protein)(± SE)	2,471 (72.4)	1,400 (82.8)	344 (12.7)	727 (69.5)
B 24	2,793	2,053	473	267
B 25	3,036	1,373	307	1,356
Mean control values (Low Protein) (± SE)	1,480 (206.5)	1,009 (201.0)	207 (12.1)	264 (0.58)

TABLE 9: Iron input and output in lambs infected with 200 H. contortus larvae three times weekly and their uninfected pair-fed controls. The lambs were fed either a high or low protein diet from the age of three months and infection started one month later.

Group	No.	Dietary iron intake (mg/d)	Total faecal iron (mg/d)	Faecal Hb-iron (mg/d)(^{59}Fe)	Non-Hb faecal iron (mg/d)	Apparent dietary Fe absorption	% of dietary iron absorbed
High Protein Infected	R71	850	126	21.0	105	745	88
	72	850	97	27.0	70	780	92
	73	850	87	9.0	78	772	91
	74	850	120	0.8	119.2	730.8	86
	$\bar{x}$	850	107.5	17.8	93.1	757	89.3
	SE	(0)	(9.26)	(7.40)	(11.49)	(11.4)	(1.38)
High Protein pair-fed controls	$\bar{x}$	850	107.3	3.9	103.6	746.4	88
	SE	(0)	(5.02)	(1.23)	(5.08)	(5.08)	(0.5)
Low Protein infected	B25	1,145	130	40	90	1,055	92
Low Protein pair-fed control	Y 3	1,145	113	2.0	111	1,034	90

TABLE 10: Carcase evaluation: female lambs infected with 200 H. contortus larvae/ three times weekly for 17 weeks and their uninfected controls. The lambs were fed either a high or low protein diet from the age of 3 months and infection started one month later.

	% Muscle	% Bone	% Fat
High Protein Infected (n=4)	47.1 (3.3)	15.7 (1.5)	37.2 (3.2)
High Protein Controls (n=4)	47.7 (1.8)	15.3 (1.7)	37.0 (2.9)
Low Protein Infected (n=3)	40.9 (3.4)	20.7 (1.9)	38.4 (4.5)
Low Protein Controls (n=4)	47.5 (1.9)	15.6 (1.1)	36.2 (1.7)

APPENDIX E

Appendix to Chapter VI

TABLE 1: Mean (± SE) haematological values and body weights of vaccinated (2 x 10,000 γ-irradiated larvae one month apart) lambs fed either a high or low protein diet, and their respective challenge controls. Vaccination started when the lambs were 8 months of age. Eight months later all lambs were challenged with 10,000 normal H. contortus larvae.

Week	Group	Hb g/dl	RCC x 10^{12}/	Hct l/l	MCH pg	MCV fl	MCHC g/dl	Bodyweight kg.
- 1	HP-Vacc	13.5(0.62)	12.1(0.58)	0.39(0.019)	11.2(0.22)	32.4(0.51)	34.6(0.49)	
	HP-CC	13.9(0.25)	12.4(0.31)	0.42(0.008)	11.2(0.16)	33.7(0.78)	33.0(0.63)	
	LP-Vacc	14.9(0.27)	12.8(0.31)	0.43(0.001)	11.6(0.21)	33.3(0.77)	34.9(0.50)	
	LP-CC	14.2(0.22)	12.6(0.30)	0.43(0.010)	11.3(0.18)	34.0(0.65)	33.3(0.48)	
0	HP-Vacc	13.0(0.62)	11.4(0.46)	0.365(0.015)	11.3(0.14)	32.0(0.35	35.3(0.87)	28.3(1.72)
	HP-CC	13.5(0.25)	12.0(0.37)	0.39(0.010)	11.3(0.21)	32.7(0.80)	34.5(0.34)	28.3(2.21)
	LP-Vacc	13.6(0.08)	12.0(0.21)	0.38(0.004)	11.3(0.21)	31.4(0.75)	36.0(0.34)	28.7(3.0)
	LP-CC	12.6(0.27)	11.4(0.37)	0.36(0.008)	11.0(0.16)	31.7(0.23)	34.9(0.23)	27.3(1.16)
+ 1	HP-Vacc	13.4(0.47)	12.1(0.40)	0.39(0.013)	11.1(0.05)	32.1(0.29)	34.5(0.36)	
	HP-CC	13.4(0.20)	12.2(0.27)	0.41(0.006)	11.1(0.14)	33.3(0.53)	32.9(0.31)	
	LP-Vacc	14.0(0.14)	12.3(0.32)	0.40(0.008)	11.4(0.29)	32.7(0.83)	34.7(0.43)	
	LP-CC	13.2(0.26)	11.7(0.36)	0.38(0.006)	11.3(0.36)	32.4(0.51)	34.6(0.83)	
+ 2	HP-Vacc	13.0(0.63)	11.7(0.49)	0.38(0.015)	11.1(0.22)	32.5(0.74)	34.3(0.77)	30.2(2.42)
	HP-CC	13.7(0.88)	12.5(0.29)	0.41(0.004)	11.0(0.24)	32.9(0.64)	33.3(0.41)	29.2(2.63)
	LP-Vacc	13.3(0.41)	12.1(0.33)	0.40(0.013)	11.0(0.26)	32.6(0.92)	33.5(0.15)	28.9(3.66)
	LP-CC	12.7(0.20)	11.8(0.30)	0.385(0.008)	10.8(0.22)	32.5(0.61)	33.1(0.41)	29.5(1.08)
+ 3	HP-Vacc	12.6(0.56)	11.5(0.49)	0.36(0.02)	10.9(0.18)	31.1(0.37)	35.0(0.22)	31.3(2.02)
	HP-CC	13.6(0.33)	12.4(0.34)	0.40(0.01)	11.0(0.21)	32.6(0.83)	33.8(0.44)	32.7(2.46)
	LP-Vacc	13.2(0.48)	11.8(0.34)	0.38(0.01)	11.1(0.30)	32.2(0.84)	34.4(0.14)	30.6(3.53)
	LP-CC	13.1(0.16)	11.9(0.32)	0.39(0.01)	11.0(0.24)	32.2(0.56)	34.0(0.2)	29.0(0.95)
+ 4	HP-Vacc	12.2(0.49)	10.9(0.44)	0.35(0.01)	11.2(0.19)	31.7(0.43)	35.0(0.31)	
	HP-CC	13.0(0.28)	11.4(0.26)	0.37(0.01)	11.4(0.13)	32.2(0.72)	35.2(0.77)	
	LP-Vacc	12.6(0.39)	11.0(0.20)	0.35(0.01)	11.4(0.30)	32.1(0.93)	35.8(0.44)	
	LP-CC	12.5(0.16)	11.3(0.32)	0.36(0.01)	11.1(0.22)	32.4(0.75)	34.5(0.76)	
+ 5	HP-Vacc	12.7(0.25)	11.3(0.29)	0.38(0.01)	11.3(0.14)	31.7(0.58)	35.5(0.49)	31.7(1.86)
	HP-CC	13.3(0.22)	11.9(0.33)	0.38(0.01)	11.2(0.23)	32.1(0.73)	34.8(0.35)	31.5(2.94)
	LP-Vacc	12.4(0.25)	10.8(0.21)	0.34(0.01)	11.3(0.21)	31.9(0.78)	35.5(0.25)	31.4(3.52)
	LP-CC	12.2(0.25)	11.0(0.15)	0.35(0.01)	11.1(0.27)	31.8(0.64)	34.8(0.31)	30.1(1.23)

Continued overleaf

TABLE 1 (contd.):

Week	Group	Hb g/dl	RCC x 10^{12}/	Hct l/l	MCH pg	MCV fl	MCHC g/dl	Bodyweight kg
+ 6	HP-Vacc	11.6(0.30)	10.6(0.30)	0.34(0.01)	11.0(0.17)	31.8(0.58)	34.6(0.41)	34.7(0.29)
	HP-CC	12.1(0.41)	11.0(0.38)	0.36(0.01)	11.0(0.31)	32.6(0.87)	33.8(1.07)	34.5(2.67)
	LP-Vacc	12.4(0.30)	10.7(0.21)	0.36(0.01)	11.6(0.26)	33.4(0.98)	34.5(0.47)	33.6(3.49)
	LP-CC	12.0(0.31)	10.9(0.13)	0.36(0.01)	11.0(0.24)	32.9(0.72)	33.2(0.13)	32.0(1.24)
+ 7	HP-Vacc	11.4(0.18)	10.5(0.20)	0.32(0.01)	10.9(0.18)	30.6(0.51)	35.5(0.38)	35.4(2.25)
	HP-CC	12.0(0.23)	11.1(0.24)	0.36(0.01)	10.8(0.24)	32.1(0.55)	33.8(0.44)	34.1(2.79)
	LP-Vacc	11.6(0.57)	10.2(0.40)	0.34(0.02)	11.3(0.25)	33.1(0.82)	34.1(0.41)	34.6(3.92)
	LP-CC	11.6(0.12)	10.6(0.14)	0.34(0.01)	11.0(0.25)	32.6(0.58)	34.0(0.58)	33.2(1.14)
+ 8	HP-Vacc	11.8(0.30)	10.8(0.33)	0.38(0.010)	11.0(0.17)	32.3(0.58)	34.1(0.27)	34.1(2.46)
	HP-CC	11.9(0.38)	11.0(0.25)	0.36(0.010)	10.9(0.29)	32.7(0.80)	33.1(0.52)	35.0(2.58)
	LP-Vacc	11.9(0.34)	10.3(0.24)	0.345(0.012)	11.6(0.26)	33.5(0.97)	34.7(0.34)	34.9(3.91)
	LP-CC	11.7(0.23)	10.6(0.18)	0.35(0.008)	11.0(0.19)	33.0(0.63)	33.2(0.115)	33.3(1.49)
+ 10	HP-Vacc	11.1(0.48)	10.2(0.47)	0.315(0.011)	10.9(0.18)	31.0(0.79)	35.3(0.57)	37.5(2.33)
	HP-CC	10.9(0.30)	10.3(0.27)	0.33(0.009)	10.6(0.255)	32.2(0.73)	33.0(0.39)	38.1(3.08)
	LP-Vacc	11.7(0.33)	10.5(0.27)	0.345(0.016)	11.2(0.16)	32.5(0.77)	34.1(0.68)	34.3(3.30)
	LP-CC	8.7(0.38)	8.3(0.47)	0.27(0.010)	10.5(0.23)	32.4(1.03)	32.6(0.82)	36.1(1.44)
+ 11	HP-Vacc	11.0(0.45)	9.8(0.51)	0.31(0.015)	11.3(0.20)	32.5(0.59)	35.2(0.85)	38.7(2.55)
	HP-CC	10.1(0.29)	10.1(0.40)	0.30(0.007)	10.1(0.62)	33.6(0.58)	34.0(0.64)	39.0(2.84)
	LP-Vacc	11.9(0.39)	10.3(0.36)	0.34(0.011)	11.6(0.17)	33.0(0.57)	35.4(0.24)	38.0(4.07)
	LP-CC	7.7(0.38)	6.4(0.51)	0.23(0.009)	12.1(0.42)	36.7(1.65)	33.3(0.51)	36.9(1.36)
+12	HP-Vacc	11.2(0.58)	10.0(0.51)	0.31(0.014)	11.2(0.25)	31.3(0.68)	36.1(0.65)	38.1(2.33)
	HP-CC	10.5(0.34)	9.4(0.36)	0.32(0.010)	11.2(0.37)	33.8(0.34)	33.2(0.97)	39.1(3.23)
	LP-Vacc	12.0(0.27)	10.3(0.20)	0.34(0.009)	11.6(0.21)	33.1(0.60)	35.3(0.36)	36.8(3.76)
	LP-CC	8.5(0.44)	6.7(0.62)	0.25(0.012)	12.9(0.54)	38.5(1.66)	33.9(0.25)	38.5(1.99)

Key: HP-Vacc = High Protein Vaccinated
HP-CC = High Protein Challenge Control
LP-Vacc = Low Protein Vaccinated
LP-CC = Low Protein Challenge Control

Week 0 - 1st Vaccination

Week +4 - 2nd Vaccination

Week +8 - Challenge